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Serological Investigation of Human Brucellosis in Pyrexia of Unknown Origin Patients

Renu Kumari[1], Raj Kumar Kalyan[#1], Shivangi Tripathi[1], Kamlesh Kumar Gupta[2], Sanjeev Kumar Verma[3]

[1]Department of Microbiology, King George's Medical University, Lucknow.

[2]Department of Medicine, King George's Medical University, Lucknow.

[3]Department of Pediatrics, King George's Medical University, Lucknow.

#Corresponding Author:

Professor, Department of Microbiology, King George's Medical University, Lucknow, U.P., India, Pin code 226003.

Email ID: Profkalyankgmu@gmail.com

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ABSTRACT

Introduction: The objective of our study was to assess the seroprevalence of brucellosis though the different serological tests. Brucellosis is the one of the major global zoonoses that affects many nations, including India.

Material and Methods: The study period for this study was two years and samples were collected from the patients attending different wards at a tertiary care hospital King George's Medical University, Lucknow in Uttar Pradesh, Northern India. The study design for this study was hospital based. Serum samples from 275 distinct cases of Pyrexia of Unknown Origin (PUO) were collected. Rose-Bengal plate test (RBPT), serum agglutination test (SAT), ELISA IgM and ELISA IgG, were used for the serological analysis of the samples.

Results: *Brucella* antibodies were detected in 70 (25.45%), 72 (26.18%), 31 (11.27%) and 5 (1.8%) of sera by RBPT, SAT for B. abortus, SAT for B. melitensis, ELISA IgM and ELISA IgG, respectively.

Conclusion: Risk factors such as contact with animals and animal products were significantly linked with brucellosis. Further epidemiology studies are warranted in such regions of endemicity to determine accurate estimates of prevalence and risk factors and to study Brucella biovars for appropriate policymaking and advocacy and awareness regarding brucellosis in Northeast India.

Keywords: *RBPT- Rose Bengal Plate test, SAT-Serum Agglutination Test, ELISA IgM- Enzyme linked immunosorbent assay Immunoglobulin M, ELISA IgG- Enzyme linked immunosorbent assay Immunoglobulin G, PUO- Pyrexia of unknown origin.*

1. INTRODUCTION

The genus Brucella contains facultative intracellular Gram-negative coccobacilli that cause human brucellosis, a neglected and reemerging zoonotic disease that has a significant impact on public health even though many countries have successfully implemented eradication and control programs for domestic animals [1-3]. It is primarily an occupational illness that has been documented in meat inspectors, farmers, veterinary professionals, slaughterhouse employees, and animal handlers. It is brought on by tiny, Gram-negative, nonspore-forming, nonencapsulated coccobacilli that are members of the genus Brucella [4]. The illness has been widely recognized since antiquity, and Hippocrates' works from 450 BC provide evidence of this. From his residence in Malta, J. A. Marston initially defined brucellosis in the 19th century in 1861 as Mediterranean gastric remittent fever (Marston, 1861) [5]. However, the bacterium was identified as Micrococcus melitensis by Sir David Bruce in 1887 when he recovered it from the spleen of a British soldier who had died of Mediterranean fever in Malta. In honor of Sir David Bruce, it was later called Brucella melitensis [6].

There are currently 12 species in the latter, and five of them—B. suis, B. ovis, B. melitensis, B. abortus, and infrequently, B. canis are more frequently linked to human illness. In the EU, B. melitensis is the most virulent and has the biggest impact on public health since it is most common in small ruminant herds [7 &8].

Direct contact with infected animals, consumption of unpasteurized dairy products, or inhalation of aerosols are the three ways that the disease is spread [9].

Human brucellosis can affect people of any age or gender. It usually shows up as a range of symptoms and indicators, such as headache, weakness, chills, chills, weight loss, intermittent or irregular fever, and overall discomfort. Differentiating this syndrome from other feverish conditions is challenging due to its vague clinical symptoms. But, if the illness is not identified and treated right away, it could develop into a chronic condition that lasts for years and causes problems like disorders of the central nervous system, hepatobiliary, cardiovascular, and osteoarticular systems [10].

Human brucellosis is still difficult to diagnose clinically, particularly in developing nations. Culture, slide or tube agglutination, indirect Coombs, enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody (IFA), and the application of molecular techniques like polymerase chain reaction (PCR) are among the Brucella-specific tests (Araj, 2003). The Standard Tube Agglutination Test (STAT), ELISA, and Rose Bengal Plate Test (RBPT) are the three most popular serological tests. Additionally, a variety of PCR assays have been created for the quick identification of Brucella species [11].

The objective of this study was to investigate the result of seroprevalence of brucellosis in patients suffering from pyrexia of unknown origin.

2. MATERIALS AND METHODS

2.1. Place of work

This study was conducted at the department of Microbiology, King George's Medical University, Lucknow.

2.2. Selection of human subjects and study area

The prospective study was conducted in Uttar Pradesh state of Northern India during the year January 2023 to September 2024. Using a structured questionnaire all cases that fulfilled the inclusion criteria were enrolled. Demographic details (Name, age, sex of the patient, occupation, address), duration of illness, date of admission, clinical symptoms, and signs, travel history, animal exposure, contact with a patient of similar illness, contact with livestock, handling of the clinical specimen.

The study was ethically approved by the Institutional Ethical Committee, King George's Medical University Lucknow (UP), India. The participants were informed about the objectives of this study and a written consent was sought before being enrolled in the study.

2.3. Sampling

A total of 275 blood samples were collected from the patients having with pyrexia of unknown origin attending outdoor and indoor department of King George's Medical University, Lucknow. The 5 ml of blood sample was aseptically collected from each subject. serum was separated from clotted blood by centrifuging for 10 minutes at 3000 rpm. Separated serum was collected in screw-caped sterilized vials and stored at -20^{o}C till used.

2.4 Serological techniques

Rose Bengal Plate Test (RBPT) and Serum Agglutination test (SAT) were the conventional serological tests used for screening of the serum samples. The RBPT kit were procured from the Linear chemicals, spain and stored at 4^{o}C until use. The presence of agglutination indicates positive result for an antibody anti Brucella concentration equal or greater than 25 IU/ml. Serum Agglutination test (SAT) antigen were procured from Atlas medical; Germany and a titre of 80 IU was considered positive. the commercially available IgG and IgM ELISA kits (Novatec, Germany) were also used to test serum samples. The IgG and IgM ELISA was performed as per the manufacturer's instructions.

3. RESULTS

In the present study, a total of 275 sera samples originated from patients with history of PUO etc. All the samples were subjected to RBPT, STAT and ELISA IgM and ELISA IgG. Upon analysis of 275 human serum samples, an overall prevalence 11.27% (table 1) of was obtained whereas 70 (25.45%), 72 (26.18%), 31 (11.27%), 5 (1.81%) samples were found positive to RBPT, STAT, ELISA IgM, and ELISA IgG, respectively, in individual test (table 1).

In case of humans, out of three age groups of <18,18-59, and >60 persons recorded highest prevalence with the values as 11 (15.71%), 52 (74.29%), 7 (10.0%), 11 (15.27%), 54 (75.0%), 7 (9.72%), 6 (19.35%), 18 (58.06%), 5 (16.12%) and 0, 2 (40.0%), 3 (60.0%) for RBPT, STAT ELISA IgM and ELISA IgG, respectively Conversel, in humans, females observed higher prevalence over males (Table 4).

Table 1 Test wise overall prevalence

Name of test	No. of patients	Prevalence
RBPT	70	25.45%
SAT	72	26.18%
ELISA IgM	31	11.27%
ELISA IgG	5	1.81%

Table 2: Clinical and demographic data of the 275 brucellosis patients analyzed

Number of the patients (%)	
Gender	
Male	150
Female	125
Age	
Children >18	69
Adult (18-59)	177
Elder (>60)	29
Clinical Data	
Fever	
Headache	275
Chills	129
Night sweats	105
Myalgia	119
Arthralgia	31
	175

Table 3: Complications

Complications	**Frequency**	**Percentage (%)**
Joint Pain		
Yes	175	63.63
No	100	36.37
Neurological		
Yes	8	2.91
No	267	97.09
Respiratory		
Yes	8	2.91
No	267	97.09
Lymphadenopathy (Generalized)		
Yes	4	1.46
No	271	98.54

Table 4. Association between demographic, clinical study, and positive patients.

	RBPT (N=70)	SAT (72)	ELISA IgM (31)	ELISA IgG (5)
Gender				
Male				
Female	35	35	14	1
	35	37	17	4
Age				
Children <18				
Adult (18-59)	11	11	6	0
Elder (>59)	52	54	18	2
	7	7	5	3
Clinical Data				
Fever				
Headache	70	72	31	5
Chills	41	40	16	1
Night sweats	30	29	13	1
Myalgia	32	36	18	1
Arthralgia	7	8	1	2
	37	39	17	4

Table 5. Association between risk factors and positive patients.

Risk factors	**RBPT**	**SAT**	**ELISA IgM**	**ELISA IgG**
Contact with animals	27	28	14	2
History of consumption of raw milk, product, animal products	16	19	10	1
Travel	1	2	1	1

4. DISCUSSION

In the present study, the seroprevalence of human brucellosis was studied in Uttar Pradesh, North India. Our findings showed an overall seroprevalence of 11.27% in the region. The present study represents the first report of highest prevalence of human brucellosis in combination of serology study in the Uttar Pradesh, North India.

The seroprevalence of brucellosis in the region was carried out using a commercial IgM ELISA assay, Rose Bengal Plate test, and Serum agglutination test. ELISA is one of the most specific and reliable diagnostic tools for brucellosis. The technique does not require any advanced infrastructure and can be used in very limited settings. Elisa technique is used to identify the individual IgM and IgG antibodies to the surface antigens, which provides a better clinical correlation for diagnosing the early stages of brucellosis, the technique can be further used for the mass screening in suspected and confirmed cases. While RBPT, SAT are used for screening of Brucellosis antibody [12].

In the present study, risk factors associated with the seropositivity of brucellosis were pyrexia of unknown origin, headache,

night sweat and joint pain. Our results are in line with several other reports which suggest these risk factors to be predominantly linked with Brucella exposure [13&14].

Based on gender-wise stratification, seroprevalence was found to be predominantly higher in females (54.83%) compared to males (45.16 %). Our reports contrast with other studies, which suggest that males owing to frequent exposure are at high risk for brucellosis [15&16]. However, the study finds that Meghalayan vendors are more likely to be exposed to brucellosis since they work closely with animals in household tasks and are mostly female [12].

Additionally, we looked at several age groups that are more susceptible to contracting Brucella. According to the study, the incidence of brucellosis is higher in the younger age range of 18 to 59 years, followed by the age group under 18 [14]. Increased brucellosis exposure in economically productive age groups is mostly associated with different agrarian practices and milk-producing animals, which is typically the main source of income for the majority of people in northern India. High seropositive rates and higher exposure to brucellosis have been associated with these risk variables in earlier research [17,18].

According to our research, contact with animals was strongly linked to 11.27% of the population enrolled in the study being seropositive for brucellosis, followed by a history of consuming raw milk, milk products, or animal products. Similarly, risk variables linked to interaction with infected animals and contaminated animal products were reported in a study by AD Pathak et al. (2014) [19].

Various immunization programs and accurate clinical diagnosis of Brucella fever with uncertain origin can help prevent this neglected zoonotic illness in humans. To reduce brucellosis cases, awareness campaigns, disease prevention, and safe livestock management should be implemented. In many nations throughout the world, brucellosis is a serious public health concern. Human brucellosis is underreported and neglected in India, but the increasing incidence of animal brucellosis is widely recognized. In 1942, India recognized human brucellosis. The actual load of the disease is yet unknown, despite the fact that many instances have been reported from various regions of the nation [2].

A limitation of our study was that molecular study were not included. Our study suggests that Lucknow, Uttar Pradesh, northern India is an endemic region for human brucellosis. However, more research from other regions of India is needed to comprehend the disease's spread, clinico-epidemiological pattern, and true prevalence. Adequate knowledge of the disease must be raised by occupational hazards such being near animals.

5. CONCLUSION

In conclusion, we report a high seroprevalence of human brucellosis as 11.27% in the north India. Risk factors such as contact with animals and animal products were significantly linked with brucellosis. Further epidemiology studies are warranted in such regions of endemicity to determine accurate estimates of prevalence and risk factors and to study Brucella biovars for appropriate policymaking and advocacy and awareness regarding brucellosis in Northeast India.

CRediT authorship contribution statement:

Renu Kumari: Conceptualization, writing original draft, Writing review & editing. **Raj Kumar Kalyan:** Visualization, Writing original draft. **Shivangi Tripathi:** Validation, Visualization. **Kamlesh Kumar Gupta:** Supervision, Validation, Visualization. **Sanjeev Kumar Verma:** Supervision, Validation, Visualization.

Declaration of competing interest: The authors declare that there are no conflicts of interest.

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