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Seroprevalence of Human Brucellosis among Patients admitted with PUO (Pyrexia of Unknown Origin) at a Tertiary Care Hospital in North India

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Abstract

Background: Brucellosis is a disease reported in many parts of the world including India and the disease is also one of the important causes of PUO (Pyrexia of Unknown Origin). The present study was conducted with an aim to determine the seroprevalence of human Brucellosis in patients reporting with PUO.

Method: A cross-sectional hospital-based study was conducted at a tertiary care medical teaching institute in Northern India. A total of 369 serum samples were collected from PUO cases and tested for human Brucellosis by using Rose Bengal Plate Agglutination Test (RBPT), Serum Tube Agglutination Test (STAT), Enzyme linked Immunoassay (ELISA).

Results: Overall seroprevalence in our study was found to be 2.98% (0.5%, 0.8%, and 1.6% by RBPT, STAT and ELISA, respectively).

Conclusion: In spite of the low prevalence of human Brucellosis, clinicians must maintain a high index of suspicion for the possibility of a patient suffering from Brucellosis while encountering a case of PUO and it is advisable to elicit history in terms of certain occupations and risk factors for Brucellosis in patients reporting with PUO.

Keywords: Human Brucellosis, Seroprevalence, PUO, Raw milk

Introduction

Fever is the most common symptom for which one visits health facility. However, in certain instances, it may be difficult to diagnose the underlying cause of fever. The cases where one is unable to detect and diagnose the cause of disease that is causing a rise in temperature above normal, comprises a large group called 'Pyrexia' or 'Fever of unknown origin'.

Among all the conditions that result in fever of unknown origin, Brucellosis constitutes an important cause. Brucella species is a bacterium which causes Brucellosis in animals like cattle, sheep, goat, *etc*. The sections of population most commonly affected include those involved in animal rearing, slaughterhouse workers, laboratory workers, and veterinarians. All the age groups and both the sexes are equally involved.[1,2] Authors from

India have reported a seroprevalence ranging between 9.3% - 16.4% among livestock populations.[3] Majority of the losses occurring in the livestock industry, *i.e.,* cattle and buffalo industry are attributed to this disease.[1]

Humans are dependent on animals for their living since time immemorial and this close interaction often results in transfer of diseases from animals to humans and Brucellosis is passed to humans via person-to-person transmission, infection from contaminated environment, occupational exposure from infected animals and food borne transmission.[4-6] Incubation period for human Brucellosis is 2-3 weeks and the most common signs and symptoms include fever, arthralgia, sweat, fatigue, malaise, anorexia, weight loss, headache and back pain.[7]

About 72.6% of the population of Jammu & Kashmir are residing in rural areas and major occupations among them include agriculture, land farming and animal rearing. Majority of the urban population is dependent on rural areas for animal products and sometimes in the process, disease is transferred to humans. Very few studies have been conducted on seroprevalence of Brucellosis among humans in Jammu.[8]

Therefore, this study was planned to assess the burden and epidemiology of Brucellosis among patients admitted with fever of unknown origin.

Materials and Methods

The present hospital based cross-sectional study was conducted at a tertiary care medical teaching institution in North India in collaboration with Sher e Kashmir University of Agriculture Science and Technology under Outreach Program for Zoonotic Diseases. The study population consisted of all the inpatients and outpatients diagnosed with PUO (Pyrexia of Unknown Origin) and fever between 1st November 2019 and 30th November 2020. All adult patients who attended Medicine OPD or those admitted in wards of Medicine with chief complains of fever and fever of unknown origin, providing consent for participation were considered eligible for the study. In order to reach the required sample, the investigator prepared a list of all the patients admitted/labelled with the diagnosis of fever and PUO in out-patient Department of Medicine. A sample size of 600 patients was calculated for the study to estimate the prevalence of Brucellosis within 2.5% of estimated 10% in and around Jammu. This was based on estimates by Sharma *et al.,* on seroprevalance of human Brucellosis in and around Jammu using different serological tests, alpha= 0.05 and power of study being 80% and non-response rate of 10%.[5] The study was commenced after seeking approval from the Institutional Ethics Committee GMC, Jammu.

Operational definition of PUO given by Durack DT, Street AC was used for the selection of potential participants for the study. Accordingly, any patient having unexplained fever, reporting to the OPD on at least three occasions and also patients admitted to the hospital for a duration of less than three days were chosen for the study.[9]

The eligible participants were briefed about the purpose of the study and after obtaining their consent to participate, a detailed history was elicited. Every patient was followed on a day-to-day basis till the patient was discharged from the hospital or an outcome was assigned to the patient. A questionnaire was used in the study for the collection of socio-demographic information of the participants, clinical presentation and information about various environmental risk factors vis-à-vis Brucellosis.

After the information collection procedure, 5 mL of blood was collected under aseptic conditions. The blood sample was immediately centrifuged in the department of Microbiology, GMC Jammu and labelled aliquots were prepared. A unique ID was assigned to every sample. The samples were stored in a deep freezer and were transported under recommended temperature conditions to Sher-e-Kashmir University of Agricultural Sciences and Technology (SKAUST), Jammu for analysis.

The blood sample was subjected to Rose Bengal Plate Agglutination Test (RBPT), Serum Tube Agglutination Test (STAT) and Polymerase Chain Reaction (PCR) test. RBPT, STAT were performed immediately, while Enzyme linked Immunoassay (ELISA) test was done after desired sample of 90 patients was achieved.

Initially, the study was planned to be conducted in GMC Jammu, but due to COVID-19 pandemic and declaration of GMC-Jammu as the dedicated COVID-19 hospital, blood samples were collected from CHC R.S Pura, CHC Bishnah and CHC Sohanjana in order to achieve the desired sample size. Despite collecting samples from additional sites under intimation to Directorate of Health Services, IECGMC Jammu and Board of Studies, a sample size of only 369 could be achieved against the proposed 600.

Statistical analysis

The data thus collected were entered on Microsoft Excel spreadsheet and were analyzed using SPSS Version 23 software. Data were grouped and tabulated. Descriptive statistics was used to calculate means, standard deviations and percentages.

Results

Table 1: Socio-demographic characteristics of the subjects with fever and PUO (N=369)

Socio demographic characteristics	Number n=369	Percentage (%)
Gender	261	70.7
Male		
Female	108	29.3
Age (in years)*	8	2.2
10-≤19		
20-≤29	103	27.9
30-≤39	114	30.8
40-≤49	63	17.1
50-≤59	53	14.4
60-≤69	17	4.6
70-≤79	7	1.9
80 and above	4	1.1
Religion	306	83.0
Hindu		
Muslim	57	15.0
Sikh	6	2.0
Occupation		
Government Job	83	22.5
Housewife	77	20.9
Private Job	62	16.8
Student	50	13.6
Farmer	39	10.6
Nomad	26	7.0
Labourer	26	7.0
Others**	6	1.6

**Defence personnel, Carpenters

Table 1 depicts the socio demographic details of patients with PUO and fever. Males outnumbered the females with a male:female ratio of 2.8:1. About 3/4th of the study participants were in the age group of 20-49 years. About 1/4th of the participants were government employees, followed by homemakers and private employees (20.9 and 16.8, respectively). Youngest patient was 12 years old, while the oldest patient was 85 years old. Mean age of males was 37.63 ± 12.7 years and the mean age of females was 39.14 ± 13.08 years.

Table 2: Distribution of environmental factors among study participants (n=369)

Environmental Factors	Number (n=369)	Percentage (%)
Consumption of milk		
Consumption of raw milk	217	58.9
Consumption of raw milk and undercooked meat	142	38.5
Others	10	2.6
Consumption of drinking water		
Consumption from piped water	184	49.9
Consumption from piped water and public tap.	68	18.4
Consumption from piped water, public tap, and open well	66	17.8
Consumption from piped water and open well	35	9.5
Animal owned		
Cattle	256	69.4
Others	34	9.2
None	79	21.4
Tending/Rearing		
Assisted in animal birth	95	25.8
Others	36	9.7
Veterinary Services and Vaccination		
Cattle vaccination	41	23.5
Veterinary services	133	76.5
Total	174	100
Consumption of Gau Mutra		
Yes	75	20.3
No	294	79.7

Nearly seven in 10 participants gave history of animal ownership, particularly cattle and 9.2% of domesticated animals were goats, sheeps and hens. About 1/5th of the participants provided no history of ownership of animals. Half the cases were directly engaged in tending/ caring of owned animals. Out of all the studied cases with fever and PUO, 78.5% owned animals. 16 (4.4%) study participants consume water from either tap water, open well and covered well water (Table 2).

Generalized weakness was the predominant symptom present in males followed by fever and headache (94%, 70%, and 33%, respectively), while anorexia and fatigue (35%, and 67% respectively) showed female predominance. Night sweats were more commonly reported in carpenters (Table 3).

Table 3: Clinical symptoms other than fever among study participants (n=369)

Socio demographic variable	Symptom's**						
Gender	**Night sweats n (%)**	**Fatigue n (%)**	**Anorexia n (%)**	**Weight loss n (%)**	**Headache n (%)**	**Arthralgia n (%)**	**Generalized weakness n (%)*** **
Male	14 (5.4)	79 (30.3)	166 (63.6)	33 (12.6)	39 (19.9)	87 (33.30	245 (93.9)
Female	6 (5.6)	38 (35.2)	72 (66.7)	22 (20.4)	12 (11.1)	34 (31.5)	90 (83.3)
Age (in years)							
10 - 29	6 (30)	36 (30.7)	68 (28.4)	16 (29)	18 (35.2)	39 (32.2)	101 (30)
30 - 59	13 (65)	71 (60.5)	154 (64.6)	35 (63.6)	24 (46.9)	69 (56.9)	208 (62)
≥60	1 (5)	10 (8.4)	16 (6.6)	4 (7.2)	9 (17.6)	13 (10.6)	26 (7.5)
Occupation							
Government job	2 (2.4)	28 (33.7)	56 (67.5)	6 (7.2)	7 (8.4)	25 (30.1)	79 (95.2)
Private job	1 (1.6)	17 (27.4)	40 (64.5)	8 (12.9)	7 (11.3)	14 (22.6)	58 (93.5)
Homemaker	6 (7.8)	22 (28.6)	49 (63.6)	18 (23.4)	11 (14.3)	26 (33.8)	62 (80.5)
Nomad	0 (0)	12 (46.2)	21 (80.8)	2 (7.7)	2 (7.7)	8 (30.8)	26 (100.0)
Farmer	6 (15.4)	14 (35.9)	22 (56.4)	9 (23.1)	8 (20.5)	17 (43.6)	34 (87.2)
Student	3 (6)	19 (38)	30 (60)	5 (10)	10 (20)	20 (40)	45 (90.0)
Labourer	1 (3.8)	5 (19.2)	15 (57.7)	5 (19.2)	5 (19.2)	10 (38.5)	26 (100.0)
Carpenter	1 (50)	0 (0)	2 (100)	2 (100)	1 (50)	1 (50)	2 (100.0)

** Multiple responses *** Percentages do not add up to 100 due to multiple responses.

Table 4: Result of serological tests for Brucellosis employed in the study (n=369)

Serological Tests							Total
	ELISA		RBPT		STAT		
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	
Number	06	363	03	366	02	367	11 (2.9%) (1.57-5.12)
Percentage (%) (95% CI)	1.6 (0.66-3.35)	98.3	0.8 (0.09-1.77)	99.1	0.5 (0.20-2.19)	99.4	

Various serological tests employed for Brucellosis in our study showed an overall seroprevalence of 2.98%.

Table 5: Sero positivity for Brucellosis among study participants (n=369)

Characteristic	Serological test		
Gender	**ELISA (n=6) N (%)**	**RBPT (n=3) N (%)**	**STAT (n=2) N (%)**
Male	5 (1.9)	3 (1.14)	2 (0.76)
Female	1 (0.9)	0 (0)	0 (0)
Age (Years)			
10-29	2 (33.3)	1 (33.3)	0 (0)
30-59	2 (33.3)	1 (33.3)	2 (100)
≥60	2 (33.3)	1 (33.3)	0 (0)
Occupation			
Private Job	0 (0)	0 (0)	1 (1.1)
Nomad	2 (7.6)	1 (3.8)	1 (3.8)
Farmer	2 (5.1)	1 (2.5)	0 (0)
Student	1 (2)	1 (2)	0 (0)
Labourer	1 (3.8)	0 (0)	0 (0)

Overall seroprevalence of Brucellosis was 2.9%. Males outnumbered the females. Majority of participants who were diagnosed with Brucellosis were either nomads or were engaged in farming (Table 4). About 20.3% of study participants gave history of consumption of cow's urine. On conducting serological tests, ELISA and STAT were positive in only one case each, respectively, while RBPT was not positive in any of the cases who consumed Gau Mautra (Table 5).

Discussion

In all health care settings including primary care, among the important clinical entities encountered, fever and fever of unknown origin constitute the major concerns around which health care is organized. More than 200 conditions have been attributed to PUO. One such disease is Brucellosis. In many instances, given the susceptibility of Brucellosis to antibiotics, it gets treated even without being diagnosed.[10]

Early in the course of present investigation, we realized the difficulty in assembling patients as per the case definition of PUO, as understood routinely in clinical practice *i.e.,* the definition of classic PUO or FUO as described by Beeson in 1961.[11] Similarly, we had to make certain modifications in the research protocol owing to COVID-19 pandemic. The extent of the effect of these changes on the prevalence estimates is difficult to quantify. However, it could be safely assumed that our prevalence estimates were lower than expected. The precision of the estimates made was also low as evident from wide confidence intervals.

Serological tests (RBPT, STAT and iELISA) for Brucellosis in our study found an overall seroprevalence of 2.98% with the seropositivity of 0.5%, 0.8% and 1.6%, respectively. The STAT titers above 1:160 were considered diagnostic, but few authors considered the titer values above 1:320 to be more specific in endemic areas.[12] Several authors reported a wide range of prevalence of human Brucellosis in different parts of country ranging from 0.8% to 26.6%.[13-16]

The age group of 20-29 years was the most commonly affected by Brucellosis followed by 40-59 years, 30-39 years and 60-69 years age groups. These results were in concordance with the reports of other authors, while Sharma HK *et al.* reported highest burden of Brucellosis in the age group >20-35 years.[16] Males were predominantly more affected than females with sero positivity of 3.83% and 0.92%, respectively. As the male to female ratio in our study was 2.4:1, lower seropositivity can be attributed to this. However, male predominance was observed in other studies too.[17] Among the different occupation groups included in our study, highest seropositivity was seen in nomads (36.3%), followed by farmers (27.2%).

Among the serologically positive cases, generalized aches (90.8%) was the most common symptom, followed by anorexia (64.4%) and the clinical results in our study were almost in agreement to studies conducted by V Jane Megid *et al*.[18] In another study conducted by Kochar DK *et al.* (2007), most common reported symptoms were joint pains (83.4%) and fever (77.7%).[19]

In our study, we attempted to determine the relation between Brucellosis and consumption of cow urine (Gou Mutra). Though we could not find any similar study in the literature, out of 20.3% of cases in our study with a history of consumption of cow's urine, 2.6% samples were found to be seropositive.

In the recent years, emphasis was laid on the development of better serological tests with better sensitivity and specificity. However, the serological tests differ in detection of various immunoglobulins (Ig) due to varying sensitivity to different Brucella infections, type, and purity of antigen, besides variation in duration of incubation period during which test remains either

positive or negative. To overcome these issues, new techniques have been developed such as ELISA which is regarded as the gold standard test to detect bovine Brucellosis. STAT and RBPT are traditional serological tests for detection of Brucellosis in humans and animals.[20]

In our study, we compared ELISA, STAT and RBPT for the diagnosis of Brucellosis. Among them, ELISA was found to be a superior diagnostic test over RBPT and STAT. Higher seropositivity was found among men compared to women. Out of 10 seropositive males, five were seropositive with ELISA, three and two with STAT and RBPT, respectively. Only one female patient was found to be seropositive with ELISA test. This correlates with 98.6% prevalence noted in males as compared to 1.03% in females with ELISA.[7] It has been opined that genus-specific PCR can also help to avoid false-negative results in patients infected with unknown species and biovars. We could not do a PCR test in our study due to the unavailability of kits.

Similar studies conducted in Karnataka have reported higher seropositivity rates in comparison to our study 2.26%, 2.26%, 19.69% by RBPT, SAT and indirect ELISA, respectively; 4.79%, 4.41% and 4.41% among PUO cases by RBPT, SAT and 2ME, respectively.[20,21] In another study conducted to detect Brucellosis among PUO patients, the serum samples tested were positive 4.25%, 3.54%, 6.02% and 4.96% by RBPT, SAT, indirect ELISA and IgG ELISA, respectively.[22]

Conclusion

In spite of the assertions made above, the present study indicates that the problem of Brucellosis might not be as acute as expected. We had expected the prevalence to be higher among certain sections of the population dealing with cattle rearing and farming. Changes in the sampling methodology and our inability to study desired sample size has resulted in underestimation of the problem. However, despite this low prevalence, clinicians need to maintain guard on the possibility of a patient suffering from Brucellosis while encountering a patient reporting with fever/PUO. It is also advisable to elicit history in terms of certain occupations and risk factors for Brucellosis in patients reporting with PUO. The strength of our study lies in the fact that there have been very few studies conducted in a limited scale in the region of Jammu.

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Nil

Conflict of Interest:

None

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