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Original Article

Seroprevalence and associated risk factors of Human brucellosis from a tertiary care hospital setting in Central India

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Summary

Brucellosis is an important zoonotic disease and has public health importance. In the present study, we studied the prevalence and associated risk factors of human brucellosis in the central Indian population from tertiary care health settings. A prospective observational study was conducted from March 2015 to February 2018 in patients attending the outpatient department (OPD) of Central India Institute of Medical Sciences (CIIMS), Nagpur. A total of 7026 individuals suspected of brucellosis were recruited based on prespecified inclusion criteria, additional risk factors, and clinical symptoms. Baseline, demographic and clinical characteristics were likewise recorded. Sera samples from recruited individuals were collected and subjected to anti-brucellosis antibody (IgM) detection using a commercial kit by ELISA assay. The overall seroprevalence of brucellosis reported from tertiary care health settings was 11% (772/7026). The majority of positive cases were from the states Madhya Pradesh (58.1%), followed by (Maharashtra (38.8%) and Chhattisgarh (2.9%). Adult age (20-60) and female groups were more vulnerable. Clinical symptoms like fever, arthralgia, and myalgia risk factors like animal exposure, consumption of raw milk, vegetable, and meat were significantly associated with brucellosis in the recruited population. Among the positive cases, high seroprevalence was associated with animal handlers (66.8%) compared to other occupationally exposed groups. The present study shows a high seroprevalence of brucellosis in health care settings. We emphasize regular screening of the disease in clinical settings to develop epidemiological data and initiate appropriate control measures.

Keywords: Human Brucellosis, Seroprevalence, Risk Factors, ELISA

Introduction

Brucellosis has been regarded as an important yet neglected zoonotic disease (Agasthya et al., 2007). Globally, more than 500,000 new cases are reported every year, with the annual incidence varying from < 2 to > 500 per 1,000,000 population among different regions (Renukaradhya et al., 2002). The disease is

usually transmitted to humans through exposure to infected live stocks and consumption of raw or unpasteurized milk and milk products contaminated with *Brucella* species (Tembhurne et al., 2017). It is one of the causes of fever of prolonged duration in endemic areas and one of the important causes of pyrexia of unknown origin (Pathak et al., 2014).

Human brucellosis is often misdiagnosed due to overlapping clinical manifestations with other bacterial infections (Aworh et al., 2013) and therefore remains underreported in most clinical settings. Although brucellosis is associated with febrile illness, in rare episodes, it can infect other organs such as the kidney, heart, and brain and establish chronic infection (Agasthya et al., 2012). Neurobrucellosis is among rare complications of the disease, wherein brucella bacilli can infect the Central Nervous system (CNS), leading to a high neurological sequel and morbidity rates (Tembhurne et al., 2017).

Brucellosis in India is often regarded as a re-emerging zoonotic disease of public health importance due to the high agrarian population and their exposure to livestock. Due to this reason, the disease was included in the list of high prioritized zoonotic infections in the roadmap to combat zoonoses in India (RCZI) initiative by India (Sekar et al., 2011). Thus screening and accurate diagnosis of brucellosis are critical for initiating appropriate public health control measures.

Various studies in different states in India have reported brucellosis as a commonly encountered disease in occupationally exposed individuals with a prevalence rate of 17-34%. (Pathak et al., 2014; Wu et al., 2013). While incidences of human brucellosis have been reported in different epidemiological studies, there is still limited data regarding estimates of brucellosis, especially from tertiary care health settings in India. Such data is critical concerning regular screening of the disease in clinical settings to develop epidemiological data and initiate appropriate control measures. In the present study, we studied the prevalence and associated risk factors with human brucellosis in a central Indian population from a tertiary care health setting.

Materials and Methods

Study Design and Participant Recruitment

A total of 8102 participants attending the outpatient department (OPD) wards of Central India Institute of Medical Sciences (CIIMS) from March 2015 to February 2018 were prospectively enrolled. Each participant was selected using pre-specified inclusion criteria with added risk factors like unconventional food intake habits (consuming raw milk/food products) and animal exposure. Participants were screened based on clinical

symptoms suggestive of brucellosis including fever, joint pain, joint swelling, chest pain, headache, back pain, and night sweating. A detailed medical history of the recruited individuals was collected using a structured questionnaire prepared beforehand by a team of expert clinicians and scientists. Baseline factors like age, gender, and other risk factors were recorded. Out of the 8102 recruited individuals, 380 were excluded due to the refusal for blood collection. Of the 7722 available participants, a total of 696 samples were excluded for the study, which included participants with Incomplete clinical data (120), Hemolyzed samples (90), Incomplete Baseline data (150), Pregnant women and children (55), Children below 10 years (85), Blood samples not available (100), Mixed infection. A total of 7026 participants were finally included in the seroprevalence study (Figure 1).

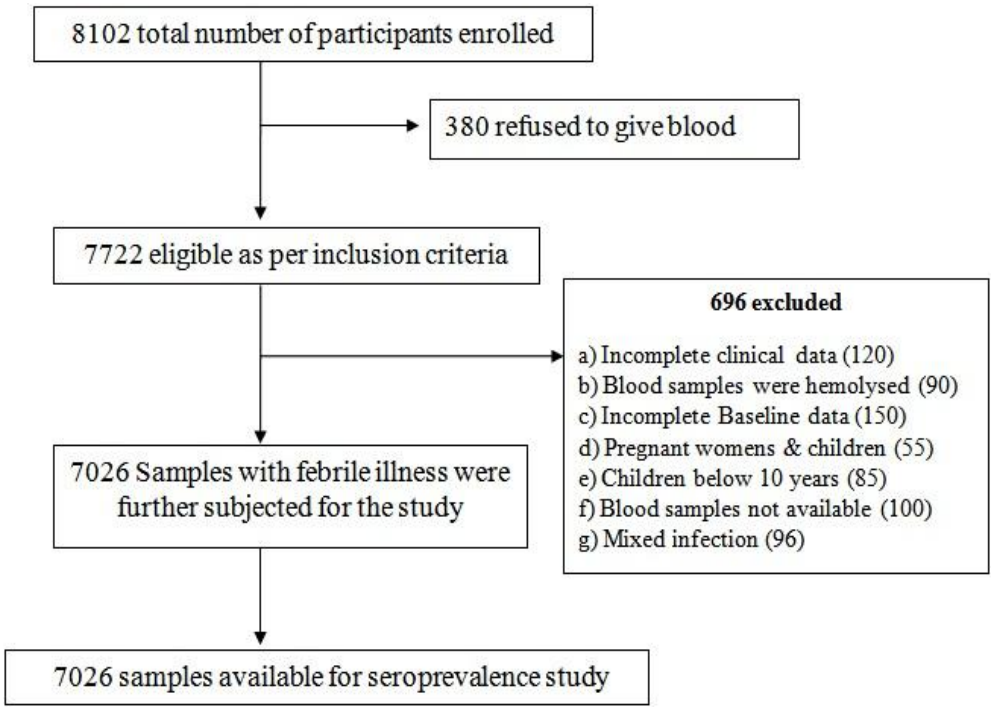

Fig.1. Study flow diagram for participant's recruitment

Sample collection

2-3 ml blood was collected by a venous puncture in plain vacutainer tubes and allowed to clot at 37^0C. Serum was collected by centrifugation of blood samples at 2000 rpm for 10 min at 4^0C and immediately stored at -20^0C until further use.

ELISA IgM

Detection of immunoglobulin M (IgM) antibodies using Enzyme-linked Immunosorbent assay (ELISA) was performed using a commercial kit (Novatec Immunodiagnostica GmbH, Germany) as per the manufacturer's instructions as described elsewhere. IgM titers above 0.7 were considered positive. Sensitivity and specificity, as given by the manufacturer, were > 95% for Immunoglobulin G (IgG) and > 95% for IgM, respectively.

Data Analysis

Descriptive and analytical statistics were used to summarize the data obtained. The frequencies (percentage) of demographics, clinical factors, and risk factors were measured on a nominal scale. The association of baseline characteristics of the study population in brucella positive and negative cases were determined by the chi-square test in MedCalc statistical software (version 10.1.2.0). Results were considered significant if the p-value was < 0.05.

Results

In the present study, we investigated the seroprevalence of brucellosis in the central India region. Out of 8102 recruited, serum samples of 7026 subjects were available for seroprevalence study. Overall and state-wise seroprevalence of brucellosis is mentioned in Table 1.Overall seroprevalence was 11% (772/7026) from hospital settings in central India. All recruited cases belonged to three states from Central India, which included Maharashtra, Madhya Pradesh, and Chhattisgarh. When overall positivity was stratified according to state-wise distribution, more than half of positive cases from Madhya Pradesh (58.1) were found exposed to brucellosis, followed by Maharashtra (38.8%) & Chhattisgarh (2.9%) (Figure 2**).**

Age and sex-wise stratification of positive cases are indicated in Table 2. More than half of positive cases (53.1%) in the younger economically productive age group (20-40) were found exposed to brucellosis compared to other age groups (P > 0.001). Around a quarter of positive cases in the age group (40-60) were also found exposed to brucellosis. Based on gender-wise distribution, females were more vulnerable to their female counterparts for brucellosis, although the difference was not statistically significant.

Table 1. Overall and state-wise seroprevalence of Brucellosis from Central India

	Sero-Positive-IgM (%)			
Total cases (n)	*Overall*	*Maharashtra*	*Madhya Pradesh*	*Chhattisgarh*
7026	772 (11%)	300 (38.8%)	449 (58.1%)	23 (2.9%)

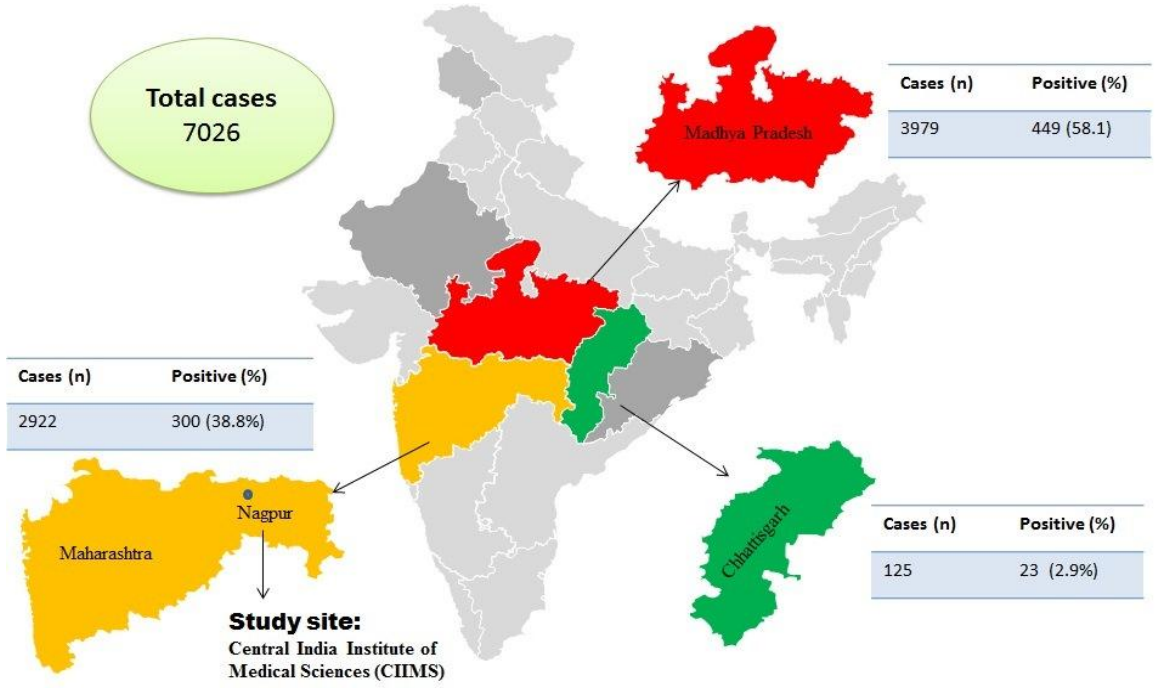

Fig. 2. State-wise stratification of Brucella seropositivity in the study population

Among the clinical history recorded on presentation, fever was the most common symptom associated with 95% of brucellosis cases. Apart from fever, Headache (88%), Arthralgia (90%), Myalgia (86%), chronic fatigue syndrome (90%) were also major symptoms associated with brucellosis (Table 3). Among the various risk factors analyzed, direct animal exposure and consumption of raw milk and vegetables along with improperly cooked meat consumption were major factors significantly ($P < 0.0001$) associated with the occurrence of brucellosis in the study population (Table 4).

Table 2. Age and gender-wise distribution among study subject after brucella diagnosis

Characteristics	Levels	No. (%)		Chi-square Value	Chi-square coefficient	DF	P-value
		Positive (n=772)	Negative (6254)				
Age in years	*< 20*	53(6.87)	531(8.49)	**36.669**	**0.072**	**3**	**< 0.0001**
	20-40	307(39.77)	1951(31.20)				
	40-60	303(39.25)	2418(38.66)				
	≥ 60	109(14.12)	1354(21.65)				
Gender	*Male*	420(9.89)	3828(90.11)	**13.027**	**0.043**	**1**	**0.0003**
	Female	352(12.67)	2426(87.33)				

We also stratified the study population according to occupation to study vulnerable groups associated with brucellosis. Table 5 shows the occupation wise distribution of Brucella IgM titers in the study population. Among the stratified groups, animal handlers, which included zookeepers and veterinarians, showed the highest seropositivity (66.8%) rates, followed by miscellaneous groups (25.7) and farmers (7.3%) with more or less similar mean IgM levels (Figure 3).

Discussion

Brucellosis remains the most common zoonotic disease worldwide. Though associated with minimal mortality, its importance is realized in the substantial morbidity associated with it, in humans and animals (Patil et al., 2016). In the present study, we studied the prevalence and associated risk factors with human brucellosis in the central Indian population from tertiary care health settings using commercial IgM ELISA assay. ELISA has been used as a rapid, sensitive, and specific assay. The latest researches showed that ELISA is more reliable for diagnosing brucella infection when compared to the Rose Bengal precipitation test (RBPT) and Serum agglutination test (SAT) (Mantur et al., 2010). ELISA is capable of readily identifying the individual IgM and IgG antibody to the surface antigens, which allows permitting a better clinical correlation, which could help for diagnosing the early stages of brucellosis; therefore, ELISA has been used for mass screening in suspected and confirmed cases. (Agasthya et al., 2007).

Table 3. Clinical presentation recorded among positive brucellosis cases

Clinical history at presentation	Positive cases (n)	%
Headache	680	**88**
Loss of Appetite	463	54
Behavioural change	77	10
Vomiting	231	30
Fever	733	**95**
Arthralgia	687	90
Stomach Pain	154	20
Diarrhoea	92	20
Rashes	68	9
Anorexia	153	20
Chronic fatigue syndrome	693	**90**
Dizziness	324	42
Weakness	424	55
Night Sweating	384	48
Myalgia	663	**86**

Table 4. Risk factors associated with brucellosis in the study population

Risk Factors	Levels	Positive	Negative	Chi-square Value	Chi - Square co efficient	DF	P values
Exposure to animals	*Direct*	661(85.62)	2778 (44.42)	465.184	0.249	1	< 0.0001
	Indirect	111(14.38)	3476 (55.58)				
Consumption of raw milk	*Yes*	577(89.32)	69(10.68)	4454.215	0.623	1	< 0.0001
	No	195(3.06)	6185(96.94)				
Consumption of dairy products	*Yes*	575(16.58)	2894(83.42)	217.611	0.173	1	< 0.0001
	No	197(5.54)	3360(94.46)				
Consumption of raw vegetables	*Yes*	734(25.7)	2122(74.3)	1062.45	0.362	1	< 0.0001
	No	38(0.91)	4132(99.09)				
Consumption of improper cooked meat	*Yes*	535(33.86)	1045(66.14)	1087.35	0.366	1	< 0.0001
	No	237 (4.35)	5209 (95.65)				

Table 5. Total positivity of Mean Brucella IgM levels in Occupational wise seropositivity of Brucellosis in the study population

Occupational category	*Total Positive (772)*	*(%)*	*Mean Brucella IgM levels*
Farmers	57	7.3	0.949
Animal handlers[a]	516	66.8	0.881
Miscellaneous[b]	199	25.7	0.881

[a] veterinarians, zoo –keeper [b] Housewives, labours, students

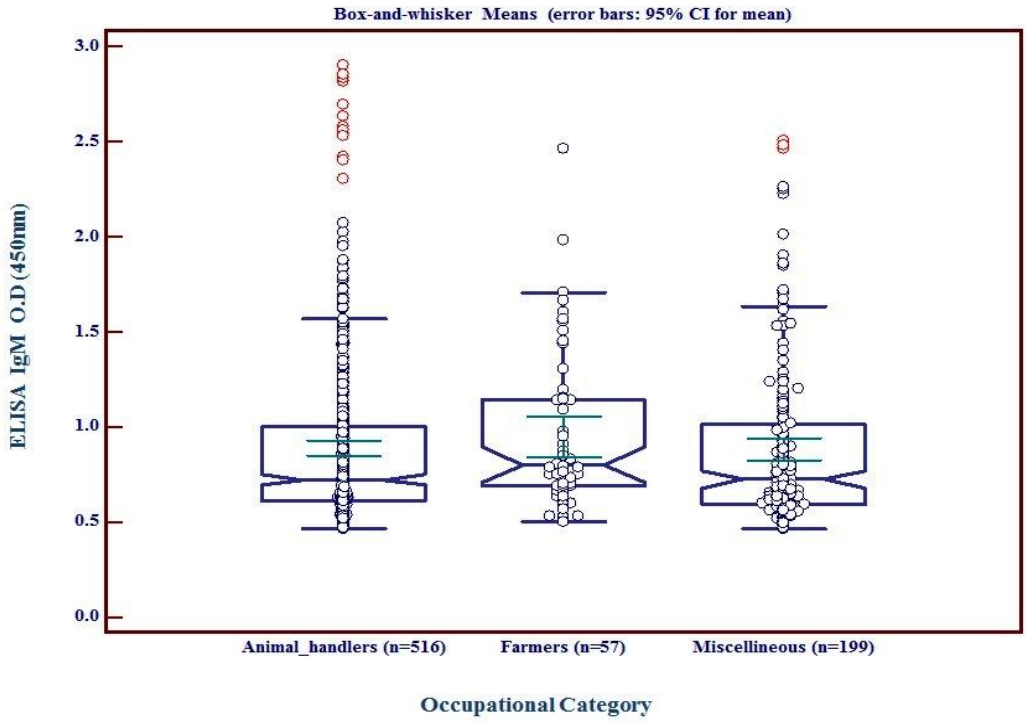

Fig. 3. Box whisker plots showing occupation wise distribution of IgM titers in the study population

In the present study, seroprevalence documented from tertiary care settings in central India was 11%. The majority of positive cases for brucellosis were from the state of Madhya Pradesh followed by Maharashtra, Chhattisgarh. The population-based studies from different parts of India in a tertiary care center reports the seroprevalence of brucellosis between 5-18%. Bansal et al. (2019) reported 16.7% seroprevalence in a tertiary care setting in Rajasthan. Similar study by Patil et al. (2019) shows 5.1% seropositivity among patients admitted over a decade in hospitals of North Karnataka. No studies till now have been reported for human brucellosis in the central Indian population. Thus our study is the first report, which shows a high

prevalence of brucellosis from health care settings in central India.

Based on clinical symptoms, fever was widely associated in > 90% of brucellosis cases in our study which is in agreement with several studies that have shown febrile illness to be common and major clinical symptoms associated with exploring brucellosis (Appannanavar et al., 2012; Pathak et al., 2014). In our study, among different age groups, younger, economically productive groups (20-40 years) were more exposed to brucellosis. On the contrary, some studies have reported brucellosis to be more prevalent in the age group between 40-60 years in Karnataka (Mangalgi et al., 2016). In our present study, it was found that females are more vulnerable than males for brucellosis, although the difference was not statistically significant. Several studies have indicated gender as significant risk factor for brucellosis (Makita et al., 2011). Males are apparently about six times more likely to be brucellosis positive than females (Mrunalini et al., 2004). This is because of the occupations described in majorly male-dominated. However, close proximity to animals in domestic chores among females can also be linked to exposure to brucellosis, as observed in our study.

It was observed from this study that animal handlers, which include veterinarians and zookeepers, were at relatively higher risk for brucellosis than farmers and any other occupation group. These factors may explain the variability of brucellosis seropositivity among individuals. In the different studies, according to previous reports, veterinarians and zookeepers along due to direct exposure with animals make them more vulnerable to developing Brucellosis in humans (Tsend et al., 2014; Tumwine et al., 2015; Ramos et al., 2008).

The limitations of the study include a lack of parallel sampling from animals to establish transmission and associated risk factors from animals. Another limitation includes the lack of serological investigation using conventional screening tests like the RBPT and STAT. This avoids variability in seroprevalence data as both these tests are associated with sensitivity and specificity limitation and often fail to discriminate between the true positive and false-positive serological results.

To the best of our knowledge, this is the first study that reports the prevalence of brucellosis in the central Indian population. Studies targeting specific regions are available, but the real situation may be far from reported yet. According to Renukaradhya et al. (2002), brucellosis may be endemic in India. Our study is the first attempt to understand the prevalence of brucellosis in a tertiary care setting. The non-existence against the brucella vaccines for humans implies that controlling this zoonotic

disease in animals will directly lead to prevention in humans (especially concerning biosecurity (Monath, 2013). Prevention is possible through public health awareness programs and safe livestock practices. Regular surveillance with high clinical suspicion and screening of populations at risk would be essential in understanding the real magnitude of human Brucellosis in endemic regions. Consideration of clinical symptoms and multiple targeting approaches is needed as the results vary from test to test.

Conclusion

In conclusion, we report the higher prevalence of brucellosis from a tertiary care setting in central India. Proper awareness, diagnosis of this disease is important, especially in developing countries such as India, where humans and animal interfaces are in close association in routine life. We emphasize regular screening of the disease in clinical settings to develop epidemiological data and initiate appropriate control measures.

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Ethical Approval

The study was approved by the Institutional Ethical Committee of the Institute. All clinical investigations were conducted according to the principles expressed in the declaration of Helsinki 1975, as revised in 1983. Each participant was interviewed independently, and the collected data were kept confidential. Study numbers were used instead of participants' names to ensure confidentiality. Written consent forms were obtained from recruited participants after the oral explanation of the study.

Conflict of interest statement

All authors declare no conflicts of interest.

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