

# Seroepidemiological survey of human brucellosis in and around Ludhiana, India

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Studies have been done on public health significance of brucellosis using serology with little or no emphasis to risk factors. Therefore, this study was designed to investigate seroprevalence of brucellosis and assess epidemiological variables associated with human brucellosis. After obtaining verbal consent, 241 peripheral blood samples were collected from occupationally exposed groups with and without pyrexia of unknown origin. A structured questionnaire was prepared to gather risk factors, such as occupation, age, sex, history of consuming raw milk and other unpasteurised dairy products, direct contact with domestic animals, general knowledge about the route of transmission and awareness level. Purposive sampling was used to select the key informants. All serum samples were first screened by Rose Bengal Plate Test (RBPT) and further analysed by Standard Tube Agglutination Test (STAT). The results revealed that 24.5% were positive by RBPT and diagnosis was established in 26.6% using STAT with a titre range between 80 and 1,280 IU/ml. Among occupational groups, prevalence was 17.8% in veterinarians and pharmacists but was not statistically significant. The most common clinical symptoms at presentation were fever, headache, back pain, arthralgia and myalgia. No female reactor was found and the mean age and standard deviation of seropositive patients was  $34.69 \pm 10.97$  years. Risk factors such as residence in rural area, participation in vaccination of animals and eating during working hours were significantly associated ( $P < 0.05$ ) with brucellosis by univariate and multivariate analysis. In conclusion, to deal with occupation-related disease like brucellosis, awareness on risk factors must be part of extension education campaign. Besides, regular surveillance of the disease needs to be integrated into control and prevention programme at a local and national level.

Keywords: *human brucellosis; risk factors; Rose Bengal Plate Test; Standard Tube Agglutination Test; Ludhiana, India*

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**B**rucellosis is the most common zoonotic disease that leads to considerable morbidity and loss of man-days across the globe and thus perpetuates poverty (1). The disease presents as an acute or persistent febrile illness with a diversity of clinical manifestations (1). The disease occurs worldwide, except in those countries where bovine brucellosis (*Brucella abortus*) has been eradicated, which means absence of any reported cases for at least five years (2). The Mediterranean countries of Europe, northern and eastern Africa, Near East countries, India, Central Asia, Mexico and Central and South America are especially affected. Although *B. melitensis* has never been detected in some countries, there are no reliable reports that it has ever been eradicated from small ruminants (2). Furthermore, brucellosis is also considered as a re-emerging problem in many countries such as Israel, Kuwait, Saudi Arabia, Brazil and Colombia, where there is an increasing

incidence of *B. melitensis* or *B. suis* biovar 1 infection in cattle (3).

In human, consumption of contaminated food and occupational contact are the major risks of infection. The main routes of infection are consumption of unpasteurised dairy products, small ruminants, camel milk and milk products like cheese and sour milk. It has been shown that the organism can survive pickling and inadequate smoking (4,5). Contact with infected materials such as aborted fetuses, placentas, urine, manure, carcass and salvaged animals has been reported in some countries to cause human brucellosis in 60–70% of cases (6). Infection by contact has been reported to be common among veterinarians, abattoir workers, farmers, rendering-plant workers, packing-house employees, animal handlers and others who work with animals and their products (7).

In India the prevalence of animal brucellosis has been well studied (8–11). In Punjab the apparent overall

prevalence of brucellosis was reported to be 12.09% (12). Hence, close association between human and animals, stray cattle, consumption of unpasteurised milk and dairy products and inappropriate waste disposal are some of the principal factors perpetuating infection in humans. So far, some studies have been done on public health significance of brucellosis using serology with little or no emphasis to risk factors (13–16). The purpose of this study was to investigate the seroprevalence of brucellosis in and around Ludhiana and assess the possible risk factors associated with human brucellosis.

## Methodology

### Study area

Ludhiana is a city in the Indian state of Punjab. It is the largest city in the state, with an estimated population of 1,398,467 in 2010. The city is located at 30°54'N 75°51'E/30.9°N 75.85°E. It has an average elevation of 244 m (798 ft). It has a semi-arid climate which ranges from April through June in the city, tends to be very hot and very dry with average highs in May and June hovering around 40°C. The monsoon season which runs from July through September sees a slight decrease in average temperatures but an increase in humidity. The bulk of the city's annual precipitation is received during the monsoon season. October and November interestingly enough is dry; more similar to a summer month than a monsoon month, although November is noticeably cooler than a summer month. Average temperatures though tend to decrease during the course of each of these months. December through February, which forms the winter months, is relatively mild with warm days and chilly nights. March is more of a sharp transitional month from winter to summer.

Besides industry, Ludhiana is a major Agri-Products producer. It is a big centre for Dairy product packaging. In association with these dairy intensifications to produce more milk, human–animal interaction increases because of various husbandry practices on the farm. The serological status of the workers and associated risk factors in such intensified dairy farms were the concerns of present study.

### Study subjects

Occupationally exposed individuals with or without pyrexia of unknown origin (PUO) and suspected cases referred by physicians in Ludhiana and its surrounding villages were included in this study using purposive sampling.

### Study design and sampling

In this cross-sectional study, first the purpose of the investigation was explained to the study subjects and verbal consent to participate was obtained from the participants. This was followed by a collection of 241

peripheral blood samples from August 2008 to May 2009. A structured questionnaire was prepared and 152 individuals were interviewed to gather risk factors such as occupation, age, sex, history of consuming raw milk and other unpasteurised dairy products, any disease conditions or symptoms, direct contact with domestic animals, general knowledge about the route of transmission, awareness level, sanitation habits, etc. Purposive sampling was used to select the key informants.

### Laboratory procedure

Serum samples were first screened by Rose Bengal Plate Test (RBPT) and further analysed by Standard Tube Agglutination Test (STAT). A titre of 80 IU/ml or greater was considered positive (17,18). Rose Bengal and plain *Brucella* antigen required for this test were procured from Punjab Veterinary Vaccine Institute, Ludhiana, Punjab.

### Statistical analysis

Information on the results of laboratory tests and data collected on risk factors were analysed by statistical package for social sciences (SPSS Inc., window version 11.0.1, Chicago, Illinois, USA). A *P*-value <0.05 was used as a cut-off point for a variable to enter the multivariate analysis using a backward stepwise Likelihood Ratio (LR) model.

## Results

Of the total 241 human sera samples screened by RBPT, diagnosis was established in 64 (26.6%) using STAT with titre range between 80 IU/ml–1280 IU/ml (Table 1). Among occupational groups seropositivity was found 17.8% in veterinarians and pharmacists but was not statistically significant (Table 2). Although assessment of medical history at presentation revealed 26 cases with fever and symptoms, only 24 (10%) were seropositive (Table 3). The most common clinical symptoms at presentation were fever, headache, back pain, arthralgia and myalgia (Table 4). No female reactor was found. The prevalence of brucellosis was significantly higher in rural compared to urban areas (Table 5). Age range of the cases was 20–76 years, with mean and standard deviation (SD) of 39.24 ± 11.6 years. The mean and SD of seropositive patients was 34.69 ± 10.97 years, and the age group of

Table 1. Results of serological tests for brucellosis

Tests	Serological tests			
	RBPT		STAT	
	Positive	Negative	Positive	Negative
Number	59	182	64	177
Percentage	24.5	75.5	26.6	73.4

**Table 2.** Seroprevalence of brucellosis based on occupation

Occupation	Number of samples	Standard Tube Agglutination Test positive (%)
Veterinarians and pharmacists	126	43 (17.8)
Para-veterinarians	16	3 (1.3)
Animal attendants <sup>a</sup> and dairy farmers	78	13 (5.4)
Miscellaneous <sup>b</sup>	21	5 (2.1)
Total	241	64 (26.6)

<sup>a</sup>Milkers, labourer in feeding and cleaning section.

<sup>b</sup>House wives, drivers, electrician, accountant, postman, store-keeper, security guards.

26–35 years was the most commonly affected with brucellosis (Table 6).

Twelve factors were included as possible risk factors for the occurrence of brucellosis and only eight factors were significantly associated with brucellosis at  $P$ -value  $<0.05$  in the univariate analysis (Table 7). Age group and level of education were not associated significantly ( $P >0.05$ ). Knowledge about zoonosis was highly significant ( $P <0.01$ ) and the odds ratio was 0.25. Eating habit was not associated with brucellosis. Individuals who deal with parturient domestics were about 3.7 times more likely to develop brucellosis than who did not handle cases of parturient dam (OR = 3.7;  $P <0.05$ ). The odds of brucellosis was 4.0 and 6.8 times higher in those groups handling 1–2 dystocia cases weekly and one case per two weeks, respectively, in comparison to those handling one case monthly ( $P <0.01$ ). Raising animals was found to be a significant risk factor ( $P <0.01$ ), but when the type of animal was considered the odds for presenting with brucellosis were not significant. Participation in vaccination of animals against brucellosis was also significantly associated with brucellosis; about 5.3 times higher than those who did not participate ( $P <0.05$ ). Individuals who had occupation-related hazard were about 4.6 times more

**Table 3.** Relationship of brucellosis with symptom

History at presentation	Number presented	Seropositive (%)
Fever only (pyrexia of unknown origin)	7	1(0.4)
Fever plus symptoms <sup>a</sup>	26	24(10)
No fever but symptoms <sup>a</sup>	15	11(4.6)
Asymptomatic	193	28(11.6)
Total	241	64(26.6)

<sup>a</sup>Symptoms like headache, back pain, arthralgia, myalgia, fatigue, weight loss, night sweating.

**Table 4.** Distribution of patients on the basis of clinical signs and symptoms.

Clinical signs and symptoms	Number presented	Standard Tube Agglutination Test positive (%)
Fever	33	25 (10.4)
Headache, back pain, arthralgia and myalgia	34	28 (11.6)
Fatigue, weight loss	10	8 (3.3)
Night sweating	5	5 (2.1)
Orchitis	1	1 (0.4)

likely to be seropositive than those who did not get such mishap ( $P <0.05$ ). The odds of brucellosis to occur were also 4.9 times higher among those who ate during working hours than who did not ( $P <0.01$ ). Thus, the main risk factors identified were lack of awareness about zoonosis, contact with parturient animal, raising animals, participation in vaccination, occupation-related mishap and eating during working hours.

All risk factors that had  $P$ -value  $<0.05$  in univariate analysis were included in multivariate logistic regression analysis using a backward stepwise LR model. This step simultaneously considers the individual and joint effects of many risk factors. Only three factors were significantly associated with brucellosis at  $P$ -value  $<0.05$  in the multivariate analysis (Table 8). That is risk factors such as residence in rural area, participation in vaccination of animals and eating during working hours were significantly associated with brucellosis.

## Discussion

Bovine brucellosis has been reported to be endemic in all states of India and appears to be on the increase in recent times, perhaps due to increased trade and rapid movement of livestock. The predominance of natural bull service in rural India, especially in buffaloes, has been suggested as another important factor in the maintenance and spread of infection (19).

The prevalence of brucellosis has been widely reported (16,20) in different regions like in Orissa (6.8%) (21) and in Andhra Pradesh (11.51%) (22). In the present study, seroprevalence documented was 26.6% using STAT. The

**Table 5.** Ecological distribution of brucellosis

Residence	Number of samples	Rose Bengal Plate Test positive (%)	Standard Tube Agglutination Test positive (%)
Rural	220	57 (23.7)	62 (25.73)
Urban	21	2 (0.8)	2 (0.83)
Total	241	59 (24.5)	64 (26.6)

**Table 6.** Age-wise seroprevalence of brucellosis

Age (years)	Number of samples	Rose Bengal Plate Test positive (%)	Standard Tube Agglutination Test positive (%)
≤15	Nil	Nil	Nil
16–25	42	14 (5.8)	14 (5.8)
26–35	73	26 (10.8)	27 (11.2)
36–45	33	4 (1.7)	5 (2.1)
46–55	75	13 (5.4)	16 (6.6)
56–65	17	2 (0.8)	2 (0.8)
≥66	1	0	0
Total	241	59 (24.5)	64 (26.6)

higher incidence of brucellosis reported in this study may be due to the diagnosis of high number of seropositive suspected cases with awareness presented to the department (32.9%). In addition during dairy farm visit, out of 47 exposed groups without awareness 31.9% were positive. In contrast, only 3.9% were seropositive on random sampling during brucellosis awareness campaign. Intensification in the dairy sector can also be another possible justification for higher prevalence in the present finding. Moreover, most researchers establish diagnostic titres of STA test as  $\geq 1:160$ , which was not the endpoint for this study (16,20,23,24).

There was no statistically significant association of the disease among the different occupation groups included in this study. Although slight difference was observed with regard to classification of exposed occupational groups, the findings of this study is partially in agreement with others. Mrunalini et al. (22) reported 25.24% in veterinarians, 23.3% in para-veterinarians, 12.62% in farmers, 11.65% in shepherds and 6.8% in other occupational groups. In Eritrea, Omer et al. (25) reported the highest prevalence (7.1%) among dairy farm workers and owners in randomly selected dairy-cattle farms, followed by veterinary personnel (4.5%) and inhabitants in pastoralist areas (3.0%) using RBPT and CFT. There was no evidence for significant differences between the three populations. In contrast, Hussein et al. (26) reported significant difference of brucellosis infection among occupational groups.

In the present study, 28 (11.6%) cases with no symptom at presentation were seropositive. This means 43.8% of seropositive cases had no clinical suspicion of brucellosis which is found to be less compared to 88.7% reported by Mantur et al. (16). Al-Fadhli et al. (27) also reported the major features on presentation, irrespective of the course of the disease, as fever, sweating, headache, rigours, arthralgia, myalgia and low back pain. In the present study, there was one patient complaining of orchitis; interestingly the patient was positive for *Brucella* antibody. Even though we did not attempt, Mantur et al. (16)

demonstrated *Brucella* agglutinins in testicular fluid of patients with epididymo-orchitis. The finding of disease profile in the present study is somewhat similar to that reported by different researchers (20,22).

In this study, female participation was less. This may be because of social dominance of males that made females less concerned in seeking medical help. In addition, it may be due to lack of awareness among females or less number of females participating in potentially dangerous activities like handling dystocia cases in the present study area. All seropositive cases were male patients. However, in various other studies conducted by other researchers it has been found that males were more commonly affected than females (22,27–29). In contrast, Hussein et al. (26) reported relatively higher incidence among females than males. The absence of female reactor in this study could probably be due to the smaller number of female ( $n=4$ ) brucellosis suspects studied as compared to males ( $n=237$ ). Otherwise, there is no sex-wise discrimination of brucellosis infection; both male and female are equally susceptible if provided exposure to potential risk factors. Al Sekait (30) also suggested that gender does not influence the immune response to *Brucella*.

The prevalence of brucellosis was significantly higher in rural areas compared to urban areas. This can be associated with increased human–animal interaction in rural areas, which may be because of intensification of dairy sector to supply milk to the industries in Ludhiana. In contrast, the findings of Hussein et al. (26) revealed higher prevalence of brucellosis in rural areas than urban areas that was not statistically different. Age range of exposed groups in this study was 20–76 years with mean and SD of  $39.24 \pm 11.6$  years. The mean and SD of the age of seropositive patients was  $34.69 \pm 10.97$  years. The age group 26–35 years was the most commonly affected followed by 46–55 years and 16–25 years. With the exception of age group 36–45 years, this study was almost in agreement with reports of other workers (16,22,28,29).

Although the age group of 26–35 years appears to be the most commonly affected, the different age groups included in our study were not significantly associated with brucellosis. In Saudi Arabia, Al Sekait's (30) seroepidemiological survey revealed that seropositivity was strongly associated with increasing age ( $P < 0.001$ ), occupation and low socioeconomic status. Al Sekait (30) also reported higher seroprevalence of *Brucella* antibody in rural compared with urban areas.

Eating habit, that is being vegetarian or non-vegetarian, was not associated with the disease. Moreover, frequency of drinking raw milk, daily or occasionally, was not significantly associated with brucellosis in the present study. Although raw milk is a potential source of *Brucella* organisms, the eating habit of residents in the present study areas is unique. The practice of modern dairy farming in the study area might be one factor for

**Table 7.** Univariate analysis of some risk factors for brucellosis

Risk factors	Categories	Cases	Controls	OR <sup>a</sup>	95% CI <sup>b</sup>	P-value
Age group	16-25 years (1)	14	14	4.000	0.718-22.282	0.107
	26-35 years (2)	27	23	4.696	0.905-24.359	
	36-45 years (3)	5	10	2.000	0.404-13.173	
	46-55 years (4)	16	33	1.939	0.369-10.205	
	>55 years (5)	2	8	0.25		
Education	Illiterate (1)	1	3	2.000	0.090-44.350	0.312
	Below matric (2)	15	20	4.500	0.484-41.447	
	Matric (3)	26	41	3.805	0.433-33.434	
	Graduate (4)	21	18	7.000	0.769-63.723	
	Postgraduate (5)	1	6	0.167		
Residence	Rural (1)	62	71	7.423	1.649-33.407	0.009
	Urban (2)	2	17			
Knowledge about zoonosis	Yes (1)	44	79	0.251	0.105-0.598	0.002
	No (2)	20	9			
Eating habit	Vegetarian (1)	47	52	1.914	0.952-3.850	0.069
	Non-vegetarian (2)	17	36			
Frequency of drinking raw milk	Daily (1)	9	13	1.251	0.472-3.220	0.218
	Occasionally (2)	29	28	1.872	0.924-3.795	
	Not at all (3)	26	47			
Contact with parturient animal	Yes (1)	59	67	3.699	1.31-10.424	0.013
	No (2)	5	21			
How often were dystocia cases treated?	Daily one case (1)	5	5	3.600	0.867-14.952	0.003
	1-2 cases weekly (2)	20	18	4.000	1.552-10.309	
	One case/two weeks (3)	17	9	6.800	2.334-19.813	
	One case monthly (4)	10	36			
Raising animals	Yes (1)	55	55	3.667	1.605-8.379	0.002
	No (2)	9	33			
Participation in vaccination	Yes (1)	10	3	5.247	1.381-19.929	0.015
	No (2)	54	85			
Occupation-related hazard	Yes (1)	9	3	4.636	1.202-17.883	0.026
	No (2)	55	85			
Eating during working hrs	Yes (1)	12	4	4.846	1.484-15.823	0.009
	No (2)	52	84			

<sup>a</sup>Odds Ratio, <sup>b</sup>Confidence Interval.

lack of association between raw milk drinking and brucellosis. In contrast, Al-Fadhli et al. (27) reported raw milk as the major source of infection.

Dealing with parturient domestics (OR = 3.7) and raising animals (OR = 3.67) were significant risk factors in study areas in and around Ludhiana where dairy

**Table 8.** Multivariate analysis of epidemiological variables

Risk factors	OR	95% CI	P-value
Residence (1)	9.619	1.653–55.994	0.012
Participation in vaccination (1)	7.972	1.268–50.135	0.027
Eating during work hours (1)	4.725	1.136–19.653	0.033

farming is most commonly practiced. Meki et al. (29) also reported that workers in occupations dealing with animals had a 2.4-fold higher risk of brucellosis than those in occupation not dealing with animals. In their study, breeding animals was also as a significant risk factor. In Eritrea, among dairy farm workers, a higher risk was associated with the presence of sheep in the farm (OR = 13.2, CI = 2.2–76.7). Furthermore, in the pastoral area, a high risk was linked to having close contact with animals (OR = 6.32, CI = 0.88–∞), while a reduced risk was seen for contact with cattle (OR = 0.18, CI = 0–1.30) (25).

Participation in vaccination of animals against brucellosis was significantly associated with brucellosis; the odds were 5.3 (CI = 1.38–19.93) times higher than those who did not participate ( $P < 0.05$ ). The values of OR also indicated that individuals who had occupation-related hazards such as sharp instrument cut, accidental injection of needle and splash of parturient dam discharges into eye/mouth were about 4.6 (CI = 1.20–17.88) times more likely to be seropositive than those who did not have such mishaps ( $P < 0.05$ ). The odds of brucellosis to occur were also 4.9 (CI = 1.48–15.82) times higher among those who ate during working hours than who did not ( $P < 0.01$ ). This result strongly supports the personal observation during a dairy farm visit in and around Ludhiana that clinicians usually enjoy a cup of tea and sweet food in-between their working hours on the spot. Accordingly, contaminated hand increases the chance of infection.

In the present study, residence in rural area, lack of awareness about zoonosis, contact with parturient animal, raising animals, participation in vaccination, occupation-related mishap and eating during working hours were identified as the main risk factors. This finding is reasonably in agreement with Ali et al. (23) who reported contact with animals (32%); occupation, mainly farmers or butchers (18%); raising animals in the vicinity of residence (14%); and drinking unpasteurised milk (4%) as risk factors for brucellosis. Al Sekait (30) seroepidemiological survey also identified direct contact with domestic animals and consumption of raw products of animal origin as the main risk factors.

In conclusion, to deal with occupation-related disease like brucellosis, knowledge of risk factors is so vital in control and prevention programmes. Thus, an extension education campaign, particularly in high-risk areas, such as veterinary practitioners and dairy farms, could aid in

decreasing the incidence of brucellosis. In addition, regular surveillance of the disease needs to be integrated into control and prevention programme at a local and national level.

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