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Original Research Article

Serological evidence of human brucellosis with female predominance in livestock-exposed communities of rural Haryana, India

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ABSTRACT

Background: Human brucellosis is a neglected zoonotic infection transmitted through direct animal contact. It frequently eludes clinicians since its presentation is often non-specific and consequently leads to underdiagnosis. Haryana is predominantly an agrarian state with close human and livestock interactions. There is a dearth of systematic population-based data on human brucellosis. This study was aimed to determine the seroprevalence of brucellosis among febrile patients within livestock exposed population and identify associated risk factors.
Methods: A cross-sectional hospital-based study was conducted over 1 year, from November 2024 to November 2025, at a tertiary care centre in Budhera, Haryana. Individuals residing within 15 km of the hospital presenting with fever, headache or malaise, giving history of contact with livestock were included. Serum specimens were tested for anti-*Brucella* antibodies using Rose Bengal Plate Test (RBPT) and IgM enzyme-linked immunosorbent assays (ELISA). Structured interviews were used to collect data. Associations between risk factors and seropositivity were evaluated by multivariate analysis.
Results: Of 173 participants (mean age 32.95±18.55 years; 55.5% male), RBPT detected antibodies in 19 (10.98%) and ELISA in 22 (12.72%). Seropositivity was significantly associated with unpasteurised milk consumption, assisting animal birthing, handling foetuses and contact with animal excreta (OR 6.3-18.8, $p<0.01$). ELISA and RBPT showed substantial concordance ($\kappa=0.91$). Women involved in livestock care had disproportionately higher seropositivity.
Conclusions: Human brucellosis among febrile patients was primarily linked to domestic exposures to high-risk activities. Integrating exposure histories with clinical assessment, promoting pasteurisation of milk, enhancing animal vaccination and strengthening One Health surveillance are critical for early diagnosis in endemic areas.

Keywords: Human brucellosis, Livestock-exposure, Seroprevalence, Risk factors, Zoonoses

INTRODUCTION

Brucellosis is a widespread zoonosis, commonly caused by *B. abortus*, *B. melitensis*, and *B. suis.* Direct exposure to infected animals, animal tissue or fluids or consumption of unpasteurised dairy products and milk are common routes for transmission to humans. People involved in agriculture, animal husbandry, dairy farming and other related occupations are the most susceptible to acquire infection. Brucellosis in humans often presents with vague and non-specific febrile illness like intermittent fever, fatigue, arthralgia, and weight loss, thus masquerading as other endemic infections. Hence, the overlapping clinical presentations often leads to delay in diagnosis, inappropriate treatment and progression to chronicity. Owing to its persistent under diagnosis, limited surveillance and inadequate integration within public health programs, the World Health Organization (WHO) has included brucellosis under neglected zoonotic diseases. Thus, a high index of clinical suspicion in vital for early diagnosis, especially in endemic regions.[1]

The global burden of brucellosis still remains largely unaccounted for. Recent studies have predicted over 2 million new infections annually from around the world, which far exceeds the officially reported numbers. These data from low- and middle-income countries (LMICs) point to a glaring diagnostic and reporting gap with regard to human brucellosis.[1,2] There are wide geographical variations in seroprevalence based on livestock density, animal rearing practices, dietary habits and cultural factors. Parts of sub-Saharan Africa have reported seropositivity rates up to 13%, among patients of febrile illness.[3,4] In India as well, available literature and data depict pronounced regional heterogenicity. Community based studies from rural Punjab have reported a brucellosis seroprevalence of 2.24% in the general population. The risk was significantly higher among people consuming raw milk and people involved in animal birthing activities.[5] In contrast, seropositivity exceeding 17% have been documented among patients with undifferentiated febrile illness, in hospital-based studies conducted in north India.[6]

Northern states of India, including Haryana, portray a unique epidemiological context owing to their predominantly agrarian economy and high human-livestock interactions. Although systematic population-based studies assessing human brucellosis in Haryana are limited, evidence from animal surveillance is suggestive of endemicity. Bovine serosurveys from Haryana, with reported prevalence ranging from approximately 6% to 8%, are highly suggestive of sustained animal reservoir with ongoing zoonotic transmission of brucellosis within the region.[7] Studies from various endemic regions of India, have documented seroprevalence of human brucellosis exceeding 10% among people involved in farming, dairy handling and animal care activities.[8]

The diagnosis of human brucellosis in India is heavily reliant on serological assays like the RBPT, standard tube agglutination tests (STAT) and ELISA. However, testing with above tests remain largely restricted to tertiary care centres and there is no uniform national screening strategy. This often leads to significant underdiagnosis of brucellosis in rural health care settings. Assessing the seroprevalence of human brucellosis among agricultural communities with close animal contact in Haryana is therefore critical. Incorporating demographic and lifestyle factors will improve understanding dynamics of transmission as well as identify high-risk groups.

Such evidence is of paramount importance for strengthening clinical awareness, planning interventions and supporting integration with One Health strategies in order to reduce the burden of brucellosis, especially in endemic regions.

METHODS

A cross-sectional hospital-based study was carried out at the Faculty of Medicine and Health Sciences and Shree Guru Gobind Singh Hospital, which is a 800 bedded tertiary care centre in Budhera, Haryana, to determine the seroprevalence of human brucellosis among live-stock exposed people attending the centre. The study was conducted over a one-year period, from November 2024 to November 2025. During this study period, the study sample size thus included all consecutive patients meeting patient selection criteria mentioned subsequently. The total sample size therefore was 173. The study protocol was approved by the institutional ethics committee (Letter no. SEC/FMHS/M.Sc/02) for research project.

Patient selection

Residents who had been residing in villages within 15 km radius from village Budhera, provided history of contact with animals or consumption of animal products and presented at the hospital with fever, malaise or head ache were included in the study. Individuals residing in far flung villages beyond 15 km radius, were pregnant or unwilling to participate were excluded from the study. Participation was voluntary and an informed consent was obtained from all eligible subjects prior to enrolment in the study.

Structured patient interviews

All consenting participants underwent a structured questionnaire-based interview, conducted in local language to collect information on age, gender, education, marital status, clinical history and dietary habits. Participants were asked to recall from a list of potential high-risk exposures to livestock or animal products during the 6 months preceding onset of symptoms.

Sampling and serological tests

A phlebotomist collected 6 mL of venous blood from each study participant from median cubital vein, into a plain vacutainer tube, after which the clotted samples were centrifuged at 3000 rpm for 5-10 minutes to separate the serum. Clear sera were collected in sterile vials, labelled with unique identification numbers and stored at -20°C until analysis. All samples were tested for *Brucella* antibodies using the RBPT and ELISA. All kit reagents and samples were brought down to room temperature before testing as per manufacturer's instructions.

RBPT

The RBPT was conducted using Brucel-RB (Tulip Diagnostics (P) Ltd., Goa, India). Briefly, one drop of positive control, 50 µL of normal saline and 50 µL of test serum were placed on separate circles on a glass slide. To this, one drop of well-mixed Rose Bengal antigen was added to each circle and the contents were thoroughly mixed with separate sticks. The glass slide was then gently rocked for 4 minutes and examined against a white background. Visible agglutination was considered positive (anti-*Brucella* antibodies ≥25 IU/mL). Absence of agglutination was recorded as negative (anti-*Brucella* antibodies <25 IU/mL).

ELISA for Brucella IgM antibodies

Presence *Brucella* IgM antibodies were determined using the Novalisa *Brucella* IgM ELISA (Gold Standard Diagnostics Frankfurt GmbH, Germany) kit. Briefly, commercially available microplates coated with antigens were provided by manufacturer. On this *Brucella* IgM antibodies from the patient serum samples were captured. Horseradish peroxidase-labeled conjugate was added to bind the captured antibodies. After a washing step, the immune complex was visualized with Tetramethylbenzidine substrate induced colour change. The reaction was then stopped with sulfuric acid. To quantify the antibody concentration absorbance was measured at 450/620 nm using an ELISA plate reader.

Statistical analysis

The data collected through questionnaires were entered into Microsoft excel (Version 2013) and analysed using SPSS software (Version 29.0). Demographic characteristics of study participants were summarized in frequency tables. The categorical variables were presented as frequencies and continuous data as mean ± standard deviation. Multivariable logistic regression analysis was performed on statistically significant risk factors for seropositivity to identify independent risk factors. Associations between risk factors and seropositivity were assessed by calculating odds ratios with 95% confidence intervals, considering a $p<0.05$ as statistically significant.

RESULTS

During the study period, 181 patients presenting with fever, headache, or malaise met the inclusion criteria. Of these, eight patients declined to provide consent and were excluded. This left 173 participants to be included in the study. Among them, 96 (55.5%) were male and 77 (44.5%) were female. The age of the enrolled participants ranged from 3 to 92 years, with a mean of 32.95±18.55 years.

Among 173 participants, *Brucella* antibodies were detected in 19 (10.98%) individuals by RBPT, whereas ELISA identified anti-*Brucella* IgM in 22 (12.72%) participants. Among the ELISA-positive participants, 19 (86%) were female and 3 (14%) were male. The ages of seropositive individuals ranged from 8 to 52 years (mean=33.45±13.21 years). Majority of the positive cases were concentrated in the 36-45 year age group. Participants younger than 8 years and older than 52 years, had tested negative by both RBPT and IgM ELISA tests. Although ELISA detected 2 additional specimens positive for *Brucella* antibodies, it showed excellent concordance with RBPT, with an overall agreement of 98.3%. The Cohen's kappa coefficient was 0.91, indicating substantial agreement between RBPT and ELISA. Using ELISA as reference test, RBPT demonstrated sensitivity of 86.4% and specificity of 100% for the detection of *Brucella* antibodies. Detailed demography of study population is illustrated in Table 1.

Exposure to unpasteurised milk (cattle, buffaloes, goat) or milk products, milking animals (cattle, buffaloes, goat), assisting with animal birthing, handling animal foetuses and contact with animal excreta were all significantly associated with seropositivity with *Brucella* IgM antibodies. The strongest associations were observed for unpasteurised milk consumption and animal birthing or foetus handling, with odds ratios ranging from 6.3 to 18.8 ($p<0.01$). Study participants who handled animal excreta also exhibited a substantially increased likelihood of seropositivity as depicted in Table 2.

Table 1: Demographic profile of seropositive and seronegative study participants.

Demographic variables		*Brucella* antibody seropositive, 22 (12.7 %)	*Brucella* antibody seronegative, 151 (87.3 %)
Gender	Male	3 (13.64)	96 (63.58)
	Female	19 (86.36)	55 (36.42)
Formal education	None	3 (13.64)	14 (9.27)
	Primary level	12 (54.52)	51 (33.77)
	Secondary level	7 (31.82)	68 (45.03)
	Graduation level	0 (0)	18 (11.92)
Marital status	Married	17 (77.27)	53 (35.10)
	Unmarried	5 (22.73)	98 (64.90)
Occupation	Home maker	11 (50)	69 (45.70)
	Farming	0	9 (5.96)
	Animal rearing	0	1 (0.66)
	Farming and animal rearing	2 (9.10)	13 (8.61)
	Student	3 (13.64)	38 (25.17)
	Others	6 (27.27)	21 (13.91)
Diet	Vegetarian and milk products	19 (86.36)	94 (62.25)
	Vegetarian without milk products	0 (0)	18 (11.92)
	Non-vegetarian and milk products	3 (13.64)	35 (23.18)
	Non-vegetarian without milk products	0 (0)	4 (2.65)

Table 2: Multivariable analysis of risk factors for *Brucella* IgM seropositivity.

Exposure parameters	Seropositive, n (%)	Seronegative, n (%)	OR (95% CI)	P value
Unpasteurised milk consumption	15 (78.95)	47 (31.13)	18.8 (4.2-85)	<0.0001
Unpasteurised milk product consumption	15 (78.95)	47 (31.13)	18.8 (4.2-85)	<0.0001
Undercooked/ raw meat consumption	0 (0)	3 (1.99)	-	-
Milking animal	17 (84.97)	71 (41.02)	9.58(2.1-42.9)	0.002
Animal birthing	13 (68.42)	18 (11.92)	16 (5.4-47.4)	<0.0001
Animal foetus handling	13 (68.42)	18 (11.92)	16 (5.4-47.4)	<0.0001
Animal excreta handling	16 (84.21)	69 (45.70)	6.34 (1.8-22.6)	0.01

DISCUSSION

This study portrays a substantial seroprevalence of human brucellosis among febrile patients from agricultural communities in the study area. With anti-*Brucella* antibodies detected in 12.72% study population by IgM ELISA, the findings place brucellosis among the important but frequently overlooked causes of undifferentiated febrile illness in this region. These findings are consistent with reports from other endemic areas of northern India and comparable agrarian settings worldwide.[2,5] Higher rates of detection as observed by ELISA as compared with RBPT illustrates the additional diagnostic value of enzyme-based assays for earlier diagnosis or the detection of low titre of antibodies. However, the excellent concordance and of ELISA and RBPT along with high specificity of RBPT, as observed in the study, reinforces the utility of RBPT as a rapid screening tool in resource limited settings.[9]

Brucella IgM seropositivity was disproportionately higher among female study participants. The economically productive age group of 36-45 years showed a relatively higher seropositivity. These findings reflect regional cultural practices and gender-specific roles in household-level animal care activities, milking of animals and handling of animal reproductive tissue in rural Haryana. The strong associations identified between seropositivity and consumption of unpasteurised cow, buffalo or goat milk, assisting with animal birthing, handling aborted foetuses and exposure to animal excreta corroborate established transmission pathways documented both in India and internationally This reinforces the critical role of behavioural and occupational exposures in disease acquisition.[5,10] A striking finding of the present study was the marked female predominance among seropositive individuals, with women accounting for over 86% of confirmed cases. Similar female preponderance has been reported in several community-based studies from India and other endemic regions, particularly where women play a central role in household animal husbandry rather than commercial farming.[11,12] However, contrasting findings have been reported from several sero-epidemiological studies in India, where the sero-prevalence of *Brucella* antibodies was higher in male study participants. Recent cross-sectional studies from Punjab have registered overall seropositivity at 17.52%, with male participants having higher (20.83%) seropositivity as compared to females (9.77%).[11] Similarly, in a study conducted within a rural population of Maharashtra, males constituted 78.5% of the screened subjects and accounted for 85.7% of seropositive cases. While in contrast, only 14.3% of positive cases were female (overall seroprevalence 1.83%).[12] These discrepancies maybe attributed to variations in occupation profile and cultural practices. In the above studies, involvement of male participants in livestock handling, slaughtering and raw animal product processing, substantially increased their risk of *Brucella* exposure. The contrasting female predominance among seropositive individuals, as observed in this study, may be reflective of gender-specific roles in household activities pertaining to animal rearing. The higher involvement of women in domestic animal care including: milking and birthing activities may collectively increase their risk profile and thus warrant further investigation.

Additionally, in rural sociocultural context of the region, women are widely involved in the traditional practice of preparing cow dung cakes for domestic fuel. The handling of animal excreta are often without protective measures and involve repeated exposures. This route appears to be epidemiologically relevant, since *Brucella spp.* have been documented in shed cattle faeces and to persist in environment under favourable conditions. Recent field-based surveys have confirmed the presence of *Brucella* in bovine faecal matter, which is suggests a possible biological pathway through contact with cow dung and fomites.[13] These findings underscore the vitality of considering local cultural practices when assessing the risk factors for zoonotic diseases.

Socio-economic and demographic analysis further emphasise the impact of lifestyle related exposure in the transmission of brucellosis (Table 1). *Brucella* IgM antibodies were significantly higher among subjects who were: married, with lower levels of formal education and homemakers. They in turn constitute the group of people who are more likely to be involved in domestic animal care. Also, the absence of seropositivity among study population who had received at least graduate-level education, strongly suggests that the awareness and risk perception among these groups may influence risk of exposure. Dietary patterns also showed a clear association; with the majority of seropositive study population

reporting consumption of unpasteurised milk and milk products. Similar associations between low educational status and traditional dietary habits with risk of seropositivity have been documented from Punjab, Maharashtra, north-east India and endemic regions in the Middle East and Africa.[8,14]

Multivariate analysis of levels of exposure to risk factors establishes robust evidence to support established routes of transmission. As illustrated in Table 2, the consumption of unpasteurised milk and milk products emerged as one of the strongest predictors of seropositivity. The findings are consistent with several global observational studies and systematic reviews.[15-17] The fact that like milking, assisting with animal birthing and handling of aborted foetuses, were substantially associated with infection, reflects the high bacterial load of *Brucella* in the reproductive tissue and body fluids. Moreover, unprotected handling of animal excreta, an often-overlooked activity and widely prevalent in rural settings of India, was found to be independently associated with seropositivity. Thus, underscoring the need for consideration of handling of animal excreta as a one of the vital routes of transmission especially in Indian rural context.[16,17]

Limitations

The hospital-based design may limit generalisability to the broader community. There also the possibility of recall bias owing to its interview-based exploration of risk factors. Though the ELISA detected IgM antibodies from patient serum, molecular confirmation was not performed due to biosafety concerns. Despite its limitations, the study provides data from an under represented region of India and explores the varied plausible modes of transmission based on regional cultural practices.

CONCLUSION

The study demonstrates a noteworthy burden of human brucellosis among febrile patients from live-stock communities in Haryana. The seropositivity is largely driven by close interactions among humans and animal, traditional dietary practices and culturally embedded domestic activities. The findings strongly emphasise the need to contextualise the epidemiology of zoonotic disease like brucellosis with local demographic and cultural frameworks. The incorporation of history of livestock exposure and delving into individual animal rearing activities, especially handling of animal waste, may considerably improve case detection. Findings of the study further emphasise the interconnectedness of animal health, human behaviour and environmental exposure, reinforcing the necessity of a One Health approach. Strengthening programs for animal vaccination, reinforcing surveillance and fostering of collaborations among veterinary and public health sectors are pivotal for reducing the burden of brucellosis. Targeted health education for women involved in household care of domestic animals and promotion of pasteurisation of milk are critical interventions in endemic rural regions.

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