Full title

The prevalence and risk factors for human *Brucella* species infection in a cross-sectional survey of a rural population in Punjab, India

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Abstract

Background

Brucellosis is an important neglected zoonosis. Effective cattle vaccines are available but infrequently used in India where rural households commonly own one or two cattle as sources of protein and income. We assessed prevalence of infection and risk factors in humans.

Methods

We conducted a cross-sectional sero-survey in randomly selected individuals in sixty villages in Punjab sampled probability proportional-to-size. . Infection prevalence was assessed by positive RBT or IgG ELISA. Risk factors were adjusted for potential confounding using multivariable analyses.

Results

Of 1927 subjects approached 93% participated. Age-standardised prevalence for *Brucella* infection was 2.24% (95% CI 1.61-3.11). Over 60% households kept cattle, 10% assisted with calving or abortions. Nearly all individuals consumed boiled cow/buffalo milk from their own or neighbours' cattle . 3.3% consumed goats milk. . There was a 2.18 increased odds (95% CI: 0.96-4.95) of infection, with calving/abortions and an increased odds (4.26, 95% CI: 1.33-13.6), with goats but not bovine milk consumption.

Conclusions

An association with calving/abortions and goats milk consumption was seen. *Brucella* vaccination of household livestock would reduce risk to humans in such settings. Additional measures include biosecurity training around calving/abortions; education to boil all milk and for health-care workers to test for brucellosis.

Keywords:

Brucellosis epidemiology; India mass vaccination/cattle; seroepidemiologic studies; zoonses epidemiology.

Introduction

Brucellosis is a globally important zoonotic disease [1, 2] but not prioritized by international health-systems despite significant impacts on human and animal health. [1] The Gram-negative intracellular bacteria of the *Brucella* genus are endemic in livestock in low income settings, and easily transmitted to humans via direct contact with ruminants, or via raw dairy products [3]. In livestock Brucellae cause abortions and reduced milk yields [4]. In humans they cause debilitating non-specific illness, including fever, fatigue, weight loss, headaches, and arthralgia persisting for months to years if untreated. Focal joint involvement such as sacro-ilitis causing disability is common [3]. Neurobrucellosis (cranial or motor defects, seizures, psychosis and meningitis) and *Brucella* endocarditis can also occur. Brucellosis is often not recognised by health workers, and laboratory tests often unavailable [5, 6].

India is the world's leading milk producer [7] and has the largest cattle population globally after the "white revolution": a stepped change to the national dairy industry in the 1960s when farmer-owned milk co-operatives to produce and market dairy products were established [8]. The prevalence of *Brucella* in large ruminants varies widely between states [9]. A study in Punjab noted a 12% prevalence in village herds [10]. Punjab produces the most milk per person [11] , and over 70 million rural households derive income or employment from the dairy sector. Poor smallholders are also commonplace, generally living in villages alongside more well-off dairy farmers [12].

Effective live-attenuated cattle vaccines against brucellosis are available and used in a number of countries [13, 14] but infrequently in India [9, 15]. To provide evidence for vaccination (eg via the new *Brucella*-free village schemes [16]), we assessed the prevalence of human infection in the rural population of Punjab and the factors putting individuals at risk. Few serological surveys have been carried out in representative samples of the general population. Additionally, analyses using recent high quality serological testing based on enzyme-linked immunosorbent assays (ELISA) together with Rose Bengal testing (RBT) [17 18] are limited.

Materials and methods

We carried out a population based cross-sectional study from December 2015 to July 2017. Participants were recruited using multi-stage stratified random sampling. Four out of seven blocks (average block size of 0.1 million) in Ludhiana district, Punjab were selected to represent accessible rural sub-districts. Sixty villages (average village size of 1000-2000 persons) were then selected using probability proportional to size (from the 2011 Census).

Within villages, healthcare is overseen by one or more anganwadis (village health workers), who maintain household registers. These registers were used to select 20 households in each village using simple random sampling. If there was more than one anganwadi in a village, 10 households were selected from each of two randomly selected anganwadis. Once a household was recruited, all household members were recorded and stratified by age (5-14, 15-24, 25-34, 35-44, 45-54, 55+). One male and one female were then randomly selected using a modified Kish Grid [19] to achieve equal representation of age-sex groups and examine age-specific sero-prevalence of *Brucella* infection. At the village level, household members were randomly selected until an age-sex group was saturated (Figure 1).

Socio-demographic and household-level risk factors for *Brucella sp.* infection were collected using structured questionnaires administered to the household head. Information on individual-level risk factors (e.g. contact with livestock and dairy consumption practices) was collected from subjects or guardians of child subjects. Interviews were in Punjabi, recorded on tablets using the Open Data Kit application.

A 4ml venous blood sample was obtained from each participant, kept in a cool box and transported to Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (GADVASU). Clotted samples were centrifuged at 2500 rpm for 10 minutes the same or next day, and the serum stored at -20^{o}C until tested. Where available, we also collected an unboiled milk sample from households, transported in a cool box and stored at -20^{o}C within 12 hours until sufficient numbers were available for batch testing.

Serological Testing

An additional ELISA test to the widely used RBT is recommended to obtain high sensitivity[18].RBT testing on human serum samples was conducted at GADVASU with additional training by staff from the World Organization for Animal Health (OIE) brucellosis reference laboratory at the Animal and Plant Health Agency (APHA), UK. Parallel testing of the first hundred serum samples were done in GADVASU and at the Public Health Research Laboratory Post-Graduate Institute of Medical Education and Research (PGIMER). The rest were then tested at PGIMER. RBT was carried out with reagents supplied by the Punjab Vaccine Institute (PVI) in India which follows the OIE protocols for manufacturing and standards. A sub-set of samples was tested with RBT antigen supplied by the APHA for quality assurance. Slides were examined manually according to the OIE method for agglutination [14]. Inconclusive slides were evaluated by a second independent reader for confirmation. All sera were also tested using commercially available IgG ELISA kits (Demeditec, Germany) which had a 100% specificity (n=88) and 100% sensitivity (n=9)

noted by the manufacturer using a CE-marked equivalent ELISA product) as standard. As per the manufacturer's standard kit protocol, optical densities (ODs) were read at 450nm. For each plate, samples were also interpreted as positive if ODs were >20% over the manufacturer's cut-off standard, negative as <20% under the cut-off, and inconclusive if in-between. All inconclusive ELISA samples were classified as negative for the purposes of this study.

Training to carry out the milk testing was also conducted at GADVASU by scientists from the APHA using reagents supplied by them. All milk samples were tested using the commercially available BRUCELISA – 160M, an indirect ELISA produced and standardised in accordance with the OIE for the detection of *Brucella abortus* antibodies in bovine milk samples. Sample ODs were read at 405nm as per the manufacturers' instructions. For each plate, samples were interpreted as positive if ODs were >50% of the median positive control. All positive samples were retested. Those with two positive results were classified as positive.

Statistical Analysis

The outcome of interest was seropositivity for *Brucella* infection by either RBT or IgG ELISA or both. The estimated population prevalence of *Brucella* infection was standardized to the age and sex distribution of the rural population in the 2011 Indian census for Ludhiana, Punjab. Sampling weights were calculated using the Census Population Fraction_{ij}/Study Population Fraction_{ij}, where i=age category and j=sex.

The distribution of participants by category of livestock, dairy risk factors and demographic characteristics were examined. Data on participants' durable household assets and amenities were used to create the same wealth quintiles using principal components analysis as in India's Family Health Survey (National Family Health Survey [20]. Crude and age-sex adjusted odds ratios (ORs) were calculated for *Brucella* infection by each factor using logistic regression with random effects, to account for clustering at the village and household level. Accounting for the highest level of clustering provides correct standard errors when clustering at lower levels also exists [21]. Age, sex and wealth were considered *a priori* confounders. Other variables associated with *Brucella* infection at a p-value of ≤ 0.2 were considered for their role as confounders in a multivariate model.

Forward selection was used to build a multivariable model to obtain adjusted ORs, controlling for confounding. After including prior confounders, other variables were added to the model and retained if there was evidence of an independent association with the outcome and/or they changed the ORs of other key variables in the model by $\geq 10\%$.

Collinearity was assessed by examining if the addition of any variable inflated the standard errors (SEs) of other factors by ≥10%.

To explore associations between human seropositivity and animal seropositivity a subgroup analysis was conducted amongst participants providing a milk sample from household livestock. All analyses were conducted in STATA 15.0 (StataCorp, College Station, TX).

Results

Household refusal rate was 1.81% (20/1104). Out of 1927 individuals selected, 1801 individuals (93.5%) were successfully enrolled.. Of the 1801 individual serological samples tested, 41 had evidence of *Brucella* infection based on either RBT and/or IgG positivity (11 by RBT alone, 23 by ELISA alone, and 7 by both tests; a further 25 had inconclusive ELISA tests and were classified as seronegative for *Brucella*). The overall standardised seroprevalence was 2.24% (95% CI: 1.61-3.11). Prevalence varied by age. Those in the youngest age group had the lowest prevalence and those in the middle age groups had the highest (Figure 2).

The non-specific clinical symptoms that can occur with Brucellosis (i.e. fever of ≥2 weeks and at least one other physical symptom) within the last year were reported by 8.6% (154/1801) of individuals, of which only 3.25% (5/154) were seropositive. Of all those who were seropositive for *Brucella* infection, only 12.2% (5/41) reported experiencing these symptoms in the last year.

The majority were Sikh (93.5%), had completed at least five years of education (i.e. primary school; ages 6 to 10 years), (63.4%), and kept large ruminants in the house (61.0%) (Table 1). About a quarter (23.7%) reported the household head worked in agriculture. The rest either worked in non-agriculture labour or other roles (e.g. homemakers or retired). Although there was a slightly higher prevalence of *Brucella* infection in those with a household head working in agriculture (3.8%) as compared to those with a household head working in non-agricultural (1.8%) or other roles (1.9%), evidence for this association disappeared once adjusted for age and sex. Distributions of other population characteristics are reported in Table 1.

At the household level, 98.8% of individuals reported never buying packaged (i.e. pasteurized) milk, and just over half reported sourcing cow/buffalo milk (60.8%) and other dairy products (57.4%) from their own household as opposed to other sources (neighbouring household, shop, street vendor, or collector).

About a quarter of individuals reported having contact with livestock (26.6%), and milking large ruminants (25.0%) and only 11% ssisted with the calving/abortion of large ruminants

in the previous year (Table 2). A breakdown of livestock contact by sex (Supplementary table 1) indicated females were more likely than males to be in contact with milk or livestock but less likely to assist with calving/abortions. There was only weak evidence that contact with livestock or milking was associated with a higher crude odds ratio of *Brucella* infection, further attenuated after adjusting for age and sex. Only for those who reported assisting with calving and abortion in the previous year was there good evidence for increased odds of infection compared to those who did not (OR 2.61, 95% CI: 1.20-5.67, p=0.02).

Consumption of dairy products was very common, with the majority of individuals (94.4%) reporting consuming cow/buffalo milk daily. However, 95.2% of individuals reported always boiling milk before consuming and 94.5% reported boiling the milk used to make other common dairy products (i.e. bauli, cheese, ice cream) (Table 3). Evidence was lacking that not always boiling milk, or not always boiling the milk used to make other dairy products was associated with *Brucella* seropositivity but numbers were small. There was however evidence that consumption of goat/sheep milk was associated with *Brucella* seropositivity, after adjusting for age and sex despite the very small proportion who did not report boiling milk before consumption (OR 3.27, 95% CI: 1.07-9.93, p=0.06) (Table 3).

The *a-priori* confounder of quintiles of the asset-based index of wealth slightly attenuated the OR for assisting with calving/abortion, but slightly increased the association between *Brucella* and consumption of goat/sheep milk. Household head occupation was included as it changed the OR of assisting with calving/abortion by greater than 10%, and was not collinear with other factors in the model, including helping with calving and abortion. Adding household head occupation to the model however further attenuated the association between *Brucella* seropositive status and calving/abortion, although there still remained some evidence of an association. Additionally, this further increased the association between exposure to *Brucella* and consumption of goat/sheep milk (see Supplementary table 2). Other demographic and population characteristics were evaluated, but did not present any strong associations.

The final multivariable logistic regression model controlled for age, sex, wealth, assisting with calving/abortion of large ruminants, consumption of goat/sheep milk, and household head occupation. After adjustment for these factors, there was still some evidence of an association between *Brucella* infection and livestock and dairy factors. The odds of infection among those who reported assisting with calving/abortion was 2.18 (95% CI: 0.96-4.95, p=0.07) times greater than those who did not, and the consumption of goat/sheep milk was associated with a 4.26 (95% CI: 1.33-13.6, p=0.03) times greater odds of infection (Table 4).

We were able to examine the presence of *Brucella spp*. antibodies in household milk samples from lactating livestock (including cattle and buffalo) for only 37.7% of households (409/1084), representing 41.1% of participants (741/1801) as not all had lactating livestock or unboiled milk samples. Livestock sampled included 409 large ruminant household bulk milk tanks (containing milk from one or more cows and/or buffalo) Of these household milk samples, 8.3% (34/409) were positive. Of the 741 human participants living in these households, only two had *Brucella* antibodies and had a positive livestock milk sample. The crude odds of infection among those who had positive livestock compared to those who did not was 1.56 (95% CI: 0.34-7.03 and p=0.57), (Table 5). Due to the low number of infections, a multivariable model was not constructed.

Discussion

This cross-sectional serological survey found a 2.2% prevalence of *Brucella spp*. infection in a random sample of the rural general population in Punjab, India where ownership of a few large ruminants kept close to the household is moderately high. Seroprevalence was associated with a history of assisting with calving and abortions, rather than through cow's milk or cow's milk products, consistent with most subjects reporting boiling milk. Seropositivity based on a positive RBT or IgG ELISA test or both was used here as a proxy marker of the risk of disease.

In addition there was evidence of a high risk with goat milk consumption but numbers were small. The rural population studied here mostly keep cattle/buffaloes; only a very small proportion keep small ruminants (3%) unlike many other settings. It is also interesting that despite the much higher presence of cattle/buffalo and much higher level of consumption of milk and dairy from cow/buffalo vs. goats, consumption of goat milk emerged as having a stronger association. This may be because consumption of milk from goats although uncommon is less likely to be boiled. It is sometimes held by traditional healers that raw goats' milk is beneficial if ill, as noted in other settings in India where goat and sheep are more common[22].

The long-standing "white revolution" that started the 1960s has successfully provided domestic sources of protein from pasteurised milk and milk products as well as a sustainable source of income [8]. However despite the establishment of dairy cooperatives, the majority of milk in India is still marketed through informal channels [23]. Rural employment patterns are changing in Punjab, but we found that sourcing of milk was still predominantly from household or neighbouring livestock or informal vendors. Boiling is common, however there

is scope for raw milk ingestion (e.g. via cream which is not boiled), as well as a considerable risk from unsafe husbandry practices and exposures from rural livestock keeping.

We were unable to show a direct link at the household level between positive cattle based on milk samples and risk of infection. The numbers were small as milk was not available from all households or all cattle. Cattle may also only be intermittently shedding. There may also be some potential under-ascertainment. Direct information on sensitivity is not available, but the milk ELISA complies with the OIE minimum analytical sensitivity requirements when used in standard samples and has a diagnostic specificity of 99.93% based on over 4000 non-infected animal samples. (J McGiven, APHA, Personal Communication).

In addition to the difficulty of identifying household infected herds other limitations include the use of prevalence of infection as a proxy measure of risk and potential underreporting of raw milk consumption given the longstanding public health advice to boil all milk. These would both however only act to underestimate any risk of *Brucella* from dairy products or animal husbandry. It should, in addition, be noted that the predominant Brucella species in sheep and goats is *Brucella melitensis* and that the serological tests used in this study would not differentiate between antibodies induced by *B. abortus* vs. *B. melitensis*.

This study suggests a higher risk of infection with goat or sheep milk consumption, despite the small numbers, that requires further confirmation. If confirmed, our results are compatible with the population being exposed to both species of Brucella through different routes: direct contact with cattle (*B. abortus*) and consumption of sheep/goat milk (*B. melitensis*).

Our findings are consistent with the literature e.g. the well-recognised risk from soft cheeses from such milk in Turkey [5]. In many settings, human infection can be traced to either direct or indirect contact to infected livestock and their products [6] with the predominant routes of transmission via the consumption of raw dairy products such as unpasteurized milk, soft cheeses, and yogurt [5] and contact with livestock—particularly during delivery and abortion [6, 9]. The latter seemed more important in this study with over 10% reporting assisting with calving and abortion in the previous year. Infection may also occur through contamination of wounds as well as through inhalation of airborne animal manure particles; however, these routes are more usually noted in occupationally exposed farmers, veterinarians and abattoirs, than in the general population [9, 24].

A recent systematic review of brucellosis incidence found wide variation in estimates both between and within endemic countries.Demographic, environmental and socio-economic characteristics (including types of livestock systems and any animal vaccination programmes) were important factors associated with infection [25]. The seroprevalence seen in our study is moderately low. In our setting only B *abortus* has so far been culture-

confirmed in cattle samples and in human cases[26]. Although there may be scope for some misclassification (it is somewhat unclear the basis on which the manufacturers of the ELISA tests used here advise the cut-offs), the low prevalence here is likely to be due to lower contact rates and lower prevalence of small ruminants, than that seen in highly endemic settings. In Kyrgyzstan the seroprevalence was nearly 9%, based similarly on RBT and IgG ELISA tests but where small ruminant (goats and sheep) keeping is also common [27]. In Togo using the same testing procedures, a prevalence of 2.4% in 255 randomly sampled pastoralists was noted. Nearly all (97%) owned or looked after both large and small ruminants, but *B. melitensis* was not found in random samples of animals tested at the same time [28]. *B. melitensis*, which infects sheep and goats, is responsible globally for most human infections, followed by *B. abortus* in cattle, and *B. suis* in pigs [3]. There is a small additional literature on seroprevalence in humans based often on RBT testing only. For example about a 2% prevalence of infection in humans was noted in settings from Argentina, Mexico, and Ethiopia, but is higher in settings like Iran, Iraq and Turkey [25].

Brucellosis was first reported in India in the early 1900s, and it is now considered endemic in most parts of the country [24] with reports based, often on convenience sampling, indicating that the disease is widespread in cattle, but appears to be rising concomitantly with increasing trade and movement of livestock [9, 10]. However high quality estimates of human incidence, and prevalence of past infection, are limited [9, 25]. Brucella prevalence in cattle is also heterogeneous[9], thus the findings in this study in humans is not necessarily generalizable across a large country like India. A study conducted in Karnataka estimated seroprevalence in humans to be 1.8% using RBT and serum tube agglutination (SAT) from a total of 26,946 serum samples, but the representativeness of this population was not specified [29]. Another recent study conducted using convenience sampling of differentially exposed populations in Jammu, found an overall seroprevalence of 4.96% using similar tests [30]. Although the tests used in these studies have been important for surveillance and screening, there is scope for additional testing using ELISAs [2].

Where risk of brucellosis to humans is partially controlled by pasteurising or boiling milk, human cases are predominantly due to occupational exposure. Indeed, a study conducted in Ludhiana showed a greater than three-fold increased risk from dealing with parturient domestic animals. It estimated the prevalence of infection in a convenience sample only of occupationally exposed groups (veterinarians, para-veterinarians, animal attendants, and farmers), some of whom were under investigation for fever, to be 24.5% by RBT and 26.6% by SAT [31].

In summary this study indicates that the general rural population in Punjab, India are at risk of brucellosis mostly via household livestock keeping rather than via food-borne routes apart from possibly goats milk consumption. The resulting morbidity and chronic disability may be partly ameliorated if clinical suspicion is high, and testing and treatment available but can play only a limited role compared to effective, well resourced and quality-assured animal vaccination programmes.

The findings reinforces the importance of the use of available effective animal vaccines to reduce the burden of disease in both animals and human in this and other low-income settings. Live animal vaccines S19 for *B*. *abortus* and Rev1 for *B*. *melitensis* have been shown to have high efficacies in calves and sheep/goats respectively [13]. Limited availability of these vaccines and training in their safe delivery need to be rectified. Robust programmes offering them to young animals before reproductive age are also required, perhaps added to existing animal vaccination programmes for foot and mouth disease and haemolytic septicaemia [15], together with information and training on how to protect against infection. More information on the *Brucella* species prevalent in small ruminants, although uncommon in Punjab, may also be useful including informing animal vaccination programmes more widely.

Finally, diagnosis of acute disease in humans is possible with serological testing for high or rising antibody titres, and maybe more recent molecular tests but medical awareness should be raised and testing guidelines for fever, sacroiliatis, seronegative arthopathies, valvular or unusual neurological presentations should include testing for brucellosis.

Authors' contributions

PM and RK conceived the study, designed the protocol and are overall guarantors for the study. IB, WB, HH implemented the study with AK, SB and VS with advice from JM,JG, JB and PK. IB, WB, PND and PM carried out the analyses and interpretation of the data. WB, IB and PM drafted the initial manuscript. RK, MK, JM, JG, JSG and GSG critically revised the manuscript for intellectual content. All authors read and approved the manuscript.

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Competing interests

All authors declare that they have no conflicts of interest

Ethical approval

Approval was obtained from the ethics committees at PGIMER, Chandigarh, India, the London School of Hygiene and Tropical Medicine (LSHTM) and the Royal Veterinary College (RVC) in the UK. Informed, written consent for interviews and blood samples was obtained from all participants, or their legal guardian for minors.

Legends for figures

Figure 1. Sampling strategy for recruiting individuals in Ludhiana, Punjab, India.

Figure 2. Seroprevalence of *Brucella Spp* by Age Category in Ludhiana, Punjab, India.

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Table 1. Sample distribution, Crude and Baseline Odds Ratios for *Brucella* infection in Ludhiana, Punjab (n=1801)

Variable	Frequency (%)	%Sero-positive	Crude OR[1] (95% CI)	P-Value*	Baseline OR[2] (95%CI)	P-Value*
Age				0.31		0.21
5-14	298 (16.6)	1.0	*1*		*1*	
15-24	298 (16.5)	1.7	1.68 (0.40-7.10)		1.70 (0.40-7.20)	
25-54	906 (50.3)	2.8	2.80 (0.84-9.33)		2.87 (0.86-9.61)	
55+	299 (16.6)	2.7	2.71 (0.71-10.3)		2.75 (0.72-10.5)	
Sex				0.44		0.37
Female	897 (49.8)	2.0	*1*		*1*	
Male	904 (50.2)	2.5	1.28 (0.68-2.38)		1.33 (0.71-2.49)	
Education level[a]				0.39		0.57
None	298 (16.6)	3.4	*1*		*1*	
<6 years complete (i.e. primary)[b]	359 (19.9)	2.0	0.57 (0.21-1.53)		0.74 (0.26-2.13)	
≥6 years complete (i.e. secondary)[b]	1142 (63.4)	2.1	0.61 (0.29-1.30)		0.63 (0.27-1.46)	
Household Head Occupation				0.07		0.17
Agriculture/Dairy	426 (23.7)	3.8	*1*		*1*	
Non-agriculture Labour	686 (38.1)	1.8	0.46 (0.21-0.98)		0.52 (0.24-1.13)	
Other[c]	689 (38.3)	1.9	0.49 (0.23-1.04)		0.54 (0.25-1.14)	
Religion				0.04		0.11
Sikh	1684 (93.5)	2.3	*1*		*1*	
Hindu	102 (5.7)	1.0	0.44 (0.06-3.24)		0.43 (0.06-3.18)	
Muslim	15 (0.8)	13.3	6.70 (1.43-31.3)		5.76 (1.22-27.3)	
Asset Based Index of Wealth[d]				0.16		0.23
Bottom quintile	396 (22.0)	1.5	*1*		*1*	
Second quintile	391 (21.7)	2.6	1.72 (0.61-4.79)		1.68 (0.60-4.71)	
Third quintile	335 (18.6)	1.5	0.99 (0.30-3.31)		0.99 (0.30-3.30)	
Fourth quintile	328 (18.2)	1.8	1.22 (0.39-3.83)		1.16 (0.37-3.67)	
Top quintile	351 (19.5)	4.0	2.73 (1.03-7.23)		2.51 (0.94-6.69)	
Household Size[a]				0.84		0.68
1-3 members	389 (21.6)	2.1	*1*		*1*	
4-5 members	824 (45.8)	2.2	1.06 (0.45-2.46)		1.24 (0.53-2.92)	
>5 members	582 (32.3)	2.6	1.26 (0.53-3.01)		1.48 (0.61-3.56)	
Large Ruminants kept in the house				0.11		0.14
No	702 (39.0)	1.6	*1*		*1*	
Yes	1099 (61.0)	2.7	1.76 (0.87-3.55)		1.67 (0.82-3.38)	
Small Ruminants kept in the house				0.13		0.22
No	1743 (96.8)	2.2	*1*		*1*	
Yes	58 (3.2)	5.2	2.58 (0.75-8.88)		2.36 (0.68-8.16)	

[a]Missing values for school n=2 (0.1%); household size n=6 (0.3%). [b]Primary school: ages 6-10, Secondary school+: ages >10 [c]Other includes those reporting as homemakers (n=309), retirees (n=121), unemployed (n=155) or sick (n=104). [d]Wealth index created using principal components analysis on the following household assets and amenities: Drinking water source; type of toilet facility; hand washing facility; household electrification; type of flooring; type of roofing; type of walls; household internet access, cooking facility; cooking fuel; and ownership of an electric fan, 1rigerator, radio, television, computer/laptop, smartphone, mobile phone, landline, bicycle, scooter, car/jeep, auto, and a tractor. [1]Crude ORs calculated from univariate logistic regression models with random effects at village level. [2]Baseline ORs calculated using crude models adjusting for age and sex. *P-values calculated from likelihood ratio test.

Table 2. Distribution of Livestock Exposure Measures, Crude and Baseline Odds Ratios for *Brucella* infection in Ludhiana, Punjab (n=1801)

Variable	Frequency (%)	% Seropositive	Crude OR[1] (95% CI)	P-Value*	Baseline OR[2] (95% CI)	P-Value*
Contact large ruminant livestock (≥1 per year)				0.07		0.22
Never	1321 (73.4)	1.9	*1*		*1*	
Ever[a]	480 (26.6)	3.3	1.79 (0.94-3.40)		1.54 (0.78-3.03)	
Milking Large Ruminants				0.09		0.22
Never	1350 (75.0)	1.9	*1*		*1*	
Ever[b]	451 (25.0)	3.3	1.76 (0.92-3.36)		1.55 (0.78-3.08)	
Assisting with Calving/Abortion of Large Ruminants (<12 months)				0.002		0.02
Never	1603 (89.0)	1.9	*1*		*1*	
Yes[c]	198 (11.0)	5.6	3.11 (1.52-6.37)		2.61 (1.20-5.67)	

[a]Of which, 469 individuals report at least 1 livestock contact in the past 12 months. [b]Of which, 450 individuals report milking at least once in the past 12 months. [c]Of which, 10 report assisting with both calving and abortion. [1]Crude ORs calculated from univariate logistic regression models with random effects at village level. [2]Baseline ORs calculated using crude models adjusting for age and sex. *P-values calculated from likelihood ratio test.

Table 3. Distribution of Dairy Exposure Measures, Crude and Baseline Odds Ratios for Brucella infection in Ludhiana, Punjab (n=1801)

Variable	Frequency (%)	% Seropositive	Crude OR[1] (95% CI)	P-Value*	Baseline OR[2] (95% CI)	P-Value*
Consume Cow/Buffalo Milk Daily				0.66		0.55
Yes	1700 (94.4)	2.2	*1*		*1*	
No[a]	101 (5.6)	3.0	1.31 (0.39-4.40)		1.48 (0.44-4.99)	
Boiling Cow/Buffalo Milk[b]				0.07		0.12
Always	1715 (95.2)	2.1	*1*		*1*	
Not Always	74 (4.1)	5.4	2.72 (0.93-7.96)		2.63 (0.89-7.78)	
Consume Other Dairy Products (i.e. Bauli, cheese, ice cream)				0.59		0.49
Ever	1725 (95.8)	2.3	*1*		*1*	
Never	76 (4.2)	1.3	0.58 (0.08-4.36)		0.53 (0.07-3.97)	
Boiling Other Dairy Products (i.e. Bauli, cheese, ice cream)[a]				0.55		0.60
Always	1701 (94.5)	2.3	*1*		*1*	
Not Always	24 (1.3)	4.2	1.87 (0.24-14.4)		1.82 (0.23-14.2)	
Consume Goat/Sheep Milk				0.03		0.06
Never	1741 (96.7)	2.1	*1*		*1*	
Ever[c]	60 (3.3)	6.7	3.49 (1.16-10.6)		3.27 (1.07-9.93)	

[a]Of which, 12 reported never having consumed milk. [b]Missing values for Boiling milk n=12 (0.7); boiling other dairy n=76 (4.2). [c]Of which, 93.3% (n=56) report always boiling goat/sheep milk. [1]Crude ORs calculated from univariate logistic regression models with random effects at village level. [2]Baseline ORs calculated using crude models adjusting for age and sex. *P-values calculated from likelihood ratio test.

Table 4. Adjusted Odds Ratios for Brucella infection in Ludhiana, Punjab (n=1801)

Variable	Adjusted OR[1] (95% CI)	P-Value*
Assisting with Calving/Abortion of Large Ruminants (<12 months)		0.07
Never	*1*	
Yes	2.18 (0.96-4.95)	
Consume Goat/Sheep Milk		0.03
Never	*1*	
Ever	4.26 (1.33-13.6)	
Age		0.73
5-14	*1*	
15-24	1.39 (0.33-5.94)	
25-54	1.86 (0.54-6.47)	
55+	1.88 (0.47-7.47)	
Sex		0.86
Female	*1*	
Male	1.06 (0.54-2.09)	
Asset Based Index of Wealth		0.31
Bottom quintile	*1*	
Second quintile	1.75 (0.62-4.99)	
Third quintile	1.01 (0.30-3.41)	
Fourth quintile	1.09 (0.34-3.56)	
Top quintile	2.35 (0.83-6.68)	
Household Head Occupation		0.48
Agriculture/Dairy	*1*	
Non-agriculture Labour	0.63 (0.27-1.48)	
Other	0.66 (0.30-1.44)	

[1]Adjusted ORs calculated using logistic regression models with random effects at village level, adjusting for all variables listed in table. *P-values calculated from likelihood ratio test.

Table 5. Distribution of Large Ruminant Milk Samples and Crude Odds Ratios for Brucella infection in Ludhiana, Punjab (n=741)

Variable	Frequency (%)	Seropositive Humans (%)	Crude OR[1] (95% CI)	P-Value*
Household Livestock				0.57
Negative	678 (91.5)	2.06	*1*	
Positive	63(8.5)	3.17	1.56 (0.34-7.03)	

[1]Crude ORs calculated from univariate logistic regression models with robust standard errors for clustering. *P-values obtained from Wald's test.

Figure 1. Sampling strategy for recruiting individuals in Ludhiana, Punjab, India.

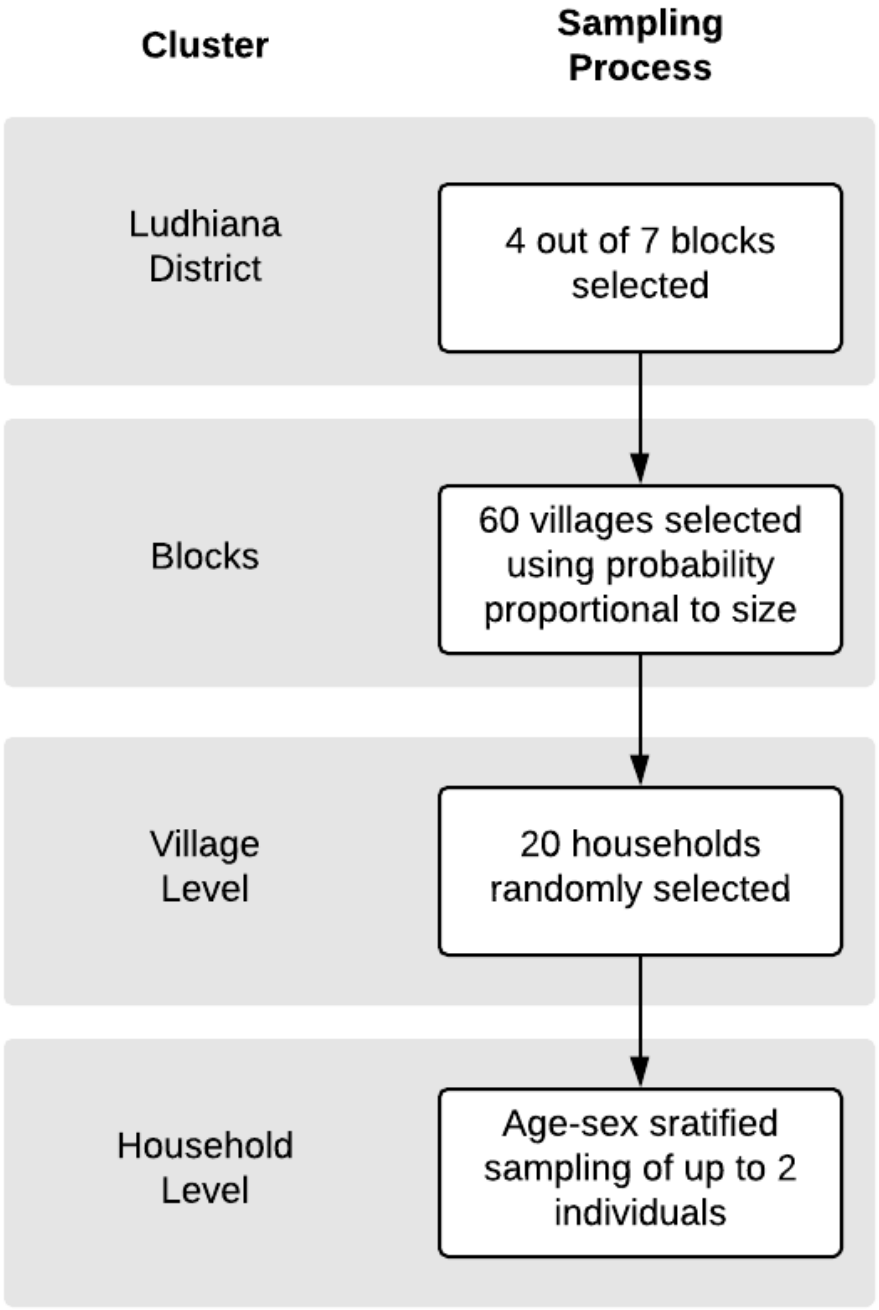
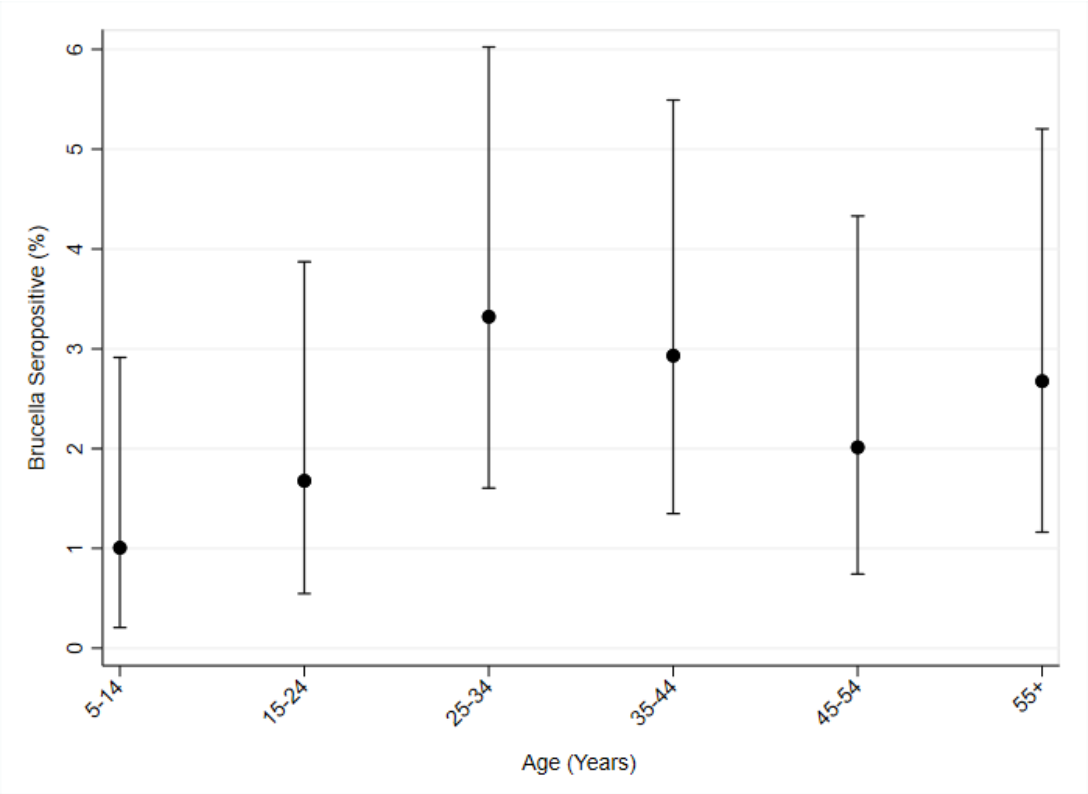


Figure 2. Seroprevalence of *Brucella Spp* by Age Category in Ludhiana, Punjab, India.



Supplementary material

Supplementary Table 1. Livestock contact by sex (row%) in Ludhiana, Punjab (n=1801)

	Total N	Male N (%)	Female N (%)	P-value
Contact large ruminant livestock (≥1 per year)				<0.001
Never	1321	711 (53.8)	610 (46.2)	
Ever	480	193 (40.2)	287 (59.8)	
Milking large ruminants				<0.001
Never	1350	739 (54.7)	611 (45.3)	
Ever	451	165 (36.6)	286 (63.4)	
Assisting with Calving/Abortion of Large Ruminants (<12 months)				<0.001
Never	1603	745 (46.5)	858 (53.5)	
Yes	198	159 (80.3)	39 (19.7)	

Supplementary Table 2. Baseline intermediate and final models for the adjusted Odds Ratios for *Brucella* infection in Ludhiana, Punjab (n=1801). Model building process

Variable	Model 1[1] OR (95% CI)	P-Value*	Model 2[1] OR (95% CI)	P-Value*	Final Model[1] OR (95% CI)	P-Value*
Assisting with Calving/Abortion of Large Ruminants (<12 months)		0.02		0.04		0.07
Never	*ref*		*ref*		*ref*	
Yes	2.62 (1.19-5.75)		2.41 (1.08-5.38)		2.18 (0.96-4.95)	
Consume Goat/Sheep Milk		0.07		0.04		0.03
Never	*ref*		*ref*		*ref*	
Ever	3.23 (1.05-9.90)		3.97 (1.25-12.6)		4.26(1.33-13.6)	
Age		0.56		0.65		0.73
5-14	*ref*		*ref*		*ref*	
15-24	1.51 (0.36-6.43)		1.41 (0.33-6.03)		1.39 (0.33-5.94)	
25-54	2.16 (0.63-7.42)		1.97 (0.57-6.82)		1.86 (0.54-6.47)	
55+	2.11 (0.54-8.28)		1.97 (0.50-7.78)		1.88 (0.47-7.47)	
Sex		0.85		0.87		0.86
Female	*ref*		*ref*		*ref*	
Male	1.07 (0.55-2.08)		1.06 (0.54-2.07)		1.06 (0.54-2.09)	
Asset Based Index of Wealth	--			0.21		0.31
Bottom quintile			*ref*		*ref*	
Second quintile			1.80 (0.63-5.11)		1.75 (0.62-4.99)	
Third quintile			1.03 (0.30-3.48)		1.01 (0.30-3.41)	
Fourth quintile			1.18 (0.37-3.83)		1.09 (0.34-3.56)	
Top quintile			2.65 (0.95-7.38)		2.35 (0.83-6.68)	
Household Head Occupation	--		--			0.48
Agriculture/Dairy					*ref*	
Non-agriculture Labour					0.63 (0.27-1.48)	
Other					0.66 (0.30-1.44)	

[1]ORs calculated using logistic regression models with random effects at village level, adjusted for all variables listed in respective column.*P-values calculated from likelihood ratio test.