Tropical Animal Health and Production

Epidemiology of Bovine Brucellosis in Hisar-India: Identification of Risk Factors and Assessment of Knowledge, Attitudes and Practices among Livestock Owners.

--Manuscript Draft--

Manuscript Number:	TROP-D-20-01879R1
Full Title:	Epidemiology of Bovine Brucellosis in Hisar-India: Identification of Risk Factors and Assessment of Knowledge, Attitudes and Practices among Livestock Owners.
Article Type:	Regular Articles
Corresponding Author:	Adamu Saleh Saidu, DVM, MSc., Ph.D. Lala Lajpat Rai University of Veterinary and Animal Science Hisar, Haryana, India INDIA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Lala Lajpat Rai University of Veterinary and Animal Science
Corresponding Author's Secondary Institution:	
First Author:	Adamu Saleh Saidu, DVM, MSc., Ph.D.
First Author Secondary Information:	
Order of Authors:	Adamu Saleh Saidu, DVM, MSc., Ph.D.
	Professor Nand K. Mahajan, Ph.D
	I. I. Musallam, DVM, MSc., Ph.D.
	H. R. Holt, DVM, MSc., Ph.D.
	J. Guitian, Ph.D
Order of Authors Secondary Information:	
Funding Information:	Indian Council of Agricultural Research (IN) (Grant No: [5510-C(b)-VPHE-1-ICAR].) — Dr. Adamu Saleh Saidu

Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation

Revised manuscript clean version

Click here to view linked References

Click here to access/download;Revised manuscript clean version;Brucella Risk factors_India_Adamu_30-4-

Epidemiology of Bovine Brucellosis in *Hisar*-India: Identification of Risk Factors and Assessment of Knowledge, Attitudes and Practices among Livestock Owners

A. S. Saidu*[1,3], N. K. Mahajan[1], I. I. Musallam[2], H. R. Holt[2], J. Guitian[2]

[1]*Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences, LUVAS, Hisar-125004, Haryana, India*

[2]*Veterinary Epidemiology, Economics and Public Health Group, Department of Pathobiology and Population Sciences, The Royal Veterinary College, AL9 7TA, Hertfordshire, United Kingdom*

[3]*Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri, P.M.B., 1069, Maiduguri-600230, Borno State, Nigeria*

**Corresponding email address: adamudvm13@gmail.com*

ORCID ID: https://orcid.org/0000-0003-4356-268X

Abstract

Brucellosis caused by facultative intracellular bacteria, *Brucella*, remains a global threat to both animal and human health. In this study we aimed to identify potential risk factors of bovine brucellosis and to assess the knowledge, attitudes, and practices (KAPs) of livestock keepers in Hisar, India. A standardized questionnaire was used to collate information regarding potential risk factors of bovine brucellosis and livestock owners' KAPs. A total of 127 livestock keepers were involved. Serum samples from their animals (n= 635) were tested for the presence of antibodies against *Brucella* by Rose Bengal Plate Test (RBPT) and indirect Enzyme-linked immunosorbent assay (iELISA). Out of these 78 (61.4%) of the herds had at least one seropositive animal and 302 (47.6%) of the cattle were seropositive. Univariate and multivariate analysis revealed significant associations between intensive farm type (OR= 4.6, 95% CI: 1.6 - 16.7, P= 0.009), hygienic disposal of aborted fetuses (OR= 0.3, 95% CI: 0.08 – 0.9, P=0.04) and herd seropositivity for brucellosis. The majority, 96 (75.6%) of the respondents were males aged 18-50, and 82 (64.6%) owned a small-backyard farm. Only 51 (40.2%) of the participants knew about brucellosis, out of them, 54.9% (28/51) could not identify clinical signs of brucellosis. Six (11.8%) participants indicated abortion as the most noticeable clinical sign, 45.1% indicated that consumption of raw milk is associated with high risk of contracting brucellosis. A large proportion of respondents confirmed that milk from their animals was regularly consumed (86.6%) and sold (59.8%) to other people. These results suggest that bovine brucellosis is endemic in Haryana, where *Brucella*-contaminated milk is likely being regularly sold. Brucellosis control efforts in Haryana should include education programs to raise awareness of the disease and means to control it in cattle and to prevent zoonotic transmission.

Keywords: Bovine-Brucellosis, Hisar, iELISA, India, RBPT, Risk-Factors

1.0 **Introduction**

Brucellosis is one of the most widespread zoonotic infections worldwide, and considered to be responsible for a high disease burden in most low-income countries (Deka et al. 2019). The disease remains an important neglected zoonotic threat in these countries due to its dual effects on livestock and human health (Franc et al. 2018). Brucellosis is endemic in many parts of India and is assumed to pose a substantial economic and public health burden (Renukaradhya et al. 2002; Chand and Chhabra, 2013). India has the largest buffalo population and second-largest cattle population in the world with estimated figures of 105.3 million and 199.08 million respectively in 2012 (Census, 2012). The dairy sector is essential for the livelihood of millions of people and has contributed to the growth of Indian Gross Domestic Product (GDP) over the years. However, brucellosis has continued to be responsible for significant losses, which have been estimated to amount to $US 3.43 billion annual loss for the livestock sector and 177,601 Disability Adjusted Life Years (DALYs) (Singh et al. 2015; Singh et al. 2018).

In India, the first investigation of '*contagious abortion'* in livestock, associated with brucellosis was carried out by the then Imperial Veterinary Research Institute (now Indian Veterinary Research Institute), Mukteswar, in Northern India (Anonymous, 1918). Since then, serological evidence of the infection has been reported from various states and the disease is considered endemic throughout India, especially in the Northern states which have large livestock populations that move freely without any restriction (Polding, 1942; Mahajan et al. 1986; Zaki et al. 1981; Mahajan and Kulshreshtha, 1991; Isloor et al. 1998a; Chand and Chhabra, 2013; Saidu et al. 2020). An extensive long-term sero-epidemiological survey of brucellosis was conducted by the Project Directorate Animal Disease Monitoring and Surveillance (PD-ADMAS) in 24 states of India between 1994 – 2001 (Isloor et al., 2001). As part of the survey, a total of 47,775 bovines comprising 38,319 cattle and 9,456 buffalo from 24 states and a union territory in India were sampled using convenience sampling and tested by RBPT and standard tube agglutination test (STAT). The results revealed 5.0% apparent prevalence in cattle and 3.0% in buffaloes

(Renukharadya et al. 2002). In the state of Madhya Pradesh, which shares borders with Haryana, a survey by Mehra et al. (2000) tested a total of 1,860 serum samples collected from cows, buffaloes, heifers and bulls for antibodies against *Brucella* using STAT. The reported overall seroprevalence in cattle and buffaloes was 6.3% (95% CI: 5.5-7.7). The survey showed a higher prevalence in cattle in organized farms 6.8% (111/1,629) compared to that of unorganized farms 5.1% (12/231). More recently, a number of smaller serosurveys have been carried out in different states of India. A study in Vidarbha region of Maharashtra state estimated the seroprevalence of bovine brucellosis at 9.1%. In Punjab, a study conducted in 32 villages detected the presence of antibodies against *Brucella* in milk samples from 18.3% of the studied animals (Aulakh et al. 2008). In this study, the prevalence in the central zone of the state was significantly higher, 23.2% ($\chi^2 = 11.34$, $p < 0.01$) than in the sub-mountainous zone (14.2%) and the arid irrigated zone (5.8%).

The state of Haryana has a large livestock population that represents a major source of income for rural families. Although previous studies have shown the presence of bovine brucellosis in Haryana, little is known regarding its frequency and distribution (Chand et al. 2014; Mahajan and Kurulshethra, 1991). Furthermore, previous studies of cattle brucellosis in this part of India did not attempt to assess livestock owners' KAPs in relation to brucellosis. Hence, this study aimed to identify potential risk factors for *Brucella* infection in cattle herds in *Hisar* district, Haryana, and to assess the knowledge, attitudes and practices (KAPs) of livestock owners regarding brucellosis and explore milk and dairy products processing and consumption practices in the area. The findings of this study are expected to provide information useful for the formulation of cattle brucellosis control programs and public health policies in that region.

2.0 Materials and Methods

2.1 Study Area

The study was conducted in *Hisar* district in the state of Haryana which is a north-central state neighboring New Delhi (Figure 1). The state has a total area of 44,212 km^2 and a population of 27,761,063, the total livestock population of Haryana is 8.81 million, with buffalo accounting for 69% followed by cattle 20.5% (1.8 million heads), according to the 2012 census data (Census, 2012). The primary livestock management system in Haryana is semi-intensive with mostly small herds.

2.2 Study Design and Herd Selection

A non-random survey involving 127 cattle herds with clinical history suggestive of brucellosis was conducted between June and December, 2018. Livestock owners visiting the Lala Lajpat Rai University of

Veterinary and Animal Sciences (LUVAS) college clinic (n=127) between June and August 2018, representing herds located around Hisar, were recruited. Herd selection was therefore based on convenience and not probabilistic. Informed consent was sought from each of the interviewed livestock owners with participants informed that their identity would remain confidential.

Herds that presented with a history suggestive of brucellosis (i.e., history of abortion, infertility or retained placenta) were assessed by vets and clinicians at the LUVAS-clinic and considered suspected cases and were selected subsequently. During the study period, the clinic was visited by the investigators twice every week (24 visits in total) and a total of 127 herds were included (average of 2 herds per day). During these visits all eligible herds in a given day were included in the study, and individual animals were selected. In each of the selected herds, 5 individual animals were selected strictly based-on either clinical signs indicative of brucellosis such as abortion, infertility, hygroma and low milk-yield or based on previous history of brucellosis. If the number of animals with clinical signs indicative of brucellosis in a herd is more than 5, only the first 5 animals were selected, and if the number is less than 5, other asymptomatic animals were selected haphazardly until the target of 5 animals per herd was reached. The majority of herds are located in Hisar district of Haryana and few of them are located in the nearby villages (Table 1).

When a livestock owner was selected, samples were collected immediately at the clinic after being consented and follow up visit was made to the herd to sample other animals.

2.4 Sample Collection

Whole blood samples were aseptically collected from the jugular vein of animals using 16- gauge needle and immediately transferred into the plain glass screw-capped bottle (HiMedia, India). The tubes were gently inverted 6-8 times; blood samples were allowed to clot in an upright position for at least 30 minutes but not longer than 2 hours before centrifugation. Blood samples were centrifuged at 2,500 rounds ×g for 15 minutes within one hour of collection. Then, the serum was harvested into a plastic screw-capped serum-vial, labeled and stored at -20ºC in the Veterinary Public Health and Epidemiology laboratory until used.

2.5 Serological Screening

Collected serum samples (n= 635) were tested by both, RBPT and iELISA. A sample was considered positive, only if it was positive by both tests, as per OIE recommendation. Rose Bengal colored antigen was procured from the IVRI-ICAR (Bareilly, UP, India). It is a qualitative test of macroscopic agglutination performed with only one dilution, and which mainly detects IgG1, but not IgG2 antibodies (Cardoso et al.

2006). The standard procedure for performing RBPT as described by Nicoletti (1967) was used. The results of the RBPT were interpreted as negative or no agglutination, or positive with agglutination.

All serum samples, regardless of their RBTP status, were tested by means of a commercial indirect ELISA assay (Arsh Biotech Pvt. Ltd., India) that uses lipopolysaccharides (LPS) as an antigen. An animal was considered positive only when it was positive by both tests. An ELISA reader (Gen-5 software, BioTek-Synergy-2, USA) was used to measure the absorbance at 450nm wavelength and results were interpreted as per manufacturer's instructions.

2.6 Questionnaire Administration and Data Collection

A standardized, structured questionnaire from Musallam *et al*. (2015) was modified for this study. It comprised of three parts with close-ended questions.

Part A of the questionnaire was designed to capture information on farm structure, characteristics, production and management practices. It included questions on farm type, herd-size, mixing of animals with small ruminants and other cattle, the introduction of new species, quarantine of new animals, separation of aborted cattle from the herd and hygienic disposal of aborted materials. These variables were considered in the risk factors analysis.

Part B of the questionnaire was designed to capture information on the KAPs of the livestock owners regarding brucellosis and included questions on: the knowledge about the clinical signs of brucellosis in ruminants, potential transmission routes from animals to humans, clinical signs of brucellosis in animals and humans, livestock owners' practices in the case of cow's normal parturition and management of suspected animals or abortion cases, all questions were closed ended in which the participants were asked to choose from a predefined set of answers *"high risk/moderate risk/no risk*" for questions related to transmission routes from animals to humans and *"most farmers/some farmers/no one"* for questions related to disease management practices. Parts A and B of the questionnaire were administered among those individuals responsible for rearing livestock.

Part C of the questionnaire was designed to capture information on milk consumption, handling and processing with close ended questions in which the participants were asked to choose from a predefined set of answers: "*regularly/ sometimes/never*". Part C of the questionnaire was administered among people responsible for the processing of milk and dairy products in the farm/household.

2.7 Data Management and Analysis

Data from the questionnaire were entered into the Microsoft Excel (Version 2019 for Windows 10), and imported into statistical package for social sciences (SPSS^{R} Version 20.0 for Windows^{R}, SPSS Inc., Chicago, USA) and "R" software (R_Statistical package 3.0.2 R Development CoreTeam, http://www.rproject.org) for further statistical analysis. Descriptive statistics were carried out using Microsoft Excel (Version 2019 for *Windows^{R} 10).

2.7.1 Risk Factors Analysis

The associations between the serological status of the herd (as binary outcome; positive vs negative) and potential risk factors (namely: farm type, median herd-size, mixing of animals with small ruminants and other cattle, the introduction of new cows, quarantine of new animals, separation of aborted cattle from the herd, and hygienic disposal of aborted fetuses) were assessed. Firstly, univariate analysis was carried out for those variables and the serological status of the herd using chi-squared (χ^2) test of association. Only those variables with $P < 0.2$ from the univariate analysis were considered as candidates for multivariate analysis. Candidate variables were assessed for collinearity employing Cramer's phi-prime (Ø) statistic; variables were considered collinear if (Ø > 0.7). When a pair of variables was found to be collinear, only the more biologically plausible variable was kept for further analysis using logistic regression. Multivariate logistic regression was used to assess the association between selected potential risk factors identified as candidate variables and the serological status of the herd as binary outcome (positive vs. negative). A manual backwards stepwise procedure was used for the least significant variables when $P \geq 0.05$, the analysis was then repeated using forward selection starting with variables with lowest p-value in the univariate analysis to ensure that the same results were obtained. Only variables with $P < 0.05$ were retained in the final model. Univariate analysis was carried out using SPSS^{R} Version 20.0 and multivariate logistic regression was carried out using the function *glm* implemented in R package *survival*.

2.7.2 KAPs Analysis

A herd was classified as exposed or not exposed to a particular practice based on the responses provided to questions on the "likely course of action of livestock keepers in the village"as opposed to the likely action by the interviewed livestock keeper themself. Descriptive statistics for the variables included in the KAPs analysis were carried out in Microsoft Excel, Version 2019.

2.8 Ethical Approval

The study was approved by the LUVAS *Institutional* A*nimal Ethical Committee (IAEC)* No.VCC/IAEC/265-93; dated 15/02/2018.

3.0 Results

3.1 Serological Status

From the 127 herds included in the survey, 635 individual animals were tested for brucellosis. Out of these, 321 (50.6%), 308 (48.5%) and 302 (47.6%) individual animals were positive by RBPT, iELISA, and both tests, respectively. Thus, 302 (47.6%) animals representing 78 (61.4%) herds were considered seropositive against *Brucella* spp., as per the criteria set for *Brucella* seropositivity in this study.

3.2 Demographic Information

Questionnaires were filled in all the 127 farms. Most participants were males (75.6%) within the median age of 24, with the majority of herds being backyard (64.6%) with a median herd size of 9 (Table 2).

3.3 Risk Factor Analysis

The results of the univariate analysis for the associations between the potential risk factors and the serological status of the farm against *Brucella* spp infection presented as binary outcome (i.e., seropositive vs. seronegative) revealed three variables that were significantly associated with the outcome, namely: Farm type, Herd size (above or below the median) and hygienic disposal of the placenta (Table 3). The multivariate logistic regression model retained two variables that were significantly associated ($P<0.05$) with the serological status of the herd namely: intensive farm type (OR= 4.6, 95% CI: 1.6 - 16.7, P= 0.009) and hygienic disposal of the aborted fetuses (OR= 0.3, 95% CI: 0.08 – 0.9, P=0.04), Table (4).

3.4 Knowledge of Brucellosis

When asked if they had heard about a disease called brucellosis, 51 (40.2%) of the participants responded "yes". Of those that had heard of the disease, 31.4% said they had heard about the disease from the media, 47.0% from local veterinarians and 21.6% from other farmers. Twenty-five (49.0%) of those who heard about brucellosis were sure that cattle/buffalo could be infected with brucellosis, 15 (29.4%) were sure that sheep/goats can be infected with brucellosis, and 11 (21.6%) were sure that other animals like dogs could be infected with brucellosis (Table 5). Regarding their knowledge of the clinical signs of brucellosis in animals, 28 (54.9%) did not mention any clinical signs of brucellosis, but 6 (11.8%) participants indicated that abortion is the most noticeable clinical sign. Only 4 (7.8%) participants additionally identified infertility and weight loss and 3 (5.9%) mentioned a drop-in milk production. Other responses to the knowledge about brucellosis are presented in Table 6.

3.5 Livestock Owners' Attitude and Practices Regarding Brucellosis

When the 51 participants who reported that they knew about brucellosis were asked about the level of risk associated with different transmission routes, 23 (45.1%) participants indicated that consumption of unpasteurized milk is associated with a high risk of brucellosis. When asked about the consumption of other unpasteurized dairy products, 12 (23.5%) participants considered it to be a high-risk practice, though

20 (39.2%) considered it low. Participants' responses concerning risk of human infection associated with different infection routes are presented in Figure 2.

Participants' opinions regarding the actions that most livestock owners take when they have an infected or suspected animal with brucellosis are presented in (Table 7).

When asked about measures that most farmers take when an animal is suspected of having brucellosis, participants declared that most farmers would: treat the animal: 7 (11.8%), call the local veterinarian 4 (7.8%), prayers and incantations / keep in *Gaushalas* 18 (35.3%) and separate the animal from others 6 (11.8%), vaccination of their herds did not appear to be an option that most farmers considered (Table 8).

3.6 Livestock owners' Practices related to consumption and Processing of Milk and Dairy Products

Most of the interviewed respondents 110 (86.6%) confirmed that milk from their animals was regularly consumed in their household and around 60% declared that they regularly sold raw milk from their animals to other people in the community, whereas 29 (22.8%) respondents purchased milk from other farmers. More than one-third of the participants 49 (38.6%) boil raw milk before it was consumed. A considerable percentage, 71 (55.9%) of the respondents boiled the milk before being proce-ssed into the local dairy products: *curd/kheer, shrikhand/gulab jamun*, *paneer and ghee/lassi* (yoghurt, cr -eam, cheese and butter, respectively) (Figure 3).

4.0 Discussion

We found that a very high proportion of herds with a history suggestive of brucellosis in Hisar had *Brucella* seropositive animals (61.4%). Previous studies had reported varying ranges of herd-level prevalence of bovine brucellosis in different states of India (Nagalingam et al. 2012; Renukharadya et al. 2002); Mehra et al. 2000) and Isloor et al. 1998a). Estimates from these studies are lower than the percentage of seropositive herds (47.6%) found in this study. For example, a recent study in neighboring Punjab state estimated animal-level seroprevalence to be 15.1% with around a third of dairy farms having at least one seropositive animal (Holt et al. 2021). However, comparisons should not be made as our study is based on suspect herds that were not randomly selected and therefore is not aimed at providing prevalence estimates for the general dairy herd population. In addition, diversity of Indian states.

Our assessment of KAPs revealed that practices that can increase the risk of livestock keepers being infected by *Brucella* in infected herds are common. It is common for livestock keepers to assist their animals when calving without personal protective equipment (PPE), and this is in concordance with Renukaradhya et al. 2002. Other practices such as disposing aborted fetuses into the water canals and streets or feeding them to dogs can contribute to spread of infection within and between herds. Previous studies in other endemic areas provide evidence of association between these practices and high prevalence of infection for example in some districts of Pakistan (Arif et al. 2017) or in Nyagatare District, Eastern Province, Rwanda (Mushonga et al. 2018). However, the sampling technique employed in this study implies that the study herds may not be representative of the state of Haryana, India.

Our finding of a strong association between hygienic disposal of placenta and lower probability of infection is in agreement with the results of the above studies. The neglect of hygienic practices can be in part attributed to limited awareness of the disease in livestock and risk of human exposure, which is likely to contribute to the spread and maintenance of infection in Haryana.

In addition to (lack of) hygienic disposal of placenta, intensive farming is also associated with a higher probability of the herd being seropositive (OR= 4.6, 95% CI: 1.6- 16.7, P= 0.009). Placenta and birth fluids from infected animals are highly infectious and their unhygienic disposal will increase the chance of spread of *Brucella* organisms from infected animals to susceptible animals, especially under intensive management practices. While intensive management may favor disease transmission, the association may be confounded by level of awareness of the disease and implementation of hygiene measures. This may explain some contradictory findings in the literature: Kazi et al. (2005) reported a higher rate of *Brucella* antibodies in rural (unorganized) farms (5.0%) than in organized farms (2.5%) in Bangladesh. However, Mehra et al. (2000) reported a higher prevalence of brucellosis in organized farms (large) vs. unorganized (backyard) farms (cattle and buffaloes) in Madhya Pradesh. Our (adjusted) estimates are compatible with the risk of infection in Hisar being higher for intensive farms, when adjusted for potential differences in hygienic practices (disposal of placenta) across farming systems. Only herds with clinical signs compatible with brucellosis were studied and ascertainment of disease status was based on testing 5 animals in each herd. Misclassification of "infected" herds as non-infected could have biased downwards our estimates of strength of association.

Regarding the routes of human exposure, the majority 76 (59.8%) of the participants did not know that brucellosis can be transmitted from animals to humans. This may contribute to the lack of PPE use during high-risk practices, such as assisting in parturition and disposal of aborted materials. This finding is consistent with a previous study by Nagalingam et al. (2012) and Renukharadya et al. (2002), who reported that lack of awareness could be a significant risk factor for brucellosis among the livestock owners in India. Recent estimates of human seroprevalence of brucellosis in Punjab state were 2.2% (95% CI: 1.6 to 3.1) and 9.7% (95% CI: 7.4% to 12.3%) in the general population and persons in direct contact with cattle and buffalo in dairy farms, respectively (Holt et al. 2021; Mangtani et al. 2020). Assisting with calving and/or abortion in dairy cattle was identified as a risk factor for human exposure and approximate 20% of people who assisted with calving and/or abortion on a seropositive farm had evidence of exposre to *Brucella* spp. As the dairy sector in Punjab and neighbouring Haryana State is very similar it is likely that people are also exposed to *Brucella* spp. in this setting, particularly those assisting with calving and abortion in dairy farms with a history suggestive of brucellosis. Therefore, activities to disseminate knowledge regarding brucellosis, particularly surrounding the risk of assisting with calving and abortion without PPE and efforts to improve dairy farmers' access to PPE in this setting are warranted. Level of awareness of brucellosis among livestock keepers varies considerably across different endemic regions; studies carried out among livestock keepers of Nigeria, Jordan and Egypt have shown high level of awareness (Agada et al. 2018, Musallam et al. 2015, Holt et al. 2011).

The risk of *Brucella* infection from consumption of dairy products depends not only on the number of *Brucella* organisms in milk but also the processing steps of each product which involve changes in pH and moisture content and different heat treatments (Falenski et al. 2011; Zuniga et al. 2005; European Commission, 2001).

Our results suggest that even those aware of brucellosis know little about its transmissibility as only a few indicated high-risk of infection if raw milk or unpasteurized dairy products are consumed. Their knowledge of zoonotic routes of infection through contact with infected foetal membranes and direct contact with infected animals was even lower.

The awareness of clinical signs of brucellosis in animals among livestock owners in this study was considered poor with more than 55% of the respondents not being aware of the clinical signs of brucellosis in cattle. However, a small proportion of them indicated that abortion is the most noticeable clinical sign. Similarly, Onunkwu et al. (2018) and Mushonga et al. (2018), had reported a consistent finding of very poor awareness level among the participants in Nigeria and Rwanda respectively, the reason being lack of awareness coupled with poor knowledge of clinical signs. On the other hand, studies in Jordan and Nigeria reported a high level of awareness of brucellosis among livestock keepers (Musallam et al. 2015, Agada et al. 2018).

Our study revealed that the majority of the participants always consumed and sold milk produced from their household animals. In a few cases, they processed milk into local dairy products as a means of livelihood. This reflected their dependence on milk and milk products at the community level. Regarding the risk of infection with *Brucella* through the consumption of raw milk, our findings showed that it is not negligible; although 38.6% of the livestock owners confirmed to regularly boil milk before consumption, almost half (48.8%) reported doing it only occasionally. The risk of infection with *Brucella* could be lower through the consumption of other local dairy products, as more than half of participants confirmed that they boil raw milk from their animals or other sources when making local dairy foods like *curd*/*kheer*, *lassi*, *gulab jammun*, *paneer* and *ghee*. However, dairy product consumption from large ruminants did not appear to be an important transmission route for human brucellosis in Punjab state, although consumption of goats milk was identified as a risk factor for human exposure (Holt et al. 2021; Mangtani et al. 2020).

Important hygienic practices such as separation of animals suspected of being infected and burying or burning of aborted fetuses/placentas, appear to be applied by only a few livestock owners. Similarly, mixing and cohabitation of cattle with other small ruminants and cattle from other sources were found to be a common practice in this area. Still mixing of animal species in a single herd or at watering and grazing points were widespread management practices among the livestock owners in the study area. Mixing of animals at grazing and watering points might contribute to the between-herd transmission of brucellosis in Hisar and other districts of Haryana.

There is a paucity of documented information on the true prevalence of the disease in the Northern India. Animal brucellosis is problematic in Hisar. The study confirmed around half (47.0%) of livestock owners were aware of brucellosis through veterinarians and around a third of them and from the Media (31.4%), therefore these routes could be targeted for knowledge dissemination to farmers.

Recommendations

The high proportion of study herds with evidence of brucellosis, the high frequency of practices posing a risk of infection for livestock keepers and their families and their limited awareness of the disease and its transmissibility to people create conditions for brucellosis to be a major cause of livestock production losses and human illness in the study area. Programs increasing awareness of the disease and promoting hygienic and safe handling during calving and disposal of abortions and afterbirths should be prioritized. For instance, the ongoing "outreach program on zoonotic diseases-ICAR" should emphasize brucellosis prevention and control as well as communicate the risks associated with consumption of raw milk.

Authors' contributions

This work was carried out in collaboration between all authors. Author ASS designed the study, did the sampling, administer the questionnaire, collected the blood samples and wrote the first draft of the manuscript. Authors NKM and JG supervised the whole research and reviewed the manuscript. Authors IIM and HRH designed the questionnaire template and managed the data analyses of the study and reviewed the manuscript. Author HRH did the literature searches and English editing of the final manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

We appreciate the sponsors of the "*Outreach Programme on Zoonotic Diseases (OPZD)*" of the Indian Council of Agricultural Research (*ICAR*) and the Indian Council for Cultural Relations (*ICCR*), New Delhi, Government of India for their funding and supports under the Grant No: [5510-C(b)-VPHE-1-ICAR].

Acknowledgements

The Authors thank the Indian Council for Agricultural Research (*ICAR*) and the Indian Council for Cultural Relations (*ICCR*), New Delhi, Government of India for their funding and supports throughout the research, also to the livestock owners involved in this study for their participation and compliance during the questionnaire interview and blood sample collection.

References

Agada, C. A., Ogugua A. J. and Anzaku, E. J., 2018. Occurrence of brucellosis in small ruminants slaughtered in Lafia central abattoir, Nasarawa State, Nigeria. Sokoto Journal of Veterinary Science, 16,16-23.

Alton, G. G., Jones, L. M. and Pietz, D. D., 1975. Laboratory Techniques in Brucellosis. WHO monograph series no. 55. Geneva, Switzerland, WHO, 975, 188p.

Arif, Shumaila, Thomson, Peter, C. Hernandez-Jover, Marta, McGill, David, M., Warriach, Hassan Mahmood and Heller Jane, 2017. Knowledge, attitudes and practices (KAP) relating to brucellosis in smallholder dairy farmers in two provinces in Pakistan. PLoS ONE, 12 (3),1-19. e0173365. 10.1371/journal.pone.0173365.

Aulakh, H. K., Patil, P. K., Sharma, S., Kumar, H., Mahajan, V. B. and Sandhu, K. S., 2008. A Study on the Epidemiology of Bovine Brucellosis in Punjab (India) Using Milk-ELISA. Acta Veterinaria, 77(3), 393-399.

Bamaiyi, P. H., Khairani-Bejo S. and Zainal-abidin, M., 2015. The economic impact attributable to brucellosis among goat farms in Peninsula Malaysia and cost benefit analysis. Research Opinion of Animal and Veterinary Science, 5, 57-64.

Cardoso, P.G, Macedo, G.C., Azevedo, V., Oliveira SC. (2006). Brucella spps noncanonical LPS: Structure, biosynthsis and interaction with host immune system. *Microbiol Cells Factories,* **5**: 13.

Census, 2012. The 19TH Livestock Census-2012. All India Report. Ministry of Agriculture Department of Animal Husbandry, Dairying and Fisheries Krishi Bhawan, New Delhi.

Chand, P. and Chhabra, R., 2013. Herd and individual animal prevalence of bovine brucellosis with associated risk factors on dairy farms in Haryana and Punjab in India. Tropical Animal Health Production, 45(6), 1313-1319. doi:10.1007/s11250-013-0362-y.

Chand, P., Chhabra, R. and Juhi, N., 2014. Vaccination of adult animals with a reduced-dose of *Brucella abortus* S19 vaccine to control brucellosis on dairy farms in endemic areas of India. Tropical Animal Health Production, 47, 29–35. doi:10.1007/s11250-014-0678-2.

Ducrotoy, M. J., Bertu, W. J., Ocholi, R.A., Gusi, A.M., Bryssinckx, W., Welburn S. and Moriyo´n I., 2014. Brucellosis as an emerging threat in developing economies: Lessons from Nigeria, PLoS Neglected Tropical Diseases, 8, e3008, doi: 10.1371/journal.pntd.0003008.

Ducrotoy, M., Bertu W.J., Matope G., Cadmus S., Conde-Álvarez R., Gusi A.M., et al., 2017. Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control, Acta Tropica, 165, 179–193.

European Commission, 2001. Brucellosis in Sheep and Goats (*Brucella melitensis*). SANCO.C.2/AH/R23/2001. Report of the Scientific Committee on Animal Health and Animal Welfare. Belgium, Brussels: European Commission.

Falenski A, Mayer-Scholl A, Filter M, Göllner C, Appel B, Nöckler K, 2011. Survival of Brucella spp. in mineral water, milk and yogurt. Int J Food Microbiol 145: 326–330.

Food and Agricultural Organisation, 2009. *Brucella melitensis* in Eurasia and the Middle East. Proceeding of a Joint Technical Meeting FAO/WHO/OIE, Rome, Italy. Available at: http://www.fao.org/docrep/012/i1402e/i1402e00.pdf. Accessed February 20, 2015.

Gilbert, M., Mitchell, A., Bourn, D., Mawdsley, J., Clifton-Hadley, R. and Wint, W. 2005. "Cattle movements and bovine tuberculosis in Great Britain," Nature, 435(7041): 491–496.

Godfroid, J., Scholz, H.C., Barbier, T., Nicolas, C., Wattiau, P., Fretin, D., Whatmore, A.M., Cloeckaert, A., Blasco, J. M., Moriyón, I., Saegerman, C., Muma, J. B., AlDahouk, S., Neubauer, H. and Letesson, J. J., 2011. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. Preventive Veterinary Medicine, 102, 118–131.

Holt HR, Bedi JS, Kaur P, Mangtani P, Sharma NS, Gill JPS, et al. (2021) Epidemiology of brucellosis in cattle and dairy farmers of rural Ludhiana, Punjab. PLoS Negl Trop Dis 15(3): e0009102. https://doi.org/10.1371/journal.pntd.0009102.

Holt, H.R., Eltholth, M., M., Hegazy, Y. M., El-Tras, W. F., Tayel, A. A., Guitian, J., 2011. Brucella spp. infection in large ruminants inan endemic area of Egypt: cross-sectional study investigating seroprevalence, risk factors and livestock owner's knowledge, attitudes and practices (KAPs). BMC Public Health, 11, 341.

Imadidden, I. Musallam, Mahmoud N. Abo-Shehada, and Javier Guitian., 2015. Knowledge, Attitudes, and Practices Associated with Brucellosis in Livestock Owner in Jordan. American Journal of Tropical Medicine and Hygiene, 93(6), 1148–1155.

Islam, M.A., Khatun, M. M., Were S. R., Sriranganathan, N., Boyle, S. M., 2013. A review of *Brucella* seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities. Veterinary Microbiology*,* 166, 317-326**.**

Isloor, S. Renukaradhya, G. J., Veeregowda, B. M., Rajasekhar, M., 2001. Bovine Brucellosis Research. In: Animal report (2000-2001) of Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS), Bangalore, India, 4-10.

Isloor, S., Renukaradhaya, G. J. and Rajasekhar, M., 1998a. A serological survey of bovine brucellosis in India. Review of Science and Technology, 17(3), 781-5.

Kalorey, D.R., Ingle, V. C. and Kurkurse, N. V., 2000. Seroprevalence of brucellosis in livestock and humans in Vidarbha region. Indian Jourmal of Animal Science, 70, 149 -150.

Kang'ethe, E. K., Ekuttan, C. E., Kimani, V. N. and Kiragu, M.W., 2007. Investigations into the prevalence of bovine brucellosis and the risk factors that predispose humans to infection among urban dairy and non-dairy farming households in Dagoretti Division, Nairobi, Kenya. East African Medical Journal, 84, S96–S100.

Kazi, M. R. Amin, M. Bahanur Rahman, M. Siddiqur Rahman, Jae-cheol Han, Jin-ho Park, Joon-seok Chae., 2005. Prevalence of *Brucella* antibodies in sera of cows in Bangladesh. Journal of Veterinary Science, 6(3), 223–226.

Lakew, A., Hiko, A., Abraha, A., Hailu, S. M., 2019. Sero-prevalence and community awareness on the risks associated with Livestock and Human brucellosis in selected districts of Fafan Zone of Ethiopian_Somali National Regional State. Veterinary and Animal Science, 7(2019), 100047. http s://doi.org/10.1016/j.vas.2019.100047.

Lindahl, E., Sattorov, N., Boqvist, S. and Magnusson, U., 2015. A study of knowledge, attitudes and practices relating to brucellosis among small-scale dairy farmers in an urban and peri-urban area of Tajikistan. PLoS One, 10, e0117318.

Mahajan, N. K. and Kulshreshtha, R. C., 1991. Comparison of serological tests for *Brucella melitensis* infection in sheep. Tropical Animal Health Production, 23(1), 11-16.

Mahajan, N. K., Kulshreshtha, R. C. and Vasudevan, B., 1986. Brucellosis cause of abortion in sheep and its public health significance. International Journal of Zoonoses, 13, 174-179.

Mangtani Punam, Isha Berry, Wendy Beauvais, Hannah R Holt, Amit Kulashri, Satinder Bharti, Vivek Sagar, Patrick Nguipdop-Djomo, Jasbir Bedi, Manmeet Kaur, Javier Guitian, John McGiven, Paviter Kaur, Jatinder P Singh Gill, Gagandeep Singh Grover, Rajesh Kumar, The prevalence and risk factors for human *Brucella* species infection in a cross-sectional survey of a rural population in Punjab, India, Transactions of The Royal Society of Tropical Medicine and Hygiene, 114 (4), 255–263. https://doi.org/10.1093/trstmh/trz133.

McGiven, J. A., Nicola. A., Commander, N. J., et al., 2012. An evaluation of the capability of existing and novel serodiagnostic methods for porcine brucellosis to reduce false positive serological reactions. Veterinary Microbiology, 160, 378-386.

McGiven, J. A., Tucker, J. D., Perrett, L. L., Stack, J. A., Brew, S. D. and MacMillan, A. P., 2003. Validation of FPA and cELISA for the detection of antibodies to *Brucella abortus* in cattle sera and comparison to SAT, CFT and iELISA. Journal of Immunological Methods, 278, 171–78.

Mehra, K. N., Dhanesar, N. S. and Chaturvedi, V. X., 2000. Seroprevalence of brucellosis in bovines of Madhya Pradesh. Indian Veterinary Journal, 11, 571 - 573.
mithuns (*Bos frontalis*). Preventive Veterinary Medicine, 69, 145-151.

Moreno, E., 2014. Retrospective and prospective perspectives on zoonotic brucellosis. Frontier Microbiol -ogy, 5, 213.

Morgan, W. J. B., MaCkinnon, D. J. and Cullen, G.A., 1969. The Rose Bengal Plate Agglutination Test in the diagnosis of brucellosis. Veterinary Records, 85, 636-641.

Mushonga, B., Dusabe, J., Kandiwa, E., Bhebhe, E., Habarugira, G. and Samkange, A., 2017. Artificial insemination in Nyagatare District: Level of adoption and the factors determining its adoption. Alexandria Journal of Veterinary Sciences, 55(1),1- 7. https://doi.org/10.5455/ajvs.273226.

Nagalingam Mohandoss, Rajeswari Shome, Vinayagamurthy Balamurugan, Bibek Ranjan Shome, Krishnamsetty NarayanaRao, Vivekananda Shrikrishna Isloor and Krishnamsett Prabhudas., 2012. Molecular typing of *Brucella species* isolates from livestock and human. Tropical Animal Health Production, 44, 5-9.

Ndazigaruye, Gervais Mushonga, Borden Kandiwa, Erick Samkange, Alaster Segwagwe, Basiamisi E., 2018. Prevalence and risk factors for brucellosis seropositivity in cattle in Nyagatare District, Eastern Province, Rwanda. Journal of South African Veterinary Association, 89, 1625. doi: 10.4102/jsava.v89i0.1625.

Nicoletti, P., 1967. Utilization of card test in brucellosis eradication. Journal of American Veterinary Medical Association, 151, 1778-1781.

OIE, Terrestrial Manual, 2004. Bovine Brucellosis. Chapter 2.3.1. Manual of Standards Diagnostic Tests and Vaccines for Terrestrial Animals. 5th edn, Paris, France.

OIE, Terrestrial Manual, 2016. Bovine Brucellosis. Chapter 2.1.4. Brucellosis (*Brucella abortus*, *B. melit - ensis & B. suis*). By the world assembly of delegates of the OIE, Paris, France.

Onunkwo, J. I., Njoga, E. O., Njoga, U. J., Ezeokafor, E., Ekere, S. O., 2018. *Brucella* seropositivity in chicken and risk factors for *Brucella* infection at the animal-human interface in Anambra State, Nigeria. International Journal of One Health, 4, 28-34.

Pfeiffer, D., Cortes, C., Otte, E. and Morris, R. 1988. Management factors affecting the prevalence of brucellosis in traditionally managed cattle herds in northern Colombia. Acta Veterinary Scandinavia, 84, 133–135.

Polding, J.B., 1942. Brucellosis in India. Indian Journal of Veterinary Sciences, 13, 27-34.

R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/. Accessed February 1, 2015.

Rajkhowa, S., Rajkhowa, H. and Bujarbaruh, K. M., 2005. Seroprevalence of brucellosis in mithuns (*Bos frontalis*). Preventive Veterinary Medicine, 69, 145-151.

Renukaradhya, G. J., Isloor, S. and Rajasekhar, M., 2002. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. Veterinary Microbiology, 90(1–4), 183–195.

Rossetti, C. A., Arenas-Gamboa, A. M. and Maurizio, E., 2017. Caprine brucellosis: A historically neglected disease with significant impact on public health, PLoS Neglected Tropical Diseases, 11, e0005692.

Saidu, A. S., Mahajan, N. K., Mahavir S., Mittal, D., Bangar, Y., and Chhabra, R., 2020. Serological evidence of Brucellosis in selected gaushalas of Haryana. Indian Journal of Animal Sciences, 90 (7): 963–966, July 2020/Article.

Singh, B. B., Kostoulas, P., Gill, J. P. S. and Dhand, N. K. 2018. Cost-benefit analysis of intervention policies for prevention and control of brucellosis in India. PLoS Neglected Tropical Diseases, 12(5), e0006488.

Singh, B. B., Dhand, N. K. and Gill, J. P. S., 2015. Economic losses occurring due to brucellosis in Indian livestock populations. Preventive Veterinary Medicine, 119(3), 211-215.

Tabachnick, B.G. and Fidell, L.S., 2013. Using Multivariate Statistics. Pearson, Boston.

Traxler, R. M., Lehman, M. W., Bosserman, E. A., Guerra, M. A. and Smith, T. L., 2013. A literature review of laboratory-acquired brucellosis. Journal of Clinical Microbiology, 51, 3055-62.

Tsegay, A., Tuli, G., Kassa, T., and Kebede, N., 2017. Seroprevalence and risk factors of brucellosis in abattoir workers at Debre Zeit and Modjo export abattoir, Central Ethiopia. BMC Infectious Disease*s*, 17, 101.

WHO, 2005. Brucellosis in humans and animals: WHO guidance. Geneva, World Health Organization of 2005. Heymann DL (ed.). Control of Communicable diseases manual: an official report of the American Public Health Association. 18th ed. Washington DC, WHO/APHA, 2004.

Wright, A. E. and Smith, D., 1897. On the employment of dead bacteria in the serum diagnosis of typhoidal and malta fever. British Medical Journal, 1, 1214.

Xiang, N., Shi, Y., Wu, J., Zhang, S., Ye, M., Peng, Z., Zhou, L., Zhou, H., Liao, Q. and Huai, Y., 2010. Knowledge, attitudes and practices (KAP) relating to avian influenza in urban and rural areas of China. BMC Infectious Diseases, 10, 34.

Zaki, S., Krishnappa, G., Keshavamurthy, B.S., 1981. Bovine Brucellosis in Karnataka and supplemental tests in the elimination of non-specific reactions. Mysore Journal of Agricultural Science, 15, 81-83.

Zuniga Estrada A, Mota de la Garza L, Sanchez Mendoza M, Santos Lopez EM, Filardo Kerstupp S, Lopez Merino A, 2005. Survival of Brucella abortus in milk fermented with a yoghurt starter culture. Rev Latinoam Microbiol, 47 (3-4), 88-91.

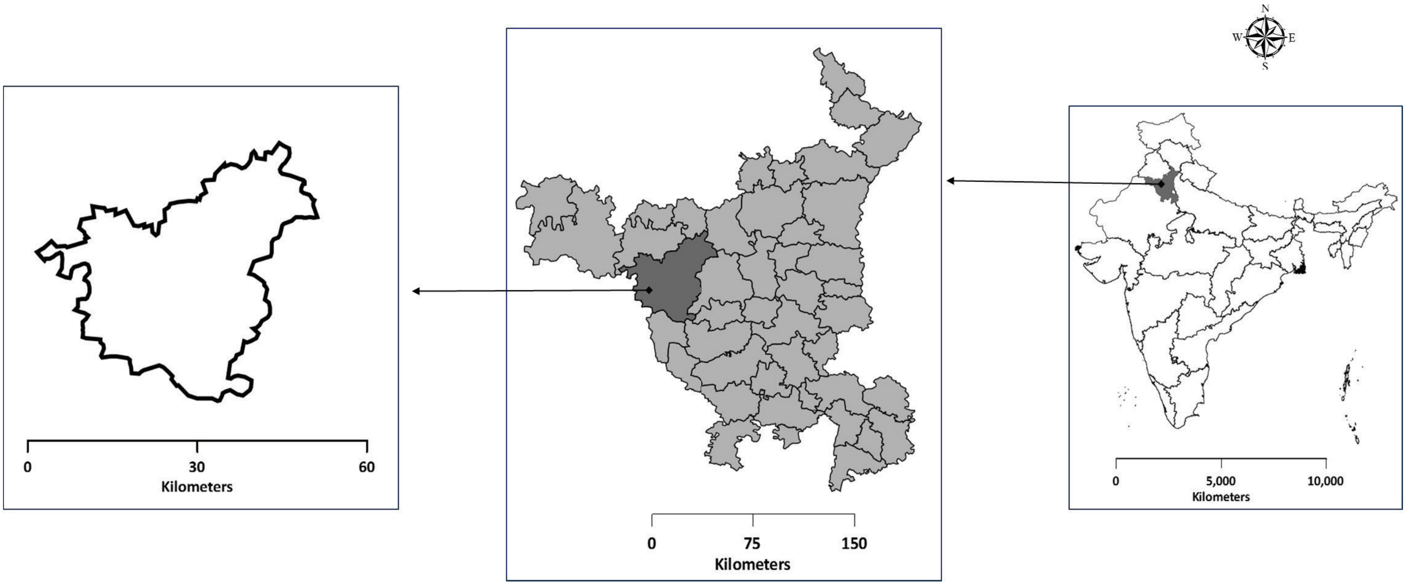

Figure 1. Map of India showing the study area (Haryana) in Hisar district and it's environ

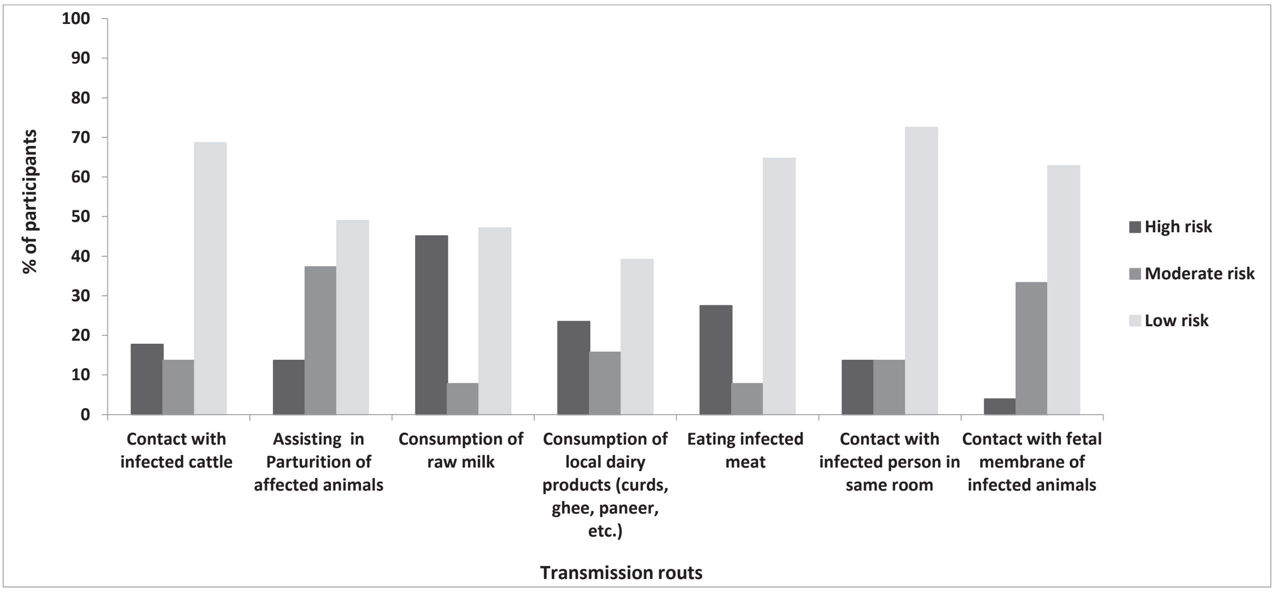

Figure 2. Participants' opinions regarding routes of brucellosis in humans (% of respondents' practices considered as low, moderate, or high risk)

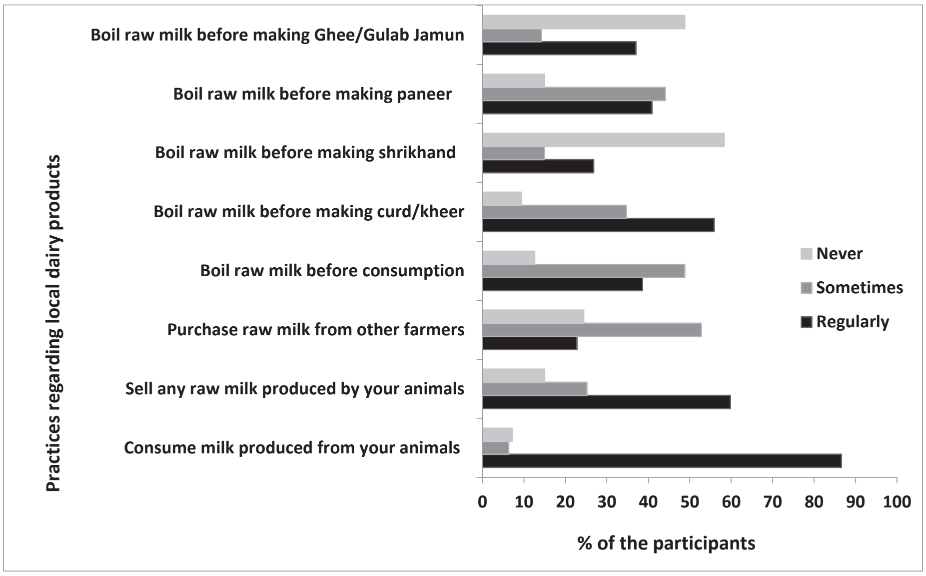

Figure 3**.** Participants' responses regarding consumption and processing of local dairy products.

Table

Click here to access/download;Table;Brucella Risk Factors_India_Adamu_Tables_25-4-2021.docx

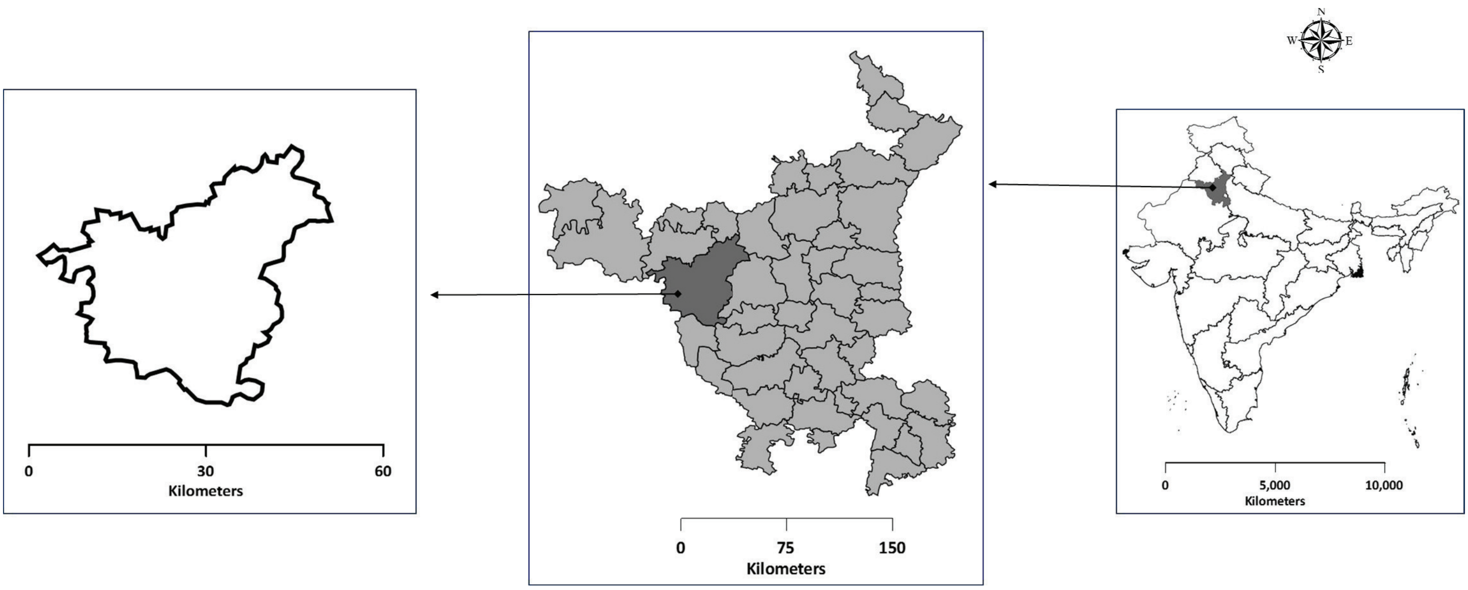

Figure 1. Map of India showing the study area (Hisar) in Haryana district and it's environ

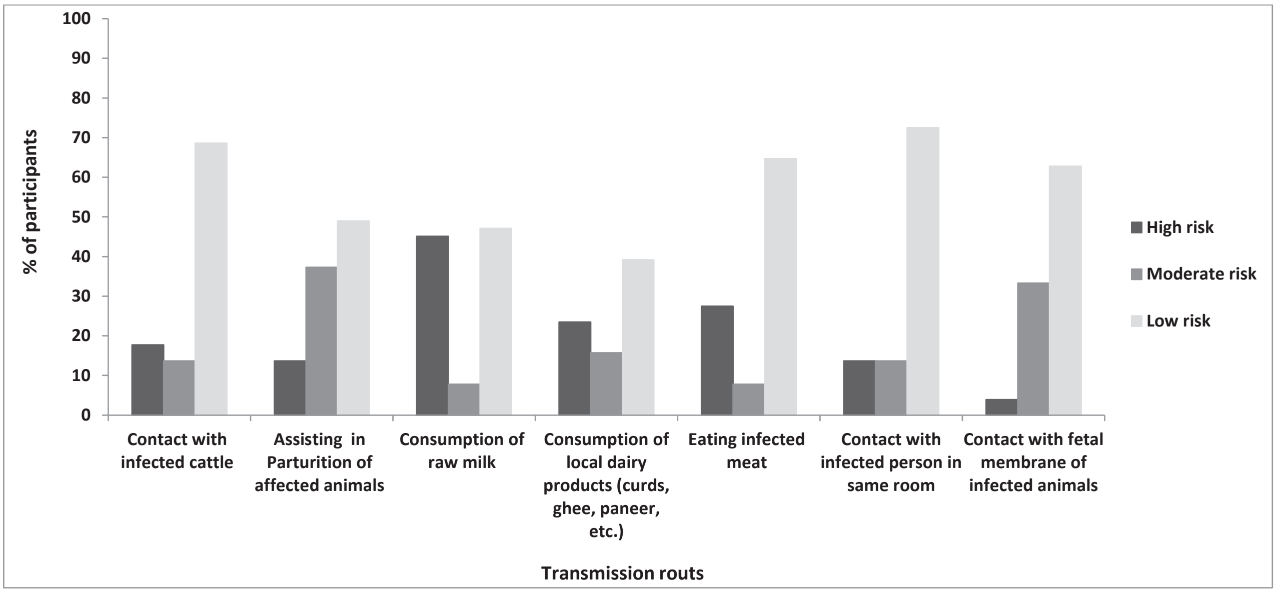

Figure 2. Participants' opinions regarding routes of brucellosis transmission to humans (% of respondents' responses considered as low, moderate, or high risk)

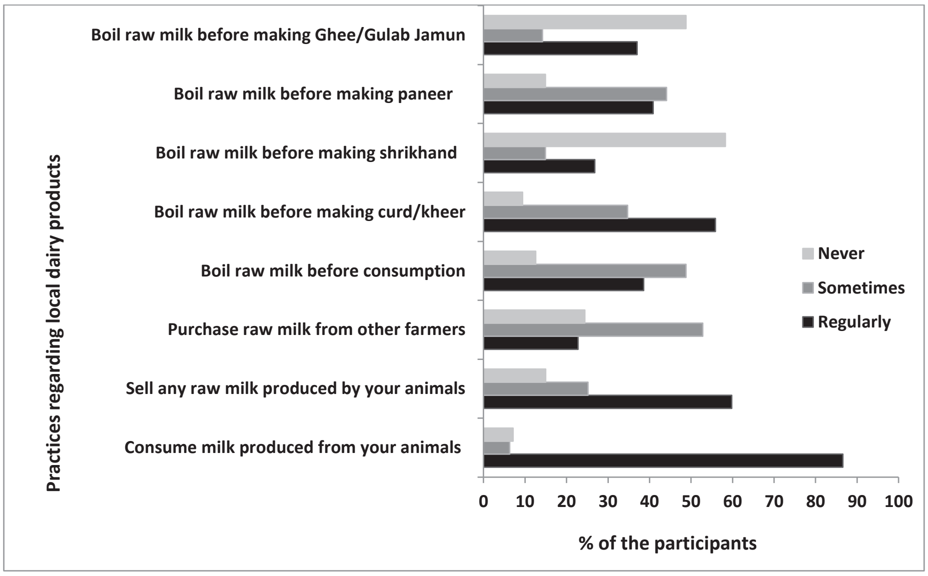

Figure 3. Participants' responses regarding consumption and processing of local dairy products.

Table 1. Number (%) of cattle herds sampled in Hisar District and the nearby villages in a Knowledge, Attitudes and Practices (KAPs) Study conducted in Hisar-India, (127 cattle herds included in a cross-sectional study conducted between June and December 2018 in *Hisar, Haryana*).

Village	Number (%) of herds sampled
Bhiwani	8 (6.3)
Fatehbad	2 (1.6)
Hisar	90 (70.9)
Sofidon/Jind	1 (0.8)
Jind	5 (3.9)
Karnal	2 (1.6)
Kurukshetra	2 (1.6)
LUVAS/Hisar	1 (0.8)
Meham	1 (0.8)
Mukhlan/Hisar	1 (0.8)
Narwana	3 (2.4)
Niyana	1 (0.8)
Rewari	1 (0.8)
Rohilla	1 (0.8)
Rohtak	1 (0.8)
Shiwani	1 (0.8)
Sirsa	6 (4.7)
Total	127(100)

Table 2. Demographic characteristics of livestock owners (n=127) participated in a knowledge, attitudes and practices (KAPs) study regarding brucellosis conducted between June and December 2018 in *Hisar, Haryana*, India.

Variables	**Category**	**Frequency (%)**
Farm Type	a) Small Backyard Farm	82 (64.6)
	b) Intensive/organized Farm	45 (35.4)
Age group of the respondents	a) 18-30	45 (35.4)
	b) 31-50	60 (47.0)
	c) > 50	22 (17.3)
Sex	a) Male	96 (75.6)
	b) Female	31 (24.4)
Herd-size	a) ≤ median of 9	111 (87.4)
	b) > median of 9	16 (12.6)

Table 3. Univariate analysis of risk factors for brucellosis in dairy herds in Hisar, India (127 cattle herds included in a cross-sectional study conducted between June and December, 2018 in *Hisar, Haryana*).

Variable	**Categories**	**Number +ve/total (%)**	**Chi square (χ^2)**	**P-value**
Farm Type	Organized	26/30 (86.7)	9.12	0.002
	Small (backyard)	52/97 (53.6)		
Herd size	≥ median of 9	37/69 (53.6)	3.873	0.049
	< median of 9	41/58 (70.7)		
Introducing new animals (Regularly)	No	40/68 (58.8)	0.416	0.322
	Yes	38/49 (77.6)		
Always mixing with cattle for drinking & grazing	No	65/108 (60.2)	0.462	0.340
	Yes	13/19 (68.4)		
Quarantine (Separation of su spected animals, always)	No	67/107 (62.6)	0.413	0.344
	Yes	11/20 (55.0)		
Separation of aborted cow from others in the herd	No	51/90 (56.7)	2.942	0.064
	Yes	27/37 (73.0)		
Hygienic disposal of placenta	No	56/101 (55.4)	*7.425	*0.006
	Yes	22/26 (84.6)		
Frequent mixing with small ruminants	No	40/71 (56.3)	1.753	0.185
	Yes	38/56 (67.9)		

Negative n=49, Positive n=78.

Table 4. Results of a multivariable logistic regression model on serological status of cattle herds against *Brucella* spp. (127 cattle herds included in a cross-sectional study conducted between June and December 2018 in *Hisar, Haryana*).

Variable (category)	**Odds ratio (OR)**	**95% CI**	**P-value**
Farm type (intensive)	4.6	1.6, 16.7	0.009
Hygienic Disposal of aborted fetuses (Yes)	0.3	0.08, 0.90	0.04

Table 5. Participants responses regarding Knowledge about Brucellosis in cattle in Hisar, India, results obtained from livestock owners (n=127) participated in a knowledge, attitudes, and practices (KAPs) study carried out between June and December 2018.

Variables	**Categories**	**Frequency (%)**
Knowledge about Brucellosis (have you heard about brucellosis?)	Yes	51 (40.2)
	No	76 (59.8)
Where did you hear about brucellosis?	a) Media	16 (31.4)
	b) Local Veterinarian	24 (47.0)
	c) Other farmers	11 (21.6)
Owners' opinions about animal species that can have brucellosis		
Animals species that can have brucellosis	a) Cattle/Buffaloes	25 (49.0)
	b) Sheep/Goats	15 (29.4)
	c) Dogs	11 (21.6)
	d) Equine/Donkeys	0
	e) Poultry	0
Animal species that owners are sure they can have brucellosis	a) Cattle/Buffaloes	13 (25.5)
	b) Sheep/Goats	10 (19.6)
	c) Dogs/Equines/Donkeys	0
	d) Poultry	0
Animal species that owners are sure they can have and transmit brucellosis	a) Cattle/Buffaloes	2 (3.9)
	b) Sheep/Goats	3 (5.9)
	c) Dogs/Equines/Donkeys	0
	d) Poultry	0

Table 6. Participants responses regarding knowledge of the clinical signs of brucellosis in cattle. Livestock owners (n=127) participated in a knowledge, attitudes, and practices (KAPs) study carried out in Hisar, India between June and December 2018.

Clinical signs	**Frequency (%)**
Abortion	6 (11.8)
Infertility	2 (3.9)
Weight loss	2 (3.9)
Reduced milk yield	3 (5.9)
Inflammation of testes	2 (3.9)
Skin lesions	1 (2.0)
Diarrhea	2 (3.9)
Lameness	2 (3.9)
Respiratory symptoms	1 (2.0)
Sudden death	2 (3.9)
Don't know	28 (54.9)

Table 7. Participants' responses (n=127) regarding livestock owner's practices associated to abortion in ruminants, data was collected from the farmers participated in a knowledge, attitudes, and practices (KAPs) study carried out, in Hisar, India, between June and December 2018.

Practices	**Frequency**		
	Regularly (%)	**Sometimes (%)**	**Never (%)**
Practices most farmers do in villages when **only one** cow aborts			
Frequently disinfect their pens/herds after abortion	16 (31.4)	29 (56.9)	6 (11.8)
Separate aborted cow	6 (11.8)	22 (43.1)	23 (45.0)
Call the local veterinarian	6 (11.8)	14 (27.5)	31 (60.8)
Slaughter aborted cow	3 (5.9)	17 (33.3)	31 (60.8)
Sell the aborted cows in market	11 (21.6)	24 (47.1)	16 (31.4)
Take aborted cows to Gaushala*	13 (25.5)	18 (35.3)	20 (39.2)
Give medicine to the affected cow	6 (11.8)	27 (52.9)	18 (35.3)
Vaccinate the aborted cow	3 (5.9)	17 (33.3)	31 (60.8)
Practices most farmers do when **more than one cow** have aborted			
Frequently disinfect their pens/herds after abortion	10 (19.6)	18 (35.3)	23 (45.1)
Separate aborted cows	13 (25.5)	24 (47.1)	14 (27.5)
Call the local veterinarian	2 (3.9)	17 (33.3)	32 (62.7)
Slaughter aborted cows	2 (3.9)	9 (17.6)	40 (78.4)
Sell the cow that has aborted in the market	24 (47.1)	11 (21.6)	16 (31.4)
Take the aborted animal to the *Gaushala	18 (35.3)	11 (21.6)	22 (43.1)
Give medicine to the affected cows	7 (11.8)	16 (31.4)	28 (54.9)
Vaccinate the aborted cows	8 (15.6)	20 (39.2)	23 (45.1)
Practices farmers do when one or more of their sheep/goats have aborted			
Frequently disinfect their pens/herds after abortion	10 (19.6)	18 (35.3)	23 (45.1)
Separate the aborted sheep/goats	6 (11.8)	7 (13.7)	38 (74.5)
Call the local veterinarian	3 (5.9)	6 (11.8)	42 (82.4)
Slaughter the aborted sheep/goats at home for consumption	9 (17.6)	6 (11.8)	36 (70.6)
Sell the aborted sheep/goats in the market	6 (11.8)	8 (15.7)	37 (72.5)
Sell the aborted sheep/goats to the butcher	7 (13.7)	7 (13.7)	37 (72.5)

* ***Gaushalas:*** is a local *Hindi* name referring to cattle farm. It is an organized farm under semi-intensive system.

Table 8. Participants' responses (n=127) regarding Livestock owners' attitudes and practices associated to parturition, abortion and hygienic disposal of placental and aborted materials in cattle, in a knowledge, attitudes, and practices (KAPs) study carried out, in Hisar, India, between June and December 2018.

Practices	**Frequency**		
	Regularly (%)	**Sometimes (%)**	**Never (%)**
Practices farmers do regarding parturition and abortion in animals			
Assisting in parturition of Cows/Buffaloes	8 (15.6)	41 (80.4)	2 (3.9)
Assisting in parturition of sheep and goats	5 (9.8)	41(80.0)	5 (9.8)
Wearing protective gloves when assist in parturition of cows.	21(41.2)	17 (33.3)	13 (25.5)
Wearing protective mask when assist in parturition of sheep and goats	24 (47.0)	13 (25.5)	14 (27.5)
Practices farmers do if one of their cows aborted/suspected of brucellosis			
Separate the cow that has aborted from the others	13 (25.5)	24 (47.1)	14 (27.5)
Call a local Veterinarian around	5 (9.8)	17 (33.3)	29 (56.9)
Slaughter the cow that has aborted at the farm/household for disposal	2 (3.9)	9 (17.6)	40 (78.4)
Sell the cow that has aborted in the market	11 (21.6)	26 (51.0)	14 (27.5)
Take the cow that has aborted to the *Gaushalas**	19 (37.3)	19 (37.3)	13 (25.5)
Give medications	2 (3.9)	17 (33.3)	32 (62.7)
Vaccinate the animals	6 (11.8)	26 (51.0)	19 (37.3)
Practices most farmers do when disposing placenta and aborted materials			
Throwing the placenta into water canals	30 (58.8)	9 (17.6)	12 (23.5)
Throwing the placenta in the street	28 (54.9)	9 (17.6)	14 (27.5)
Giving the placenta to dogs	20 (39.2)	16 (31.4)	15 (29.4)
Burying the placenta inside the ground	5 (9.8)	26 (51.0)	20 (39.2)
Burning the placenta in an open ground	26 (51.0)	11 (21.6)	14 (27.5)
Just ignore it anyhow	24 (47.1)	12 (23.5)	15 (29.4)
Wear protective gloves during disposal	26 (51.0)	10 (19.6)	15 (29.4)

* ***Gaushalas:*** is a local *Hindi* name referring to cattle farm. It is an organized farm under semi-intensive system, kept for spiritual and commercial purposes.