[Downloaded free from http://www.johb.info on Wednesday, September 27, 2023, IP: 112.66.109.236]



johb.info



Original Article

One Health Bulletin

# Seroprevalence and risk factor analysis of small ruminant brucellosis in the semi–arid region of India

Sai Simha Reddy Vakamalla[1]✉, Murthy Suman Kumar[1]✉, Himani Dhanze[1], Vinodh Kumar Obli Rajendran[2], Chandni Abdul Jabbar Rafeeka[1], Dhirendra Kumar Singh[1]

[1]*Division of Veterinary Public Health, ICAR–Indian Veterinary Research Institute, India Izatnagar 243122, India*

[2]*Division of Epidemiology, ICAR–Indian Veterinary Research Institute, Izatnagar 243122, India*

## ABSTRACT

**Objective:** To study the occurrence of brucellosis in small ruminants in a district of Southern India.

**Methods:** A total of 425 serum samples (215 sheep and 210 goats) were collected from January to March 2022 using a multistage sampling method. The serum samples were subjected to three serological tests that were employed in this study, namely RBPT (Rose Bengal Plate Test), STAT (Standard Tube Agglutination Test), and I-ELISA. The risk factors were determined by collecting data through a questionnaire and establishing correlations between the responses and seropositivity within a specific flock.

**Result:** Among the 425 samples collected, 91 samples (21.4%) were positive by RBPT, 124 samples (29.2%) by STAT and 156 samples (36.7%) by I-ELISA in sheep and goats. Sheep showed higher seropositivity in all three tests than goats. Analysis of multivariable logistic regression showed that age (>3 *vs.*<3 years; *OR*: 2.262, 95%*CI*:1.414–3.618), abortion history (yes *vs.* no; *OR*: 1.837; 95%*CI*:1.098–3.071) , vaginal discharges (yes *vs.* no; *OR*: 2.334; 95%*CI*:1.421–3.835), the migratory herd (yes *vs.* no; *OR*: 2.197; 95%*CI*:1.369–3.527), and place of disposal of the foetus (yes *vs.* no; *OR*: 2.093; 95%*CI*:1.320–3.318) were significant risk factors of brucellosis.

**Conclusions:** Livestock owners and consumers of sheep and goats should be educated about brucellosis and prevention strategies to mitigate its uncontrolled spread and lower the potential threat of human brucellosis. Choosing the right rearing practices, enhancing husbandry procedures, changing farmer's perspectives on brucellosis, and inclusion of small ruminants in brucellosis control programs are all key management approaches that can aid in the prevention and control of *Brucella* infection in small ruminants.

**KEYWORDS:** *Brucella*; Seroprevalence; Small ruminants; India

## 1. Introduction

Brucellosis is a chronic infectious zoonotic disease prevalent worldwide, particularly in developing nations, caused by *Brucella*, a gram-negative facultative intracellular bacterium which endangers public health. The World Health Organization (WHO) classifies it as the most common zoonotic disease. Despite this, brucellosis is one of the frequently neglected illnesses[1]. It is an animal illness that mostly affects domesticated livestock, with humans acting as incidental hosts. Every year around 500 000 human brucellosis cases are recorded, however, the real incidence is believed to be between 5 000 000 and 12 500 000 cases per year[2]. Countries in the Central and South America, Central Asia, the Middle East, the Indian subcontinent, the Mediterranean basin and Sub-Saharan Africa are all endemic to brucellosis[3].The occurrence rate of a

### Significance

Brucellosis is a chronic zoonotic disease that is widely prevalent in India. This study aims to determine seroprevalence of brucellosis and the risk factors responsible for the disease occurence in the study region. Consequently, this research aids in formulating prevention and control measures in the study area to reduce the disease burden.

✉To whom correspondence may be addressed. E-mail: v.saisimhareddy@gmail.com.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**For reprints contact:** reprints@medknow.com

©2023 *One Health Bulletin* Produced by Wolters Kluwer- Medknow.

**How to cite this article:** Vakamalla SSR, Kumar MS, Dhanze H, Rajendran VKO, Rafeeka CA, Singh DK. Seroprevalence and risk factor analysis of small ruminant brucellosis in the semi-arid region of India. One Health Bull 2023; 3; 14. https://www.doi.org/10.4103/2773-0344.383635

**Article history:** Received 10 May 2023 Revision 23 June 2023
Accepted 15 July 2023 Available online 17 August 2023

[Downloaded free from http://www.johb.info on Wednesday, September 27, 2023, IP: 112.66.109.236]



disease in countries where it is endemic is 10%, whereas the rate at which it results in fatalities is minimal[4]. Brucellosis is estimated to cost India 3.4 billion dollars in livestock and 9.06 million dollars in humans every year[5,6]. Among the illnesses acquired in laboratories worldwide, brucellosis is one of the most prevalent[7], a fact that can be ascribed to the low infectious dosage, which ranges from 10 to 100 bacterial cells when introduced through aerosol or subcutaneous route[8]. Several domestic species and practically all states in India are endemic to brucellosis[9]. Natural infection with brucellosis is caused by direct exposure to diseased animals or their bodily fluids, including aborted foetuses and foetal membranes that have high levels of bacteria, as well as airborne agents inhalation and consumption of dairy products contaminated with the bacteria[10]. Blood transfusion, organ transplantation, and vertical transfer *via* breastfeeding are known routes of human-to-human transmission[11,12].

Brucellosis in sheep and goats is widespread all over the world, but it remains a severe problem in low- and middle-income nations. Small ruminants are also infected with *Brucella (B.) abortus* and *B. suis*, in addition to *B. melitensis*, but clinical illness appears to be rare[13]. Signs of brucellosis in small ruminants include several reproductive problems such as abortions in the third trimester, stillbirths, underweight and little lambs, and placentitis in pregnant animals. Male animals may experience epididymitis, infertility, and orchitis, while female animals might excrete the organism in their uterine and milk secretions. These symptoms serve as indicators of infection. Addition to the physical pain it brought to animals and human, brucellosis also results in severe production loss due to increased morbidity. The detection of *Brucella* spp. can be challenging due to its long incubation period, which can last anywhere from five days to five months. The infection can manifest itself in several ways, including acute, chronic, or asymptomatic, and laboratory tests are necessary to confirm the presence of the bacteria[14]. Sheep and goats provide an income source for millions of rural Indian communities. The 20th livestock census estimates that India has 74.26 million sheep and 148.88 million goat population[15]. Prioritizing the health of small ruminants is difficult due to the haphazard approach to small ruminant husbandry and the difficulty in quantifying their disease expenses. Disease prevention and management in sheep and goats is already difficult because of a shortage of veterinary services in rural regions[16]. In India, small ruminants are a major cause of human zoonoses infection. Understanding the occurrence of zoonoses in small ruminants is critical for public health since small ruminants are reservoirs for several zoonotic diseases of important animal and human health concerns[6]. Epidemiological investigations conducted in a variety of settings have identified age, history of abortion, herd size, herd composition, practices of animal husbandry, and socioeconomic elements as the essential brucellosis risk factors[17]. The disease risk is directly linked to changes in small ruminant farming practices. The movement of flocks from one geographical place to another is a crucial influencing factor for disease transmission in small ruminants. The species that causes small ruminant brucellosis, *B. melitensis*, has been blamed for a sharp rise in human brucellosis lately[18].

The majority of brucellosis diagnostic procedures in animals rely on serology tests, such as Standard Tube Agglutination Test (STAT), Rose Bengal Plate Test (RBPT), Complement Fixation Test (CFT), and indirect Enzyme Linked Immunosorbent Assay (ELISA), as a cornerstone approach, with the Lipopolysachharide (LPS) smooth chains eliciting the strongest immune responses in diverse hosts[19]. Serological tests are simple, affordable, and potentially quick, yet they are liable to false positive findings when exposed to cross-reacting bacteria, which could be due to *Brucella* LPS's O-antigenic side chain[20]. Due to high level of sensitivity of RBPT test, it is extensively used as a screening test in herds and has been recognized globally. Although serological tests like STAT and RBPT are often employed in animals for early brucellosis screening, it is strongly suggested to add supplemental non-agglutination testing such as ELISA, which is a highly specific and sensitive test for brucellosis detection when compared to other tests[21]. Several serological tests are devised due to differences in sensitivity and specificity; however, no one test is suitable for all epidemiological studies[22,23].

Furthermore, serology alone can be unreliable in determining the risk variables associated with brucellosis, *Brucella* spp. is shed by a large number of seronegative animals, and the opposite is also true; certain animals may show seropositivity yet not excrete the organism[24,25]. Studies in developing countries that largely employed serology have fallen short of offering useful insights into long-term disease control strategies[26]. It is imperative to comprehend the animal-human-environmental interaction patterns, which is vital information for reducing zoonotic transmission risks. Hence, it is important to study the on-farm practices on the handling of sheep and goats by livestock owners and their association with the occurrence of brucellosis[27,28]. This study was undertaken in a district of India with a high density of small ruminant population to ascertain brucellosis seropositivity and risk variables for its occurrence.

## 2. Materials and methods

### *2.1. Ethical statement*

The study was approved by the Deemed University, ICAR-Indian Veterinary Research Institute (No. F.4-6/5932/21-Acad). The blood samples were obtained by veterinarians in accordance with the Animal Welfare Guidelines of Government of India. The farm/animal owners were informed about the purpose of sample

[Downloaded free from http://www.johb.info on Wednesday, September 27, 2023, IP: 112.66.109.236]



collection and due consent was obtained for the same.

### 2.2. Study area

The study was conducted in the YSR district in Andhra Pradesh and which is located at 14.47°N 78.82°E in the Rayalaseema region of the south-central part of Andhra Pradesh, India. The average rainfall in the district is 678 mm yearly. It has a semi-arid climate with high temperatures throughout the year, with temperatures stated to get above 50℃ on several days during summer. Farmers of the district rely more on animal husbandry practices such as sheep and goat rearing since crop production is not profitable. Livestock farming yields a good source of family income and livelihood for smallholder farmers. Despite a lack of water and fodder, sheep and goats can acclimatize to a diverse range of critical climatic challenges. Sheep and goats may be raised efficiently on marginal soils and make good use of crop residues as feed, which is available abundantly in the district.

### 2.3 Sampling method and sample size determination

The multi-stage sampling method was followed for sample collection. According to the 20th livestock census, in the YSR district sheep population stands at 18.6 lakh, whereas the goat population is around 5.8 lakh. The Epitools software was used in the sample size calculation. For samples size estimation parameters were as follows: sheep and goat population in the study region as per the 20th Livestock Census (Goats: 578 607; Sheep: 1 869 861) which was categorised as adults >3 years and young adults of 1-3 years of age, frequency of brucellosis reported in small ruminants from previous studies as 16% and 15% for sheep and goats, respectively at 95%*CI* and statistical power was set to 80%[15,29]. The final sample size that arrived for sheep and goats was 215 and 210, respectively.

### 2.4. Sample collection

A total of 425 serum samples (215 sheep and 210 goats) were collected from January 2022 to March 2022 (Table 1).

**Table 1.** Total number of sheep and goat samples collected.

| Species | Male | Female | Total |
|---|---|---|---|
| Sheep | 59 | 156 | 215 |
| Goat | 35 | 175 | 210 |
| Total | 94 | 331 | 425 |

### 2.5. Blood collection and serum separation

Blood collection was done using a sterile needle. The jugular vein was punctured and approximately 2-3 mL of blood was collected into the serum clot activator tube with utmost precaution to avoid haemolysis. If the serum was not separated after one to two hours at room temperature, the tubes were centrifuged at 3000 rpm for three to four minutes. After the separation of serum, it was transferred into the serum collecting tubes and stored at −20℃ in an ultra-low temperature freezer until being utilised for further laboratory tests.

### 2.6. Laboratory tests

#### 2.6.1. Rose Bengal Plate Test (RBPT)

The RBPT was performed according to the standard protocol[30]. The Rose Bengal antigen was obtained from the Division of Biological Products, Indian Veterinary Research Institute. Agglutination that showed within 4 min of reagent mixing was seen as positive, while its absence was regarded as negative.

#### 2.6.2. Standard Tube Agglutination Test (STAT)

The test was performed in accordance with the standard protocol[30] using the plain antigen procured from the Division of Biological Products, Indian Veterinary Research Institute. In sheep and goats, a titre of 40 I.U. or more was regarded as positive.

#### 2.6.3 ELISA Test Proper

The ELISA test was conducted according to the standard protocol[31]. Sera samples from goats and sheep with PP values more than 58 and 64, respectively, were classified as positive.

$$\% \text{ Positivity} = \frac{\text{O.D value of the test serum samples}}{\text{Mean O.D value of the positive control}} \times 100$$

### 2.7. Questionnaire survey

A semi-structured peer-evaluated questionnaire was used to identify the potential risk factors associated with small ruminant brucellosis encompassing individual animal characteristics, herd characteristics, housing characteristics, biosecurity measures, lambing or kidding practices, disinfection practices followed, and animal handler's behavior and detailed history were obtained while collecting samples. The survey data was classified into seven parts: 1) Individual characteristics including age, sex, parity status, and reproductive disorders history; 2) Herd characteristics, such as herd size, herd composition, type of herd, grazing system, and mode of acquisition of animals by the herd; 3) Housing characteristics; 4) Biosecurity measures; 5) Lambing/kidding practices; 6) Disinfection practices followed; and 7) Animal handler's behaviour.

### 2.8. Statistical analysis

Microsoft Excel spreadsheets were used to enter and organize the data before being uploaded to statistical analysis software (SPSS software, 28.0 version (SPSS Inc., USA). Data from questionnaires and results of serological tests were stored in the database. In order

[Downloaded free from http://www.johb.info on Wednesday, September 27, 2023, IP: 112.66.109.236]



to investigate any possible correlation between seropositivity and relevant risk variables, we utilized either the *chi*-square analysis ($\chi^2$) or Fisher's exact test. To determine the strength of association between potential risk factors and seroprevalence, we calculated the odds ratio (*OR*), while confounding factors were identified through the use of strata-specific *OR*. We only considered variables with a *P*-value of less than 0.25 in bivariate analysis, with no evidence of collinearity, for inclusion in our multivariable logistic regression analysis. Our optimal final model was chosen using the forward Wald selection approach. The goodness of fit for the final model was assessed using the Hosmer-Lemeshow test. Statistical significance was determined at a *P*-value of 0.05 or below.

## 3. Results

Among the 425 samples collected, 91 samples (21.4%) were positive by RBPT, 124 samples (29.2%) by STAT, and 156 samples (36.7%) were revealed by I-ELISA to be positive in sheep and goats. Table 2 displays the total seroprevalence values for the RBPT, STAT, and I-ELISA tests performed on sheep and goats. Pearson's *Chi*-square test was employed to ascertain associations of statistical significance between the putative risk factors and the seropositivity of small ruminant brucellosis in age, abortion history, parity status, vaginal discharges, herd size, mixed farming, migratory herd, dog presence with the flocks, act of dog consuming aborted foetus, the distance between pen and dump site, and place of disposal of the foetus. Bivariate analysis showed that brucellosis seropositivity for small ruminants was strongly correlated with all potential risk variables, as indicated by Pearson's *Chi*-square test (Table 3). Analysis of multivariate logistic regression showed age, abortion history, vaginal discharges, the migratory herd, and place of disposal of the foetus to be risk factors with statistical significance (Table 4).

**Table 2.** Brucellosis seropositivity assessed by RBPT, STAT, and I-ELISA.

| Species | Samples tested | RBPT | STAT | I-ELISA |
|---|---|---|---|---|
| Sheep | 215 | 73 (34.0) | 85 (39.5) | 91 (42.3) |
| Goats | 210 | 18 (8.6) | 39 (18.6) | 65 (31.0) |
| Total | 425 | 91 (21.4) | 124 (29.2) | 156 (36.7) |

Data are expressed as *n*(%).

### 3.1 Questionnaire survey result

Questionnaires were employed in a total of 77 sheep and goat farms in various villages in the study area to gauge their levels of knowledge about the disease. The responders were unaware that brucellosis is a zoonotic illness that may be transmitted between animals and humans. Seven flock owners (9.1%) responded that they frequently consume raw milk from their animals. None of the participants answered burying or burning aborted foetuses and foetal membranes; instead, all respondents answered that the foetuses were thrown into the bushes. In addition, there was no practice of isolation of aborted animals and quarantine of newly purchased animals and disinfection of farm premises by respondents.

### 3.2 Bivariate analysis of potential risk factors

Bivariate analysis in small ruminants showed that seropositivity was greater in animals over 3 years old (45.2%) than in animals under 3 years old (23.5%) (Table 3). Adult animals (>3 yrs) had a 2.6 times higher risk than the younger ones (*OR*=2.6; *P*<0.001). A higher parity in animals and a history of reproductive issues appeared to influence the positivity. Multiparity revealed higher seropositivity (42.2%) and odds of disease occurrence (*OR*=2.092; *P*=0.001) than in small ruminants with no multiparity. The occurrence of reproductive problems in small ruminants such as abortion (*OR*=2.342; *P*<0.001) and vaginal discharges (*OR*=4.24; *P*<0.001) is significantly associated with higher seropositivity of 43.2% and 59.2%, respectively. Seropositivity was also found to be higher in small ruminants from larger herds (47.1%, *OR*=2.711; *P*<0.001) and those following a migratory rearing pattern (55.3%, *OR*=3.2; *P*<0.001). Mixed farming practices (sheep and goats) also showed higher seropositivity of 42% and higher odds of disease occurrence (*OR*=3.2; *P*<0.001). Certain biosecurity factors were found to increase the risk of brucellosis, including the presence of dogs around the flock, observation of dogs consuming aborted foetuses, and the proximity of the farm to the dumpsite. Herds with dogs surrounding the flock showed a higher seropositivity rate of 40.5% (*OR*=3.0; *P*<0.001), while instances of dogs consuming aborted foetuses had a higher seropositivity of 40.7% and higher odds of disease occurrence (*OR*=2.78; *P*=0.001). Farms located closer to or adjacent to the dumpsite also showed higher seropositivity of 42.1% and higher odds of disease occurrence (*OR*=2.5; *P*<0.001). Finally, lambing practices, including disposal of foetuses near the farm perimeter, were found to result in higher seropositivity (48.6%) and higher odds of infection (*OR*=2.9; *P*<0.001) compared to disposal sites located further away from the farm.

### 3.3. Multivariable regression analysis of potential risk factors

To produce the probable model (*P*<0.05), multivariable logistic regression analysis was conducted by using the Forward Wald stepwise method for all variables having a *P*<0.25. Therefore, using this model for multivariable logistic regression analysis (Table 4) demonstrated a statistically significant correlation between the prevalence of small ruminant brucellosis in animals more than 3 years old, history of abortion, vaginal discharges, migratory herds and place of disposal of foetus near farm surroundings for small ruminant brucellosis.

[Downloaded free from http://www.johb.info on Wednesday, September 27, 2023, IP: 112.66.109.236]



**Table 3.** Analysis of potential risk variables using bivariate analysis.

| Variable | Response/ Category | Number of animals ($n$= 425) | Seropositivity | *OR*(95%*CI*) | *P* |
|---|---|---|---|---|---|
| Age | > 3 years | 259 | 45.2% | 2.683 | <0.001 |
| | < 3 years | 166 | 23.5% | (1.738-4.142) | |
| Parity status | Multiparous | 282 | 42.2% | 2.092 | 0.001 |
| | Primiparous | 143 | 25.9% | (1.343-3.246) | |
| History of abortion | Yes | 278 | 43.2% | 2.342 | <0.001 |
| | No | 147 | 24.5% | (1.501-3.653) | |
| Vaginal discharge | Yes | 142 | 59.2% | 4.244 | <0.001 |
| | No | 283 | 25.4% | (2.765-6.514) | |
| Herd size | >100 | 227 | 47.1% | 2.711 | <0.001 |
| | <100 | 198 | 24.7% | (1.791-4.105) | |
| Mixed Farming | Yes | 331 | 42.0% | 3.279 | <0.001 |
| | No | 94 | 18.1% | (1.857-5.791) | |
| Migratory herd | Yes | 141 | 55.3% | 3.270 | <0.001 |
| | No | 284 | 27.5% | (2.144-4.987) | |
| Dog presence with flocks | Yes | 353 | 40.5% | 3.090 | 0.001 |
| | No | 72 | 18.1% | (1.634-5.843) | |
| Act of dog consuming aborted foetus | Yes | 344 | 40.7% | 2.788 | 0.001 |
| | No | 81 | 19.8% | (1.549-5.018) | |
| Distance between pen and dump site | Just adjacent to the farm | 309 | 42.1% | 2.514 | <0.001 |
| | Far away from the farm | 116 | 22.4% | (1.538- 4.109) | |
| Place of disposal of foetus | Near farm surroundings | 216 | 48.6% | 2.931 | <0.001 |
| | Far away from farm | 209 | 24.4% | (1.938-4.431) | |

## 4. Discussion

Chronic infectious illness, brucellosis affects both animals and humans in many countries, especially in developing nations, and is considered a significant threat to public health. In developing nations, the occurrence of brucellosis has increased in recent years mainly due to inadequate management, limited resources, increased animal commerce, and frequent animal transportation. This disease has significant impacts on both veterinary and public health, leading to substantial morbidity and economic losses. Brucellosis in animals is distinguished by abortion, as well as pathogen expulsion through the milk and uterine secretions. The bacteria are concentrated in the udders of female animals, where they are excreted through milk. In male animals, epididymitis and orchitis can result in infertile conditions[32]. In humans, the disease is serious, persistent, and frequently accompanied by symptoms that are debilitating[32]. For the disease to be effectively controlled in animals, correct identification of *Brucella* infection is highly essential. An animal's reproductive history can be used as a starting point for a clinical diagnosis; however, this is an assumed diagnosis that has to be confirmed with laboratory testing. Because no single test can be used to diagnose brucellosis serologically, serological testing has to be utilised in conjunction with other methods to reduce the occurrence of false positives and false negatives[33]. Furthermore, ELISA is superior in terms of suitability, sensitivity, and specificity[34]. Numerous risk variables connected to production methods, the biology of the particular host, and environmental factors affect the likelihood of contracting brucellosis. Age, flock size and composition, farm hygiene, farm biosecurity, the frequency of interaction between sick and vulnerable animals, and climate are some of these factors[35,36]. For diseases to be effectively controlled, managed, and eradicated, awareness of risk factors that promote their spread is a fundamental prerequisite. The objective of this investigation was to identify brucellosis risk factors in small ruminants and to estimate brucellosis seroprevalence in the region. Since the government of India lacks a small ruminant immunisation policy, identifying risk variables will assist in formulating strategies to limit infection transmission in animals.

Out of the 425 serum samples collected from sheep and goats in the YSR district and screened in this study, 21.4%, 29.2%, and 36.7% of the samples showed positivity for RBPT, STAT, and I-ELISA, respectively. These results are in line with the

**Table 4.** Analysis of potential risk variables using multivariable logistic regression.

| Variable | Response/ Category | *OR* ( 95% *CI*) | *P* |
|---|---|---|---|
| Age | > 3 years *vs.*< 3 years | 2.262 ( 1.414 -3.618) | 0.001 |
| Abortion history | Yes *vs.* No | 1.837 (1.098 -3.071) | 0.020 |
| Vaginal discharge | Yes *vs.* No | 2.334 (1.421-3.835) | 0.001 |
| Migratory herd | Yes *vs.* No | 2.197 (1.369-3.527) | 0.001 |
| Place of disposal of foetus | Near farm surroundings *vs.* Far away from farm | 2.093 (1.320- 3.318) | 0.002 |

[Downloaded free from http://www.johb.info on Wednesday, September 27, 2023, IP: 112.66.109.236]



seroprevalence reported in a previous study[37] who observed a seroprevalence of 29.1% by RBPT and 22.6% by STAT. However, some authors reported higher seroprevalence with 37.6% by RBPT and 44.4% by STAT[38]. In contrast, another study recorded a total seroprevalence of 11.30% by RBPT, 11.10% by STAT, and 8.80% by I-ELISA in small ruminants, which were much lower than the current results[39].

Numerous variables, including the stage of infection, the presence of animals that test falsely positive or negative, and cross-reacting organisms, among others, may contribute to the difference in test findings. Additionally, various animal species may react to brucellosis differently. Another possibility is that the immunoglobulins of various species respond differently to the tests used to diagnose brucellosis. These factors need further study with a large number of serum samples under both controlled and natural settings. High levels of seropositivity were measured in this investigation among small ruminant flocks, which suggests the persisting endemicity of animal infections and the substantial risk of human exposure. Furthermore, the possibility that most of the herds under investigation had a history of abortion points to a higher likelihood of having animals with *Brucella* spp. infection involved in this study, which might explain the increased incidence of infection pictured. On the basis of the present study's findings, one may draw the following conclusion that I-ELISA is a reliable test with high throughput and sensitivity that can be commonly used for an effective and accurate diagnosis of *Brucella* infection, as the possibility of failing to detect an infected animal in I-ELISA is limited.

The risk factor analysis used in the current investigation was carried out using a questionnaire designed to evaluate the risk variables contributing to seropositivity for brucellosis in the study region. In the context of this analysis, the seropositivity determined by the combined findings of RBPT, STAT, and I-ELISA was employed as the outcome variable for modelling risk variables.

The study found seropositivity of brucellosis in both sheep and goats is higher in the more than 3-year age group compared to the less than 3-year age group ($P$<0.05). This is in line with the previous findings of other studies[40–42]. Age is likely the most significant risk factor for brucellosis as it is closely correlated with the probability of infection due to sexual maturity, higher coital chances and increasing frequency of interaction with other animals with age[43]. Therefore, it is important to consider the age of the animal when analysing the factors influencing the occurrence of the disease.

In this study, abortion history was observed to be a significant risk factor ($P$<0.05) for both sheep and goat flocks, consistent with previous studies[37,44]. This is because abortions can release large numbers of infectious organisms into the environment, which can contaminate the area and infect other healthy animals in the flock. Vaginal discharge was also found as a significant risk factor ($P$<0.05) for brucellosis infection in both sheep and goats. Animals infected with brucellosis often excrete *Brucella* species through vaginal discharges during lambing or kidding, which can contaminate the environment and could serve as an animal infection source. Insects such as flies may also serve as mechanical vectors for the spread of *Brucella* species throughout the farm atmosphere[45].

It was observed that the place of disposal of foetuses near the farm perimeter showed higher odds in small ruminants. This could be due to the spread of infection by the farm animals or any stray animals such as dogs dragging of the aborted foetuses which may contain up to $>10^9$ *Brucella* cells per gram. Therefore, it is recommended that the aborted foetuses and their contents be disposed of by incineration. Another alternative is to bury them deep in slaked lime away from farms and waterways[46].

The pattern of rearing small ruminants as migratory herds showed to be a significant risk factor by multivariable regression analysis. This is in concordance with results reported in previous studies who reported higher odds of being seropositive for brucellosis with statistical significance in both sheep and goat migratory flocks.[47,48] The migratory pattern of these flocks, their contact with other, possibly diseased sheep and goats throughout their transit, and the interaction of domestic and wild animals, which facilitates disease transmission, may be partly responsible for the high seropositivity observed.

## 5. Conclusions

The current study emphasizes the significance of proper disposal of aborted foetuses and its contents, which have a greater odds to higher seropositivity of brucellosis. In addition to these, the likelihood that brucellosis may spread in some areas is increased by inadequate management, ineffective prevention efforts, and unrestricted animal movement across 'open' borders. Livestock owners and consumers of sheep and goats should be educated about the disease's characteristics and prevention strategies to mitigate its uncontrolled spread and lower the potential threat of human brucellosis. The higher expenses involved in test and slaughter policy is a deterrent for effective implementation unless suitable compensation is in place. Therefore, management strategies must be established to mitigate the risks of brucellosis to re-emerge in the absence of immunization. Choosing the right rearing practices, enhancing husbandry procedures, changing farmer's perspectives on brucellosis, and inclusion of small ruminants in brucellosis control programs are all key management approaches that can aid in the prevention and control of *Brucella* infection in small ruminants. Further, successful practices to minimise human illness at the animal, environmental, and human interface require a One Health strategy that includes transdisciplinary collaboration and community engagement. Controlling human brucellosis relies on minimising the illness in its small ruminant reservoir.

[Downloaded free from http://www.johb.info on Wednesday, September 27, 2023, IP: 112.66.109.236]



## Conflict of interest statement

The authors claim there is no conflict of interest.

## Funding

This study receives no extramural funding.

## Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

## Authors' contributions

Vakamalla SSR was responsible for samples and data collection, laboratory analysis of samples, manuscript preparation. Kumar MS and Singh DK conceptualized and designed the study, conducted laboratory analysis of samples, edited and reviewed the manuscript. Dhanze H supervised the methodology and laboratory analysis. Rajendran VKO edited and reviewed manuscript, and completed data analysis to assess the risk factors. Rafeeka CA completed laboratory analysis of samples.

The manuscript has been read and approved by all the authors.

## References

[1] Franc K, Krecek R, Häsler B, Arenas-Gamboa A. Brucellosis remains a neglected disease in the developing world: A call for interdisciplinary action. *BMC Public Health* 2018; **18**(1): 1-9.

[2] Hull NC, Schumaker BA. Comparisons of brucellosis between human and veterinary medicine. *Infect Ecol Epidemiol* 2018; **8**(1):1500846.

[3] Zheng R, Xie S, Lu X, Sun L, Zhou Y, Zhang Y, et al. 2018. A systematic review and meta-analysis of epidemiology and clinical manifestations of human Brucellosis in China. *Biomed Res Int* 2018; **2018**: 5712920

[4] Ghanbari MK, Gorji HA, Behzadifar M, Sanee N, Mehedi N, Bragazzi NL. One health approach to tackle Brucellosis: A systematic review. *Trop Med Health* 2020; **48**(1): 1-10.

[5] Singh B, Dhand NK, Gill J. Economic losses occurring due to Brucellosis in Indian livestock populations. *Prev Vet Med* 2015; **119**(3-4): 211-215.

[6] Singh B, Khatkar M, Aulakh R, Gill J, Dhand N. Estimation of the health and economic burden of human Brucellosis in India. *Prev Vet Med* 2018; **154**: 148-155.

[7] Yagupsky P. Preventing laboratory-acquired brucellosis in the era of MALDI-TOF technology and molecular tests: A narrative review. *Zoonotic Dis* 2022; **2**(4): 172-182.

[8] Bagheri Nejad R, Krecek RC, Khalaf OH, Hailat N, Arenas-Gamboa AM. Brucellosis in the Middle East: Current situation and a pathway forward. *PLoS Negl Trop Dis* 2020; **14**(5): e0008071.

[9] Renukaradhya GJ, Isloor S, Rajasekhar M. Epidemiology, zoonotic aspects, vaccination and control/eradication of Brucellosis in India. *Vet Microbiol* 2022; **90**(1-4): 183-195.

[10]Moreno E. Retrospective and prospective perspectives on zoonotic Brucellosis. *Front Microbiol* 2014; **5**: 213.

[11]Kaya S, Elaldi N, Deveci O, Eskazan AE., BekcibasiM, Hosoglu S. Cytopenia in adult Brucellosis patients. *Indian J Med Res* 2018; **147**(1): 73-80.

[12]Bosilkovski M, Edwards MS. Brucellosis: Epidemiology, microbiology, clinical manifestations, and diagnosis.

[13]Mantur BG, Amarnath SK. Brucellosis in India—A review. *J Biosci* 2008; **33**(4): 539-547.

[14]Al–mashhadany DA. The role of milk ring test in monitoring brucellosis among cow milk in Erbil Governorate/Kurdistan Region/Iraq. *IJBPAS* 2018; **7**(5): 802-819.

[15]Department of Animal Husbandry & Dairying. (n.d.). *Provisional Key Results of 20th Livestock Census.* [Online] Aailable from: https://dahd.nic.in/division/provisional-key-results-20th-livestock-census. [Accessed on 16 June 2022].

[16]Kumar A. Brucellosis: Need of public health intervention in rural India. *Prilozi* 2010; **31**(1): 219-231.

[17]Kothalawala KAC, Makita K, Kothalawala H, Jiffry AM, Kubota S, Kono H. Association of farmers' socioeconomics with bovine Brucellosis epidemiology in the dry zone of Sri Lanka. *Prev Vet Med* 2017; **147**: 117-123.

[18]Sonekar CP, Kale S, Bhoyar S, Paliwal N, Shinde S, Awandkar S, et al. Brucellosis in migratory sheep flock from Maharashtra, India. *Trop Anim Health Prod* 2018; 50(1): 91-96.

[19]Nielsen K. Diagnosis of Brucellosis by serology. *Vet Microbiol* 2002; **90**(1-4): 447-459.

[20]Weiner M, Iwaniak W, Zlotnicka J, Szulowski K. Diagnosis of bovine Brucellosis using traditional serological techniques and fluorescence polarisation assay. *Bull Vet Inst Pulawy* 2010; **54**(2010): 485-488.

[21]Shome R, Padmashree B, Krithiga N, Triveni K, Sahay S, Shome B,et al. Bovine brucellosis in organized farms of India-An assessment of diagnostic assays and risk factors. *Adv Anim Vet Sci* 2014; **2**(10): 557-564.

[22]Mert A, Ozaras R, Tabak F, Bilir M, Yilmaz M, Kurt C, et al. The sensitivity and specificity of *Brucella* agglutination tests. *Diagn Microbiol Infect Dis* 2003; **46**(4): 241-243.

[23]OIE. *Manual of Diagnostic tests and vaccines for terrestrial animals.* [Online] Paris: World Organization for Animal Health; 2018. Available from: 140318-reference.pdf (fao.org). [Accessed on 25 March 2023 ]

[Downloaded free from http://www.johb.info on Wednesday, September 27, 2023, IP: 112.66.109.236]



[24]Porter SR, Czaplicki G, Mainil J, Guattéo R, Saegerman C. Q Fever: Current state of knowledge and perspectives of research of a neglected zoonosis. *Int J Microbiol* 2011; **2011**: 248418.

[25]Van den Brom R, Van Engelen E, Roest H, Van der Hoek W, Vellema P. *Coxiella burnetii* infections in sheep or goats: An opinionated review. *Vet Microbiol* 2015; **181**(1-2): 119-129.

[26]Godfroid J, Dahouk SA, Pappas G, Roth F, Matope G, Muma J, et al. A "One Health" surveillance and control of Brucellosis in developing countries: Moving away from improvisation. *Comparat Immunol Microbiol Infect Dis* 2013; **36**(3): 241-248.

[27]Kanani A, Dabhi S, Patel Y, Chandra V, Kumar OV, Shome R. Seroprevalence of brucellosis in small ruminants in organized and unorganized sectors of Gujarat state, India. *Vet World* 2018; **11**(8): 1030.

[28]Natesan K, Kalleshamurthy T, Nookala M, Yadav C, Mohandoss N, Skariah S, Sahayet al. Seroprevalence and risk factors for brucellosis in small ruminant flocks in Karnataka in the Southern Province of India. *Vet World* 2021; **14**(11): 2855.

[29]Shome R, Kalleshamurthy T, Rathore Y, Ramanjinappa KD, Skariah S, Nagaraj C, et al. Spatial sero-prevalence of brucellosis in small ruminants of India: Nationwide cross-sectional study for the year 2017–2018. *Transbound Emerg Dis* 2021; **68**(4): 2199-2208.

[30]Alton GG, Jones LM, Pietz DE, World Health Organization. *Laboratory techniques in Brucellosis*. [Online] Available from: https://apps.who.int/iris/handle/10665/38676. [Accessed on 10 August 2022].

[31]Reddy DA, Kumari G, Rajagunalan S, Singh DK, Kumar A, Kumar PP. Seroprevalence of caprine Brucellosis in Karnataka. *Vet World* 2014; **7**(3): 182.

[32]Gholizadeh SS, Zali MH, Hashempour A, Ahmadi EM. Investigation of Brucellosis in cattle and sheep in Urmia-Iran. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi* 2013; **24**(3): 133-134.

[33]Montasser AM, Affi MM, El-Bayoumy EM, Abdul-Raouf UM, Mohamad HA. Efficiency of serological tests for detection of Brucellosis in ruminant at south provinces of Egypt. *Global Vet* 2011; **6**(2): 156-161.

[34]Fadeel MA, Wasfy MO, Pimentel G, Klena JD, Mahoney FJ, Hajjeh, R. A. Rapid enzyme-linked immunosorbent assay for the diagnosis of human Brucellosis in surveillance and clinical settings in Egypt. *Saudi Med J* 2006; **27**(7): 975-981.

[35]McDermott JJ, Arimi SM. Brucellosis in sub-Saharan Africa: Epidemiology, control and impact. *Vet Microbiol* 2002; **90**(1-4): 111-134.

[36]Radostits OM, Gay C, Hinchcliff KW, Constable PD. A textbook of the diseases of cattle, horses, sheep, pigs and goats. *Vet Med* 2007; **10**: 2045-2050.

[37]Abnaroodheleh F, Emadi A, Dadar M. Seroprevalence of brucellosis and chlamydiosis in sheep and goats with history of abortion in Iran. *Small Ruminant Res* 2021; **202**: 106459-106459.

[38]Esendal OM, Yardımc H, Keskin O, Altay G. The use of conventional tests and Coombs test in the serological diagnosis of bovine, ovine and caprine Brucellosis. *Ankara Univ Vet Fak* 2001; **48**(1): 97-102.

[39]Sadhu DB, Panchasara HH, Chauhan HC, Sutariya DR, Parmar VL, Prajapati HB. Seroprevalence and comparison of different serological tests for Brucellosis detection in small ruminants. *Vet World* 2015; **8**(5): 561.

[40]Ullah Q, Jamil T, Melzer F, Saqib M, Hussain MH, Aslam MA, et al. Epidemiology and associated risk factors for Brucellosis in small ruminants kept at institutional livestock farms in Punjab, Pakistan. *Front Vet Sci* 2020; **7**: 526.

[41]Suryawanshi S, Tembhurne P, Gohain S, Ingle V. Prevalence of *Brucella* antibodies in sheep and goats in Maharashtra. *Indian Res J Extens Educat* 2016; **14**(4): 75-77.

[42]Tegegn AH, Feleke A, Adugna W, Melaku SK. Small ruminant Brucellosis and public health awareness in two districts of Afar Region, Ethiopia. *J Vet Sci Technol* 2016; **7**(335): 2.

[43]Gul ST, Khan A. Epidemiology and epizootology of Brucellosis: A review. *Pakistan Vet J* 2007; 27(3): 145.

[44]Alhamada AG, Habib I, Barnes A, Robertson I. Risk factors associated with *Brucella* seropositivity in sheep and goats in Duhok Province, Iraq. *Vet Sci* 2017; **4**(4): 65.

[45]Coelho A, García-Díez J, Góis J, Rodrigues J, Coelho A. Farm practices and risk factors which influence the high prevalence of Brucellosis in small ruminant flocks in Northeast Portugal. *Vet Ital* 2019; **55**(4): 355-362.

[46]WHO. *Brucellosis in Humans and Animals*. Geneva: WHO; **2006.** pp. 65.

[47]Gompo TR, Shah R, Tiwari I, Gurung YB. Sero-epidemiology and associated risk factors of Brucellosis among sheep and goat population in the southwestern Nepal: A comparative study. *BMC Vet Res* 2021; **17**(1): 1-10.

[48]Sadhu D. Assessment of seroprevalence and risk factors associated with brucellosis in goat. *Int J Agric Sci* 2016; **8**(51): 2290-2294.

## Publisher' s note

The Publisher of the *Journal* remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.