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Epidemiology of Abortion in Small Ruminants: High prevalence of Brucellosis and Improved Detection by Indirect ELISA

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A B S T R A C T

Abortions in small ruminants are an important cause of economic loss and public health concern due to zoonotic pathogens such as *Brucella* spp. A cross-sectional survey of 64 flocks comprising 11530 sheep and goats in Kadapa district of Andhra Pradesh state, India revealed 71.87% abortion prevalence at flock level, and animal level prevalence rates of 4.85 % in sheep and 3.07% in goats. Significant risk factors associated with abortions were large flock size ($p<0.01$), extensive rearing system ($p<0.05$), communal grazing practices ($p < 0.01$), improper disposal of aborted materials ($p < 0.05$), and the presence of reproductive disorders ($p < 0.01$). Abortions were predominantly recorded during winter and in late gestation among small ruminants. Serological analysis of 120 samples collected from recently aborted animals showed brucellosis seroprevalence of 36.6%, 40.83%, and 48.33% by RBPT, STAT, and i-ELISA, respectively, with higher seropositivity observed in sheep than in goats. Among the serological tests evaluated, Indirect ELISA detected a higher proportion of seropositive animals than RBPT and STAT indicating its greater utility for the detection of brucellosis in small ruminants. In conclusion, the high prevalence of brucellosis among aborted small ruminants highlights the importance of continuous serological surveillance, enhanced flock management, and strengthened biosecurity measures to mitigate reproductive losses, improve animal health, and reduce zoonotic transmission.

Introduction

Small ruminants play a crucial role in the livelihood and socio-economic well-being of rural populations, especially in developing countries (Saboor *et al.,* 2024). The sheep and goat contribute to 13.86 and 27.78% of

the total livestock population respectively of India. However, reproductive disorders remain a major constraint to their productivity and profitability. Among these, abortion represents a significant cause of economic loss due to fetal loss, repeat breeding, increased treatment costs and the potential spread of infectious agents within the flock (Sharma *et al.,* 2008; Ali *et al.,* 2019). Abortions can be caused by various infectious agents such as bacterial, viral, protozoal and additionally by non-infectious agents such as toxins, genetic issues, metabolic and nutritional disorders (Yadav *et al.,* 2021). Herds with low fertility often exhibit higher abortion incidences due to poor reproductive health and management factors (Alemayehu *et al.,* 2021). Furthermore, infectious agents such as *Brucella spp.* contribute significantly to both decreased fertility and increased abortion by causing placental damage and reproductive failure (Rossetti *et al.,* 2022).

Brucellosis is a major neglected zoonotic disease worldwide, causing significant economic losses and posing serious public health concerns. It is endemic in many regions of the world, including Central and South America, Central Asia, the Middle East, the Indian subcontinent, the Mediterranean region, and Sub-Saharan Africa (Vakamalla *et al.,* 2023). It is endemic in several domestic animal species and across nearly all states of India. The bacteria causing brucellosis belong to the family *Brucellaceae* and are Gram-negative, non-motile, non-spore-forming coccobacilli that are aerobic and facultative intracellular bacteria (Leclercq *et al.,* 2020). Currently, nine *Brucella* species are recognized, among them, *B. melitensis* primarily infects sheep and goats (Talukdar *et al.,* 2020). Clinical signs of brucellosis in small ruminants primarily involve abortions during the second half of pregnancy, stillbirths, low-birth-weight offspring, weak lambs, and placentitis in pregnant females. In males, the disease may cause epididymitis, orchitis, and infertility, whereas infected females can shed the organism through uterine discharges and milk secretion (Ganter, 2015). Small ruminants serve as the primary reservoirs for *Brucella melitensis* and play a key role in transmission to humans. The infection predominantly occurs to humans through occupational or direct contact with infected animals, particularly during parturition via conjunctival or oronasal exposure or by consuming contaminated dairy products (Mahboub *et al.,* 2013).

Detection of *Brucella* spp. is often difficult because of its prolonged incubation period, ranging from five days to several months, and the infection may present in acute, chronic, or asymptomatic forms, thereby requiring laboratory confirmation for accurate diagnosis (Al Mashhadany, 2018). Various serological tests, including Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT), Complement Fixation Test (CFT), Milk Ring Test (MRT), and Enzyme-Linked Immunosorbent Assay (ELISA), are commonly used for detecting antibodies against *Brucella*. Although RBPT and STAT are commonly used as preliminary screening tests for brucellosis in animals, the inclusion of supplementary non-agglutination assays such as ELISA is recommended due to their higher sensitivity and specificity for accurate detection of brucellosis (Shome *et al.,* 2014). However, due to variations in their sensitivity and specificity, no single test is adequate for all epidemiological investigations, and a combination of tests is often recommended (OIE, 2018).

The present study was aimed to assess the seroprevalence and to identify the risk factors associated with brucellosis in small ruminants in selected epidemiological units of Kadapa district, Andhra Pradesh state of India.

Materials and Methods

Sampling plan and sampling size

The present study focused on the epidemiological assessment of abortions in sheep and goats across villages in YSR Kadapa district, Andhra Pradesh, India. Using a qualitative research framework, a descriptive analytical approach was applied to identify the major factors influencing the occurrence of abortions in small ruminants. A multistage cluster sampling strategy was employed. Sixty-four sheep and goat flocks were selected from the study area and subjected to epidemiological investigation. All available abortion cases encountered in the selected flocks during the study period were included in the study. Epidemiological data were collected using a structured questionnaire. Data collection was carried out over a six-month period from August 2025 to January 2026.

Tools of data collection and observation

Data for the study were collected through structured interviews and questionnaires developed from an

extensive review of relevant literature and aligned with the study objectives. The questionnaire was designed in a simple and farmer-friendly manner to ensure clarity and ease of understanding. To evaluate farmers knowledge of abortions in sheep and goats, questions were framed around key factors including flock size (small flocks <100 and large flocks >100), grazing practices (such as sharing grazing land or water sources with other flocks or large ruminants), and the type of rearing system (semi-intensive or extensive).

The survey further incorporated aspects related to management and reproductive health, including methods of disposal of aborted foetuses or fetal membranes (eg. disposal in unused wells, left accessible to dogs, or buried) as well as the history of reproductive disorders within the flock such as retained placenta, repeat breeding, and prolapse. It also captured patterns in the occurrence of abortions with respect to season (summer, rainy, or winter) and stage of pregnancy (early, mid, or late gestation).

In addition, the questionnaire assessed post-abortion outcomes, including the animal's ability to conceive again, the results of subsequent pregnancies (normal birth, stillbirth, or reabortion), the viability of offspring, and the nutritional status of the pregnant ewe/doe.

Collection and Processing of blood samples

A 3ml of blood was transferred into a serum clot activator tubes and kept in upright positions and transferred to the laboratory in insulated ice boxes within two hours of collection. The tubes were then centrifuged at 3000rpm for 10 minutes to facilitate serum separation. The separated serum was carefully transferred into labelled Eppendorf tubes and preserved in deep freezer at –20°C until further analysis.

Sero-Diagnostic Techniques

Rose Bengal Plate Test (RBPT)

Rose Bengal antigen were obtained from Institute of Animal Husbandry and Veterinary Biologicals (IAH & VB), Hebbal, Bengaluru, and were performed following Alton *et al.,* (1957). Serum samples showing agglutination within 4 minutes were considered positive, while those without agglutination were regarded as negative for RBPT.

Standard Tube Agglutination Test (STAT)

Brucella plain antigens were obtained from Institute of Animal Husbandry and Veterinary Biologicals (IAH & VB), Hebbal, Bengaluru, and were performed following Alton *et al.,* (1957). Positivity was identified by mat formation with a clear supernatant, and samples showing agglutination at a dilution of ≥1:40 were considered positive.

Indirect ELISA

Indirect ELISA was conducted using a standardized kit from ICAR-NIVEDI, Yelahanka, Bengaluru, Karnataka, as per the manufacturer's instructions. The results were interpreted by comparing sample optical density (OD) values with control sera.

Percent positivity (PP) values that were used for the diagnostic interpretations were calculated as follows.

$$PP = \frac{Average\ OD\ value\ of\ test\ serum}{Average\ OD\ value\ of\ positive\ control} \times 100$$

Percent positivity (PP) is then calculated for each sample, with PP > 54% considered positive, PP < 54% considered negative.

Statistical analysis

Risk factor analysis was carried out using the Chi-square test to determine factors associated with abortions. Statistical significance was assessed based on p-values, where $p < 0.05$ was considered statistically significant, $p < 0.01$ as highly significant, and $p > 0.05$ as non-significant (NS). The categorical data of serological tests, viz., RBPT, STAT and i-ELISA were expressed as percentages by using MS Excel.

Diagnostic statistical analysis

The diagnostic statistical analysis was performed following the methodology outlined by Mandrekar and Mandrekar (2005).

Sensitivity: The probability that a test result will be positive when the disease is present. The true positive rate is expressed as a percentage = a / (a+c).

Specificity: The probability that a test result will be

negative when the disease is not present. The true negative rate is expressed as percentage = d / (b+d).

Overall agreement: It is expressed as the sum of true positives and true negatives divided by the total number of observations. It is used in evaluating agreement between a diagnostic test and a gold standard.

a = Number of sera samples that tested positive by both conventional and standard test

b = Number of sera samples that tested positive by conventional and negative for standard test

c = Number of sera samples that tested negative by conventional and positive for standard test

d = Number of sera samples that tested negative by both conventional and standard test

Kappa Statistics for agreement

Kappa Statistics is an inter-rater agreement statistic (Kappa) to evaluate the agreement between two classifications on ordinal or nominal scales (Landis and Koch, 1977). Agreement is quantified by the Kappa (K) or Weighted Kappa (Kw) statistics. K is 0.01 - 0.20 indicate Slight agreement, 0.21 - 0.40 Fair agreement, 0.41 - 0.60 Moderate agreement, 0.61 - 0.80 Substantial, 0.81 - 1.00 Almost perfect agreement.

Ethical Approval The study received approval from the Institutional Animal Ethics Committee of the College of veterinary science, Proddatur (Approval No. 7R-CVSc, PDTR/IAEC/2025).

Results and Discussion

A cross-sectional survey was conducted in fifteen villages involving 64 small-ruminant flocks and their respective shepherds. The surveyed population comprised 11,530 animals, including 9,419 sheep and 2,111 goats. Overall, abortion was recorded in 522 out of 11,530 small ruminants (4.52%), including 457 sheep (4.85%) and 65 goats (3.07%), with a flock-level prevalence of 71.87% (46/64). Abortion prevalence was significantly higher in large flocks (>100 animals) (80%) than in small flocks (<100 animals) (42.8%) ($p<0.01$). Extensively managed flocks showed a higher prevalence (78%) compared to semi-intensive flocks (50%) ($p<0.05$). Sheep-only flocks recorded the highest abortion prevalence (77.7%), followed by mixed flocks (69.8%) and goat flocks (50%), although the variation was not statistically significant. Flocks practicing communal grazing and shared water bodies had significantly greater abortion prevalence (82%) compared to non-sharing flocks (35.7%) ($p<0.01$). Similarly, flocks with histories of retained placenta and repeat breeding showed higher abortion prevalence (79.2%) ($p<0.05$). Disposal of aborted materials into unused wells and leaving aborted materials to dogs were significantly associated with abortion occurrence, with prevalence of 76.4% and 44.4% respectively ($p<0.05$).

Seasonal analysis revealed that abortions occurred predominantly during winter (57.47%), while most cases were recorded during late gestation (62.45%). Evaluation of subsequent fertility showed that 60.15% of aborted animals successfully reconceived, whereas 13.98% failed to conceive again. Among animals that reconceived, 63.05% had normal parturition, while reabortion and stillbirth were observed in 28.66% and 8.28% of animals, respectively.

A total of 120 serum samples collected from recently aborted sheep and goats were screened for brucellosis using RBPT, STAT, and i-ELISA. Seropositivity rates of 36.6%, 40.83%, and 48.33% were recorded by RBPT, STAT, and i-ELISA, respectively. Overall, higher seroprevalence was observed in sheep (53.13%) compared to goats (29.16%). In sheep, seropositivity rates were 42.7%, 46.87%, and 53.13% by RBPT, STAT, and i-ELISA, respectively, whereas in goats the corresponding rates were 12.5%, 16.66%, and 29.16%. i-ELISA detected the highest number of seropositive animals in both species, indicating superior sensitivity compared to RBPT and STAT. Age-wise analysis revealed higher seroprevalence in animals older than 3 years compared to younger animals

Comparative analysis of RBPT and STAT against i-ELISA demonstrated overall sensitivities of 75.86% and 84.48%, respectively, while both tests showed 100% specificity. In sheep, RBPT and STAT exhibited sensitivities of 82.35% and 88.24%, respectively, whereas lower sensitivities were observed in goats (42.85% and 54.14% respectively). Agreement with i-ELISA was 88.33% for RBPT and 92.5% for STAT, with kappa values of 0.76 and 0.84, respectively. These findings indicated that i-ELISA exhibited the superior diagnostic performance indicating its potential as a more sensitive assay for the serological detection of brucellosis.

Hamza and Bouyoucef (2013) and Kardjadj *et al.,* (2016) reported higher flock-level abortion prevalence of 75.33% and 90%, respectively, compared to the present findings. Such variation may be due to differences in geographical conditions, management systems, flock size, disease prevalence, and biosecurity measures adopted (Haif *et al.,* 2021). Large flock size (>100 animals) was significantly associated with abortion occurrence ($p<0.01$), which agrees with the findings of Kardjadj *et al.,* (2016) and Haif *et al.,* (2021) Increased animal density and closer contact among animals in larger flocks may favour pathogen transmission and environmental contamination, while maintaining hygiene and proper abortion management becomes more difficult.

Communal grazing, shared watering sources ($p<0.01$), and extensive rearing systems ($p<0.05$) were identified as important risk factors for abortion. Similar observations were reported by Teklue *et al.,* (2013), Gebretensay *et al.,* (2019), and Natesan *et al.,* (2021), who attributed the increased prevalence to poor biosecurity, communal grazing, and increased exposure to infectious agents. Seasonal variation in abortion occurrence was observed, with the highest incidence during winter, followed by the rainy and summer seasons. This finding was consistent with Mishra *et al.,* (2023), although Sultan *et al.,* (2015) reported higher prevalence during summer, suggesting that seasonal influence may vary depending on environmental and management conditions.

Improper disposal of aborted foetuses and fetal membranes was significantly associated with abortion occurrence ($p<0.05$). Similar observations were reported by Leahy *et al.,* (2020) indicating that open disposal practices may facilitate environmental contamination as well as any stray animals such as dogs dragging of the aborted foetuses might lead to the mechanical spread of infectious agents. While WHO (2006) recommended safe disposal methods such as deep burial or incineration to reduce disease transmission. Most abortions occurred during late gestation, followed by mid and early gestation, which agrees Pretzer (2008) who stated that infectious abortifacient agents commonly induce abortion during advanced pregnancy.

A significant association was also observed between reproductive disorders and abortion occurrence ($p<0.05$). Flocks with histories of repeat breeding and retained placenta showed higher abortion prevalence, similar to findings by Dechicha *et al.,* (2020) and Hussen *et al.,* (2023).This may indicate the presence of persistent reproductive infections within affected flocks.

Assessment of post-abortion fertility revealed that 60.15% animals conceived again and 63.05% delivered normally, however, 28.66% and 8.28% animal's experienced repeat abortion and stillbirth were also recorded. Similar findings were reported by Mahboub *et al.,* (2013) and Lokamar *et al.,* (2020) who associated abortion with reduced reproductive efficiency, infertility, and poor pregnancy outcomes. Nutritional deficiencies, uterine infections, and environmental stress may further impair reproductive recovery following abortion (Ali *et al.,* 2019). Overall, the findings emphasize the need for effective disease control, improved management, and adequate post-abortion care to enhance reproductive performance in small ruminant.

Brucella seropositivity in the present study was 36.6%, 40.83%, and 48.33% by RBPT, STAT, and i-ELISA, respectively, indicating higher detection of seropositive animals by i-ELISA. Comparable findings were reported by Esendal *et al.,* (2001) who observed 37.6% positivity by RBPT and 44.4% by STAT, while Padher *et al.,* (2018) recorded 46.50% positivity by i-ELISA. In contrast, lower prevalence rates in healthy and aborted small ruminants were reported by Shome *et al.,* (2006) (RBPT: 9.95%, STAT: 5.67%, i-ELISA: 7.36%) and Sadhu *et al.,* (2015) (RBPT: 11.30%, STAT: 11.10%, i-ELISA: 8.80%). Variations among studies may be due to differences in selection of animals, sampling methods, endemicity, management practices, and diagnostic sensitivity (Vakamalla *et al.,* 2023).

In sheep, seropositivity was 42.7%, 46.87%, and 53.13% by RBPT, STAT, and i-ELISA, respectively. Similar RBPT prevalence of 45% was reported by Abd (2025)]. While Basyony *et al.,* (2012) and Salman *et al.,* (2018) observed higher prevalence of 60.20% and 55% by RBPT. However, Rahman *et al.,* (2012) and Sharma *et al.,* (2016) documented much lower prevalence rates of 3.75% and 13.82% by RBPT. The higher positivity detected by i-ELISA in the present study may be due to its superior sensitivity in identifying chronic and low-titre infections.

In goats, RBPT, STAT, and i-ELISA detected 12.5%, 16.66%, and 29.16% positivity, respectively. Comparable findings were reported by Tekle *et al.,* (2021) who observed a seroprevalence of 13.3% using

the RBPT, Sulima *et al.,* (2010) who recorded seroprevalence rates of 16.02% by STAT and 24.86% by indirect ELISA, and Vakamalla *et al.,* (2023) who reported prevalence rates of 18.57% with STAT and 30.95% with indirect ELISA. Conversely, Eissa *et al.,* (2017) reported a much higher prevalence (69.3%), whereas Sharma *et al.,* (2017) recorded lower prevalence rates. Such variations may be influenced by geographical factors, husbandry practices, breed susceptibility, and differences in diagnostic tests (Amin *et al.,* 2005).

Comparative evaluation showed that i-ELISA detected the highest number of seropositive samples, followed by STAT and RBPT. Sensitivity of RBPT and STAT was 75.86% and 84.48%, respectively, with 100% specificity for both tests when compared with i-ELISA. Similar RBPT sensitivity values of 80% and 71.59% were reported by Sadhu *et al.,* (2015) and Patel *et al.,* (2017) STAT showed better agreement with i-ELISA (92.5%; κ=0.84) than RBPT (88.33%; κ=0.76), confirming STAT as the more reliable serological assay than RBPT for screening brucellosis in small ruminants.

Age-wise analysis revealed higher seroprevalence in animals older than 3 years. In sheep, i-ELISA positivity was 62.5% in older sheep compared to 48.44% in younger sheep, while in goats it was 33.33% and 27.77%, respectively. Similar observations were reported by Awandkar *et al.,* (2012), Suryawanshi *et al.,* (2016), and Vakamalla *et al.,* (2023). The increased prevalence in older animals may be attributed to cumulative and prolonged exposure to infection over time, the potential persistence of *Brucella* infection (Seakamela *et al.,* 2026), and the influence of sexual maturity and erythritol, which favour the multiplication of *Brucella* organisms (Gul and Khan, 2007).

In conclusion, the present study demonstrated that abortion is a significant reproductive problem in small ruminants, with factors such as large flock size, extensive rearing systems, communal grazing, improper disposal of aborted materials, and previous reproductive disorders significantly increasing the risk of abortion. Serological screening revealed a high prevalence of brucellosis among aborted sheep and goats, with i-ELISA detecting a greater number of seropositive animals than RBPT and STAT, indicating superior diagnostic sensitivity. The higher seropositivity observed in sheep and adult animal's highlights the role of brucellosis as an important contributor to reproductive losses in the study area. These findings underscore the need for strengthened biosecurity measures, improved flock management practices, farmer awareness and education, and routine surveillance using sensitive diagnostic tools, together with coordinated efforts among veterinary, medical, and administrative authorities to control brucellosis and reduce its zoonotic transmission.

Authors' contributions

Pendimi Megha Varna was responsible for executing the experiments, collecting clinical samples, and writing the manuscript. Kancharla Jyothi designed the experiment and contributed to the critical review of the manuscript. Sai Gunaranjan contributed to the conception of the study and was involved in reviewing manuscript. Varra Manasa supervised the methodology and laboratory analysis.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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