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Research Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2026.57.3.430-436

Investigation of zoonotic bacterial abortifacients associated with caprine abortion in Kerala, India

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Citation: Neha, R., Krupa, R. J., Justin, D. K., Amritha, A., Binu, K. M., Ambily, R., Tittu, A., Shyma, V. H., Vinodkumar, K., Tresamol, P. V. & Preena, P.(2026). Investigation of zoonotic bacterial abortifacients associated with caprine abortion in Kerala, India. *Journal of Veterinary and Animal Sciences, 57(3),430-436*
https://doi.org/10.51966/jvas.2026.57.3.430-436

Received: 06.06.2026 Accepted: 03.09.2026 Published: 30.09.2026

Abstract

Caprine abortion is an important cause of economic loss and may be associated with several zoonotic bacterial pathogens. The present study investigated the occurrence of selected zoonotic bacterial abortifacients among goats in Kerala, India. Aborted materials from 25 goats were subjected to molecular screening for *Brucella abortus, B. melitensis*, *Leptospira* spp., *Listeria monocytogenes*, and *Chlamydia abortus* by polymerase chain reaction (PCR). Additionally, sera from 90 goats with a history of abortion were screened for antibodies against *Brucella* spp. using an indirect Enzyme-linked Immunosorbent Assay (ELISA), Rose Bengal Plate Test (RBPT), and against *Leptospira* spp. using Microscopic Agglutination Test (MAT). *Brucella abortus* DNA was detected in 3 of 25 (12.0%) aborted materials, whereas none of the EDTA blood samples tested positive. Sequencing and phylogenetic analysis identified the isolate as *Brucella abortus* and demonstrated close genetic relatedness to previously reported Indian isolates. Serological screening revealed positivity rates of 1.11% and 2.22% for brucellosis by indirect ELISA and RBPT, respectively, while all samples were negative for leptospirosis by MAT. No molecular evidence of *Brucella melitensis, Leptospira* spp., *L. monocytogenes*, or *C. abortus* was detected. The findings indicate the involvement of *Brucella abortus* in caprine abortion in Kerala and highlight the importance of including brucellosis in abortion investigations and surveillance programmes.

Keywords: *Brucella*, *Chlamydia abortus, Leptospira*, *Listeria*, ELISA, RBPT, MAT

Goats are among the most important livestock species reared by smallholder and marginal farmers in India owing to their adaptability, high reproductive potential, short generation interval, and economic value (Narmatha et al., 2013). Goat farming contributes significantly to rural livelihoods and nutritional security, particularly in resource-limited farming systems (Meena et al., 2025). India possesses one of the largest goat populations in the world, with approximately 148.88 million goats recorded in the 20th Livestock Census (DAHD, 2019).

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Reproductive efficiency is a critical determinant of productivity and profitability in goat farming (Ali et al., 2019). Among reproductive disorders, abortion is considered one of the most important causes of economic loss in small ruminant production systems, resulting in the loss of viable foetuses, reduced kidding rates, increased replacement costs, and decreased overall herd productivity (Ali et al., 2019; Alemayehu et al., 2021). While abortion rates below 2% are generally acceptable in goat herds, rates exceeding 5% warrant immediate epidemiological and diagnostic investigation (Lokamar et al., 2020).

Infectious agents are considered the predominant causes of abortion outbreaks in small ruminants worldwide and are responsible for substantial reproductive and economic losses (Menzies, 2011; Gebretensay et al., 2019). Among infectious causes, bacterial abortifacients are of particular importance because several of them possess significant zoonotic potential and pose serious public health concerns. Major zoonotic bacterial pathogens associated with caprine abortion include *Brucella* spp., *Chlamydia abortus*, *Coxiella burnetii*, *Campylobacter* spp., *Leptospira* spp., *Listeria* spp., and *Salmonella* spp. (Menzies, 2011; Ramo et al., 2022). These pathogens are commonly transmitted to humans through direct contact with infected animals, aborted foetuses, placental tissues, vaginal discharges, and contaminated farm environments, whereas consumption of contaminated milk and dairy products can also contribute to zoonotic transmission (Hajibemani and Sheikhalislami, 2020). Occupationally exposed individuals, including farmers, veterinarians, animal handlers, and laboratory personnel are considered to be at greater risk of infection. Consequently, caprine abortion caused by zoonotic bacterial pathogens represents an important One Health concern involving animal health, public health, and economic sustainability of livestock production systems.

The prevalence and distribution of abortifacient pathogens vary considerably across geographical regions owing to differences in climate, husbandry practices, biosecurity measures, animal movement, and disease control programmes. Although several studies have investigated infectious causes of abortion in small ruminants worldwide (Menzies, 2011; Dorsch et al., 2021), information regarding zoonotic bacterial abortifacients associated with caprine abortion in Kerala remains limited. Among the various bacterial abortifacients, *Brucella* spp., *Leptospira* spp., *Listeria monocytogenes*, and *Chlamydia abortus* were selected considering their zoonotic importance, documented association with caprine abortion, and reported occurrence in ruminants in India, including Kerala for some of these pathogens. Such data are essential for understanding the epidemiology of these pathogens and for developing effective surveillance and control strategies. Therefore, the present study was undertaken to investigate the occurrence of *B. abortus, B. melitensis*, *Leptospira* spp., *L. monocytogenes*, and *C. abortus* associated with caprine abortion in Kerala, India, and to generate baseline epidemiological information for disease surveillance and control.

Materials and methods

A cross-sectional investigation was conducted between November 2024 and November 2025 in the Thrissur district, Kerala, India. The study included caprine cases presented to the University Veterinary Hospitals at Mannuthy and Kokkalai, as well as animals maintained in selected organized goat farms and smallholder production units. Twenty-five goats with active abortion were included for molecular investigations, while 90 goats with a previous history of abortion during the preceding 12 months were included for serological investigations. Molecular investigations were performed using DNA extracted from EDTA blood samples and aborted materials. The latter included aborted foetal stomach contents (preferred when available), placental tissues and vaginal discharge. For serological investigations, blood samples were collected and serum was separated. All samples were transported under cold chain and stored at –20°C until analysis. Information regarding age, breed, parity, stage of gestation, and abortion history was recorded whenever available.

Table 1. Primers used for PCR amplification of bacterial abortifacients

Pathogen	Target gene	Primer sequence (5′–3′)	Amplicon size (bp)	Reference
Brucella abortus	*IS711*	F: GACGAACGGAATTTTTCCAATCCC R: TGCCGATCACTTAAGGGCCTTCAT	498	Bricker and Halling (1994)
Brucella melitensis	*IS711*	F: AAATCGCGTCCTTGCTGGTCTGA R: TGCCGATCACTTAAGGGCCTTCAT	731	
Leptospira spp.	*loa22*	F: AGAGGAGAATTCAGCTCCTGAGC R: TGGTGCCTGCAGCGCAAAACGGA	525	Roshni et al. (2023)
Listeria monocytogenes	*inlA*	F: AGCCACTTAAGGCAAT R: AGTTGATGTTGTGTTAGA	760	Poyart et al. (1996)
Chlamydia abortus	*pmp*	F: ATGAAACATCCAGTCTACTGG R: TTGTGTAGTAATATTATCAAA	300	Laroucau et al. (2001)

Genomic DNA was extracted from EDTA blood and aborted samples using the DNeasy® Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions. Approximately 100 µL of blood and 25 mg of aborted materials, including foetal stomach contents, placental tissues, and vaginal discharge, were used for extraction. Molecular detection of *B. abortus, B. melitensis*, *Leptospira* spp., *L. monocytogenes* and *C. abortus* was performed by PCR using species-specific IS711 primers for *B. abortus* and *B. melitensis*, and primers targeting the *loa22*, *inlA*, and *pmp* genes for *Leptospira* spp., *L. monocytogenes*, and *C. abortus*, respectively. Amplified products were resolved on 1.5% agarose gels stained with ethidium bromide and visualized under UV illumination. Primer sequences, amplicon sizes, and references are provided in Table 1.

A representative PCR-positive amplicon was subjected to bidirectional Sanger sequencing (GeneSpec Biosciences, Kochi). The obtained sequence was analysed using BLAST and compared with reference sequences available in GenBank. The representative sequence was submitted to the GenBank database for accession number assignment. Sequence alignment and phylogenetic analysis was performed in MEGA X using the Maximum-Likelihood method with 1000 bootstrap replicates.

Serological investigations were carried out to detect antibodies against *Brucella* spp. and *Leptospira* spp. in goats with a history of abortion. Serum samples were screened for antibodies against *Brucella* spp. using an indirect ELISA kit for sheep and goat brucellosis developed by ICAR-NIVEDI, Bengaluru, according to the manufacturer's instructions (Shome et al., 2015). Briefly, serum samples were incubated in microplates coated with smooth lipopolysaccharide (sLPS) antigen, followed by the addition of anti-sheep/goat IgG-peroxidase conjugate. The reaction was visualized using O-phenylenediamine dihydrochloride (OPD) substrate, and optical density was measured at 492 nm. Results were expressed as percent positivity (PP), calculated as the average OD of the test serum divided by the median OD of the positive control and multiplied by 100. Samples with PP values greater than 54% were considered positive. Serum samples were also screened for antibodies against *Brucella* spp. using the Rose Bengal Plate Test (RBPT) as described by WOAH (2024).

Antibodies against *Leptospira* spp. were detected by the Microscopic Agglutination Test (MAT) using live antigens representing 13 serovars, namely Australis, Autumnalis, Bataviae, Canicola, Djasiman, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes, and Patoc as per the method described by Faine et al. (1999).

Results and discussion

PCR using the *B. abortus*-specific *IS711* primers detected *B. abortus* DNA in 3 of 25 (12.0%) aborted samples, whereas all EDTA blood samples tested negative. None of the samples yielded amplification for *B. melitensis, Leptospira* spp., *Listeria* spp., or *C. abortus*. A representative PCR amplification of the *B. abortus* is shown in Fig. 1.

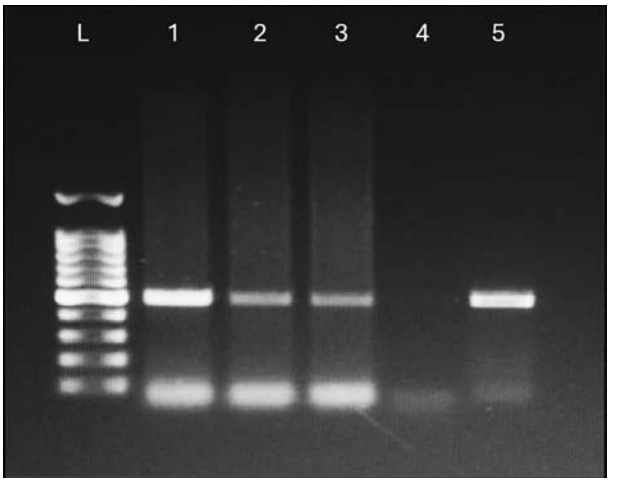


Fig. 1: Representative agarose gel electrophoresis of PCR amplification of *IS711* region of *Brucella abortus* (498 bp). Lane L: 100 bp DNA ladder; lanes 1-3: positive samples; lane 4: negative control; lane 5: positive control.

Sequencing of a representative *IS711* amplicon identified the Kerala goat isolate as *B. abortus*. The sequence was deposited in the GenBank database under accession number PZ142830. Phylogenetic analysis based on *IS711* sequences placed the isolate within a cluster comprising previously reported Indian *B. abortus* isolates, including a human isolate from Karnataka and livestock isolates from Maharashtra and Gujarat (Fig. 2).

Serological screening of 90 serum samples detected antibodies against *Brucella* spp. in one sample (1.11%) by indirect ELISA and in two samples (2.22%) by RBPT. All serum samples tested negative for antibodies against the 13 *Leptospira* serovars included in the MAT panel.

Brucella abortus was the only zoonotic bacterial abortifacient detected in the present investigation, with PCR positivity observed in 12.0% (3/25) of abortion cases. The detection of *Brucella* DNA in aborted materials suggests a possible role of brucellosis in caprine reproductive failure and highlights its zoonotic significance in livestock production systems. Similar findings have been reported in small-ruminant abortion investigations, where *Brucella* spp. was identified among the major infectious causes of abortion and reproductive losses (Alemayehu et al., 2021). Notably, *B. abortus* DNA was detected exclusively in aborted materials, while all EDTA blood samples tested negative by PCR. This finding contrasts with the observations of Wareth et al. (2015), who detected *B. abortus* DNA in serum samples of recently aborted goats and sheep using real-time PCR. The discrepancy may be

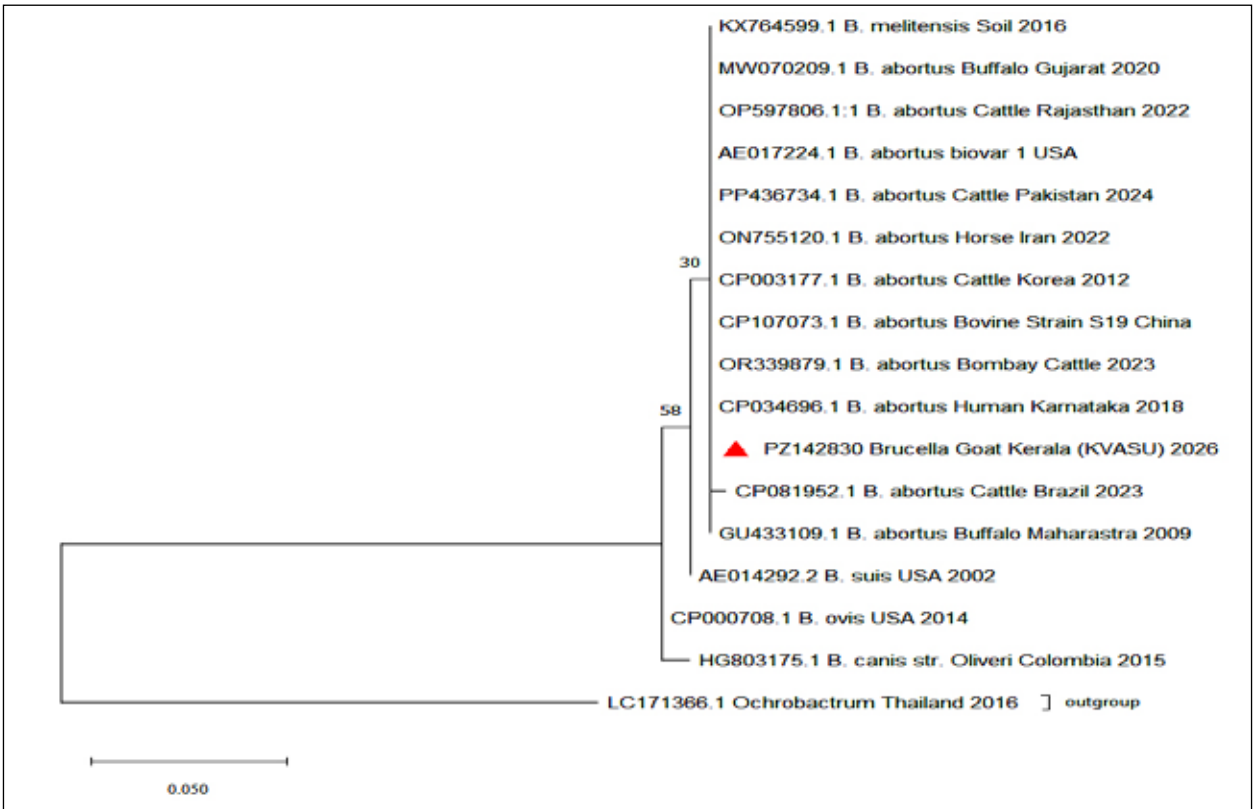


Fig. 2. Maximum-Likelihood phylogenetic tree of *Brucella* isolates based on IS711 sequences. The tree was constructed using the Tamura 3-parameter model with *Ochrobactrum* (LC171366.1) as the outgroup. Bootstrap values are shown at the nodes. The scale bar represents 0.050 substitutions per site. The red triangle indicates the *Brucella* isolate (PZ142830) identified in the present study.

attributable to differences in the stage of infection, bacterial load, timing of sample collection, or the transient nature of bacteremia. However, this observation is consistent with the findings of Modise-Tlotleng et al. (2024), who reported that foetal and placental tissues constituted the most informative specimens for molecular detection of abortifacient bacteria in ruminant abortion investigations. The preferential detection of *Brucella* in reproductive tissues may be attributed to its marked tropism for the gravid uterus, placenta, and foetal tissues, where bacterial multiplication results in placentitis, foetal infection, and abortion (Corbel, 2006; Olsen, 2010).

The detection of bacterial DNA in aborted materials in the present study may also be influenced by the type of specimens examined. Previous studies have identified foetal stomach contents, liver, spleen, placenta, and vaginal discharges among the most informative samples for laboratory investigation of infectious abortions in ruminants (van den Brom et al., 2025). In particular, foetal stomach contents are considered highly valuable diagnostic specimens because they may contain organisms and infected fluids swallowed by the foetus during intrauterine infection.

Sequence analysis of the representative IS711 amplicon identified the isolate as *Brucella abortus.* No amplification was obtained with the *B. melitensis*-specific PCR in any of the samples examined. Although *B. melitensis* is traditionally regarded as the principal cause of caprine brucellosis, infection of goats with *B. abortus* has been increasingly reported, particularly in mixed livestock production systems where goats are maintained in close proximity to cattle (Rossetti et al., 2017). Similar spillover infections have been documented in small ruminants from several countries, suggesting that host specificity among *Brucella* species is not absolute (Wareth et al., 2015). The phylogenetic clustering of the present isolate with previously reported Indian livestock and human isolates further supports the circulation of genetically related *B. abortus* strains in the country and emphasizes the zoonotic significance of the pathogen.

Serological screening of goats with a previous history of abortion revealed a low seroprevalence of brucellosis, with positivity rates of 1.11% and 2.22% by indirect ELISA and RBPT, respectively. The low seropositivity observed in the present study may indicate limited exposure of the study population to *Brucella* spp. or a low level of infection within the investigated herds. Similar low-to-moderate seroprevalence rates have been reported in goats from different regions of India and other endemic countries, reflecting variations in management practices, animal movement, herd size, and disease

control measures (Natesan et al., 2021; Shome et al., 2021). Although serological tests remain valuable tools for herd-level surveillance, their diagnostic performance may be influenced by the stage of infection, antibody kinetics, and test characteristics. The marginal increase in positivity observed by RBPT compared with ELISA may reflect differences in the sensitivity and specificity of the assays. RBPT is widely employed as a rapid and economical screening test; however, false-positive reactions may occur because of cross-reactivity with antigenically related organisms. In contrast, ELISAs are generally considered more specific and are frequently used as confirmatory serological tests for brucellosis (WOAH, 2024).

The proportion of *B. abortus* PCR-positive abortion cases (12.0%) observed in the present study was higher than the seropositivity detected among goats with a previous history of abortion. However, direct comparison of these findings was difficult because molecular and serological investigations were conducted on different animal populations and reflected different stages of infection. While PCR detected the presence of pathogen DNA and indicated current or recent infection, serological tests detected host antibody responses, which may have varied with the time elapsed since infection, immune status of the animal, and test characteristics (Corbel, 2006).

All serum samples tested negative for antibodies against the 13 *Leptospira* serovars included in the MAT panel. This finding contrasts with the report of Dhivahar et al. (2019), who recorded serological evidence of leptospiral exposure among aborted goats in Kerala and identified multiple circulating serovars, including Pomona, Australis, Hebdomadis, and Canicola. The absence of seropositive reactors in the present study may be attributable to differences in geographical coverage, sample size, herd characteristics, timing of sample collection, or variation in exposure levels among the investigated goat populations.

No molecular evidence of *Leptospira* spp., *L. monocytogenes*, or *C. abortus* was detected in the aborted materials examined in the present study. This finding contrasts with earlier reports from Kerala, where *C. abortus* had been isolated from caprine abortion cases (Mani et al., 2011), and serological evidence of leptospiral exposure among aborted goats has also been documented (Dhivahar et al., 2019). In India, Singh et al. (2026) reported the detection of *B. abortus*, *B. melitensis*, and *Chlamydia* spp. in goats with reproductive disorders, with *Chlamydophila* spp. being the most frequently detected pathogen. Internationally, Şevik (2024) identified *C. abortus* as the predominant abortifacient pathogen, followed by *C. burnetii* and *L. monocytogenes* in ovine and caprine foetuses in Turkey, whereas Modise-Tlotleng et al. (2024) reported *Brucella* spp. as the predominant bacterial abortifacient in ruminant abortions in Botswana, with no detection of *Leptospira* spp. or *L. monocytogenes*. The absence of *Leptospira* spp., *L. monocytogenes*, and *C. abortus* in the present study may reflect differences in geographical distribution, sample type, stage of infection, management systems, or pathogen prevalence in the investigated population.

Conclusion

The present study detected *B. abortus* as the only zoonotic bacterial abortifacient among the pathogens investigated in caprine abortion cases from Kerala. In contrast to reports from other regions where *C. abortus*, *L. monocytogenes*, and other bacterial agents have been implicated in reproductive losses, none of these pathogens were detected in the samples examined. The findings should, however, be interpreted in light of certain limitations, including the relatively small number of abortion cases investigated, sampling from a single district, and the restricted panel of bacterial pathogens examined. Furthermore, serological screening was confined to *Brucella* spp. and *Leptospira* spp. owing to the availability of validated diagnostic assays, precluding the assessment of exposure to *L. monocytogenes* and *C. abortus*. The detection of *B. abortus* in goats highlights the need for integrated surveillance of brucellosis in mixed livestock production systems, particularly in regions where cattle and goats are reared in close proximity. These findings also support broader investigations involving larger sample sizes, multiple geographic regions, and a wider range of abortifacient pathogens to better understand the epidemiology of infectious abortion in small ruminants. Such information is essential for developing evidence-based surveillance and control programmes for reproductive diseases in Kerala.

Acknowledgement

The authors acknowledge the support of the Department of Veterinary Epidemiology and Preventive Medicine, the Central Instrumentation Facility, College of Veterinary and Animal Sciences, Mannuthy and the University Veterinary Hospitals, Mannuthy and Kokkalai, KVASU. The authors thank Dr. Rajeshwari Shome, ICAR-NIVEDI, Bengaluru, for assistance with brucellosis ELISA testing. The authors also acknowledge the National Collection of Veterinary Type Cultures (NCVTCC), ICAR-NRCE, Hisar, for providing the *Listeria monocytogenes* reference culture, and thank Dr. Rajesh Kumar Vaid for his assistance. The authors further thank the goat farm owners and field veterinarians for their cooperation during sample collection.

Conflicts of interest

The authors declare that they have no conflict of interest.

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