

Zoonotic bacteria associated with reproductive disorders in cattle and buffaloes: One Health implications

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ABSTRACT

Bovine reproductive disorders caused by infectious agents not only result in significant economic losses but also pose potential zoonotic risks, emphasizing the relevance of a One Health perspective. This study investigated the occurrence of four major zoonotic bacteria—*Brucella* spp., *Leptospira* spp., *Listeria monocytogenes*, and *Coxiella burnetii* in cattle and buffaloes affected by reproductive disorders such as abortion, retention of foetal membranes (RFM), and metritis in Gujarat, India. Clinical specimens collected from affected animals were analysed using PCR-based assays to determine pathogen involvement and identify species-specific patterns. *Brucella*, *Leptospira*, and *Coxiella* were frequently detected across reproductive disorder cases, whereas *Listeria* was not detected in any of the cases. Aborted animals showed a higher tendency for *Brucella* and *Coxiella* positivity, while RFM cases were more often associated with *Coxiella* and *Leptospira*. Evidence of mixed infections was also noted, with *Brucella*–*Coxiella* co-infections more common in cattle. Buffaloes exhibited a greater predisposition to *Leptospira*, whereas cattle were more frequently affected by *Coxiella*, indicating distinct host–pathogen relationships. Overall, the findings highlighted the widespread involvement of multiple zoonotic pathogens in bovine reproductive disorders and reinforced the need for integrated surveillance, targeted control measures, and strengthened veterinary–public health collaboration. The study provided valuable insights to support One Health-based strategies for managing zoonotic bacterial infections in livestock populations.

Keywords: Bovine abortion, Brucellosis, Leptospirosis, Q fever, Retention of foetal membrane, Zoonotic diseases

Reproductive disorders and infectious abortion in cattle and buffaloes cause significant economic losses and considerable psychological stress to farmers. The financial impact of a single abortion is estimated at USD 555 in the U.S.A. and around USD 89 in India (De Vries 2006, Deka *et al.* 2018). Conditions such as retained foetal membranes (RFM) further reduce herd productivity by delaying uterine involution, prolonging the interval to first service, lowering conception rates, and increasing susceptibility to secondary infections, ultimately affecting milk yield and overall fertility (Laven and Peters 1996, Kashoma and Ngou 2021). These impacts highlight the need for early identification and effective control of infectious causes of reproductive failure.

Several important zoonotic pathogens identified through One Health risk assessments—particularly, *Brucella* spp., pathogenic *Leptospira* spp., *Coxiella burnetii*, and *Listeria monocytogenes*—are strongly associated with reproductive

disorders in cattle and buffaloes (Salyer *et al.* 2017, Robi *et al.* 2024). Their circulation in dairy herds, therefore, presents a dual challenge by affecting reproductive performance and posing risks to human health, especially for farmers, veterinarians, and dairy workers.

Bovine brucellosis remains endemic in many regions of India, causing late-term abortions, infertility, and prolonged shedding of the organism in milk and reproductive discharges (Rehman *et al.* 2025, Sharma *et al.* 2025). Leptospirosis, a widespread zoonosis, impairs bovine fertility and productivity while exposing farm workers to infection through contaminated urine, milk, and foetal fluids (Kadar *et al.* 2021, Sohm *et al.* 2023). *Coxiella burnetii*, the causative agent of Q fever, can lead to sporadic abortions, weak offspring, and metritis; its environmental persistence contributes to sustained zoonotic risk (Vidal *et al.* 2017, Gisbert *et al.* 2024). *Listeria monocytogenes* is an emerging food-borne and zoonotic pathogen affecting both animal and human health, causing abortion, neonatal losses, and systemic illness (Matle *et al.* 2020; Jibo *et al.* 2022).

Despite the recognized importance of these pathogens, information on their involvement in reproductive disorders

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in India, particularly, within unorganized dairy systems—remains limited. The present study therefore, investigated the presence of these major zoonotic pathogens in cattle and buffaloes with reproductive disorders in Gujarat to support integrated surveillance and control strategies that could address both livestock health and public health risks.

MATERIALS AND METHODS

Study location and plan: The study was conducted in Gujarat, where a pilot-scale Brucellosis control project was being implemented by the National Dairy Development Board, Anand. In the study, FTA® cards (GE Healthcare, Whatman) were used to detect pathogenic zoonotic organisms involved in bovine reproductive disorders, such as abortion, retained foetal membrane (RFM), and metritis. Information on individual animals, such as species (cattle and buffalo), breed, and parity (lactation number) was recorded. A total of 485 FTA® cards were collected from 211 villages across 23 districts in Gujarat by local veterinarians who were called upon by farmers to attend to these cases. Clinical specimens (vaginal discharge, placenta, uterine discharge, and nasal discharge) were collected from ailing animals based on the availability of clinical lesions/samples and spotted onto FTA® cards. The cards were then allowed to dry at room temperature for a minimum of 4 hours. The dried cards were individually placed in zip-lock pouches to prevent any potential cross-contamination. Subsequently, they were submitted to the laboratory along with the sample details via India Post.

Nucleic acid extraction: DNA was extracted from FTA® Classic cards (Whatman, GE Healthcare) using the QIAamp DNA Blood Mini Kit (Qiagen, Germany). Briefly, three to five punches from the sample-spotted area of each FTA® card were incubated with ATL buffer and Proteinase K for lysis, followed by DNA purification using QIAamp spin columns, according to the manufacturer's instructions. DNA was finally eluted in 100 µL of Buffer AE and stored at -20°C until further analysis. To minimize carryover contamination, the punch was cleaned with isopropyl alcohol and sterilized between samples. In addition, one extraction of negative control (consisting of blank Whatman filter paper punches) was included after every five samples and processed alongside the test samples.

Detection of bovine growth hormone (bGH) gene: To assess the quality of DNA obtained from the FTA® cards, a real-time PCR test targeting the *bovine growth hormone (bGH)* gene was undertaken (Wang *et al.* 2007). The reaction mixture contained 12.5 µL of Premix Ex Taq Master Mix (TaKaRa Bio), 0.5 µL of each primer (4.5 µM), 1 µL of probe (3 µM), 0.4 µL of ROX Reference dye II (50x concentrated), 5.1 µL of nuclease-free water (NFW), and 5 µL of DNA template. Real-time PCR was performed using either Quant Studio 7 flex or 7300 (both by ABI), with the following cycling conditions: 95°C for 60 seconds, followed by 45 cycles of 5 seconds at 95°C and 35 seconds at 60°C. To check for contamination during DNA extraction, a negative control using empty punches

of Whatman filter paper was included. Further, a positive control with bGH TOPO TA plasmid and a non-template control with nuclease-free water were also included. Samples with a Ct value less than 35 were deemed to have passed the quality control check and were used for further analysis, whereas samples with a Ct value greater than 35 or no amplification were considered to have failed the quality control check and were excluded.

Detection of *Brucella* species: To detect *Brucella* species, a real-time PCR assay targeting the *bcs31* gene, was employed (Mukherjee *et al.* 2015; Sarangi *et al.* 2020). The reaction and thermal profiles were the same as for the bGH real-time PCR test. In each run, duplicate positive controls consisting of DNA extracted from the *Brucella abortus* S19 strain (vaccine strain), as well as a negative control consisting of nuclease-free water were included. Samples with a Ct value less than 38 were considered positive for brucellosis.

Detection of *Coxiella burnettii*: For the detection of *Coxiella burnettii*, a PCR assay targeting the *IS1111* gene as previously described was employed (Sarangi *et al.* 2021). QIAGEN Hotstar PCR mastermix was used for PCR setup, and the PCR reaction was performed using a Veriti thermal cycler (ABI). The reaction mixture consisted of 10 µL of Hotstar Master Mix (Qiagen), 1 µL each of forward and reverse primers (10 pmol/ µL), 3 µL of NFW, and 5 µL of extracted DNA template in a single final reaction volume of 20 µL. The thermal profile used in the assay consisted of one cycle of initial denaturation at 95°C for 180 s, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s, followed by one cycle of final extension at 72°C for 300 s. The PCR amplified product was visualized by agarose gel electrophoresis. In each run, one positive control (DNA extracted from a previously confirmed positive sample) and three negative controls (non-template controls) were included. Any sample showing amplification at 295 bp was considered positive.

Detection of *Listeria monocytogenes*: To detect *Listeria monocytogenes*, a PCR assay targeting the *hlyA* gene was used with the primers as previously described (Sarangi *et al.* 2021). The reaction mixture and thermal profile were the same as described above, except that the annealing temperature was set at 55°C for 35 s. In each run, one positive control (DNA extracted from *Listeria monocytogenes* strain ATCC 19111) and three negative controls (non-template controls) were included. Any sample showing amplification at 456 bp was considered positive.

Detection of *Leptospira* species: A PCR assay targeting the 16S rRNA gene of *Leptospira* species was used (Mérien *et al.* 1992; Sarangi *et al.* 2021). The reaction mixture and thermal profile were as described above, with the annealing temperature set at 55°C for 30 seconds. Each run included one positive control (DNA extracted from a previously confirmed positive sample) and three negative controls (non-template controls). Any sample showing amplification

at 330 bp was considered positive. Subsequently, all *Leptospira*-positive samples were further screened with a PCR assay targeting the *lipL32* gene to detect pathogenic *Leptospira* species (Ahmed *et al.* 2006). Sample showing amplification at 476 bp was considered positive.

Statistical analysis: The prevalence of pathogens in the clinical specimens collected from cattle and buffaloes, along with their 95% confidence intervals, was determined using the Epitools statistical software. The Chi-square and Fisher's exact tests were used to compare the results obtained in the study, and a difference was considered significant if the p-value was less than 0.05. SPSS software (Version 2016) was used for the analysis.

RESULTS AND DISCUSSION

A total of 485 FTA® spotted cards received from the field were processed in the laboratory. Following initial quality control by detection of the *bGH* gene, 445 cards from 401 different cattle and buffaloes were considered suitable for further analysis. Detection of infectious agents was performed using PCR/real-time PCR assays. An animal was considered positive for a specific organism if any clinical specimen collected from it tested positive. *Brucella* spp., *Leptospira* spp., and *Coxiella burnetii* were detected in 18.5%, 22.2%, and 28.9% of animals, respectively. However, *Listeria monocytogenes* DNA could not be detected in any of the samples (Table 1).

In the present study, *Brucella* spp. was the most common pathogen detected in abortion cases and less frequently in RFM cases. The pattern was similar across cattle and buffaloes, with no significant interspecies

differences (Table 2). Among cattle, *Brucella* DNA was detected more frequently in exotic breeds (31.4%) than in crossbred (17.3%) and indigenous cattle (14.7%), although these differences were not statistically significant. Likewise, *Brucella* positivity varied only marginally across lactation groups, ranging from 14.8% to 28.6%, without any consistent trend. These findings were consistent with previous reports identifying *Brucella abortus* as a major cause of bovine abortion in organized dairy herds (Sarangi *et al.* 2021, Modise-Tlotleng *et al.* 2024, Şevik 2025). The findings reaffirmed that brucellosis remains a major contributor to bovine abortion in endemic regions, with important public health implications considering that the infected animals shed pathogen in milk, urine, and in reproductive materials (Rehman *et al.* 2025; Sharma *et al.* 2025).

Bovine leptospirosis, caused by pathogenic *Leptospira* species, is a highly contagious disease affecting reproductive performance, fertility, and milk production (Kadar *et al.* 2021, Aymée *et al.* 2024). In this study, *Leptospira* spp. DNA was detected in 22.2% of animals using *16S rRNA*-based PCR, with comparable detection rates among abortion and RFM cases. A significant association of *Leptospira* infection in animals with bovine reproductive disorder has been previously reported (Ikpe *et al.* 2021, Sohm *et al.* 2023). A significantly higher prevalence was observed in buffaloes than cattle (35.9% vs. 15.6%; $p = 0.034$), corroborating earlier serological observations (Mai *et al.* 2021). Within cattle, *Leptospira* positivity ranged from 11.8% in exotic breeds to 20.6% in indigenous breeds and showed no consistent association

Table 1. Prevalence of *Brucella* spp., *Leptospira* spp., *Listeria monocytogenes* and *Coxiella burnetii* according to reproductive disorders

Disease/ Condition	Total animal screened	<i>Brucella</i>	<i>Leptospira</i>	<i>Listeria</i>	<i>Coxiella</i>
Abortion recorded	108	34 (31.48)	27 (25.00)	0 (0.0)	33 (30.56)
Retention of foetal membrane recorded	312	46 (14.74)	72 (23.08)	0 (0.0)	83 (26.60)
Metritis cases	9	1 (11.11)	1 (11.11)	0 (0.0)	4 (44.44)
Total	401	71 (18.45)	89 (22.19)	0 (0.0)	116 (28.93)
Both RFM and Abortion recorded	28	7 (25.00)	11 (39.29)	0 (0.0)	4 (14.29)

Table 2. Species-wise prevalence of zoonotic bacterial pathogens in cattle and buffaloes with reproductive disorders

Species	Condition	Total animal screened	<i>Brucella</i>	<i>Leptospira</i>	<i>Listeria</i>	<i>Coxiella</i>
Cattle	RFM	208	34 (16.35)	31 (14.90)	0 (0.00)	63 (30.29)
	Abortion	65	22 (33.85)	14 (21.54)	0 (0.00)	25 (38.46)
	Both abortion & RFM	12	4 (33.33)	4 (33.33)	0 (0.00)	3 (25.00)
	Metritis	9	1 (11.11)	1 (11.11)	0 (0.0)	4 (44.44)
	Total	270	53 (19.63)	42 (15.56)	0 (0.00)	89 (32.96)
Buffalo	RFM	104	12 (11.54)	41 (39.42)	0 (0.00)	20 (19.23)
	Abortion	43	12 (27.91)	13 (30.23)	0 (0.00)	8 (18.60)
	Both abortion & RFM	16	3 (18.75)	7 (43.75)	0 (0.00)	1 (6.25)
	Total	131	21 (16.03)	47 (35.88)	0 (0.00)	27 (20.61)

with lactation number. Since *16S rRNA* PCR cannot differentiate saprophytic from pathogenic *Leptospira* species (Mérien *et al.* 1992, Gentilini *et al.* 2015), positive samples were further tested using *LipL32* gene-based PCR. Only 22 of 89 samples were positive, indicating pathogenic *Leptospira* prevalence of 6.5% in abortion cases, 5.8% in RFM, and 10.7% in animals with both abortion and RFM. The marked difference between the two assays perhaps reflected the presence of non-pathogenic strains or limitations of single-gene PCR systems (Roy *et al.* 2014). Future studies employing real-time PCR targeting *LipL32* and identification of circulating serovars are recommended.

In this study, around 30% animals were tested positive for *C. burnetii*, which is consistent with prior studies from Hungary (25.9%) but lower than reports from Ethiopia, Switzerland, and India (Kreizinger *et al.* 2015, Vidal *et al.* 2017, Sarangi *et al.* 2021, Robi *et al.* 2024). *C. burnetii* infection was significantly more common in cattle than buffaloes ($p = 0.013$), which was consistent with previous reports, suggesting species-related differences in susceptibility or management practices (Dhaka *et al.* 2020; Ferrara *et al.* 2022; Naveena *et al.* 2022). Among cattle, crossbred animals showed a higher prevalence (37.8%) than exotic (25.5%) and indigenous breeds (17.7%), although statistical significance was not observed. This finding aligned partially with the previous observations (Keshavamurthy *et al.* 2019). Across lactation groups, *C. burnetii* positivity varied from 25.9% to 50.0%, with the highest prevalence observed in sixth to ninth lactation animals; however, no clear trend with parity was evident. As *C. burnetii* can be shed for prolonged periods in milk and reproductive secretions, and persists for a longer period in the farm environment (Lee *et al.* 2025), the findings underscored its importance as an under-recognised cause of reproductive disorders in India.

Despite the known association of *Listeria monocytogenes* with bovine abortion, none of the samples were positive, which was in contrast to earlier reports from India and other countries (Barkallah *et al.* 2014, Sarangi *et al.* 2021; Končurat and Sukalić 2024). This likely reflects regional or management-related differences and suggested that listeriosis was not a major cause of reproductive loss in the study area.

Overall, at least one microorganism was detected in 57.9% cases, which is consistent with previous studies using molecular techniques to detect microorganisms (Yang *et al.* 2012; Sarangi *et al.* 2021). Single infections were most common, while dual infections occurred in 43 animals and triple infections in 2 cases (Tables 3 and 4). Dual infection of *Brucella* and *Coxiella* was more common in cattle than buffaloes, which is consistent with previous findings (Modise-Tlotleng *et al.* 2024), suggesting potential inter-pathogen interactions that warrant further investigation. Infection with one pathogen may predispose animals to additional infections. Further, an infection with multiple pathogens can result in more severe disease outcomes and can change host immune response, resulting in higher morbidity, mortality and pathogen transmission during interaction between the pathogens (Yang *et al.* 2012; Middlebrook *et al.* 2022). The detection of multiple zoonotic agents in animals with abortion or RFM illustrates the complexity of reproductive disorders and highlights the occupational risks for farm workers, veterinarians, and others handling infected animals or reproductive materials (Robi *et al.* 2024).

In conclusion, this study demonstrated the frequent occurrence of zoonotic bacterial infections-*Brucella abortus*, pathogenic *Leptospira* spp., and *Coxiella burnetii*-in cattle and buffaloes with reproductive disorders in Gujarat, India. *Brucella* was the most common pathogen in abortion cases, while *Coxiella* and *Leptospira*

Table 3. Species-wise distribution of single and mixed zoonotic bacterial infections

Pathogen Combination	Cattle (n=270)	Buffalo (n=131)	Total
Only <i>Brucella</i>	28	15	43
Only <i>Leptospira</i>	28	36	64
Only <i>Coxiella</i>	62	18	80
<i>Brucella</i> + <i>Leptospira</i>	5	4	9
<i>Brucella</i> + <i>Coxiella</i>	18	2	20
<i>Leptospira</i> + <i>Coxiella</i>	7	7	14
<i>Brucella</i> + <i>Leptospira</i> + <i>Coxiella</i>	2	0	2
Total	150	82	232

Table 4. Distribution of single and mixed zoonotic bacterial infections according to reproductive disorders

Pathogen combination	RFM only	Abortion only	Both RFM & Abortion	Metritis	Total
Only <i>Brucella</i>	28	13	2	0	43
Only <i>Leptospira</i>	48	9	7	0	64
Only <i>Coxiella</i>	58	17	3	2	80
<i>Brucella</i> + <i>Leptospira</i>	1	4	4	0	9
<i>Brucella</i> + <i>Coxiella</i>	9	9	1	1	20
<i>Leptospira</i> + <i>Coxiella</i>	11	2	0	1	14
<i>Brucella</i> + <i>Leptospira</i> + <i>Coxiella</i>	1	1	0	0	2
Total	156	55	17	4	232

were prevalent in both abortion and RFM cases. Dual and mixed infections were observed in a significant proportion of animals, underscoring the multifactorial nature of reproductive disorders. The findings emphasized that reproductive infections in livestock have direct implications for human health, highlighting the need for integrated surveillance, biosecurity measures, and control strategies under a One Health framework. Targeted interventions considering both species-specific risks and zoonotic potential are crucial to safeguard livestock productivity and public health.

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