ORIGINAL ARTICLE

Veterinary Research Forum. 2026; 17 (3): 153 - 160

doi: 10.30466/vrf.2025.2052553.4653

Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Identification of *Chlamydia abortus*, *Coxiella burnetii*, and *Brucella* species from ruminant fetal abomasal contents using molecular methods

Ayfer Güllü Yücetepe[1]*, Sadiye Dılşa Ateş[1], Osman Yaşar Tel[1], Özden Dellal[1], Oktay Keskin[1], Ahmet Murat Saytekin[1], Sibel Kızıl[2], Ufuk Ülker[3], Efsun Melike Çeçen[2], Sevil Erdenliğ Gürbilek[1]

[1] Department of Microbiology, Faculty of Veterinary Medicine, Harran University, Şanliurfa, Türkiye; [2] Department of Microbiology, Faculty of Veterinary Medicine, Kirikkale University, Kirikkale, Türkiye; [3] Department of Microbiology, Faculty of Veterinary Medicine, Bozok University, Yozgat, Türkiye.

Article Info

Article history:

Received: 04 February 2025
Accepted: 20 May 2025
Available online: 15 March 2026

Keywords:

Brucella
Chlamydia
Coxiella burnetii
One Health concept

Abstract

This study aimed to detect *Coxiella burnetii*, *Chlamydia abortus*, and *Brucella* species in the abomasal contents of aborted ruminant fetuses from the Central Anatolia region of Türkiye using PCR between 2020 and 2023. The abomasal contents of a total of 97 aborted fetuses from cattle, sheep, and goats with a history of abortion, collected between the years 2020 and 2023, were tested in this study. As a result of PCR analysis of 97 abomasal contents, four (4.10%; 95.00% confidence interval [CI]: 1.33 - 10.82) of them were *C. abortus*, including three sheep and one goat. Two (2.10%; 95.00% CI: 0.36 - 7.96) of them were *C. burnetii*, including one sheep and one cow. A total of 60 (61.90%; 95.00% CI: 51.40 - 71.37) samples from 47 cattle, nine sheep, and four goats were determined by *Brucella* genus-specific PCR. Following multiplex PCR analysis of the positive *Brucella* spp. samples, 39 (65.00%; 95.00% CI: 51.52 - 76.55) samples were identified as *B. abortus*, including two sheep, one goat, and 36 cattle. Additionally, 19 (31.70%; 95.00% CI: 20.60 - 45.09) isolates were identified as *Brucella melitensis*, including five sheep, two goats, and 12 cattle. In two sheep samples, both *B. melitensis* and *C. abortus* were identified from the same animals. In conclusion, *Brucella* spp. were the predominant abortion-causing pathogens, with *C. abortus* also contributing significantly. Effective control strategies under the One Health approach are essential to prevent the uncontrolled spread and inter-species transmission of these zoonotic agents in the region and country.

© 2026 Urmia University. All rights reserved.

Introduction

The interactions between human, animal, and environmental factors play a significant role in the etiology and epidemiology of infectious diseases. Abortions, causing considerable economic losses in livestock, have a very complex etiology. In addition, their diagnosis generally requires lengthy and risky procedures for laboratory staff.[1] The major zoonotic bacterial diseases are characterized by abortions. They generally have a wide host range, including cattle, sheep, goats, and dogs. The etiological agents of these diseases are mostly *Chlamydia abortus*, *Coxiella burnetti*, and *Brucella* spp. These are most significant infections that occur in numerous countries worldwide.[1,2-4] Infected animals can shed the organism in their feces, milk, and especially in the membranes of the placenta and birth fluids.

During birth, billions of bacteria are excreted with the birth products of infected ruminants. The bacteria can easily be transmitted to humans through direct contact with infected animals, the ingestion of contaminated food or water, and inhalation of infectious aerosols.[5,6] It has been reported that the presence of abortion organisms can be detected in various biological samples, including placental cotyledons, abomasal content, vaginal swabs, and samples of tissue (liver, lung, brain, and tongue) from the fetus.[7-9]

The *C. burnetti* is an obligate intra-cellular pathogen that requires live media in order to be cultured, such as embryonic chicken eggs and/or cell cultures. Furthermore, the culture work with this pathogen should be performed under biosafety level 3 conditions. All of these factors have made the use of serological and molecular methods more advantageous for the diagnosis of the disease.[10,11]

The *C. abortus* is one of the causative agents of chlamydiosis, causing a contagious, infectious, and zoonotic disease being characterized by placentitis and abortion in cattle, particularly in sheep and goats.

***Correspondence:**

Ayfer Güllü Yücetepe. PhD
Department of Microbiology, Faculty of Veterinary Medicine, Harran University, Şanliurfa, Türkiye
E-mail: ayfergullu@harran.edu.tr

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0) which allows users to read, copy, distribute and make derivative works for non-commercial purposes from the material, as long as the author of the original work is cited properly.

Materials, such as semen, placenta, vaginal discharge, and fetal organs, can be used for laboratory diagnosis. Bacteria of the genus *Chlamydia* are obligate intracellular microorganisms that can only be isolated from living cells.[12] Although various serological tests are used to diagnose the infection, previous conducted studies have shown that the sensitivity and specificity of these tests may vary.[13,14] Among the conventional methods, techniques based on the detection of nucleic acids, such as PCR, have been found to be more successful in detecting pathogens.[15]

Brucellosis is another important zoonotic infection that causes significant economic damage and affects many animal species. The diagnosis of the disease is conducted using a variety of methods, including bacteriological, serological, and molecular techniques. While these methods have their respective advantages, their success rates vary. Molecular diagnostic tests based on PCR have been described as an effective method for identifying different species, biotypes, and strains of the pathogen.[16] Gene targets being widely used and internationally accepted in molecular diagnostics were selected. The *com1* gene of *C. burnetii* was chosen as it encodes a highly conserved outer membrane protein, providing high species specificity and reducing the risk of cross-reactivity. This gene is commonly used in PCR-based detection because it enables sensitive and reliable identification of the organism in clinical and field samples.[17] For *C. abortus*, primers targeting the *CpaOMP1* gene, encoding outer membrane protein 1, were used. This gene is recognised for its species-level specificity, allowing clear distinction of *C. abortus* from other *Chlamydia* species. The use of *CpaOMP1* in molecular diagnosis increases accuracy, especially in mixed or challenging clinical cases.[18] For *Brucella* spp., the *bcsp31* gene was used for genus-level detection. This gene encodes a 31.00-kDa immunogenic cell surface protein present in all *Brucella* species and is regarded as a standard target for PCR identification, due to its high degree of conservation and demonstrated sensitivity and specificity in various studies.[19] The Bruce-ladder multiplex PCR, in particular, targets several species-specific genetic regions, enabling the identification and differentiation of multiple *Brucella* species and commonly used vaccine strains within a single assay.[20] These gene targets are selected based on their specificity, conservation, and validation in previous studies. Using these markers provides a sensitive, reliable, and internationally comparable molecular approach for detecting these important zoonotic pathogens.

In this study, it was aimed to detect *C. burnetii*, *C. abortus,* and *Brucella* species in aborted fetal abomasal contents of ruminants collected from different regions of the Central Anatolia region in Türkiye at the molecular level.

Materials and Methods

Sampling. In this study, a total of 97 abomasal contents of aborted fetuses from 69 cattle, 19 sheep, and nine goats with a history of abortion were used. The abomasal contents of aborted fetuses were obtained from sheep and goats during the late stages of pregnancy, and from cattle during the 3rd month of pregnancy. Samples were collected between the years 2020 and 2023 from farms in different regions, far away from each other, of the Central Anatolia region in Türkiye and sent to the Department of Microbiology Laboratory, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, Türkiye for diagnostic purposes.

Positive control strains. *C. abortus S26/3, C. burnetii, B. abortus* 544, and *B. melitensis* 16M strains in the culture collection of the Department of Microbiology Laboratory, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, Türkiye were used as positive controls.

DNA extraction from abomasal contents. A commercial isolation kit, GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, USA), was used for DNA extraction from abomasal contents. The DNA extraction was conducted following the manufacturer's protocols. Briefly, each of 1.00 mL abomasal content was subjected to enzymatic digestion with 20.00 µL of proteinase K in 180 µL of enzymatic digestion buffer, followed by incubation at 56.00 ˚C for 30 min. Subsequently, 20.00 µL of RNase solution was added and the mixture was incubated at 37.00 ˚C for 10 min. Then, 200 µLof lysis buffer was added and mixed for 15 sec until a complete homogeneous solution was achieved. The whole content was then mixed with 400 µL of 50.00% ethanol and transferred to the spin column. Following centrifugation at 10,000 *g* for 1 min, the spin column was washed with 500 µL of wash buffer one and then wash buffer two. After centrifugation, the DNA was eluted in 50.00 µL of elution buffer and centrifuged at 13,000 *g* for 60 sec. The eluted DNA was transferred to a sterile Eppendorf tube. The amount of DNA was determined using a spectrophotometer (NanoDrop ND-2000, Thermo Fisher Scientific) at 260 - 280 nm (112.30 ± 0.30 ng µL^{-1}). The obtained DNAs were stored at – 20.00 ˚C until the PCR test was performed.

Coxiella burnetii. Primer pairs amplifying 74 bp of the *com1* gene (GenBank No. AF318146.1), being known to be a highly protected region among *C. burnetii* strains and designed to be used in a real-time PCR, were used in conventional PCR, as shown in Table 1.[17] The PCR mixture was prepared with a total volume of 25.00 µL in each tube, including 12.50 µL master mix (Qiagen multiplex, Hilden, Germany), 1.50 µL of each primer pair, 7.50 µL water, and 2.00 µL template DNA. The PCR-grade water was used as a negative control. The mixture was amplified in a Thermal Cycler (Thermo Fisher Scientific) for a total of 30 cycles at

95.00 ˚C for 15 min initial denaturation step, followed by denaturation at 94.00 ˚C for 30 sec, annealing at 58.00 ˚C for 90 sec, first synthesis at 72.00 ˚C for 3 min (first synthesis), and final synthesis at 72.00 ˚C for 10 min. The amplicons were subjected to electrophoresis (EC300 XL; Thermo Fisher Scientific) in a 4.00% agarose gel (Sigma-Aldrich, St. Louis, USA). At the end of process, the presence of specific bands was investigated with a gel imaging device (UVCI-1100: Major Science, Taoyuan City, Taiwan).

Chlamydia abortus. Classical PCR was applied to the DNAs isolated from abomasal contents by modifying the real-time PCR method reported by Pantchev *et al*.[18] The PCR mixture was prepared in a total volume of 25.00 µL in each tube and consisted of 12.50 µL master mix (Qiagen multiplex), 1.50 µL of each primer, 8.50 µL water, and 1.00 µL template DNA. The PCR-grade water was used as a negative control. The amplification process was completed as follows: Initial denaturation at 95.00 ˚C for 5 min, followed by 45 cycles consisting of denaturation at 94.00 ˚C for 15 sec, initial binding at 60.00 ˚C for 30 sec, initial synthesis at 72.00 ˚C for 30 sec, and the final cycle at 72.00 ˚C for 7 min. The amplified DNAs were subjected to electrophoresis in a 3.00% agarose gel. At the end of the process, the presence of specific bands was investigated with the gel imaging device.

Genus-specific and species-specific multiplex PCR (Bruce-ladder). For genus-specific DNA amplification, primers were used to amplify a 223 bp long target region on a gene encoding the bcsp31 protein, being present in all *Brucella* species, in accordance with the protocol described by Baily *et al*.[19] Nine pairs of species-specific primers ranging in size from 152 to 2,524 bp from different gene regions were used for multiplex PCR (Bruce-ladder) as illustrated in Table 1.[20,21] The multiplex PCR was used for identification of different species of *Brucella* spp. The multiplex PCR method described by Mayer-Scholl *et al*. was used.[16] The PCR mixture was prepared in a total volume of 25.00 µL in each tube, including 12.50 µL Qiagen master mix, 0.20 µM of each of nine primer pairs, 9.00 µL water, and 1.00 µL template DNA. The PCR-grade water was used as a negative control. The amplification process was performed at 95.00 ˚C for 15 min for initial denaturation, followed by denaturation at 94.00 ˚C for 30 sec, initial binding at 58.00 ˚C for 90 sec, initial synthesis at 72.00 ˚C for 3 min (first synthesis), and final synthesis at 72.00 ˚C for 10 min, for a total of 30 cycles. The amplicons were subjected to electrophoresis in a 1.50% agarose gel and at the end of the process, the presence of specific bands was investigated by gel imaging device.

Statistical analysis. Descriptive statistical analysis was performed using SPSS Software (version 24.0; IBM Corp., Armonk, USA). Prevalence rates were calculated as percentages, and 95.00% confidence intervals (CI) were determined using standard binomial proportion calculations. No comparative statistical tests were applied.

Table 1. Primers used in PCR and multiplex PCR methods.

Primers	Primer sequence (5'-3')	Amplicon size (bp)	References
*CpaOMP*1[a]	F: GCAACTGACACTAAGTCGGCTACA R: ACAAGCATGTTCAATCGATAAGAGA	82	18
*com*1[b]	FAF216: GCACTATTTTTAGCCGGAACCTT RAF290: TTGAGGAGAAAAACTGGATTGAGA	74	17
*Bcsp*31[c]	B4F: TGG CTC GGT TGC CAA TAT CAA B5 R: CGC GCT TGC CTT TCA GGT CTG	223	19
BMEI0998 [1-3, 5-13] BMEI0997	F: ATCCTATTGCCCCGATAA-GG R: GCTTCGCATTTTCACTGTAGC	1,682	20
BMEI0535[1-13] BMEI0536	F: GCGCATTCTTCGGTTATGAA R: CGCAGGCGAAAACAGCTATAA	450	20
BMEII0843 [2-10, 13] BMEII0844	F: TTT-ACA-CAG-GCA-ATC-CAG-CA R: GCGTCCAGTTGTTGTTGATG	1,071	20
BMEI1436[1-4,6-13] BMEI1435	F: ACGCAGACGACCTTCGGTAT R: TTTATCCATCGCCCTGTCAC	794	20
BMEII0428[1-10,12,13] BMEII0428	F: GCCGCTATTATGTGGACTGG R: AATGACTTCACGGTCGTTCG	587	20
BR0953 [3, 5-8]	F: GGAACACTACGCCACCTTGT R: GATGGAGCAAACGCTGAAG	272	20
BMEI0752[13]	F: CAGGCAAACCCTCAGAAGC R: GATGTGGTAACGCACACCAA	218	20
BMEII0987[1-5, 7-13]	F: CGCAGACAGTGACCATCAAA R: GTATTCAGCCCCCGTTACCT	152	20
Bmispec[8]	F: AGATACTGGAACATAGCCCG R: ATACTCAGGCAGGATACCGC	510	21

a: *Chlamydia abortus*; b: *Coxiella burnetii*; c: *Brucella* spp.; 1: *Brucella abortus*; 2: *Brucella melitensis*; 3: *Brucella suis*; 4: *Brucella ovis*; 5: *Brucella canis*; 6: *Brucella neotomae*; 7: *Brucella inopinata*; 8: *Brucella microti*; 9: *Brucella pinnipedialis*; 10: *Brucella ceti*; 11: *Brucella abortus* S-19; 12: *Brucella abortus* RB51; 13: *Brucella melitensis* Rev-1.

Results

As a result of PCR analysis of DNAs from 97 abomasal contents (Fig. 1), four (4.10%; 95.00% confidence interval [CI]: 1.33 - 10.82) of them were *C. abortus*, including three sheep (3.09%) and one goat (1.03%), Two (2.10%; 95.00% CI: 0.36 - 7.96) of them were *C. burnetii*, including one sheep (1.03%) and one cow (1.03%). A total of 60 (61.90%; 95.00% CI: 51.40 - 71.37) samples from 47 cattle, nine sheep, and four goats were determined by *Brucella* genus-specific PCR. Following multiplex PCR analysis of the positive *Brucella* spp. samples, 39 (65.00%; 95.00% CI: 51.52 - 76.55) samples were identified as *B. abortus*, including two sheep, one goat, and 36 cattle. Additionally, 19 (31.70%; 95.00% CI: 20.60 - 45.09) isolates were identified as *B. melitensis*, including five sheep, two goats, and 12 cattle, as shown in Table 2.

Two (3.30%; 95.00% CI: 0.58 - 12.54) samples being positive for *Brucella* spp. by the specific PCR analysis were found to be negative by the Bruce-ladder. No vaccine strain was detected in the multiplex PCR analysis. Two sheep samples were found to be positive for both *B. melitensis* and *C. abortus*. A total of 68.00% (95.00% CI: 57.69 - 76.94) of the aborted fetuses abomasal contents were found to contain the pathogens that were the subject of the search.

Discussion

Humans, animals, and the environment play a critical role in the emergence and transmission of various infectious diseases. Zoonoses represent a significant public health concern as they directly impact human health and potentially lead to mortality. Globally, the most prevalent zoonotic diseases have a greater impact on poor livestock

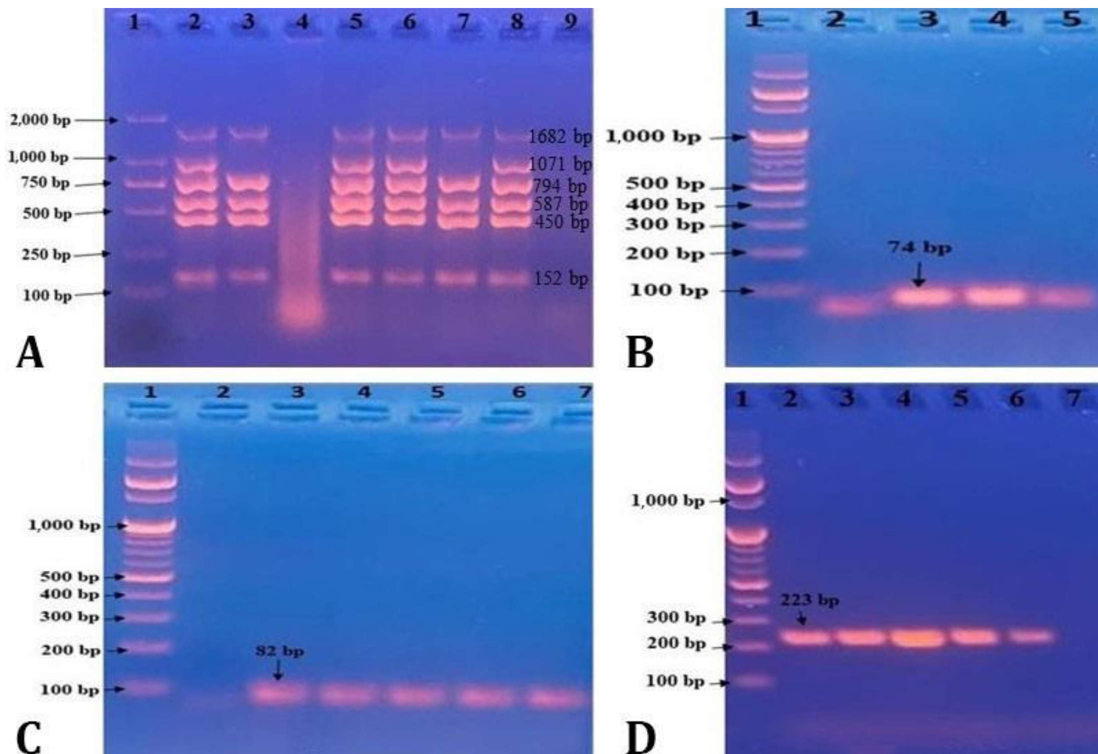

Fig. 1. Polymerase chain reaction gel images. **A)** Multiplex PCR gel image; 1: Marker (Qiagen GelPilot Mid Range); 2: Positive control *Brucella melitensis* 16M; 3: Positive control *Brucella abortus* 544; 4: Negative abomasal content sample; 5, 6, and 8: Field samples positive for *B. Melitensis*; 7: Field sample positive for *B. Abortus*; 9: Negative control; **B)** *Coxiella burnetii* conventional PCR gel image; 1: Marker (Qiagen GelPilot Mid Range); 2: Negative control; 3: *C. burnetii* positive control; 4 and 5: Positive field samples; **C)** *Chlamydia abortus* conventional PCR gel image; 1: Marker; 2: Negative control; 3: *C. abortus* S26/3 positive control; 4, 5, 6, and 7: Positive field samples; and **D)** *Brucella* spp. conventional PCR gel image; 1: Marker; 2: *Brucella* spp. positive control; 3, 4, 5, and 6: Positive field samples; 7: Negative control.

Table 2. Polymerase chain reaction results of analyzed animal samples.

Species	No.of animals	*Brucella* spp.	*Brucella melitensis*	*Brucella abortus*	*Chlamydia abortus*	*Coxiella burnetii*
Sheep	19	9	5	2	3	1
Goat	9	4	2	1	1	-
Cattle	69	47	12	36	-	1
Total	97	60 (61.90%)	19 (31.70%)	39 (65.00%)	4 (4.10%)	2 (2.10%)

keepers in low- and middle-income countries. They cause an estimated 2.40 billion cases of human disease and 2.70 million deaths each year.[22] Innovative approaches are essential to promote the ongoing dissemination of multidisciplinary research findings in zoonotic bacterial diseases. The global One Health approach, emphasizing interdisciplinary collaboration across human, animal, and environmental health sectors, offers a promising framework to support public health efforts.[23] Effective public health measures are needed to prevent underdiagnosis and the development of severe cases. Many medical professionals are unaware of the One Health approach, and some zoonoses, such as Q fever, can lead to underdiagnosis, development of severe cases, and lack of appropriate treatment, factors that can contribute to patient mortality. The widespread lack of knowledge about this disease among healthcare professionals underscores the need to integrate the One Health concept into the medical education, residency programmes, and continuous professional development.[24] Therefore, the implementation of One Health strategies is strongly recommended for the effective prevention and control of potential zoonotic diseases.

In comprehensive studies conducted in Türkiye and various regions worldwide regarding the detection of abortifacient agents in ruminants, brucellosis, chlamydiosis, and Q fever have been identified as the main diseases among the potential zoonotic infections causing abortions.[8,15,25,26] The diagnostic materials used to detect pathogens include abomasal contents and internal organs, vaginal discharges, placenta, cotyledons, milk, and blood serum from aborted fetuses. These materials are analyzed through cultural, serological, and molecular techniques.[7,8,27-31] Molecular diagnostic methods, such as PCR, offer considerable advantages over conventional serological and culture-based methods in terms of sensitivity, specificity, and speed. However, these methods are associated with higher initial costs due to the need for specialized equipment and reagents.[32] To avoid the challenges associated with culturing these pathogens and prevent potential false positives or negatives in serological diagnosis, molecular methods, which are relatively simpler and safer to implement, were employed in this study.

Brucella infections in cattle are typically caused by *B. abortus,* less commonly by *B. melitensis,* and occasionally by *B. suis.* In sheep and goats, *Brucella* infections are predominantly caused by *B. melitensis*, with *B. abortus* being less frequently identified.[31] In this study, *B. melitensis* was detected in 12 (25.50%) cattle, while *B. abortus* was identified in two (3.10%) sheep and one (1.50%) goat. Such heterologous infections may be more frequent on farms where multiple animal species are co-housed. Several studies have reported similar findings, indicating that inter-species transmission plays a significant role.[33-35] In a study, *B. abortus* was identified by PCR in one (8.00%) sheep and one goat (50.00%) among 15 positive *Brucella* samples from abortions in small ruminants.[33] In a farm where both small and large ruminants are kept together and share the same pastures, *B. abortus* was molecularly confirmed in sheep and goats. The authors concluded that *B. abortus* can be a problem for small ruminants.[35] Büyük and Şahin confirmed 105 isolates as *B. abortus* and one isolate as *B. melitensis* using a *Brucella* species-specific PCR kit from 265 milk, 261 vaginal fluid, and 97 fetal tissue samples from 284 non-vaccinated cows against brucellosis.[34] Based on their findings, the isolation rate of *Brucella* spp. was 25.72% among cows in the Kars region, Türkiye, confirming the endemic presence of the disease.

In our study, a total of 60 (61.90%) samples were found to be positive for *Brucella* spp., including 47 cattle, nine sheep, and four goats. As a result of multiplex PCR analysis, 39 (65.00%) of the samples were detected as *B. abortus*, including two sheep, one goat, and 36 cattle. Additionally, 19 (31.70%) samples were identified as *B. melitensis*, including five sheep, two goats, and 12 cattle. Of the 60 samples tested positive for *Brucella* spp. by genus-specific PCR, two could not be identified by species-specific multiplex PCR. The *Brucella* spp. are phylogenetically classified within the *Rhizobiaceae* family, in the α-2 sub-group of the *Proteobacteria* class, including the *Rhizobium* and *Ochrobactrum* genera.[36] Genus-specific PCR methods have been developed for the detection of *Brucella* spp. using highly conserved *16S rRNA* and regions on a gene encoding the bcsp31 protein.[19,37] However, it has been reported that these regions may occasionally cross-react with *O. anthropi* and *O. intermedium*, being genetically close to the *Brucella* genus.[37,38] In addition, the majority of the virulence factors known as molecular determinants, such as cell envelope components, and secretion and regulatory systems, are also present in soil bacteria associated with *Brucella*.[39] The inability of Bruce-ladder PCR to identify two *Brucella* spp. previously detected by genus-specific PCR may be attributed to the genetic similarity between *Brucella* and *Rhizobiaceae.*

A case of multi-etiological abortion involving both *B. melitensis* and *C. abortus* in a sheep was reported for the first time in Türkiye in 2023.[40] In this study, *B. melitensis* and *C. abortus* were co-detected as causative agents in two cases of sheep abortions. A study conducted in various countries worldwide revealed the prevalence of *Brucella* and *Chlamydia* infections in sheep and goat flocks. Among the twenty investigated flocks, three exhibited mixed infections with brucellosis and chlamydiosis.[41] Another study simultaneously identified five positive samples for *C. abortus* and *B. melitensis* using immunohistochemistry and PCR.[42] This demonstrates that multiple abortifacient agents can be identified simultaneously in a single animal, having important implications for diagnosis, treatment, and epidemiological research.

The *C. abortus* is one of the primary causes of abortion, typically occurring during the last 2 - 3 weeks of pregnancy. It is characterised by pre-mature births, weak lambs with low birth weight, and rapid neonatal death. The disease predominantly affects intensively managed flocks during the lambing season, and remains a leading cause of abortion in many countries worldwide.[12] In this study, *C. abortus* was detected in aborted animals during the final month of gestation, resulted in pre-mature and weak lambs with low body weight. Further studies in different animal species are recommended to determine the contribution of *C. abortus* in abortions in small ruminants, together with other agents.[43] In this study, *C. abortus, C. burnetii,* and *Brucella* species in abortion cases among various animal species, including sheep, goats, and cattle were investigated.

Many researchers have used PCR as one of the molecular techniques to detect *C. burnetii* and *C. abortus*, both of which cause abortion in ruminants.[25,44] In a study conducted in Türkiye, 227 aborted samples were tested, and *C. burnetii* DNA was detected by PCR in a total of four samples (1.80%), including two (1.50%) of 132 cattle samples and two (2.70%) of 72 sheep samples.[30] In another study, the significance of detecting *C. burnetii* in fetal abomasal contents was demonstrated. The *C. burnetii* DNA was detected by PCR in four (3.92%) of 102 cattle, five (11.11%) of 45 sheep, and two (40.00%) of five goats.[28] In this study, *C. burnetii* was detected by PCR in two (2.10%) of 97 samples, one sheep (1.03%), and one cow (1.03%). As a result of PCR analysis of the stomach contents of 65 aborted bovine fetuses, two (3.00%) bovine fetuses were positive for *C. abortus*.[27] Kalender *et al.* analyzed 71 aborted sheep and goat fetus samples by PCR and reported that seven (9.80%) of the samples were positive for *C. abortus*.[45] Kılıç *et al.* reported that three out of 47 clinical samples from cattle were positive for *C. abortus* by PCR.[46] In a study conducted in Konya, Türkiye, *Chlamydia* DNA was detected by PCR in six (3.50%) of the fetal stomach contents of 172 aborted sheep.[47] In this study, *C. abortus* was detected in four (4.10%) of 97 samples, including three (3.09%) sheep and one (1.03%) goat, by PCR analysis of fetal abomasal contents. In comparison, the study conducted by Aras *et al.* found that cattle samples were not positive for *C. abortus*.[27] Moreover, goat samples were not positive for *C. burnetii*. The detection rates in this study were lower than those reported by Kalender *et al.* and Kılıç *et al.*, but they were consistent with the findings of Güler *et al.*[45-47]

The lower detection rates of *C. abortus* and *C. burnetii* observed in this study, compared to similar studies, were mainly attributed to the reliance on abomasal contents; the maternal samples, such as placenta and vaginal swabs, where these pathogens are more likely to be found, were not analyzed. Other contributing factors may include the sampling method, transportation conditions, and timing of the abortion. Nevertheless, the detection of these zoonotic pathogens, even at low rates, remains an important finding that should not be over-looked. This study analyzed 97 samples from a specific region. While this provides valuable insight, a larger sample size and the inclusion of samples from more diverse regions would improve the generalizability of the findings. Although molecular methods, such as PCR, were used due to their sensitivity and specificity, they may not be able to detect all strains or variants of the pathogens. In addition, low pathogen loads in some samples may lead to false-negative outcomes.

It has been reported that placental cotyledons yield higher detection rates of *C. burnetii* than fetal fluids.[7] However, Gülaydın *et al.* found that abomasal contents were more suitable for the detection of bacterial pathogen DNA in the sample material used for analyses.[8] In this study, 68.00% of bacterial pathogen DNA was identified in abomasal contents; the majority of which belonged to *Brucella* species. The stage of infection and type of sample collected are important considerations in diagnosing pathogens. If pregnancy is terminated, placental infection may facilitate bacterial transmission to the fetus through the amniotic-oral route. This process occurs when bacteria penetrate the placenta, contaminate the amniotic fluid, and are subsequently swallowed by the fetus. Another possible route for bacterial presence in fetuses and subsequent abortion is hematogenous spread *via* the umbilical vessels.[48] Given these factors, abomasal contents from 97 aborted fetuses (sheep, cattle, and goats) were selected as the sample type in this study. The decision to use abomasal contents was based on multiple considerations. The advantage of using abomasal contents is that they may contain vaginal secretions, placental fluids, and birth fluids, potentially providing a more comprehensive representation of pathogen exposure.[49] The abomasal contents are easily accessible at necropsy of aborted fetuses and allow efficient sampling without additional invasive procedures. This approach also reduces the risks associated with handling, potentially hazardous biological materials, such as placental tissues, which could pose biosecurity concerns when handle zoonotic pathogens, such as *C. burnetii* and *Brucella* spp.

Regarding the methodology of epidemiological studies and prevalence of specific pathogens studied, it should be noted that the comparison of etiologies may vary between countries and geographical areas. For this reason, the data obtained in this study will be significant value in contributing to the prevention of quarantine practices and environmental contamination, which can be employed to prevent the uncontrolled spread of different zoonotic agents in the region and country, with a view to safeguard environmental, human, and animal health. Also, the study revealed that *Brucella, Coxiella,* and *Chlamydia* were detected in association with abortions among the tested materials, but aligning with the actual epidemiological

situation in the country, where brucellosis is endemic. The findings of this study indicate the need for design and development of effective control strategies to prevent the spread of these important zoonotic agents and inter-species transmission, as exemplified by the case of *Brucella* infections.

Acknowledgments

This study did not receive any specific funding. Ethical approval was not required, as the study was conducted on materials submitted for clinical diagnostic purposes in accordance with the Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees.

Conflict of interest

The authors declare no conflict of interest.

References

1. Gürbilek SE, Baklan EA, Sağlam G, et al. Conventional and molecular identification of *Brucella* isolates from livestock in Turkey. Ankara Univ Vet Fak Derg 2022; 69(3): 297-302.
2. Kneipp CC, Ros AM, Robson J, et al. *Brucella suis* in three dogs: presentation, diagnosis and clinical management. Aust Vet J 2023; 101(4): 133-141.
3. Singh A, Mishra AK, Gururaj K, et al. Molecular detection of *Brucella melitensis*, *Chlamydophila* spp., *Campylobacter* spp., *Brucella abortus* and *Coxiella burnetii* in genital tracts of goats. Indian J Anim Res 2023; 5074. doi: 10.18805/IJAR.B-5074.
4. Zhang H, Deng X, Cui B, et al. Abortion and various associated risk factors in dairy cow and sheep in Ili, China. PLoS One 2020; 15(10): e0232568. doi: 10.1371/journal.pone.0232568.
5. Jawad SM. Brucellosis: Infectious disease. In: Rodríguez-Morales AJ (Ed). Current topics in zoonoses. 1st ed. London, England: IntechOpen 2024; 1-8.
6. WHO. The Control of Neglected Zoonotic Diseases. 1st ed. From Advocacy to Action: Report of The Fourth International Meeting Held at WHO Headquarters. Geneva, CH. World Health Organization; 2015.
7. Pritchard GC, Smith RP, Errington J, et al. Prevalence of *Coxiella burnetii* in livestock abortion material using PCR. Vet Rec 2011; 169(15): 391. doi: 10.1136/ vr.d4693.
8. Gülaydın Ö, Öztürk C, Ekin İH, et al. Investigation of selected bacterial agents causing sheep abortion in the Van Province by RT-PCR and histopathological methods. Acta Vet Brno 2023; 92(1): 69-77.
9. Alzuguren O, Domínguez L, Chacón G, et al. Infectious abortions in small domestic ruminants in the Iberian Peninsula: optimization of sampling procedures for molecular diagnostics. Front Vet Sci 2023; 10: 1152289. doi: 10.3389/fvets.2023.1152289.
10. Marrie TJ. Q fever - a review. Can Vet J 1990; 31(8): 555-563.
11. Nicollet P, Valognes A. Current review of Q fever diagnosis in animals. Bull Acad Vét Fr 2007; 160(4): 289-295.
12. WOAH. Enzootic abortion of ewes chapter: 3.7.5. OIE, France: World Organisation for Animal Health; 2018.
13. Malal ME, Karagül MS, Akar K. Serological investigation of ovine chlamydiosis in small ruminants in Western Turkey. Acta Vet Brno 2020; 89(3): 255-261.
14. Öztürk D, Türütoğlu H, Kaya M. Seroprevalence of *Chlamydophila abortus* infection in sheep in Burdur province [Turkish]. MAE Vet Fak Derg 2016; 1(2): 17-20.
15. Tel OY, Erdenliğ Gürbilek S, Keskin O, et al. Detection of *Chlamydophila abortus* in sheep waste by PCR. In Proceedings: IV. internatıonal academic research congress. Turkey 2018; 35-38.
16. Mayer-Scholl A, Draeger A, Göllner C, et al. Advancement of a multiplex PCR for the differentiation of all currently described *Brucella* species. J Microbiol Methods 2010; 80(1): 112-114.
17. Brennan RE, Samuel JE. Evaluation of *Coxiella burnetii* antibiotic susceptibilities by real-time PCR assay. J Clin Microbiol 2003; 41(5): 1869-1874.
18. Pantchev A, Sting R, Bauerfeind R, et al. New real-time PCR tests for species-specific detection of *Chlamydophila psittaci* and *Chlamydophila abortus* from tissue samples. Vet J 2009; 181(2): 145-150.
19. Baily GG, Krahn JB, Drasar BS, et al. Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. J Trop Med Hyg 1992; 95(4): 271-275.
20. Lopez-Goñi I, García-Yoldi D, Marín CM, et al. Evaluation of a multiplex PCR assay (Bruce-ladder) for molecular typing of all *Brucella* species, including the vaccine strains. J Clin Microbiol 2008; 46(10): 3484-3487.
21. Scholz HC, Hubalek Z, Nesvadbova J, et al. Isolation of *Brucella microti* from soil. Emerg Infect Dis 2008; 14(8): 1316-1317.
22. Grace D, Mutua F, Ochungo P, et al. Mapping of poverty and likely zoonoses hotspots. In Zoonoses Project 4. Report to the UK department for international development. In Proceedings: International livestock research institute, Nairobi, Kenya; 2012; 21-32.
23. Cantas L, Suer K. Review: the important bacterial zoonoses in "One Health" concept. Front Public Health 2014; 2: 144. doi: 10.3389/fpubh.2014.00144.
24. Meurer IR, Silva MR, Roland RK, et al. Evaluation of medical professionals' knowledge about Q fever. Sci Med 2024; 34: e45474. doi: 10.15448/1980-6108. 2024.1.45474.
25. Afrisham S, Golchin M, Mohammadi E, et al. Prevalence

of *Chlamydia abortus* infection in aborted sheep and goats in Kerman province, southeast of Iran. Iran J Vet Sci Technol 2023; 15(3): 42-47.
26. El-Diasty M, Wareth G, Melzer F, et al. Isolation of *Brucella abortus* and *Brucella melitensis* from seronegative cows is a serious impediment in brucellosis control. Vet Sci 2018; 5(1): 28. doi: 10.3390/vetsci5010028.
27. Aras Z, Sayın Z, Sanioğlu Gölen G. Investigation of *Chlamydophila abortus* in abortion of cattle by PCR [Turkish]. Eurasian Vet Sci 2017; 33(2): 77-80.
28. Günaydın E, Müştak HK, Sareyyüpoğlu B, et al. PCR detection of *Coxiella burnetii* in fetal abomasal contents of ruminants. Kafkas Univ Vet Fak Derg 2015, 21(1): 69-73.
29. Kirkan Ş, Kaya O, Tekbiyik S, et al. Detection of *Coxiella burnetii* in cattle by PCR. Turk J Vet Anim Sci 2008; 32(3): 215-220.
30. Gürbilek SE, Keskin O, Yiğin A, et al. Investigation of *Coxiella burnetii* by real-time PCR in ruminant abortus cases [Turkish]. Harran Üniv Vet Fak Derg 2018; 7(1): 79-83.
31. WOAH. Brucellosis (Chapter 3. 1. 4. Infection with *B. abortus, B. melitensis* and *B. suis*. in manual of diagnostic tests and vaccines for terrestrial animals). Paris: World Organisation for Animal Health; 2022.
32. Aranaz A. Significance and integration of molecular diagnostics in the framework of veterinary practice. In: Cunha MV, Inácio J. (Eds). Veterinary infection biology: molecular diagnostics and high-throughput strategies. 1st ed. New York, USA: Humana Press 2015; 19-30.
33. Alirezaei A, Khalili M, Baseri N, et al. Molecular detection of *Brucella* species among aborted small ruminants in southeast Iran. Braz J Microbiol 2024; 55(1): 911-917.
34. Büyük F, Şahin M. Investigation of *Brucella* species from various samples of aborted cattle in Kars province (Turkey) by cultural and molecular methods and epidemiological analysis of cases [Turkish]. Kafkas Üniv Vet Fak Derg 2011; 17(5): 809-816.
35. Saleem MZ, Akhtar R, Aslam A, et al. Evidence of *Brucella abortus* in non-preferred caprine and ovine hosts by real-time PCR assay. Pakiatan J Zool 2019; 51(3): 1187-1189.
36. Jumas-Bilak E, Michaux-Charachon S, Bourg G, et al. Unconventional genomic organization in the alpha subgroup of the Proteobacteria. J Bacteriol 1998; 180(10): 2749-2755.
37. Da Costa M, Guillou JP, Garin-Bastuji B, et al. Specificity of six gene sequences for the detection of the genus *Brucella* by DNA amplification. J Appl Bacteriol 1996; 81(3): 267-275.
38. Velasco J, Romero C, López-Goñi I, et al. Evaluation of the relatedness of *Brucella* spp.and *Ochrobactrum anthropi* and description of *Ochrobactrum intermedium* sp. nov., a new species with a closer relationship to *Brucella* spp. Int J Syst Microbiol 1998; 48(Pt 3): 759-768.
39. Barquero-Calvo E, Conde-Alvarez R, Chacón-Díaz C, et al. The differential interaction of *Brucella* and *Ochrobactrum* with innate immunity reveals traits related to the evolution of stealthy pathogens. PloS One 2009; 4(6): e5893. doi: 10.1371/journal.pone.0005893.
40. Deniz İ, Oruç E. Multietiological abortion induced by *Brucella melitensis* and *Chlamydophila abortus* in a sheep fetus. Etlik Vet Mikrobiyol Derg 2023; 34(2): 205-210.
41. Abnaroodheleh F, Emadi A, Dadar M. Seroprevalence of brucellosis and chlamydiosis in sheep and goats with history of abortion in Iran. Small Rumin Res 2021; 202: 106459. doi: 10.1016/j.smallrumres.2021.106459.
42. Hailat N, Khlouf S, Ababneh M, et al. Pathological, immunohistochemical and molecular diagnosis of abortions in small ruminants in Jordan with reference to *Chlamydia abortus* and *Brucella melitensis*. Pak Vet J 2018; 38(1): 109-112.
43. Esmaeili H, Bolourchi M, Mokhber-Dezfouli MR, et al. Detection of *Chlamydia abortus* and risk factors for infection in small ruminants in Iran. Small Rumin Res 2021; 197: 106339. doi: 10.1016/j.smallrumres.2021.106339.
44. Simeonov K, Chilingirova M. *Chlamydia abortus* and *Coxiella burnetii*-related abortions in small ruminants in Bulgaria during a five-year period (2013-2018). Acta Microbiol Bulg 2018; 34(4): 236-239.
45. Kalender H, Kiliç A, Eröksüz H, et al. Identification of *Chlamydophila abortus* infection in aborting ewes and goats in Eastern Turkey. Revue Méd Vét 2013; 164(6): 295-301.
46. Kılıç A, Kalander H, Muz A. The detection of *Chlamydophila abortus* from aborted bovine fetuses using PCR and microbiological culture [Turkish]. F Ü Sağ Bil Vet Derg 2010; 24(3): 129-132.
47. Güler L, Hadimli HH, Erganiş O, et al. Field evaluation of a PCR for the diagnosis of chlamydial abortion in sheep. Vet Rec 2006; 159(22): 742-745..
48. Roest HJ, van Gelderen B, Dinkla A, et al. Q fever in pregnant goats: pathogenesis and excretion of *Coxiella burnetii*. PloS One, 2012; 7(11), e48949. doi: 10.1371/journal.pone.0048949.
49. Safarpoor Dehkordi F. Prevalence study of *Coxiella burnetii* in aborted ovine and caprine fetuses by evaluation of nested and real-time PCR assays. Am J Anim Vet Sci 2011; 6(4): 180-186.