World Journal of Experimental Biosciences

Volume 14, Number 1: 79 – 86
P-ISSN: 2313-3937, e-ISSN: 3070-0647
2026

Research article

Molecular Detection of Abortifacient Zoonotic Bacteria and Placental Gene-Expression Alterations in Ruminants

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ABSTRACT

Abortion in livestock results in high reproductive losses and poses zoonotic risks. *Brucella*, *Chlamydia abortus, Coxiella bunetii*, and *Listeria monocytogenes* infect placental tissue, stimulate inflammation and disrupt trophoblast function. Infection-related gene expression changes are known; however, the mechanisms of pregnancy loss remain unclear. The current study employs real-time PCR to detect abortifacient pathogens and assess associated placental gene-expression alterations. Placental cotyledon and uterine discharge samples were collected from cattle, sheep, and goats with a recent history of abortion. Pathogen detection was performed using real time polymerase chain reaction (RT-PCR) targeting *bcsp31*, *ompA*, *IS1111*, and *hlyA*, while placental gene expression (*IL1B*, *IL6*, *TNF-α*, *IL10*, *VEGFA*, and *PLAC1*) was quantified using SYBR Green RT-qPCR normalized to *GAPDH*. Gene expression results were calculated using the ΔΔCt method and correlated with the positive samples for the pathogens. RT-qPCR detected at least one target in 79 of 120 abortion cases; 16 cases contained dual-target mixed infections. Coxiella burnetii and Brucella spp. were detected most frequently. Placental expression changes were pathogen-specific rather than uniformly directional: inflammatory genes were increased in selected Brucella- and Coxiella-positive groups, IL10 was reduced in Brucella- and Chlamydia-positive tissues, and VEGFA and PLAC1 varied by pathogen. These molecular changes were significantly associated with pathogen status. Detection of abortifacient zoonotic bacteria was associated with pathogen-specific placental gene-expression changes in inflammatory, angiogenic, and trophoblast-related pathways. Combined molecular profiling may support the investigation of infectious abortion and its One Health significance, although the cross-sectional findings do not establish causality.

Keywords: Abortifacient pathogens; Gene expression; Placenta; RT-PCR.

Citation: Ghanim KG, Jassim AI, Bneed QZ, Yaseen MM. (2026) Molecular Detection of Abortifacient Zoonotic Bacteria and Placental Gene-Expression Alterations in Ruminants. *World J Exp Biosci* **14**:79-86. doi: 10.65329/wjeb.v14.02.03.

Received June 20, 2026; Revised August 02, 2026; Accepted: August 10, 2026; Published: September 6, 2026

1. INTRODUCTION

The impact of abortions in ruminants and obstetric vet medicine continues to be dominated by the following: Its economic and public health implications. Several bacterial pathogens have a strong preference for small ruminants, resulting in lost reproductive opportunities, fetal death, and diminished flock productivity. *Salmonella Enterica* Serovar Abortusovis is an example. It is highly specialized to sheep and is known to trigger strong abortion storms in endemic locations [1]. From a historical and endemic perspective, *Brucella Melitensis* and *Brucella Ovis* continue to be major abortifacient agents in sheep and goats over the Mediterranean, Middle East, and parts of Asia [2]. Effective surveillance in Africa and Asia documents the significant undermining of food security and livelihoods of those reliant on livestock [3]. Abortion in ruminants is an underestimation of reproductive loss due to a lack of diagnostics, incomplete reporting systems and bacterial agents.

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These pathogens present a unique and complex epidemiology of reproductive illness.

Among ruminants, abortion has many possible causes. The virulence factors of *L. monocytogenes* and *Listeria ivanovii* enable placental invasion and fetal death, broadening the differential diagnosis of infectious abortion [4],The occurrence of listeriosis in livestock also has direct public-health relevance [5], *C. burnetii*, the agent of Q fever, further complicates control because it can cause abortion in domesticated ruminants, infect people, and persist in the environment [6], The simultaneous circulation of several abortifacient pathogens therefore increases occupational and community health risks. Handling infected placental tissue and inhaling contaminated aerosols are important routes of exposure [7].

With the introduction of diagnostic practices, pathogen abortion management has become even more complicated. The traditional strategies based on culture and bacterial phenotypic characterization have low success rates. These low success rates usually result from the slow growth of bacteria or intermittent bacterial shedding from the host. This has prompted the adoption of more sensitive molecular methods or advanced strategies [8], Abortion and long-term production deficits are also a major economic problem in domestic species. Leptospirosis is also a growing zoonotic problem that has become increasingly prevalent in major livestock-producing areas. Leptospira infections can result in infertility, stillbirth, and abortion [9]. These pathogens can greatly increase the economic loss in livestock-producing cattle and increase the conversion of cattle to zoonotic animals.

This study sought to determine the presence of key abortifacient zoonotic pathogens using RT-PCR and correlate the loss of pregnancy with changes in the placental expression of selected pro-inflammatory, anti-inflammatory, and trophoblast-integrity genes in ruminant species that were aborted.

2. MATERIALS AND METHODS

2.1. Study Site, Animals, and Sampling

The study included 120 abortion cases (40 cattle, 45 sheep, and 35 goats) collected from farms in Al-Diwaniyah Province, Iraq, during the 2023–2024 reproductive seasons. An a priori power calculation performed in G*Power 3.1.9.7 for a Pearson chi-square test with three species, two outcome categories, α = 0.05, 80% power, and a medium effect size (w = 0.30) indicated a minimum of 108 cases; the final sample of 120 exceeded this requirement [10]. This research involved cattle, sheep, and goats that had recently aborted on select farms in the region. To minimize the potential impact of environmental contamination, all farms were visited 24–48 hours after the reported abortion to obtain the samples as quickly as possible. Placental cotyledons, fetal membranes, and uterine discharges were collected using aseptic techniques with sterile disposable swabs (Puritan® HydraFlock, USA; Cat No: 25-3406-H). To minimize tissue variation, a minimum of three cotyledons were sampled from each placenta. Each sample was immediately placed in a 2 mL sterile microtube containing 1 mL RNAlater Stabilization Solution (Thermo Fisher Scientific, USA; Cat No: AM7021) in order to preserve the sample's RNA. Samples were collected and transported in insulated ice boxes to maintain a temperature of 4°C with reusable cold packs (Cole-Parmer, USA) and were transported to the laboratory within 4 hours of collection. Standardized field forms were used to capture metadata such as the species of the animal, parity, gestational age, vaccination status, the geographical location of the farm, and the observed clinical signs. The protocol was reviewed by the Scientific Research Ethics Committee, College of Veterinary Medicine, University of Al-Qadisiyah. The committee determined that a formal experimental-animal protocol number was not required because sampling was limited to placental tissue, fetal membranes, and uterine discharge after naturally occurring abortions and involved no experimental intervention, treatment, or euthanasia; farm-owner permission was obtained before sampling. Procedures followed the committee's animal-welfare requirements and the WOAH Terrestrial Animal Health Code. We also observed zoonotic pathogen biosafety protocols.

2.2. DNA Extraction

Genomic DNA was extracted from both the uterine discharge and placental cotyledons using Qiagen's DNeasy Blood and Tissue Kit. 25 mg of ground placental tissue was combined with 180 µL of ATL buffer and 20 µL of Proteinase K. The mixture was incubated in a 56°C dry bath incubator overnight (Model Digital DryBath 88870001, Thermo Fisher Scientific, USA). Once lysis was complete, the mixture was supplemented with 200 µL of AL buffer and 200 µL of Merck's (Germany) absolute alcohol. This was placed in a Silica Spin Column, and the column was washed with the AW1 and AW2 buffers. The column was then eluted with 100 µL, 37°C AE buffer. This final buffer was used to maximize the DNA yield. Concentration and purity were determined, and the extracted DNA was stored at −20°C until use. A contamination-monitoring step was included as an extraction blank.

2.3. Extraction of RNA and cDNA

Total RNA from the placenta was extracted with the RNeasy Mini Kit (Qiagen®, Germany; Cat No: 74104) according to the manual. A 30 mg portion of each tissue sample was homogenized in 600 µL RLT buffer containing 1% β-mercaptoethanol (Sigma-Aldrich, USA; Cat No: M6250) using a motorized handheld tissue homogenizer (Bio-Gen PRO200, USA; Cat No: 01-01200). To remove genomic DNA, on-column DNase treatment was conducted using the RNase-Free DNase Set (Qiagen®, Germany; Cat No: 79254). RNA was assessed using a NanoDrop™ 2000 (Thermo Fisher Scientific, USA) to determine A260/A280 and A260/A230 ratios, and only RNA with a range of 1.8–2.0 was accepted. cDNA was generated using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, USA; Cat No: 4368814) using 1 µg of total RNA, and in a final volume of 20 µL. The Veriti™ 96-Well Thermal Cycler (Applied Biosystems™, USA; Cat No: 9902) was set to 25°C for 10 minutes, 37°C for 2 hours, and finally 85°C for 5 minutes. The generated cDNA was kept at –20°C.

2.4. RT-qPCR for Abortifacient Pathogen Detection

Real-time PCR (RT-qPCR) was used to detect genes of *Brucella* (*bcsp31*), *Chlamydia abortus* (*OmpA*), *Coxiella burnetii* (*IS1111*), and *Listeria monocytogenes* (*hlyA*). Each reaction was performed in a final volume of 20 µL consisting of 10 µL of 2× SYBR Green Master Mix (Thermo Fisher Scientific, USA), 1 µL of each primer (10 pmol/µL, Macrogen®, South Korea—custom synthesis), 2 µL of DNA template, and sterile nuclease-free water. Using a real-time PCR system (Applied Biosystems StepOnePlus™, USA), amplification was performed with initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension for 30 s. The target-specific annealing temperatures were 60°C for bcsp31, ompA, and IS1111 and 58°C for hlyA.

Fluorescence signals were measured during each cycle, and amplification specificity was assessed by melt-curve analysis. A no-template control (NTC), an extraction blank, and a target-specific positive control were included in every assay. Macrogen Inc. (South Korea) provided all primers (Table 1).

For each pathogen assay, a five-point 10-fold dilution series of positive-control DNA was analyzed in triplicate to generate a standard curve; assays were accepted only when amplification efficiency was 90–110% and R^2 was at least 0.98. Representative products were electrophoresed on 2% agarose gels with a 100-bp DNA ladder and produced single bands at the expected sizes. Amplicon sequencing was not used for routine confirmation.

A mixed infection was defined as detection of two or more pathogen targets in the same case. All mixed cases in this dataset were dual-target detections; no triple- or quadruple-target cases occurred.

Table 1. RT-qPCR primer sequences for bacterial targets. F, forward; R, reverse.

Pathogen/ gene (function)	Primer	Sequence (5'→3')	Amplicon size (bp)	Reference
Brucella spp. – *bcsp31*	F	GCTCGGTTGCCAATATCAATGC	151	[11, 12, 13]
	R	GGGTAAAGCGTCGCCAGAAG		
Chlamydia abortus – *ompA*	F	GCAACTGACACTAAGTCGGCTACA	82	[14, 15]
	R	ACAAGCATGTTCAATCGAT		
Coxiella burnetii – IS1111	F	GTCTTAAGGTGGGCTGCGTG	295	[16]
	R	CCCCGAATCTCATTGATCAGC		
Listeria monocytogenes – hlyA	F	TGCAAGTCCTAAGACGCCA	113	[17]
	R	CACTGCATCTCCGTGGTATACTAA		

2.5. Analysis of the Host Placental Gene Expression Levels

Gene expression of IL1B, IL6, TNF-α, IL10, VEGFA, PLAC1, and the GAPDH reference gene was quantified. IL1B, IL6, and TNF-α were selected as pro-inflammatory mediators, whereas IL10 was selected as an anti-inflammatory regulator. VEGFA is critical for placental vascular development, and PLAC1 contributes to trophoblast differentiation and maintenance of placental structure.

SYBR Green RT-qPCR was performed using a QuantStudio™ 3 Real-Time PCR System (Applied Biosystems™, USA; Cat No: A28137). Each 20 µL reaction contained 10 µL PowerUp™ SYBR Green Master Mix (Applied Biosystems™, USA; Cat No: A25742), 0.5 µL of each primer (10 pmol/µL; Macrogen®, South Korea), 2 µL cDNA, and 7 µL nuclease-free water. The cycling protocol consisted of UDG activation at 50°C for 2 min, initial denaturation at 95°C for 2 min, and 40 cycles at 95°C for 15 s and 60°C for 1 min. Melt-curve analysis was performed after amplification. Ct values were exported, and fold changes relative to pathogen-negative placentas were calculated using the 2−ΔΔCt method after normalization to GAPDH. Primer details are shown in Table 2.

Table 2. RT-qPCR primer sequences for placental gene-expression targets and reference gene. F, forward; R, reverse.

Target gene	Primer	Sequence (5′ → 3′)	Reported amplicon size (bp)	Reference
IL1B	Forward	ACCTTCATTGCCCAGGTTTCT	120	[18]
	Reverse	CTGTTTAGGGTCATCAGCCTCAA		
IL6	Forward	TGAGTGTGAAAGCAGCAAGGA	137	[18]
	Reverse	TACTCCAGAAGACCAGCAGTGG		
TNF-α	Forward	TAACAAGCCGGTAGCCCACG	277	[18]
	Reverse	GCAAGGGCTCTTGATGGCAGA		
IL10	Forward	TGCTGGATGACTTTAAGGG	186	[18]
	Reverse	AGGGCAGAAAGCGATGACA		
VEGFA	Forward	CAAACCTCACCAAAGCCAGC	186	[19]
	Reverse	CGCGAGTCTGTGTTTTTGCA		
PLAC1 (placenta-specific 1)	Forward (sense)	GTGAGCACAAAGCCACATTTC	118	[20]
	Reverse (antisense)	GCAGCCAATCAGATAATGAACC		
GAPDH	Forward	GGGATGAGGCTCAGAGCAAGAGA	118	[19]
	Reverse	AGCTCGTTGTAGAAGGTGTGGTGCC		

Five-point 10-fold pooled cDNA dilution series were analyzed in triplicate for every host-gene assay. Only assays with 90–110% efficiency, $R^2 \geq 0.98$, and a target-to-GAPDH efficiency difference below 5% were accepted for 2−ΔΔCt analysis. Representative amplicons produced a single band of the expected size on 2% agarose gels; sequencing was not used for routine confirmation.

2.6. Statistical Methods

Statistical analyses were performed using SPSS version 25.0 (IBM Corp., USA; academic license). Pathogen frequencies among cattle, sheep, and goats were compared using Pearson's chi-square test. Relative gene-expression values were calculated by the 2−ΔΔCt method after normalization to GAPDH. Distributional assumptions were assessed with the Shapiro–Wilk test; because the pathogen-group expression distributions remained non-normal after logarithmic transformation, two-sided Mann–Whitney U tests were used for pathogen-positive versus pathogen-negative comparisons, with standardized z statistics reported. Associations between pathogen status and gene expression were quantified using

Spearman's rank-correlation coefficient (ρ). Values are presented as mean ± standard error (SE), exact two-sided p values are reported, and $p < 0.05$ was considered significant. Graphs were prepared using GraphPad Prism version 9.0 (GraphPad Software, USA).

3. RESULTS

3.1. Detection of pathogens and their prevalence

RT-qPCR detected at least one bacterial target in 79 of 120 cases (65.8%), including 16 dual-target mixed infections (13.3%); 41 cases (34.2%) were negative for all four targets. Coxiella burnetii was detected in 33/120 cases (27.5%), *Brucella* spp. in 31/120 (25.8%), *Chlamydia* abortus in 20/120 (16.7%), and *Listeria monocytogenes* in 11/120 (9.2%). Sheep had the numerically highest *Brucella* positivity (31.1%), but differences among species were not significant (Pearson $\chi^2(2) = 1.365$, $p = 0.505$). Species differences were also non-significant for C. abortus ($\chi^2(2) = 0.866$, $p = 0.649$), *C. burnetii* ($\chi^2(2) = 0.036$, $p = 0.982$), *L. monocytogenes* ($\chi^2(2) = 1.573$, $p = 0.455$), and mixed infections ($\chi^2(2) = 0.604$, $p = 0.739$) (Table 3 and Fig. 1). All extraction blanks and NTCs remained negative, all positive controls amplified within their established acceptance range, and all accepted reactions showed single melt peaks and expected-size gel bands.

Table 3. Pathogen positivity rates in cattle, sheep, and goats based on RT-qPCR detection

Species	No. Tested	*Brucella* Positive n (%)	*Chlamydia abortus* Positive n (%)	*Coxiella burnetii* Positive n (%)	*Listeria Monocytogenes* Positive n (%)	Mixed Infections n (%)
Cattle	40	8 (20.0%)	5 (12.5%)	11 (27.5%)	3 (7.5%)	4 (10.0%)
Sheep	45	14 (31.1%)	9 (20.0%)	12 (26.7%)	6 (13.3%)	7 (15.6%)
Goats	35	9 (25.7%)	6 (17.1%)	10 (28.6%)	2 (5.7%)	5 (14.3%)
Total	120	31 (25.8%)	20 (16.7%)	33 (27.5%)	11 (9.2%)	16 (13.3%)

Data are presented as n (%); total sample size, n = 120. Mixed infection was defined as detection of two or more pathogen targets in the same case. Pearson chi-square tests among species: Brucella, $\chi^2(2) = 1.365$, $p = 0.505$; C. abortus, $\chi^2(2) = 0.866$, $p = 0.649$; C. burnetii, $\chi^2(2) = 0.036$, $p = 0.982$; L. monocytogenes, $\chi^2(2) = 1.573$, $p = 0.455$; mixed infections, $\chi^2(2) = 0.604$, $p = 0.739$.

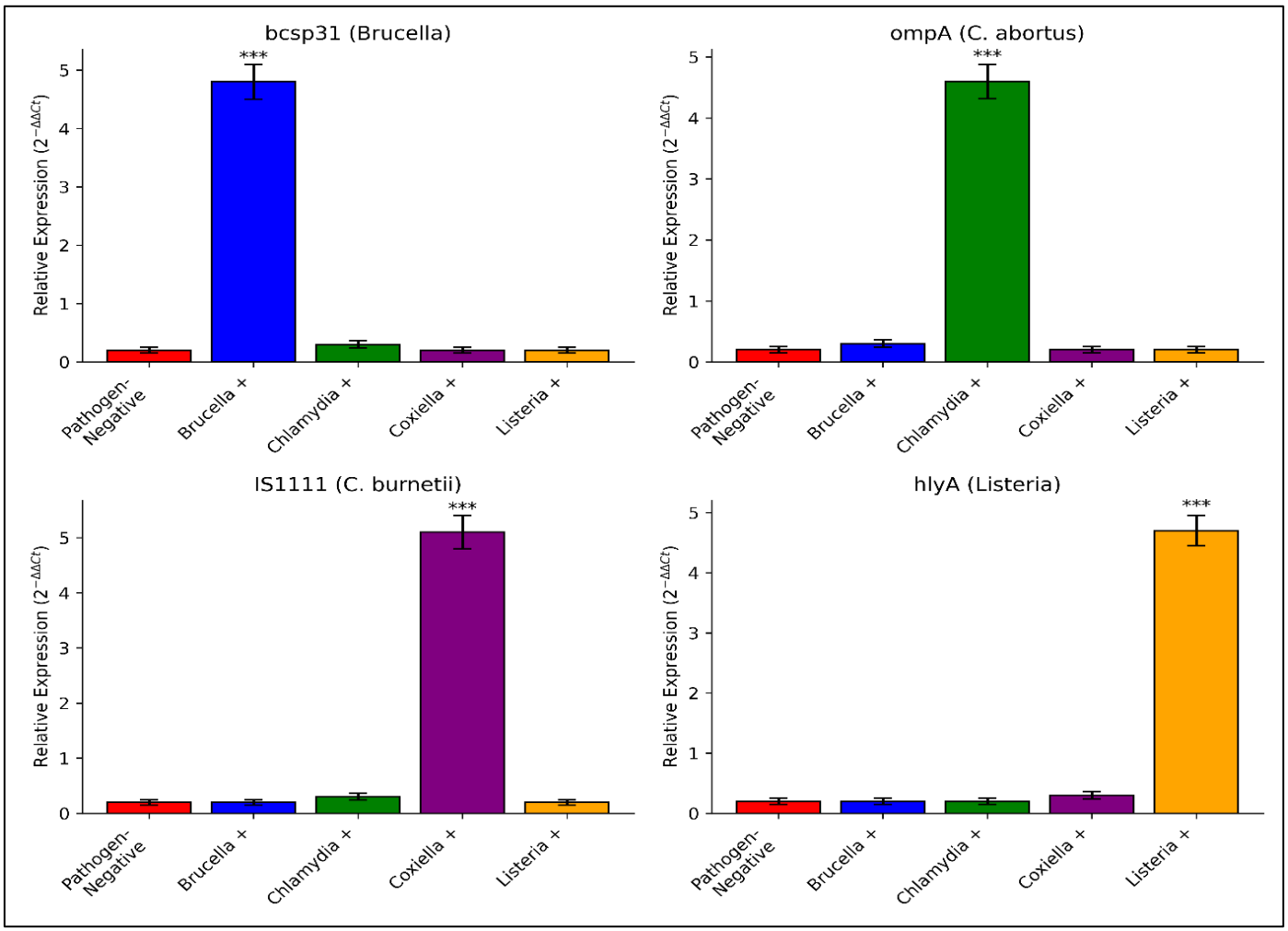

Fig. 1. Detection of virulence genes *bcsp31* (*Brucella*), *OmpA* (*C. abortus*), *IS111* (*C. burnetii*) and *hlya* (*Listeria*) using real-time PCR.

3.2. Differential expression of pro- and anti-inflammatory genes

Placental cytokine expression showed pathogen-specific patterns. Compared with pathogen-negative tissues, IL1B was higher in Coxiella-positive placentas (4.9 ± 0.20-fold, p = 0.001), lower in *Brucella*-positive (3.2 ± 0.25-fold, p = 0.030) and Listeria-positive placentas (1.6 ± 0.15-fold, p = 0.0005), and unchanged in *Chlamydia*-positive placentas (3.8 ± 0.30-fold, p = 0.400). IL6 was higher with *Brucella* (5.4 ± 0.25-fold, p = 0.020), *Chlamydia* (5.6 ± 0.20-fold, p = 0.008), and Coxiella (5.3 ± 0.20-fold, p = 0.015), but lower with *Listeria* (4.2 ± 0.15-fold, p = 0.040). TNF-α was higher with *Brucella* (5.8 ± 0.25-fold, p = 0.0008) and Coxiella (5.9 ± 0.30-fold, p = 0.0007), but lower with *Chlamydia* (1.3 ± 0.30-fold, p = 0.0005) and Listeria (1.7 ± 0.30-fold, p = 0.001). IL10 was lower with *Brucella* (2.9 ± 0.30-fold, p = 0.040) and *Chlamydia* (1.5 ± 0.30-fold, p = 0.0008), but higher with Coxiella (5.0 ± 0.25-fold, p = 0.010) and *Listeria* (5.7 ± 0.20-fold, p = 0.0005). Full standardized statistics and Spearman coefficients are provided in Table 4 (Figs. 2–5).

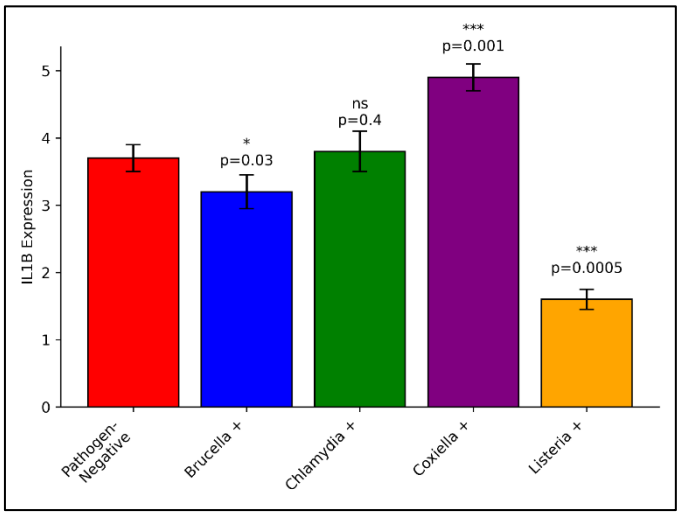

Fig. 2. *IL1B* expression levels across pathogen-positive and -negative placental tissues

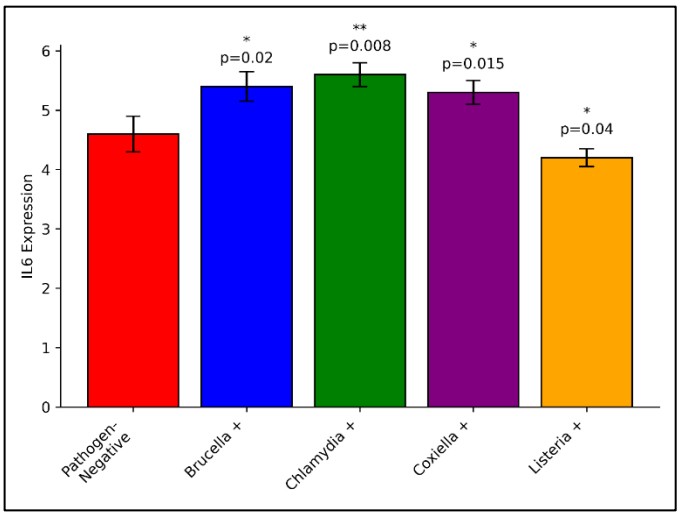

Fig. 3. *IL6* gene expression profiles in placentas infected with different pathogens

Table 4. Placental gene-expression comparisons and pathogen-status associations.

Gene	Pathogen group (n)	Mean ± SE	z	Exact p	Spearman ρ
IL1B	*Brucella* + (31)	3.2 ± 0.25	-2.170	0.030	-0.256
IL1B	*Chlamydia* + (20)	3.8 ± 0.30	0.842	0.400	0.110
IL1B	Coxiella + (33)	4.9 ± 0.20	3.291	0.001	0.375
IL1B	Listeria + (11)	1.6 ± 0.15	-3.481	0.0005	-0.466
IL6	*Brucella* + (31)	5.4 ± 0.25	2.326	0.020	0.274
IL6	*Chlamydia* + (20)	5.6 ± 0.20	2.652	0.008	0.337
IL6	Coxiella + (33)	5.3 ± 0.20	2.432	0.015	0.282
IL6	Listeria + (11)	4.2 ± 0.15	-2.054	0.040	-0.286
TNF-α	*Brucella* + (31)	5.8 ± 0.25	3.353	0.0008	0.386
TNF-α	*Chlamydia* + (20)	1.3 ± 0.30	-3.481	0.0005	-0.432
TNF-α	Coxiella + (33)	5.9 ± 0.30	3.390	0.0007	0.385
TNF-α	Listeria + (11)	1.7 ± 0.30	-3.291	0.001	-0.443
IL10	*Brucella* + (31)	2.9 ± 0.30	-2.054	0.040	-0.243
IL10	*Chlamydia* + (20)	1.5 ± 0.30	-3.353	0.0008	-0.418
IL10	Coxiella + (33)	5.0 ± 0.25	2.576	0.010	0.298
IL10	Listeria + (11)	5.7 ± 0.20	3.481	0.0005	0.466
VEGFA	*Brucella* + (31)	5.0 ± 0.20	3.320	0.0009	0.383
VEGFA	*Chlamydia* + (20)	1.1 ± 0.20	-3.540	0.0004	-0.439
VEGFA	Coxiella + (33)	3.3 ± 0.40	1.881	0.060	0.220
VEGFA	Listeria + (11)	3.6 ± 0.25	2.326	0.020	0.322
PLAC1	*Brucella* + (31)	3.7 ± 0.25	-2.054	0.040	-0.243
PLAC1	*Chlamydia* + (20)	5.2 ± 0.20	3.291	0.001	0.411
PLAC1	Coxiella + (33)	4.9 ± 0.20	2.968	0.003	0.340
PLAC1	Listeria + (11)	3.1 ± 0.20	-2.326	0.020	-0.322

Values are fold change (2−ΔΔCt). The pathogen-negative group contained 41 cases. Negative-group means ± SE were IL1B, 3.7 ± 0.20; IL6, 4.6 ± 0.30; TNF-α, 4.0 ± 0.30; IL10, 3.7 ± 0.30; VEGFA, 3.1 ± 0.15; and PLAC1, 4.2 ± 0.30. z is the standardized two-sided Mann–Whitney statistic; ρ is the Spearman coefficient for the corresponding pathogen-positive versus pathogen-negative comparison. Exact p values are two-sided.

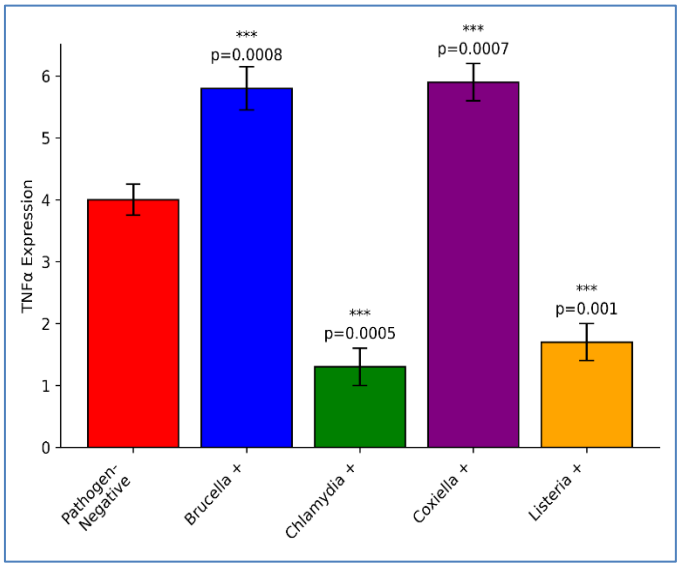

Fig. 4. TNF-α expression variations among pathogen groups compared with negative controls.

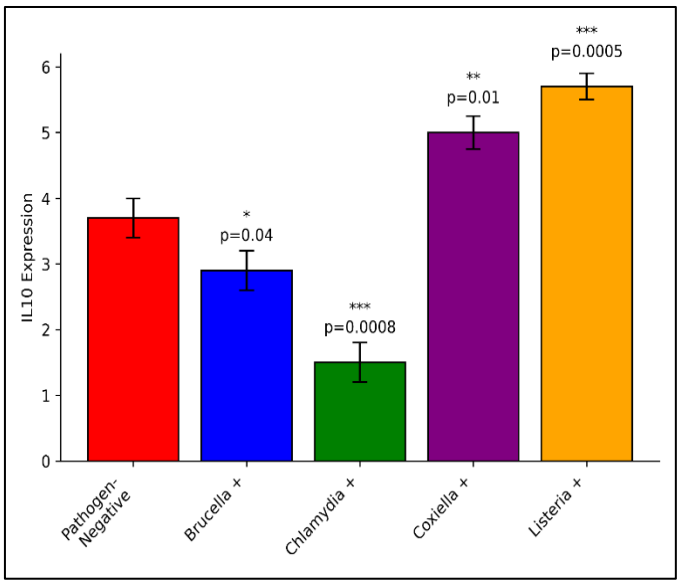

Fig. 5. *IL-10* anti-inflammatory gene expression across infection categories in placental samples.

3.3. Defensive Genes Against Pathogens and Placental Tissue Integrity

VEGFA and PLAC1 also showed pathogen-specific changes rather than uniform suppression. VEGFA was higher in *Brucella*-positive (5.0 ± 0.20-fold, p = 0.0009) and Listeria-positive placentas (3.6 ± 0.25-fold, p = 0.020), lower in *Chlamydia*-positive placentas (1.1 ± 0.20-fold, p = 0.0004), and not significantly changed in Coxiella-positive placentas (3.3 ± 0.40-fold, p = 0.060). PLAC1 was lower with *Brucella* (3.7 ± 0.25-fold, p = 0.040) and Listeria (3.1 ± 0.20-fold, p = 0.020), but higher with *Chlamydia* (5.2 ± 0.20-fold, p = 0.001) and Coxiella (4.9 ± 0.20-fold, p = 0.003). These results indicate pathogen-dependent alterations in placental angiogenic and trophoblast-maintenance pathways (Table 4; Figs. 6–7).

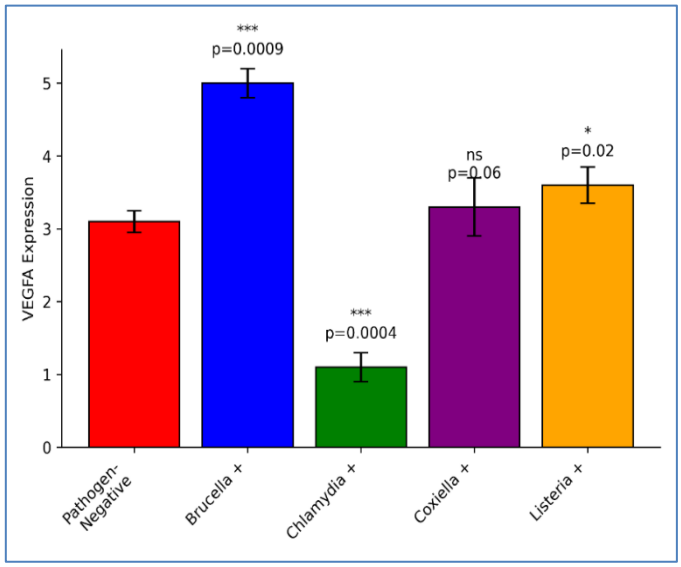

Fig. 6. Expression *of VEGFA* angiogenesis-related genes in relation to pathogen status

4. DISCUSSION

The findings demonstrated pathogen-specific inflammatory and trophoblast signatures rather than one uniform placental response. Coxiella-positive placentas showed increased IL1B and TNF-α, whereas *Brucella*-positive placentas showed increased IL6, TNF-α, and VEGFA together with reduced IL10 and PLAC1. *Chlamydia* positivity was associated with increased IL6 and PLAC1 but reduced TNF-α, IL10, and VEGFA. Listeria positivity was associated with reduced IL1B, IL6, TNF-α, and PLAC1 but increased IL10 and VEGFA. This heterogeneity is consistent with infection-associated activation of inflammatory pathways and altered trophoblast-gene regulation described in placental studies [21, 22, 23].

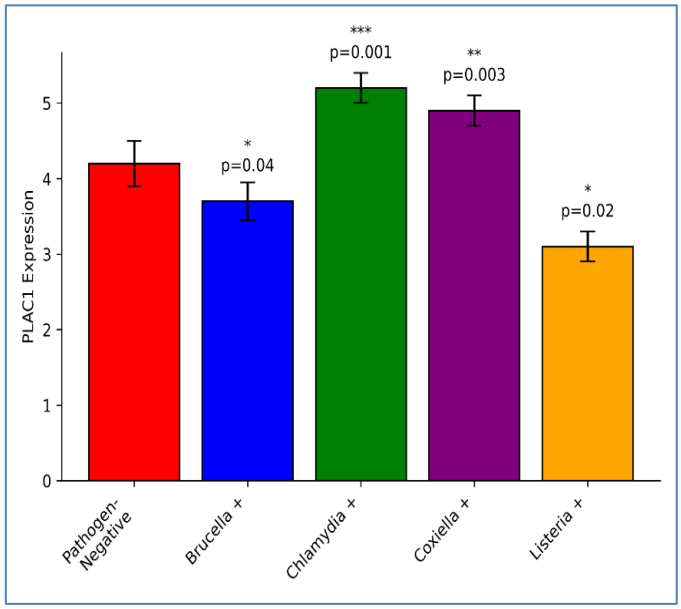

Fig. 7. Expression *of the PLAC1* trophoblast-integrity gene across pathogen-positive groups and controls

Although *Leptospira interrogans* was not tested in the present study, the pathogen-specific pattern-recognition-receptor responses described in previous studies provide only an indirect mechanistic comparison and were not used as evidence for any of the four bacterial agents examined here [24]. The present associations support disruption of the balance between pathogen recognition, cytokine regulation, angiogenesis, and trophoblast maintenance, but they do not establish a single common pathway for pregnancy loss.

The pathogen-detected RT-PCR results should be explained with caution. The presence of pathogen DNA does not mean that it was a cause of the abortion in field investigations. Co-infections may complicate identification of the primary etiological agent. Cross-pathogen abortion literature was considered only as contextual evidence and not as a direct comparator. Protozoal infections can also produce placental inflammation, impaired angiogenesis, trophoblast injury, and fetal loss [25, 26], but those organisms were not tested here and cannot support pathogen-specific conclusions. The bacterial findings were most directly aligned with reports that *C. burnetii*, *Brucella* spp., C. abortus, and *L. monocytogenes* can produce reproductive pathology and zoonotic exposure through infected placental material [2, 4, 27, 28]. The current analysis observed an association between bacterial-target detection and placental gene expression; however, the cross-sectional field design did not permit causal inference or identification of the primary etiological agent in mixed infections. Mechanistic and longitudinal studies are therefore required to establish causality [29]. A study examined vertical transmission by a viral pathogen, specifically bluetongue virus; this was a cross-pathogen comparison and not direct evidence for the bacterial agents tested here. Likewise, the reproductive effects of mastitis-associated systemic inflammation provide general inflammatory context rather than organism-specific support [7, 30]. The practical implication of these findings is therefore integrated surveillance and biosafety for *Brucella* spp., C. abortus, C. burnetii, and *L. monocytogenes* during ruminant abortion events.

5. CONCLUSION

This study showed that detection of abortifacient bacterial targets in ruminant placentas was associated with pathogen-specific changes in inflammatory, angiogenic, and trophoblast-related gene expression. *Brucella*- and Coxiella-positive tissues showed the clearest increases in selected pro-inflammatory genes, whereas IL10 was reduced in *Brucella*- and *Chlamydia*-positive placentas. VEGFA and PLAC1 changed in different directions by pathogen, indicating that placental vascular and trophoblast responses were not uniformly suppressed. These associations do not establish causality, particularly in mixed infections, but they support combining molecular pathogen detection with placental host-response profiling. Integrated One Health surveillance, abortion-event biosafety, and targeted confirmation of *Brucella* spp., *C. abortus*, *C. burnetii*, and *L. monocytogenes* remain essential for limiting reproductive losses and zoonotic exposure.

Acknowledgments

At this stage, we would like to thank the dean of the College of Veterinary Medicine at the University of Al-Qadisiyah and the staff of the laboratories for their support throughout the project.

Funding information

This work received no specific grant from any funding agency.

Conflict of interest

The authors declare no conflicts of interest.

Ethical Approval

The Scientific Research Ethics Committee of the College of Veterinary Medicine at the University of Al-Qadisiyah reviewed the study protocol. The authors submitted a scanned copy of the signed official letter from the dean of the College of Veterinary Medicine, University of Al-Qadisiyah (**No. 3089**), supporting the statement regarding ethical approval, and a copy of this letter is saved in the archive of the *World Journal of Experimental Biosciences*. The Committee determined that formal animal ethics approval was not required because the study involved only placenta, fetal membranes, and uterine discharge collected following naturally occurring abortions. No experimental procedures, animal handling, manipulation, treatment, or euthanasia were performed for this research. Permission to collect samples was obtained from the farm owners prior to sampling. All procedures were conducted in accordance with the institutional guidelines for animal welfare and the principles of the World Organization for Animal Health (WOAH) Terrestrial Animal Health Code.

Author contributions

Ghanim KG. Conceptualization; Investigation; Methodology; Data curation; and Writing – original draft.

Jassim AI. Investigation; Methodology; Formal analysis; Validation; Visualization; and Writing – review and editing.

Bneed QZ. Resources; Supervision; Project administration; Validation; and Writing – review and editing.

Yaseen MM. Conceptualization; Methodology; Supervision; Project administration; Writing – original draft; and Writing – review and editing.

All authors reviewed and approved the final manuscript and agreed to be accountable for all aspects of the work.

AI Declaration

During the preparation of this work, the authors used ChatGPT (GPT-5.6; OpenAI, 2026) to improve language, readability, and formatting. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Data availability

Data will be made available on request.

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