

Research article

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

Kamal Ghazi Ghanim¹, Ali Ismail Jassim¹, Qasim Zamel Bneed², Mohammed Mahdi Yaseen^{3*}

ABSTRACT

Abortion in livestock results in high reproductive losses and poses zoonotic risks. *Brucella*, *Chlamydia abortus*, *Coxiella burnetii*, 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 *bcs31*, *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 $\Delta\Delta C_t$ 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.

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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.

* Correspondence: Dr. Mohammed Mahdi Yaseen. E-mail: mohammed.yaseen@qu.edu.iq

Department of Public Health, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq.

Full list of author information is available at the end of the article.

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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, $\alpha = 0.05$, 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 RNeasy Lysis Solution (Qiagen®) 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 WOAHS 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 μ L of ATL buffer and 20 μ L 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 μ L of AL buffer and 200 μ L 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 μ L, 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 μ L 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 μ g of total RNA, and in a final volume of 20 μ L. 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* (*bcs31*), *Chlamydia abortus* (*OmpA*), *Coxiella burnetii* (*IS1111*), and *Listeria monocytogenes* (*hlyA*). Each reaction was performed in a final volume of 20 μ L consisting of 10 μ L of 2× SYBR Green Master Mix (Thermo Fisher Scientific, USA), 1 μ L of each primer (10 pmol/ μ L, Macrogen®, South Korea—custom synthesis), 2 μ L 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 *bcs31*, *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
<i>Brucella</i> spp. – <i>bcs31</i>	F	GCTCGGTTGCCAATATCAA TGC	151	[11, 12, 13]
	R	GGGTAAAGCGTCGCCAGA AG		
<i>Chlamydia</i> <i>abortus</i> – <i>ompA</i>	F	GCAACTGACACTAAGTCGG CTACA	82	[14, 15]
	R	ACAAGCATGTTCAATCGAT		
<i>Coxiella</i> <i>burnetii</i> – IS1111	F	GTCTTAAGGTGGGCTGCGT G	295	[16]
	R	CCCCGAATCTCATTGATCA GC		
<i>Listeria</i> <i>monocytogenes</i> – <i>hlyA</i>	F	TGCAAGTCCTAAGACGCCA	113	[17]
	R	CACTGCATCTCCGTGGTAT ACTAA		

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 μ L reaction contained 10 μ L PowerUp™ SYBR Green Master Mix (Applied Biosystems™, USA; Cat No: A25742), 0.5 μ L of each primer (10 pmol/ μ L; MacroGen®, South Korea), 2 μ L cDNA, and 7 μ L 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- $\Delta\Delta$ Ct 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	ACCTTCATTGCCAGGTTTCT	120	[18]
	Reverse	CTGTTTAGGGTCATCAGCCTC AA		
IL6	Forward	TGAGTGTGAAAGCAGCAAGGA	137	[18]
	Reverse	TACTCCAGAAGACCAGCAGTG G		
TNF- α	Forward	TAACAAGCCGGTAGCCACG	277	[18]
	Reverse	GCAAGGGCTCTTGATGGCAGA		
IL10	Forward	TGCTGGATGACTTTAAGGG	186	[18]
	Reverse	AGGGCAGAAAGCGATGACA		
VEGFA	Forward	CAACCTCACAAAGCCAGC	186	[19]
	Reverse	CGCGAGTCTGTGTTTTGCA		
PLAC1 (placental-specific 1)	Forward (sense)	GTGAGCACAAAGCCACATTC	118	[20]
	Reverse (antisense)	GCAGCCAATCAGATAATGAAC C		
GAPDH	Forward	GGGATGAGGCTCAGAGCAAG AGA	118	[19]
	Reverse	AGCTCGTTGTAGAAGGTGTGG TGCC		

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- $\Delta\Delta$ Ct 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- $\Delta\Delta$ Ct 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 $\pm$ 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	<i>Brucella</i> Positive n (%)	<i>Chlamydia abortus</i> Positive n (%)	<i>Coxiella burnetii</i> Positive n (%)	<i>Listeria monocytogenes</i> 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, $\chi^2(2) = 0.604$, $p = 0.739$.

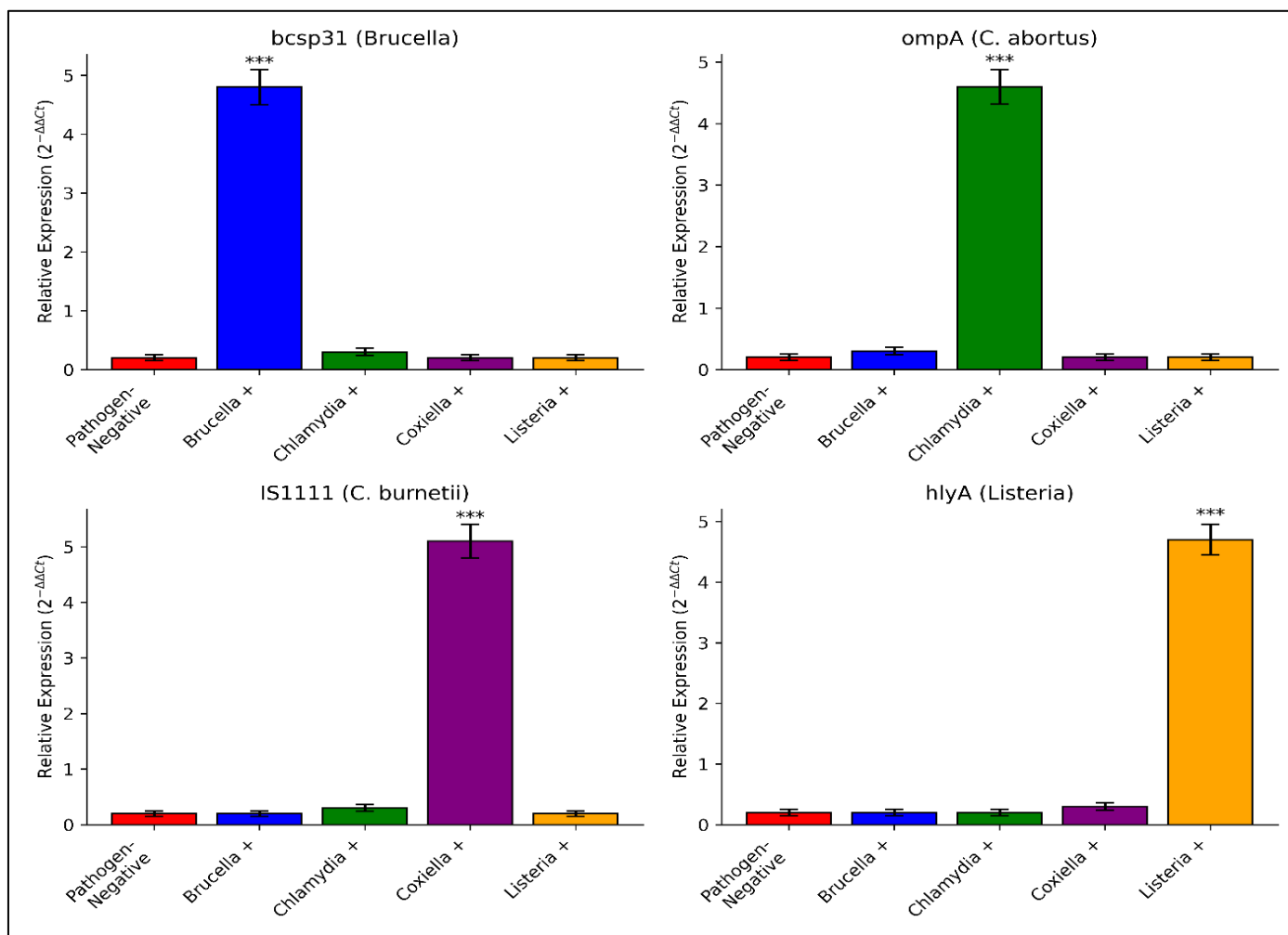


Fig. 1. Detection of virulence genes *bcs31* (*Brucella*), *OmpA* (*C. abortus*), *IS1111* (*C. burnetii*) and *hlyA* (*Listeria*) using real-time PCR.

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, $p = 0.400$).

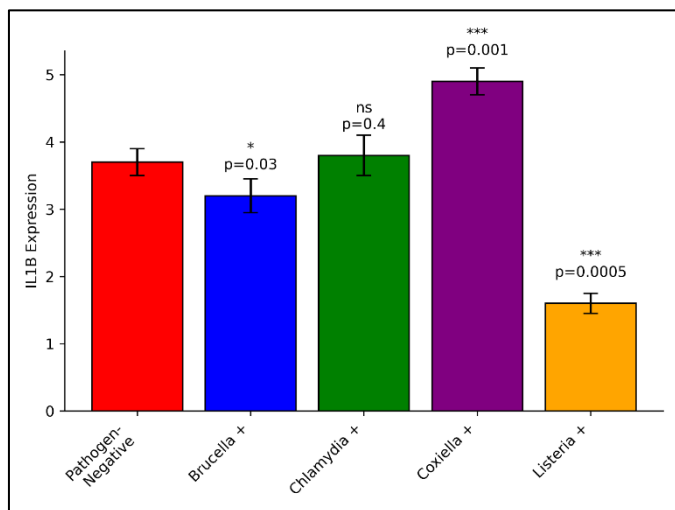


Fig. 2. IL1B expression levels across pathogen-positive and -negative placental tissues

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).

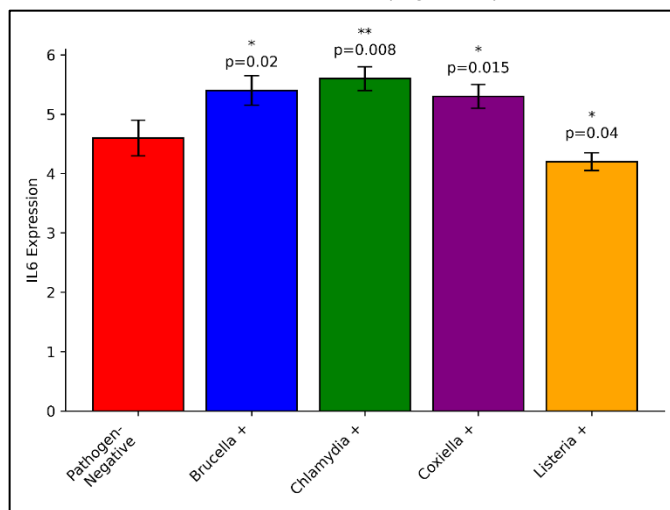


Fig. 3. IL6 gene expression profiles in placentas infected with different pathogens

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

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

Values are fold change ($2^{-\Delta\Delta Ct}$). The pathogen-negative group contained 41 cases. Negative-group means $\pm$ 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; p is the Spearman coefficient for the corresponding pathogen-positive versus pathogen-negative comparison. Exact p values are two-sided.

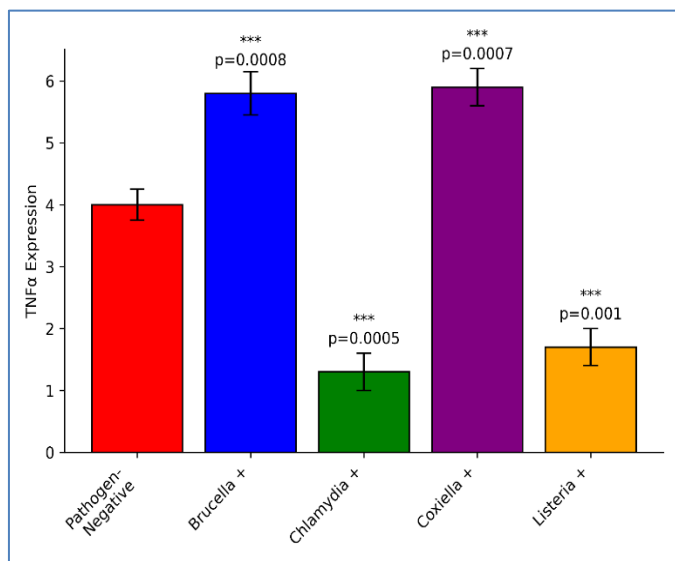


Fig. 4. TNF- α expression variations among pathogen groups compared with negative controls.

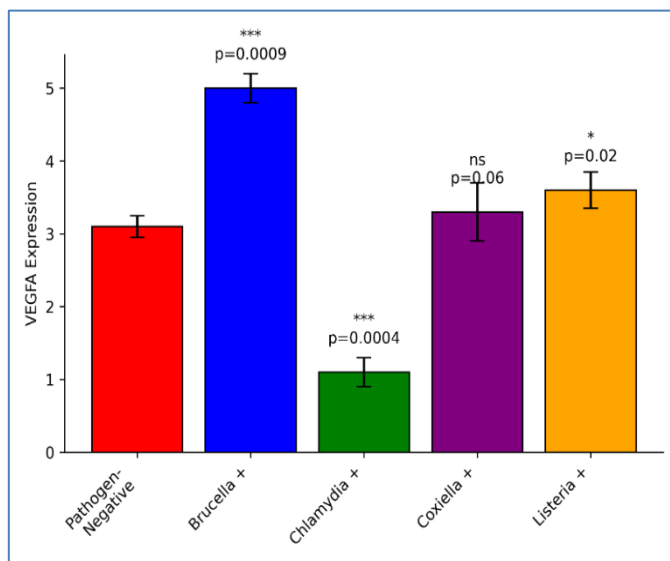


Fig. 6. Expression of VEGFA angiogenesis-related genes in relation to pathogen status

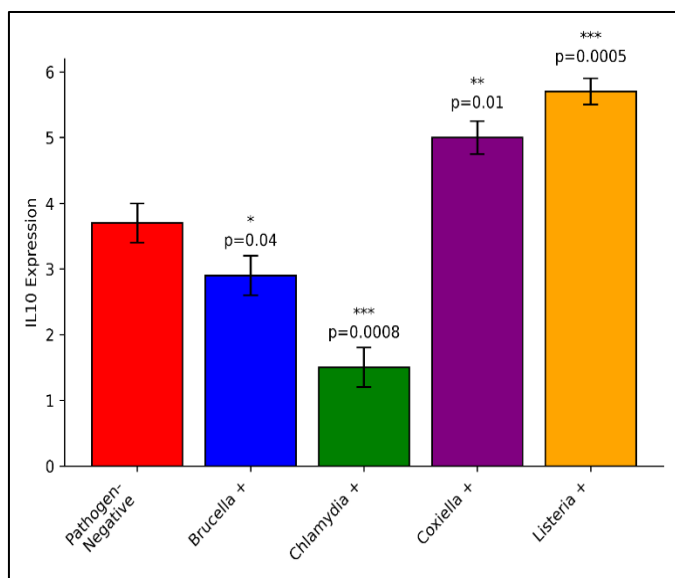


Fig. 5. IL-10 anti-inflammatory gene expression across infection categories in placental samples.

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).

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].

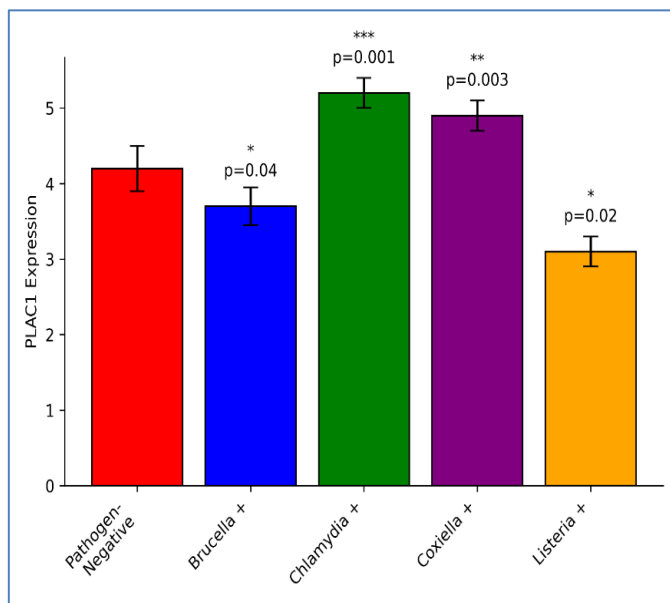


Fig. 7. Expression of the PLAC1 trophoblast-integrity gene across pathogen-positive groups and controls

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.

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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.

6. REFERENCES

- [1] Amagliani G, La Guardia ME, Dominici S, Brandi G, Omiccioli E. (2021) Salmonella Abortusovis: An epidemiologically relevant pathogen. *Curr Microbiol* 79:3. doi: [10.1007/s00284-021-02689-1](https://doi.org/10.1007/s00284-021-02689-1). PMID: [34878615](https://pubmed.ncbi.nlm.nih.gov/34878615/).
- [2] Rossetti CA, Maurizio E, Rossi UA. (2022) Comparative Review of Brucellosis in Small Domestic Ruminants. *Front Vet Sci* 9:887671. doi: [10.3389/fvets.2022.887671](https://doi.org/10.3389/fvets.2022.887671). PMID: [35647101](https://pubmed.ncbi.nlm.nih.gov/35647101/).
- [3] Semango GP, Buza J. (2024) Review of the Current Status on Ruminant Abortigenic Pathogen Surveillance in Africa and Asia. *Vet Sci* 11: 425. doi: [10.3390/vetsci11090425](https://doi.org/10.3390/vetsci11090425). PMID: [39330804](https://pubmed.ncbi.nlm.nih.gov/39330804/).
- [4] Rossi F, Giaccone V, Colavita G, Amadoro C, Pomilio F, Catellani P. (2022) Virulence Characteristics and Distribution of the Pathogen *Listeria ivanovii* in the Environment and in Food. *Microorganisms* 10: 1679. doi: [10.3390/microorganisms10081679](https://doi.org/10.3390/microorganisms10081679). PMID: [36014096](https://pubmed.ncbi.nlm.nih.gov/36014096/).
- [5] Končurát A, Sukalić T. (2024) Listeriosis: Characteristics, Occurrence in Domestic Animals, Public Health Significance, Surveillance and Control. *Microorganisms* 12:2055. doi: [10.3390/microorganisms12102055](https://doi.org/10.3390/microorganisms12102055). PMID: [39458364](https://pubmed.ncbi.nlm.nih.gov/39458364/).
- [6] Ebani VV. (2023) *Coxiella burnetii* Infection in Cats. *Pathogens (Basel, Switzerland)* 12:1415. doi: [10.3390/pathogens12121415](https://doi.org/10.3390/pathogens12121415). PMID: [38133298](https://pubmed.ncbi.nlm.nih.gov/38133298/).
- [7] Plummer PJ, McClure JT, Menzies P, Morley PS, Van den Brom R, Van Metre DC. (2018) Management of *Coxiella burnetii* infection in

- livestock populations and the associated zoonotic risk: A consensus statement. *J Vet Intern Med* **32**:1481–1494. doi: [10.1111/jvim.15229](https://doi.org/10.1111/jvim.15229). PMID: [30084178](https://pubmed.ncbi.nlm.nih.gov/30084178/).
- [8] Loy JD, Clawson ML, Adkins PRF, Middleton JR. (2023) Current and emerging diagnostic approaches to bacterial diseases of ruminants. *Vet Clin North Am Food Anim Pract* **39**: 93–114. doi: [10.1016/j.cvfa.2022.10.006](https://doi.org/10.1016/j.cvfa.2022.10.006). PMID: [36732002](https://pubmed.ncbi.nlm.nih.gov/36732002/).
- [9] Orjuela AG, Parra-Arango JL, Sarmiento-Rubiano LA. (2022) Bovine leptospirosis: effects on reproduction and an approach to research in Colombia. *Trop Anim Health Prod* **54**:251. doi: [10.1007/s11250-022-03235-2](https://doi.org/10.1007/s11250-022-03235-2). PMID: [35943610](https://pubmed.ncbi.nlm.nih.gov/35943610/).
- [10] Faul F, Erdfelder E, Buchner A, Lang AG. (2009) Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods* **41**: 1149–1160. doi: [10.3758/BRM.41.4.1149](https://doi.org/10.3758/BRM.41.4.1149). PMID: [19897823](https://pubmed.ncbi.nlm.nih.gov/19897823/).
- [11] Shell WS, Abdullah FFJ, Zamri-Saad M, Haron AW, Saad N, Bejo SK. (2017) Using real-time polymerase chain reaction as an alternative for colony counting of living *Brucella* vaccines. *Vet World* **10**: 630–637. doi: [10.14202/vetworld.2017.610-615](https://doi.org/10.14202/vetworld.2017.610-615). PMID: [28717311](https://pubmed.ncbi.nlm.nih.gov/28717311/).
- [12] Gwida MM, El-Gohary AH, Melzer F, Tomaso H, Rösler U, et al. (2011). Comparison of diagnostic tests for the detection of *Brucella* spp. in camel sera. *BMC Res Notes* **4**:525. doi: [10.1186/1756-0500-4-525](https://doi.org/10.1186/1756-0500-4-525). PMID: [22145943](https://pubmed.ncbi.nlm.nih.gov/22145943/).
- [13] Kumari R, Singh S, Behera B, Singh D, Kaur P, Kumar R. (2023) Human brucellosis: An observational study from a tertiary-care centre in North India. *Cureus*, **15**:e42980. doi: [10.7759/cureus.42980](https://doi.org/10.7759/cureus.42980). PMID: [37671228](https://pubmed.ncbi.nlm.nih.gov/37671228/).
- [14] Pantchev A, Sting R, Bauerfeind R, Tyczka J, Sachse K. (2009) New real-time PCR tests for species-specific detection of Chlamydia psittaci and Chlamydia abortus from tissue samples. *Vet J* **181**:145–150. doi: [10.1016/j.tvjl.2008.02.025](https://doi.org/10.1016/j.tvjl.2008.02.025). PMID: [18413292](https://pubmed.ncbi.nlm.nih.gov/18413292/).
- [15] Esmaili H, Hamed M, Madani SA, Barin A, Haji Agha Khiyabani F. (2024). Diagnosis of Chlamydia abortus using real-time PCR and cell culture in small ruminants with abortion. *J Med Bacteriol* **12**:9–16. doi: [10.18502/jmb.v12i4.17003](https://doi.org/10.18502/jmb.v12i4.17003).
- [16] Klee SR, Tyczka J, Ellerbrok H, Franz T, Linke S, et al. (2006) Highly sensitive real-time PCR for specific detection and quantification of *Coxiella burnetii*. *BMC Microbiol* **6**:2. doi: [10.1186/1471-2180-6-2](https://doi.org/10.1186/1471-2180-6-2). PMID: [16423303](https://pubmed.ncbi.nlm.nih.gov/16423303/).
- [17] Mendonça M, Conrad NL, Conceição FR, Moreira ÂN, da Silva WP, et al. (2012) Highly specific fiber optic immunosensor coupled with immunomagnetic separation for detection of low levels of *Listeria monocytogenes* and *L. ivanovii*. *BMC Microbiol* **12**:275. doi: [10.1186/1471-2180-12-275](https://doi.org/10.1186/1471-2180-12-275). PMID: [23176167](https://pubmed.ncbi.nlm.nih.gov/23176167/).
- [18] Yamada S, Yoshioka K, Mizuguchi H, Ohta M, Mikami T. (2009) Quantitative analysis of cytokine mRNA expression in calves experimentally infected with *Theileria parva*. *J Vet Med Sci* **71**: 49–55. doi: [10.1292/jvms.71.49](https://doi.org/10.1292/jvms.71.49). PMID: [19194076](https://pubmed.ncbi.nlm.nih.gov/19194076/).
- [19] Lopera-Vásquez R, Uribe-García F, Rondón-Barragán I. (2022) Effect of estrous cycle phases on gene expression in bovine oviduct epithelial cells. *Vet World* **15**:1665–1675. doi: [10.14202/vetworld.2022.1665-1675](https://doi.org/10.14202/vetworld.2022.1665-1675). PMID: [36185535](https://pubmed.ncbi.nlm.nih.gov/36185535/).
- [20] Gholami P, Asgarian-Omran H, Yaghmaei M, Mahmoudian J, Kianersi S, et al. (2023) Investigation of expression profile of placenta-specific 1 (PLAC1) in acute myeloid and lymphoid leukemias. *Avicenna J Med Biotechnol* **15**:167–172. doi: [10.18502/ajmb.v15i3.12926](https://doi.org/10.18502/ajmb.v15i3.12926). PMID: [37538244](https://pubmed.ncbi.nlm.nih.gov/37538244/).
- [21] Lesseur C, Jessel RH, Ohn S, Ma Y, Li Q, et al. (2022) Gestational SARS-CoV-2 infection is associated with placental expression of immune and trophoblast genes. *Placenta* **126**:125–132. doi: [10.1016/j.placenta.2022.06.017](https://doi.org/10.1016/j.placenta.2022.06.017). PMID: [35797939](https://pubmed.ncbi.nlm.nih.gov/35797939/).
- [22] Cappelletti M, Presicce P, Kallapur SG. (2020) Immunobiology of Acute Chorioamnionitis. *Front Immunol* **11**:649. doi: [10.3389/fimmu.2020.00649](https://doi.org/10.3389/fimmu.2020.00649). PMID: [32373122](https://pubmed.ncbi.nlm.nih.gov/32373122/).
- [23] do Imperio GE, Bloise E, Javam M, Lye P, Constantinof A, et al. (2018) Chorioamnionitis Induces a Specific Signature of Placental ABC Transporters Associated with an Increase of miR-331-5p in the Human Preterm Placenta. *Cell Physiol Biochem* **45**:591–604. doi: [10.1159/000487100](https://doi.org/10.1159/000487100). PMID: [29402780](https://pubmed.ncbi.nlm.nih.gov/29402780/).
- [24] Bonhomme D, Werts C. (2022) Host and Species-Specificities of Pattern Recognition Receptors Upon Infection With *Leptospira interrogans*. *Front Cell Infect Microbiol* **12**:932137. doi: [10.3389/fcimb.2022.932137](https://doi.org/10.3389/fcimb.2022.932137). PMID: [35937697](https://pubmed.ncbi.nlm.nih.gov/35937697/).
- [25] Dubey JP, Rosenthal BM. (2023) Bovine sarcocystosis: Sarcocystis species, diagnosis, prevalence, economic and public health considerations, and association of Sarcocystis species with eosinophilic myositis in cattle. *Int J Parasitol* **53**:463–475. doi: [10.1016/j.ijpara.2022.09.009](https://doi.org/10.1016/j.ijpara.2022.09.009). PMID: [36462560](https://pubmed.ncbi.nlm.nih.gov/36462560/).
- [26] Tirosh-Levy S, Savitsky I, Blinder E, Mazuz ML. (2022) The involvement of protozoan parasites in sheep abortions - A ten-year review of diagnostic results. *Vet Parasitol* **303**:109664. doi: [10.1016/j.vetpar.2022.109664](https://doi.org/10.1016/j.vetpar.2022.109664). PMID: [35131575](https://pubmed.ncbi.nlm.nih.gov/35131575/).
- [27] Devaux CA, Osman IO, Million M, Raoult D. (2020) *Coxiella burnetii* in Dromedary Camels (*Camelus dromedarius*): A Possible Threat for Humans and Livestock in North Africa and the Near and Middle East?. *Front Vet Sci* **7**:558481. doi: [10.3389/fvets.2020.558481](https://doi.org/10.3389/fvets.2020.558481). PMID: [33251255](https://pubmed.ncbi.nlm.nih.gov/33251255/).
- [28] Bayne JE, Waters KM. (2025) Biosecurity for Reproductive Disease Prevention in Sheep and Goats. *Vet Clin North Am Food Anim Pract* **41**:71–82. doi: [10.1016/j.cvfa.2024.11.009](https://doi.org/10.1016/j.cvfa.2024.11.009). PMID: [39741070](https://pubmed.ncbi.nlm.nih.gov/39741070/).
- [29] Rojas JM, Martín V, Sevilla N. (2021) Vaccination as a Strategy to Prevent Bluetongue Virus Vertical Transmission. *Pathogens (Basel, Switzerland)* **10**:1528. doi: [10.3390/pathogens10111528](https://doi.org/10.3390/pathogens10111528). PMID: [34832683](https://pubmed.ncbi.nlm.nih.gov/34832683/).
- [30] Wang, N., Zhou, C., Basang, W., Zhu, Y., Wang, X., Li, C., Chen, L., & Zhou, X. (2021) Mechanisms by which mastitis affects reproduction in dairy cow: A review. *Reprod Domest Anim* **56**:1165–1175. doi: [10.1111/rda.13953](https://doi.org/10.1111/rda.13953). PMID: [34008236](https://pubmed.ncbi.nlm.nih.gov/34008236/).

Author affiliation

1. Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq.
2. Department Medical Biotechnology, College of Biotechnology, University of AL-Qadisiyah, Iraq.
3. Department of Public Health, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq

ORCID IDs & Email:

- Ghanim KG: <https://orcid.org/0009-0002-2953-5134>
Kamal.alshamari@qu.edu.iq
- Jassim Al: <https://orcid.org/0000-0002-0583-9978>
Ali.ismail@qu.edu.iq
- Bneed QZ: <https://orcid.org/0009-0005-6606-5848>
qasim.bneed@qu.edu.iq
- Yaseen MM: <https://orcid.org/0000-0003-4285-5739>
mohammed.yaseen@qu.edu.iq