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Original Article

Molecular identifications of co-infected pathogens in aborted sheep in Garmian administration, Iraq

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ABSTRACT

Background: Abortion is one of the main factors of large financial losses in the worldwide livestock sector due to infectious and non-infectious causes.

Objective: It was conducted to estimate the molecular identification of co-infections of ovine abortions caused by *Brucella* spp., *Coxiella burnetii* (*C. burnetii*), *Mycoplasma* spp., *Listeria monocytogenes* (*L. monocytogenes*), *Chlamydia abortus* (*C. abortus*), *Leptospira* spp., *Campylobacter* spp., *Salmonella* spp., and *Toxoplasma gondii* (*T. gondii*) in aborted ewes.

Materials and Methods: Fifty blood samples were attempted directly from the jugular veins of aborted ewes (n=50) from 23 flocks in Garmian Administrations (GA), Kurdistan Region-Iraq, during August 2024 to January 2025. Polymerase chain reaction (PCR) was used to analyze the blood samples collected from 50 aborted ewes

Results: It was concluded that *Brucella* spp. (90 %) was the main cause of the abortions, followed by *Mycoplasma* spp with 72%, and *C. burnetii* with 62%. Other pathogens were not detected in any of the specimens investigated. Mixed infections with *Brucella spp*., *Mycoplasma* spp., and *C. burnetii* were observed in 42% of the specimens examined.

Conclusions: Brucellosis was the most prominent outcome associated with ewe abortions in the Garmian administration, Iraq. This study also confirmed the co-occurrence of *Brucella* spp., *C. burnetii*, and *Mycoplasma* spp. infections in aborted sheep. For the first time, the *Mycoplasma* spp. was found as a causative agent of ovine abortion in Kurdistan region by using PCR test, and no one mentioned this agent.

Keywords: Ovine abortion, abortifacient pathogens, PCR test, Garmian administration, Kurdistan region, Iraq.

1 Introduction

Abortion is the termination of pregnancy at any stage of gestation caused by infectious agents and less likely non-infectious causes and a significant problem in sheep farming [1]. Abortion in small ruminants is mostly caused by bacterial, protozoan, and viral infections [2]. Major financial losses and a serious risk to the public's health may arise from animal abortions [3]. Both direct and indirect costs are linked to economic losses. The indirect costs are associated with longer lambing intervals, which typically result in higher food costs, lower sales proceeds from mutton sales, fewer available replacement lambs, and higher veterinarian care [4]. The typical rate of abortions in sheep farms is usually less than 2% [1]. Moderate abortion rates of 2%–5% may indicate the presence of endemic diseases and usually not an epidemic of a new pathogen. Abortion rates exceeding 5 % usually indicate a critical outbreak, thus requiring investigation and, if possible, veterinary intervention [1].

Furthermore, investigations of the etiological agents of abortions play a crucial role in the subsequent mitigation and control strategies [5]. Traditionally, the aborted fetus and placenta are the focus of abortion diagnostic tests [6]. Furthermore, there are numerous tests for the diagnosis of etiological agents of infectious abortion in ewes. Reliable methods for identifying the agents are molecular approaches [7]. On the other hand, except infectious agents, non-infectious causes like poisoning, diet deficiencies, and extreme weather conditions also lead to abortion in sheep [8- 17]. Furthermore, in the worldwide livestock sector, abortion is one of the main reasons for significant economic losses [17]. Data collection is important to reaching an accurate investigation (e.g., location of the farm, stage of pregnancy at the time of abortion, age, vaccination history, animal movements, introduction of new animals, previous abortion problems,

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nutrition management and feeding changes, drugs administration, exposure to toxins or teratogenic plants, heat stress or stressful events like predators on the farm, illness during pregnancy) [1]. Most abortifacient agents are zoonotic pathogens that can also pose a risk to breeders, animal keepers or veterinarians [18]. Indeed, these pathogens can cause both abortions in women [19] and epidemic outbreaks in populations at risk [1,20]. Furthermore, there are several infectious agents which are known to cause abortions in small ruminants. The most common causes are *Brucella* spp., *Coxiella burnetii*, *Mycoplasma* spp., *Listeria monocytogenes* (*L. monocytogenes*), *Chlamydia abortus*, *Leptospira* spp., *Campylobacter jejuni*, *Campylobacter fetus* subsp. fetus (*C. fetus*), *Salmonella Abortusovis* (*S. abortusovis*) , *Toxoplasma gondii* (*T. gondii*), Border disease virus and Bluetongue virus [1,21,22]. In addition, *Mycoplasma agalactiae* pathogen leads to contagious agalactia, which can cause abortion in sheep [23,24].

The global spread of these abortifacient pathogens may differ depending on many factors, including geographical locations, climate, management techniques, flock size, vaccination, eradication strategies, and nutritional factors [25,26]. The current study aimed to investigate the most common infectious agents of ovine abortion and its risk factors among flocks of sheep.

2 Materials and Methods

2. 1 Geographical background of the study area

The research was conducted in the Garmian area, Sulaymaniyah province, Kurdistan Region of Iraq, as shown in the map (Figure 1). The region has a hot and dry climate, located in the southeast Kurdistan region of Iraq, between the latitudes (34˚15–33 = - 35˚ 11–05 =) above the equator and the longitudes (44˚ 29–41 = - 45˚ 54–20 =) of the eastern hemisphere, with a total size of 6731.73 square kilometers and is composed of the districts of Kalar, Kifri, and Khanaqin [27].

2. 2 Study area and sample collection

Fifty blood samples were attempted directly from the jugular veins of aborted ewes (n=50) from 23 flocks during August 2024 to January 2025. The samples were collected in EDTA tubes and then they were promptly transported to the laboratory of Garmian Veterinary Private Hospital in a cold package and stored at -20°C until investigation. Using a standardized questionnaire, data were collected by face-to-face personal interview from the flock owners at 23 locations (Table 1). The animal owners were interviewed about the history of reproductive failure (abortion, stillbirth, neonatal loss and repeat breeding) in the flock, flock size and flock management risk factor attributes, grazing system, presence of dog and cat, feeding practices, ownership of other livestock species such as cattle and goats. Animal level risk factors such as breed, age and body condition were recorded during sample collections. Age was determined by dentition and body condition scoring was done by body parts examination according to the method described by [28]. The study was ethically approved by College of Medicine, University of Garmian, Kurdistan Region, Iraq. Consents were taken from the animal owners and the samples were handled by veterinarians.

Tabel 1: Details of blood sample collection according to location.

No. of Flocks	Location	Total samples
1	Majed salar	2
2	Hasan Prchin	3
3	Garmik	2
4	Bwaka	3
5	Hawaraqula	1
6	Kalar	8
7	Hassan Amna/ Zamawanga	2
8	Bawakr	3
9	Kani pamw	1
10	Twlabi	1
11	Pyazajar	3
12	Gomazard	2
13	Salah Agha	1
14	Zinana	1
15	Masolkan	2
16	Kawacharmw	3
17	Rizgari	4
18	Chwarshax	1
19	Zard	1
20	Hajiawal	1
21	Twran	1
22	Chwarklaw	3
23	Girdagozina	1
Total		50

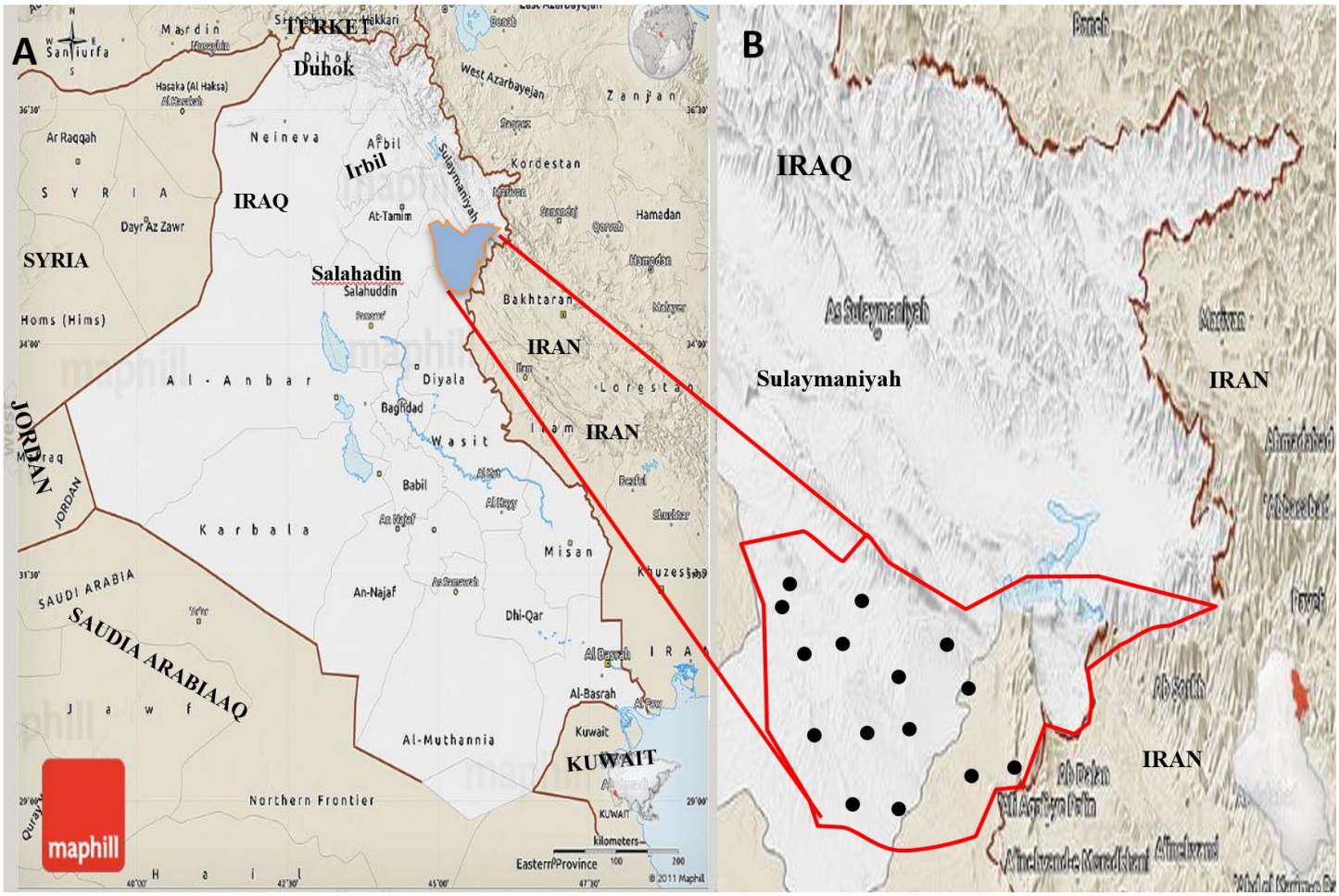


Figure 1: Geographical map of the research area around the Garmian region. (a, b) [29].

2. 3 DNA extraction

The DNA was extracted used (200 µl) of the whole blood samples with DNA extraction Kit (ADD BIO INC, daejeon, Republic of Korea) according to the manufacturer's instructions. The DNAs were eluted into 150 µl Elution buffer at room temperature where they were immediately PCRs performed.

2. 4 PCR amplification

Nine sets of primers were used in the present study (Table 2). The primers were purchased from Macrogen Company (Korea). All primers were checked in their NCBI Genbanks as shown in Supplementary materials S1, S2, S3, S4, S5, S6, S7, S8, and S9 showing alignment of the primers with the regions of the genes amplified.

The PCR mixture was prepared by adding 1.5 µl of molecular water, 5 µl of Addstart master mix (ADDBIO, Korea), 0.5 µl of each primers and 2.5 µl of DNA into a 0.2 ml PCR tube. The PCR reactions were as follows in a thermocyler (Bio-Rad C1000, Germany): Initial denaturation at 96 °C for 5 minutes, followed by 40 cycles of 95 °C for 30 seconds, annealing at 50-60 °C for 30 seconds and extention at 72 °C for 30 seconds. These were followed by final extension at 72 °C for 5 minutes.

Table 2: Details of all primers used in the study.

Names	Sequences	Gene	Size (bp)	Tm °C	causative agents	Ref
LepL32 F	ACTCTTTGCAAGCATTACCGC	LipL32 gene **JN886738.1**	660	58	*Leptospira* spp.	[30]
LepL32 R	AGCAGACCAACAGATGCAACG					
Bruc2 F	GTGCCAGCAGCCGCGGTAATAC	16srRNA AY513531.1	900-1000	52	*Brucella* spp.	[31]
Bruc2 R	TGGTGTGACGGGCGGTGTGTACAAG					
Toxo F	CAGGGAGGAAGACGAAAGTTG	Repeated region MG574979.1	522	60	*Toxoplasma gondii*	[32]
Toxo R	CAGACACAGTGCATCTGGATT					

Chlam F	GGGCTAGACACGTGAAACCTA	23S rRNA NR_077001.1	356	60	*Chlamydia* spp.	[33]
Chlam R	CCATGCTTCAACCTGGTCATAA					
Coxil2 F	GTCTTAAGGTGGGCTGCGTG	IS1111 gene MN025541.1	295	60	*Coxiella burnetii*	[34]
Coxil2 R	CCCCGAATCTCATTGATCAGC					
Campf1 F	GCTAAGGGTGAGGTTGATGGG	SAB gene AF048699.1	220	50	*Campyolobacter* spp.	[35]
Campf1 R	AAGTTATTCTTCAAATCTACC					
LisPrs F	GCTGAAGAGATTGCGAAAGAAG	Prs gene AF497228.1	370	53	*Listeria* spp.	[36]
LisPrs R	CAAAGAAACCTTGGATTTGCGG					
MycAll F	GCTGCGGTGAATACGTTCT	16s rRNA U02968.1	160	50	*Mycoplasma* spp.	[37]
MycAll R	TCCCCACGTTCTCGTAGGG					
Sal Inva2 F	ACAGTGCTCGTTTACGACCTGAAT	Inva gene M90846.1	244	60	*Salmonella* spp.	[38]
Sal Inva2 R	AGACGACTGGTACTGATCGATAAT					

2. 5 Gel electrophoresis

Agarose gel (1%) was prepared by adding 1 g of agarose powder (AddBio. Korea) into 100 ml of Tris Base EDTA (TBE) 1 x buffer (AddBio, Korea). The amplified PCR products were run on agarose gel electrophoresis.

2. 6 Gel purification

A gel purification kit (AddPrep Gel Purification Kit, AddBio, Korea) was used to purify the specific PCR product bands from the gel electrophoresis. The PCR products were excised from the gel and put in a 1.5 ml Eppendorf tube containing binding buffer. Following the manufacturer's procedure, using spin columns, centrifugations, washing buffers, the samples were ultimately eluted with the 50 µl elution buffer provide by the kit.

2. 7 DNA sequencing and analysis

The purified PCR products (20µl) were sent with either forward or reverse primers (10µM) for sequencing using the Sanger method on an ABI 3730 ×l DNA Analyzer (Macrogen Inc., Seoul, South Korea).

2. 8 Statistical Analysis

All data were analyzed using SPSS. The Chi-square test was employed to determine significant differences between infected species within and between the regions. Additionally, multiple correspondence analysis was used to clarify the relationship between categorical variables. The odds ratio (OR) and relative risks (RR) were calculated to identify positive cases within specific regions. These measurements were then compared against the evidence of the diseases. In addition, Mc Nemar test, and Haldane- Anscombe were used.

3 Results

Out of the 9 primers used for detecting the abortion agents, only *Brucella*, *Coxiell*a and *Mycoplasma* were positive in the blood of the animals while other pathogens including *Toxoplasma*, *Chlamydia*, *Campylobacter*, *Salmonella*, *Liesteria and Leptospira* were negative as shown in (Figure 2). The sequencing results of *Brucella*, *Coxiella* and *Mycoplasma* blasted in NCBI were shown in supplementary Figures S11, S12, and S13, respectively.

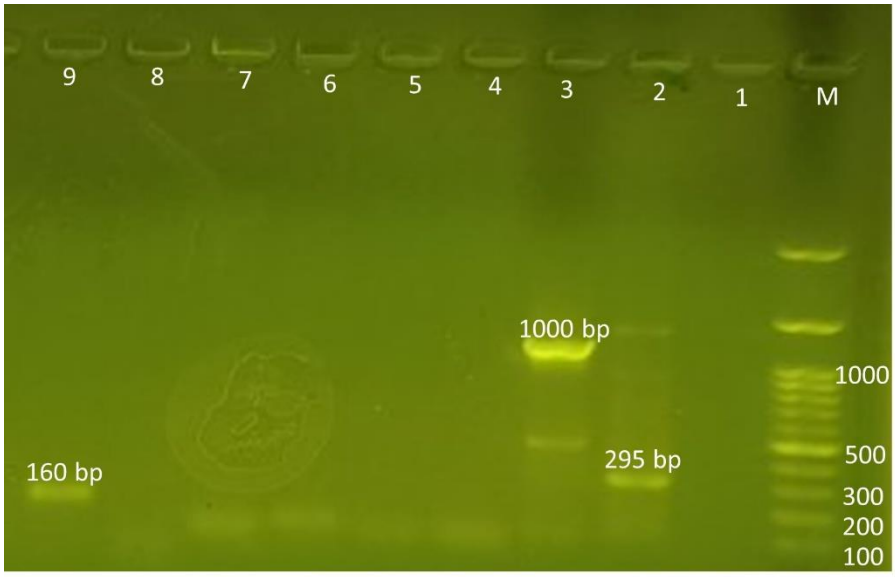
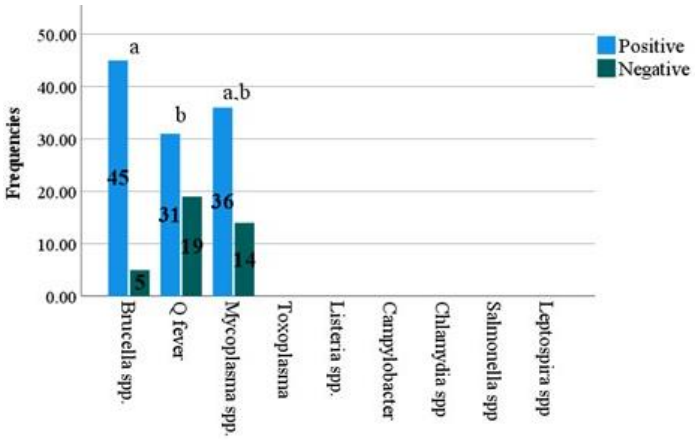


Figure 2: Agarose gel electrophoresis (1 %) stained with Prime Safe Dye. Lanes: M- DNA Marker 100 bp, 1- *Toxoplasma*, 2- *Coxiella*, 3- *Brucella*, 4- *Chlamydia*, 5- *Campylobacter*, 6- *Listeria*, 7- *Leptospira*, 8- *Salmonella*, 9- *Mycoplasma*.

Based on the PCR results, out of 50 blood samples, 45 (90 %), 36 (72 %), and 31 (62 %) were positive by *Brucella* spp., *Mycoplasma* spp., and *C. burnetii*, respectively (Figure 3).



*Test: Mc Nemar

Figure 3: Occurrence of ovine abortions according to the pathogens of *Brucella spp*, *Mycoplasma spp*, *C. burnetti*. (Different letter means statistically significant).

In this study, single, double and triple infections in aborted ewes were found (Tables 3). The rate of single infection by *Brucella* spp., and *Mycoplasma* spp., were 5 (10%), and 4 (8 %) respectively. Furthermore, Co- infections with *Brucella* spp., and *C. burnetti* were reported at rate 9 (18 %). While, *Brucella* spp., and, *Mycoplasma* spp., and *Mycoplasma* spp., and *C. burnetii* were 10 (20%), and 1 (2 %) respectively. In addition, triple Co-infections with *Brucella* spp, *Mycoplasma* spp., and *C. burnetti* were found in 21 ewes (42 %). The details of the results are given in (Table 3).

Table 3: Co-infection of ovine abortions.

Pathogens	Number of positive (%)
Brucella spp + *Mycoplasma* spp. *C. burnetii*	21 (42%)
Brucella spp + *C. burnetii*	9 (18%)
Brucella spp + *Mycoplasma* spp	10 (20%)
Mycoplasma spp. *C. burnetii*	1 (2%)
Total	41 (82%)

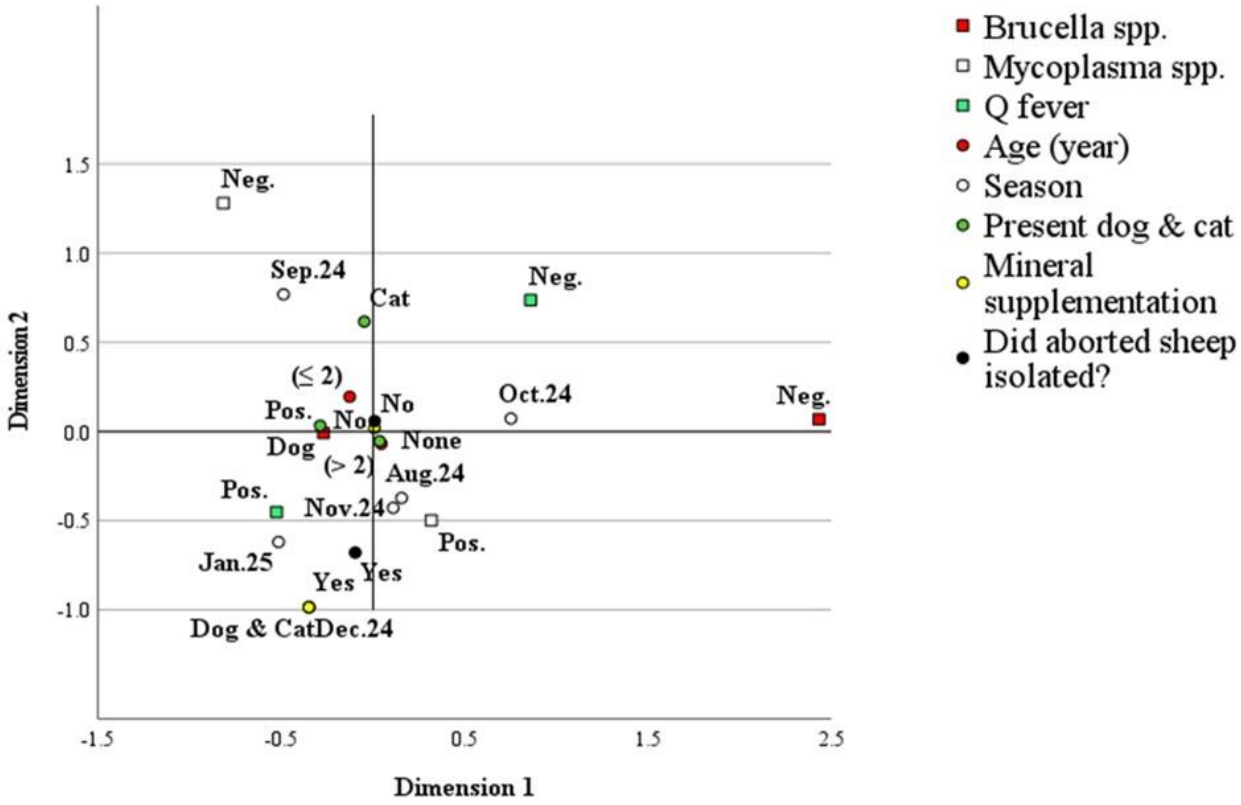


Figure 4: Multiple correspondence analysis.

Figure (4) showed that a high rate of abortion due to *Brucella* spp. which detected in group of animals did not isolated during lambing time.

Table (4) illustrated there were no statistically significant correlations between *Brucella* spp. infection and the majority of the parameters examined, including age, history of stillbirth, mineral supplements, abortion timing, isolation, pet ownership, and season. Regardless of individual characteristics, the infection rate is high in all groups, indicating that *Brucella* is common in the flock.

Table 4: *Brucella spp* infection according to independent variables.

Independent Variables		*Brucella* spp.		Total	Fisher`s exact test [P-value]	Relative risk	Odd ratio
		Positive	Negative				
Age (year)	(≤ 2)	12 [92.3]	1 [7.7]	13 [100.0]	1	1.03 [0.9-1.3]	1.5 [0.14-14.3]
	(> 2)	33 [89.2]	4 [10.8]	37 [100.0]		Ref.	Ref
History of stillbirth	Yes	1 [50.0]	1 [50.0]	2 [100.0]	0.19	0.54 [0.14-2.2]	0.09 [0.005-1.7]
	No	44 [91.7]	4 [8.3]	48 [100.0]		Ref	Ref
Mineral supplementation	Yes	1 [100.0]	0 [0.0]	1 [100.0]	0.35	1.32 [0.6-2.96]	3.8 [0.3-47.9]
	No	44 [89.8]	5 [10.2]	49 [100.0]		Ref	Ref
Time of abortion	Mid	1 [100.0]	0 [0.0]	1 [100.0]	0.35	0.77 [0.34-1.7]	0.27 [0.02-3.4]
	Late	44 [89.8]	5 [10.2]	49 [100.0]		Ref	Ref
Repeat breeding in the flock	Yes	29 [90.6]	3 [9.4]	32 [100.0]	1	1.02 [0.84-1.24]	1.2 [0.18-8]
	No	16 [88.9]	2 [11.1]	18 [100.0]		Ref.	Ref.
Did aborted sheep isolated?	Yes	4 [100.0]	0 [0.0]	4 [100.0]	1	0.95 [0.66-1.4]	0.7[0.07-7.2]
	No	41 [89.1]	5 [10.9]	46 [100.0]		ref	Ref
Present dog & cat	Both	1 [100.0]	0 [0.0]	1 [100.0]	0.53	0.75[0.34-1.7]	0.27[0.02-3.5]
	Dog	3 [100.0]	0 [0.0]	3 [100.0]		0.9[0.6-1.4]	0.5[0.05-5.7]
	Cat	4 [80.0]	1 [20.0]	5 [100.0]		0.81[0.5-1.31]	0.33[0.05-2.2]
	None	37 [90.2]	4 [9.8]	41 [100.0]		Ref	Ref
Season	Jan.25	5 [100.0]	0 [0.0]	5 [100.0]	0.54	ref	Ref
	Aug.24	2 [100.0]	0 [0.0]	2 [100.0]		0.9[0.5-1.7]	0.5[0.02-11.1]
	Sep.24	14 [93.3]	1 [6.7]	15 [100.0]		1.03[0.7-1.5]	1.3[0.1-16.5]
	Oct.24	9 [69.2]	4 [30.8]	13 [100.0]		0.8[0.5-1.2]	0.3[0.03-3.6]
	Nov.24	11 [100.0]	0 [0.0]	11 [100.0]		1.1[0.8-1.5]	2[0.11-37.8]
	Dec.24	4 [100.0]	0 [0.0]	4 [100.0]		0.97[0.6-1.6]	0.8[0.04-16.9]

Table (5) exposed that there was no significant between the most independent variables and *Mycoplasma* infection rate. The only variable that could affect infection is seasonality (P = 0.05), with the highest incidence occurring in October, and November.

Table 5: *Mycoplasma spp*. infection according to independent variables.

Variables		*Mycoplasma* spp.		Total	Fisher`s exact test [P-value]	Relative risk	Odd ratio
		Positive	Negative				
Age (year)	(≤ 2)	8 [61.5%]	5 [38.5%]	13 [100.0%]	0.95	0.8 [0.51-1.3]	0.51 [0.13-1.98]
	(> 2)	28 [75.7%]	9 [24.3%]	37 [100.0%]		Ref.	Ref.
History of stillbirth	Yes	2 [100.0%]	0 [0.0%]	2 [100.0%]	1	1.1[0.6-1.9]	1.3[0.12-13.4]
	No	34 [70.8%]	14 [29.2%]	48 [100.0%]		ref	ref
Mineral supplementation	Yes	1 [100.0%]	0 [0.0%]	1 [100.0%]	1	0.9[0.4-2.1]	0.8[0.07-9.9]
	No	35 [71.4%]	14 [28.6%]	49 [100.0%]		ref	ref
Time of abortion	Mid	1 [100.0%]	0 [0.0%]	1 [100.0%]	1	0.9[0.4-2.1]	0.8[0.07-9.9]
	Late	35 [71.4%]	14 [28.6%]	49 [100.0%]		ref	ref
Repeat breeding in the flock	Yes	24 [75.0%]	8 [25.0%]	32 [100.0%]	0.53	1.13 [0.77-1.7]	1.5 [0.42-5.3]
	No	12 [66.7%]	6 [33.3%]	18 [100.0%]		ref	ref
Did aborted sheep isolated?	Yes	4 [100.0%]	0 [0.0%]	4 [100.0%]	0.65	1.2[0.8-1.8]	2.3[0.2-21.2]
	No	32 [69.6%]	14 [30.4%]	46 [100.0%]		ref	ref
Present dog & cat	Dog & Cat	1 [100.0%]	0 [0.0%]	1 [100.0%]	0.38	0.9[0.4-2]	0.7[0.1-8.3]
	Dog	2 [66.7%]	1 [33.3%]	3 [100.0%]		0.8[0.4-1.7]	0.5[0.1-3.5]
	Cat	2 [40.0%]	3 [60.0%]	5 [100.0%]		0.6[0.2-1.4]	0.3[0.05-1.3]
	None	31 [75.6%]	10 [24.4%]	41 [100.0%]		Ref	Ref
Season	Jan.25	4 [80.0%]	1 [20.0%]	5 [100.0%]	0.05	Ref	Ref
	Aug.24	2 [100.0%]	0 [0.0%]	2 [100.0%]		1.1[0.5-2.2]	1.2[0.07-19.6]
	Sep.24	4 [26.7%]	11 [73.3%]	15 [100.0%]		0.4[0.17-0.99]	0.17[0.02-1.16]
	Oct.24	11 [84.6%]	2 [15.4%]	13 [100.0%]		1.1[0.66-1.9]	1.6[0.2-12.7]
	Nov.24	11 [100.0%]	0 [0.0%]	11 [100.0%]		12.3[0.8-2.1]	4.8[0.35-65.7]
	Dec.24	4 [100.0%]	0 [0.0%]	4 [100.0%]		1.2[0.65-2.1]	2[0.13-29.8]

In addition, independent variables like age, history of stillbirth, mineral supplements, and timing of abortion, seclusion, pet ownership, and season are the majority of factors that do not significantly correlate with *C. burnetii* infection. Age is the sole significant predictor (P = 0.002), however relative risk indicates that infection rates are comparable across age groups. Overall, the flock has a high prevalence of *C. burnetii* infection, and no significant risk factors were found (Table 6).

Table 6: *C. burnetii* infection and independent variables.

Variables		*C. burnetii* infection		Total	Fisher`s exact test [P-value]	Relative risk	Odd ratio
		Positive	Negative				
Age (year)	(≤ 2)	8 [61.5%]	5 [38.5%]	13 [100.0%]	0.002	0.99 [0.6-1.6]	0.97 [0.27-3.57]
	(> 2)	23 [62.2%]	14 [37.8%]	37 [100.0%]		Ref	Ref
History of stillbirth	Yes	0 [0.0%]	2 [100.0%]	2 [100.0%]	0.28	0.4[0.1-2.2]	0.2[0.02-1.9]
	No	31 [64.6%]	17 [35.4%]	48 [100.0%]		ref	ref
Mineral supplementation	Yes	1 [100.0%]	0 [0.0%]	1 [100.0%]	1	1.1[0.5-2.5]	1.3[0.11-15.2]
	No	30 [61.2%]	19 [38.8%]	49 [100.0%]		ref	ref
Time of abortion	Mid	1 [100.0%]	0 [0.0%]	1 [100.0%]	1	1.1[0.5-2.5]	1.3[0.11-15.2]
	Late	30 [61.2%]	19 [38.8%]	49 [100.0%]		ref	ref
Repeat breeding in the flock	Yes	19 [59.4%]	13 [40.6%]	32 [100.0%]	0.76	0.89 [0.57-1.38]	0.73 [0.22-2.44]
	No	12 [66.7%]	6 [33.3%]	18 [100.0%]		ref	ref
Did aborted sheep isolated?	Yes	3 [75.0%]	1 [25.0%]	4 [100.0%]	1	1.23 [0.67-2.27]	1.93 [0.19-20]
	No	28 [60.9%]	18 [39.1%]	46 [100.0%]		ref	ref
Present dog & cat	Dog & Cat	1 [100.0%]	0 [0.0%]	1 [100.0%]	0.99	1.1[0.5-2.5]	1.3[0.11-15.6]
	Dog	2 [66.7%]	1 [33.3%]	3 [100.0%]		0.99[0.5-2.1]	0.98[0.15-6.5]
	Cat	3 [60.0%]	2 [40.0%]	5 [100.0%]		0.9[0.5-1.9]	0.9[0.2-4.4]
	None	25 [61.0%]	16 [39.0%]	41 [100.0%]		ref	ref
Season	Jan.25	5 [100.0%]	0 [0.0%]	5 [100.0%]	0.27	ref	ref
	Aug.24	1 [50.0%]	1 [50.0%]	2 [100.0%]		0.6[0.2-1.6]	0.2[0.009-2.9]
	Sep.24	10 [66.7%]	5 [33.3%]	15 [100.0%]		0.8[0.5-1.2]	0.3[0.03-3.2]
	Oct.24	5 [38.5%]	8 [61.5%]	13 [100.0%]		0.5[0.2-0.9]	0.11[0.01-1.2]
	Nov.24	6 [54.5%]	5 [45.5%]	11 [100.0%]		0.6[0.35-1.13]	0.2[0.02-2.1]
	Dec.24	4 [100.0%]	0 [0.0%]	4 [100.0%]		0.9[0.6-1.6]	0.8[0.04-16.9]

4 Discussion

Accurate and timely detection of abortion cases caused by zoonotic infections in livestock is critical for the welfare of people and animals, national economies, a healthy environment, and the livelihoods of communities that depend on livestock husbandry [39]. In general, the diagnostic method of infectious abortions among sheep by clinical signs is difficult. Thus, the PCR test was applied in the present study because of its accuracy in sensitivity, and this test seems to be a practical and reliable tool for the diagnosis of small ruminant brucellosis. In addition, investigation of etiological agents of ovine abortions is important for enhancing disease control and management [39]. Nevertheless, the sensitivity and specificity of PCR vary between studies because of depend on the primers that they used, DNA extraction and PCR kit, and the amount of DNA in the sample; if its low (DNA) requirement increase the number of cycling [40]. However, among the nine pathogens investigated, only three bacterial pathogens, include *Brucella* spp., *C. burnetii*, and *Mycoplasma* spp. were detected. It means most ovine abortions were bacterial pathogens [41,4].

The results showed that *Brucella* spp. were the most commonly found pathogen, followed by *Mycoplasma* spp., and *C. burnetii*.

Several studies conducted throughout Iraq have shown that brucellosis is common in humans as well as in cattle, sheep, goats, camels, and other livestock [42,43,44,45,46,47,48,49]. Besides, according to the studies' findings, brucellosis infection rates have sharply increased in both humans and animals [50]. The majority of the studies are based on serology and Rose Bengal test in Iraq.

In addition, the findings demonstrate *Brucella* has potentially a dominant role as an important infections in livestock abortions in Sulaimani province, northern Iraq, underscoring the possible threat to Iraq's health and way of life. There was no significant difference according to the independent variables in infection rate of ovine brucellosis. This might be due to our sample size being low and our samples being collected of selected aborted sheep. Our results were also in agreement with the results of other researchers [51,52] who mentioned that the high degrees of sensitivity and specificity of the PCR assay, together with its speed, versatility in sample handling, and risk reduction for laboratory personnel, make this technique a very useful tool for the diagnosis of brucellosis. In other countries, for instance, in southeast Iran, *Brucella* spp. were found in 15 (30%) of the 50 aborted fetuses that were examined using a PCR test [53]. Whereas, according to [54] study the incidence was 10.5 % in a study on ovine serum using Rose Bengal test (RBPT) and 8.5 % in milk samples using the milk ring test. In addition, according to [53] study revealed that *Brucella* species that were identified were not restricted to the animals that were their principal hosts. These suggest that small ruminants may be susceptible to cross-species transmission. In addition, in Syria [55], Turkey [56], Saudi Arabia [57], Jordan [58], Egypt [59], and Bangladesh [60] *Brucella* infection was recorded. It means, this disease is endemic in several regions of the world [61]. According to researches, both etiological agents of ovine abortions and prevalence rate were changed to geographical distribution for instance, a review of the PCR results of field specimens found that bacterial pathogens (*Brucella* spp., *Mycoplasma* spp., and *C. burnetii*) are the most common causes of ovine abortions. In contrast, in Israel and Spain, protozoan parasites like *Neospora caninum* infection found the most common causes of ewe abortions, while bacterial pathogens were rarely reported [62,63]. Moreover, poor management and husbandry techniques by farmers, such as grazing several flocks of sheep on the same pasture and sharing rams for mating across different herds, are likely the reason for the high prevalence of brucellosis [64].

According to the findings of the current study, 72 % of the specimens were diagnosed as positive for *Mycoplasma* spp. by PCR test. Moreover, by the current study's findings, for the first time, it was confirmed that *Mycoplasma* spp. is also one of the other causes of abortion in sheep in Iraq. In addition, according to [24] study, contagious agalactia, which can result in abortion in sheep, is mostly caused by *Mycoplasma agalactiae*. He also found that 24 (30.8%) cases were infected with *M. agalactiae*. It is also characterized by mastitis with reduction of milk production, abortion, keratoconjunctivitis, and arthritis [65]. Our results disagree with the study of [23] the rate which was 3 %. In addition, according to the current study there was no statistically significant relationship between such independent variables as age, stillbirth, mineral supplements, timing of abortion, presence of dogs and cats, and recurrent breeding with the infection. This is in agreement with [24] study. In the present study, the rate of *C. burnetii* was 62 % among aborted ewes. While, the rate is in disagreement with studies of [66] and [23] which were 5.55% and 2%, respectively. According to the current study, the infection rate was higher that group of animals that had more than two years. According [67] study, he recorded *C. burnetii* in aborted ovine and caprine. Findings of the present study, co-infection of Brucellosis and Q fever were 18 %. While it was 47 % according to the [39] study. Furthermore, in different location of Iraq, Other pathogenic of ewe abortion illness were recorded, such as *C. abortus* [68,69,70,71], *T. gondii* [72]. Additionally, the number of abortions in the flock as well as the prevalence of abortions could be greatly increased if the flock contains at least one of the four infectious agents being studied. In the Kurdistan region, Iraq, various agro-ecological and production systems, this suggesting that several infectious agents were involved in the abortion of small ruminants, which had a cumulative effect on small ruminant reproduction. In agreement with the current findings, [73,74,75] that highlight the significant importance of co infection as the cause of abortion in flocks of small ruminants. Furthermore, in study of [16] demonstrated the impact of infectious illness, the role of management, and agroecological. He also reported that improving management can lead to a decrease in abortion rates among small ruminants. Nevertheless, Management of abortions includes the correct and timely diagnosis of the causative agent of the disorder, as well as the strategic administration of therapeutic agents, aiming to prevent abortions in flocks with confirmed infection with an abortifacient agent, especially if no appropriate vaccinations had been carried out before the mating season. Health management also aims to prevent the main metabolic disorders of pregnant sheep (i.e., pregnancy toxaemia and hypocalcaemia), as well as to monitor flocks for the development of these disorders [76]. Moreover, in the current study, almost all interviewed sheep owners also confirmed that they did not have dogs and cats, but according to the findings of [16] revealed that the presence of dogs and cats in the household could increase the risk of abortion significantly. Numerous reasons could be responsible for the high prevalence rates in our study: (i) the reason might be due to owners during the injection, such as vaccination or administering other drugs, use of the same needle for injection for all animals. (ii) Most aborted ewes were no isolated from the flock and the aborted fetuses, placentas, the vaginal discharge of infected dams were source of the infections. Besides, all agents transfer those germs into the environment. Animal populations may become infected by them [16]. In general, the diagnostic methods, methods and time of sampling, infection rate, and various national control measures, including vaccinations, antibiotic use, farmer education, the quality of available vaccines, and breeding characteristics, may all contribute to variations in the abortion agent results in the studies mentioned previously [3].

5 Conclusions

Investigating the etiological pathogens of abortions is crucial for control strategies; however, it can be challenging due to the

disorganized management of the breeding flocks in the region. Brucellosis was the most prominent outcome associated with ewe abortions in the Garmian administration, Iraq. This study also confirmed the co-occurrence of *Brucella* spp., *C. burnetii*, and *Mycoplasma* spp. infections in aborted sheep. For the first time, the *Mycoplasma* spp. was found as a causative agent of ovine abortion in Kurdistan region by using PCR test, and no one mentioned this agent. The majority of ovine abortifacient agents have been zoonotic diseases. The significance of taking preventative hygiene precautions should be understood by humans, particularly pregnant women, who have intimate contact with lambing sheep or goats. To decrease the effects of these diseases, effective prevention and control strategies are crucial. These include improved animal health programs, improved diagnostics, and vaccination. To protect livestock health and enhance rural farmers' livelihoods, it is essential to do ongoing studies on the pathogens and gain a better knowledge of how they spread, particularly for diseases like brucellosis, mycoplasmosis, and Q fever. Additional research is required to investigate whether these pathogens can be found in other animal species and humans suffering from abortions.

Declaration of interests

The author declare that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data statement

Data available in Supplementary materials and up on request from the corresponding author.

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