Investigation of *Chlamydia abortus* and *Brucella Species* in Abortion Cases of Sheep by Serological and Molecular Methods

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SUMMARY

Chlamydiosis and brucellosis, which cause abortion cases in animals, are among the major zoonotic agents that are widely distributed across the world. This study aimed to identify *Chlamydia abortus (C. abortus)* by using serological and molecular methods and *Brucella* spp. by using conventional bacteriological, serological, and molecular methods in blood and vaginal swab samples collected from aborted sheep in the Siirt region in Turkiye. In the study, 200 vaginal swab samples and blood samples were collected from 350 sheep with a history of abortion. The 350 serum samples collected from the aborted sheep were tested for *C. abortus* by ELISA and *Brucella* spp. by the Rose Bengal Plate Test (RBPT) and the Serum Agglutination Test (SAT). Furthermore, vaginal swab samples collected from the same sheep were analysed by conventional and molecular methods for *Brucella* and molecular methods for *C. abortus*. As a result of the serological analysis, it was found that *C. abortus* was 11.42% and *Brucella* spp. was 22.28% and 11.71% in terms of RBPT and SAT, respectively. However, as a result of the Polymerase Chain Reaction (PCR) analysis, no positive results were obtained for *C. abortus*. Conventional bacteriological and molecular analysis of vaginal swab samples indicated that *B. melitensis* was identified in 11 (5.5%) isolates. According to biotyping tests, *B. melitensis* biotype 1 was identified in 2 isolates and *B. melitensis* biotype 2 in 7 isolates. By using the disk diffusion method, all *B. melitensis* isolates (100%) were found to be susceptible to doxycycline, tetracycline, gentamicin, streptomycin, and ciprofloxacin. Consequently, it was determined that *C. abortus* and *B. melitensis* may play a role in abortion cases in the Siirt region. It is thought that the sensitivity of the isolates obtained to the antibiotics used will contribute to the treatment of *Brucella* species, which pose a significant risk to public health.

KEY WORDS

Abortion, *Brucella melitensis*, *Chlamydia abortus*, Vaginal swap.

INTRODUCTION

Chlamydia abortus (C. abortus) and *Brucella* spp. are major pathogens that are widely distributed across the world and cause infections in many hosts, including sheep, goats, and cattle. Infections induced by *C. abortus* and *Brucella* spp. result in abortion and infertility in animals, leading to significant economic losses (1).

Brucella in small ruminants is considered a global significant risk factor due to the fact that symptoms are not specific to the disease and can be transmitted to humans. Therefore, early diagnosis of *Brucella* infections in herds is of great importance in terms of implementing protection-control programmes and eliminating the disease. Although conventional methods are accepted as the 'Gold Standard' in the definitive diagnosis of the agents that cause brucellosis, serological and molecular methods have been preferred today. In this context, the Rose Bengal Plate Test and the Serum Agglutination Test using *Brucella abortus* antigen are widely used (2).

C. abortus, an obligate intracellular pathogen, causes enzootic abortion in sheep and chlamydial abortion in goats, pigs and cattle. In addition, *C. abortus*, which has a wide host range, can cause abortions in horses, guinea pigs, mice and rabbits (3). It is very difficult to eradication *C. abortus*, which usually causes miscarriage in the last 2-3. weeks of pregnancy, and it poses a major risk to public health (4). Vaginal discharge and placental tissue from aborted sheep, as well as infected lamb posts and foetuses, constitute a source of infection for humans and animals in the flock (5). Since *C. abortus* is an obligate intracellular pathogen, its isolation is difficult and time-consuming. Therefore, serological and molecular methods are more commonly used for diagnosis. In particular, the ELISA test is widely preferred due to its higher sensitivity and specificity. Besides serological tests, molecular methods are also preferred techniques due to their higher sensitivity and reliability (6).

A number of national and international studies have been conducted on *Brucella* spp. and *C abortus.* When the studies conducted in this context were examined, the prevalence of Brucella species was found to be between 0.11% and 16.3% (7, 8), while the prevalence of C. abortus was determined to be between 8.38% and 24.1% (9, 10).

The aim of this study is to identify *Brucella* spp. and *C. abortus*, which pose a great risk to both animal and public health, in aborted sheep in the Siirt region. For this purpose, it is aimed to detect the presence of *C. abortus* in the blood serum and vagi-

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nal swab samples of aborted sheep by serological and molecular methods, to reveal the presence of brucellosis in these samples by conventional, serological, and molecular methods, and to determine the antibiomicrobial susceptibilities of the obtained *Brucella* isolates.

MATERIALS AND METHODS

Material

Ethical approval was obtained from the Local Ethics Committee of Animal Experiments at Siirt University (the decision number: 2021.01.02).

The study included a total of 350 blood serum samples (Table 1) and 200 vaginal swab samples (Table 2) from aborted sheep in city centre and districts of Siirt province. The blood samples were centrifuged at 10.000 x g for 10 minutes, and the serum parts were stored at -20 °C until they were used in the tests. The vaginal swab samples were stored in Stuart's medium at -20 °C until the isolation of *Brucella* spp. and the extraction of DNA.

Method

Brucella spp. Isolation

The vaginal swab samples collected from aborted sheep were inoculated on *Brucella* selective agar (Oxoid CM0169, England) and blood agar (Oxoid CM0271, England) for the identification of *Brucella* species and incubated in both an aerobic and a 5-10% CO_2 environment at 37°C for 5-7 days. After inoculation, the swab samples were transferred to a tube containing 5 ml of Farrell broth [(Brucella Broth (BD BBL, 211088, Canada)] for pre-enrichment and incubated aerobically for 5-7 days. Following incubation, Farrell Broth was subcultured on Farrell agar [(Brucella Medium (Oxoid CM0169, Canada)] and blood agar and incubated under the same conditions and for the same periods. The *Brucella* spp. suspected colonies were identified according to results of Gram stain, catalase, oxidase, and urease tests (11).

Serum agglutination test (SAT) for brucellosis

Brucella antigen required for SAT was procured from Pendik Veterinary Control and Research Institute, Istanbul, Turkiye. The test was run according to the instructions of the institute.

Rose Bengal Plate Test (RBPT) for Brucellosis

All serum samples were analysed with RBPT antigen procured from Pendik Veterinary Control and Research Institute, Istanbul, Turkiye, in accordance with the recommended test procedure.

DNA Extraction

The genomic DNA of *Brucella* spp. suspected isolates was extracted by modifying the phenol-chloroform/isoamyl alcohol extraction method reported by Sambrook and Russell (12). Furthermore, the DNA Mini kit (PureLink™, K182002, USA) was used to obtain the genomic DNA from 200 vaginal swab samples for molecular identification of *C. abortus*.

Table 1 - Distribution of sheep blood sera taken from city centre and districts of Siirt province according to herd size and number of samples.

Where samples were taken	Number of samples	Size of herds from which samples were taken	Total number of animals
City centre	126	11	9480
Kurtalan	49	12	1953
Pervari	20	2	400
Şirvan	34	3	866
Eruh	23	2	1400
Tillo	60	3	1800
Baykan	38	2	1100
Total	**350**	**35**	**16999**

Table 2 - Distribution of sheep vaginal swab samples taken from Siirt centre and districts according to herd size and number of samples taken.

Where samples were taken	Number of samples	Size of herds from which samples were taken	Total number of animals
City centre	51	11	9480
Kurtalan	49	12	1953
Pervari	20	2	400
Şirvan	3	3	866
Eruh	23	2	1400
Tillo	29	3	1800
Baykan	25	2	1100
Total	**200**	**35**	**16999**

Table 3 - Primers and target genes used in the Bruce-Ladder PCR method (11).

Brucella species	BA	BM	BO	BS	BC	S19	REV1	Primer	Sequence (5'—3')	Target gene	Amplicon Size (bp)
Gene distributions of *Brucella* species	•	•		•	•	•	•	BMEI0998f	ATCCTATTGCCCCGATAAGG	Glycosyltransferase , *wbo*	1682
								BMEI0997r	GCTTCGCATTTTCACTGTAGC		
		•	•	•	•		•	BMEII0843f	TTTACACAGGCAATCCAGCA	Outer membrane protein, *omp31*	1071
								BMEII0844r	GCGTCCAGTTGTTGTTGATG		
	•	•	•	•		•	•	BMEI1436f	ACGCAGACGACCTTCGGTAT	Polysaccharide deacetylase	794
								BMEI1435r	TTTATCCATCGCCCTGTCAC		
	•	•	•	•	•		•	BMEII0428f	GCCGCTATTATGTGGACTGG	Erythritol catabolism , *eryC*	587
								BMEII0428r	AATGACTTCACGGTCGTTCG		
	•	•	•	•	•	•	•	BMEI0535f	GCGCATTCTTCGGTTATGAA	Immunodominant antigen , *bp26*	450
								BMEI0536r	CGCAGGCGAAAACAGCTATAA		
				•	•			BR0953f	GGAACACTACGCCACCTTGT	ABC transporter junction protein	272
								BR0953r	GATGGAGCAAACGCTGAAG		
							•	BMEI0752f	CAGGCAAACCCTCAGAAGC	Ribosomal protein S12, *rpsL*	218
								BMEI0752r	GATGTGGTAACGCACACCAA		
	•	•	•	•	•	•	•	BMEII0987f	CGCAGACAGTGACCATCAAA	Transcriptional regulator, CRP family	152

BA: *B.abortus*, **BM:** *B. melitensis*; **BO:** *B. ovis*; **BS:** *B. suis*; **BC:** *B. canis*; **S19:** *B. abortus* S19 (Vaccine strain); **REV1:** *B. melitensis* (Vaccine strain).

PCR Analysis

Detection of *Brucella* spp.

Species-level identification of the *Brucella* spp. isolates was performed by using the Bruce-ladder PCR, a multiplex PCR. Table 3 shows the primers and target genes used in the Bruce-ladder PCR. Bruce-ladder PCR consisted of 1 l MgCl2 (20 mM), 5 l 5XLongAmp™ Taq Reaction Buffer, 1 l LongAmp® Taq DNA Polymerase (5 U), 0.75 l dNTP (10 mM), 1 l of each primer (12 pieces) (20 pmol), and 3 l template DNA (50 ng/ l). The thermal condition of the Bruce-ladder PCR was determined by initial denaturation at 95 °C for 3 min, followed by 30 denaturation cycles lasting for 35 s (primer annealing at 95 °C for 45 s at 62 °C, extension at 65 °C for 3 min), and final extension at 65 °C for 10 min. Amplified products were analysed on 1.5% agarose gel. The band sizes obtained were evaluated based on the information in Table 3.

Detection of *C. abortus*

In the study, species-specific primers were used to analyse *C. abortus* PCR (Table 4).

Commercial mastermix (PN0047, A.B.T®, Ankara, Turkiye) was used for the preparation of the reaction mixture. The reaction was performed using 12.5 µl mastermix, 5 µl genomic DNA, and 1.5 µl of each primer. At the stage of amplification, the PCR protocol was optimised according to the instructions of the manufacturer company that synthesized the primers (Table 4). During the optimisation process, initial denaturation was performed at 94 °C for 10 minutes, followed by 35 cycles of predenaturation at 94 ± 30 seconds, primer annealing at 56 °C for 60 seconds, extension at 72 °C for 60 seconds, and final extension at 72 °C for 10 minutes.

Biotyping *of Brucella* spp.

Biotyping *of Brucella* spp. was made according to the criteria reported by Alton et al., (14). The isolates were analysed according to their CO_2 requirement, H_2S production, growth in the presence of basic fuchsin and thionine, and agglutination with A and M monospecific antisera.

Antibiotic Susceptibility Test

Disk Diffusion Method

Antibiotic susceptibility of *Brucella* isolates was analysed by using disk diffusion method in accordance with EUCAST guidelines (15). In this context, streptomycin (Oxoid, 5 µg, England), oxytetracycline (Oxoid, 30 µg, England), doxycycline (Oxoid, 30 µg, England), cefoperazone (Oxoid, 75 µg, England), ciprofloxacin (Oxoid, 5 µg, England), rifampicin (Oxoid, 5 µg, England), gentamicin (Bioanalyse, 10 µg, Turkiye), and trimethoprim-sulfamethoxazole (Bioanalyse, 25 µg, Turkiye) discs were used. The isolates were suspended in physiological saline and adjusted at a density of McFarland 0.5. The 100 µl of the suspension was inoculated onto Mueller Hinton agar (Merck, 103872, Germany) with 5% sheep blood. After the media were dried for 5-10 minutes at ambient temperature, antibiotic disks were placed. The media were incubated under aerobic conditions at 37 °C for 48 hours (11). Following incubation, the diameter of the inhibition zone around the disk was measured and susceptibility was evaluated according to EUCAST (15) guidelines.

ELISA

A commercially available ELISA test kit (CHLMS-MS, ID.vet, France) was used for serological identification of *Chlamydia*

Table 4 - Primer and target gene used for *Chlamydia abortus.*

TargetGene	Oligonucleotide (5'-3')	Amplicon Size (bp)	Reference
pmp	CpsiA (5'-ATGAAACATCCAGTCTACTGG-3) CpsiB (5'-TTGTGTAGTATTATTATCAAA-3)	300 bp	13

abortus according to the manufacturer's instructions. The results were read on an ELISA reader with a 450 nm wavelength filter.

Statistical Analysis

Pearson's Chi-square (x2) method was used to evaluate the differences between the methods used in the study. Statistical significance was assessed at $p<0.05$.

RESULTS

While 78 (22.28%) samples yielded positive results in 4 minutes after RBPT for serological identification of brucellosis, a total of 41 (11.71%) serum samples with titres between 1/20 and 1/320 were evaluated as positive for brucellosis as a result of SAT on 350 blood serum samples.

As a result of the bacteriological tests, 16 isolates were suspected of *Brucella* spp. As a result of Bruce-Ladder PCR, nine of the isolates showed band profiles of 152, 450, 587, 794, 1.071, and 1.682 bp and these isolates were considered as *B. melitensis* (Figure 1). Furthermore, *B. melitensis* was identified in two vaginal swab samples other than the culture-positive vaginal swab samples by PCR analysis with genomic DNAs obtained as a result of DNA extraction from Farrell broth enrichment medium using the Phenol Chloroform/Isoamyl Alcohol method. In total, *B. melitensis* was identified in 11 (5.5%) of the 200 vaginal swab samples analysed.

Biotyping and biochemical analysis indicated that while 2 (22.22%) of 9 isolates were identified as *B. melitensis* biotype 1,7 (77.77%) isolates were identified as *B. melitensis* biotype 2.

All isolates were susceptible to doxycycline, tetracycline, gentamicin, streptomycin, and ciprofloxacin. While 1 isolate was evaluated as resistant to trimethoprim/sulfamethoxazole, 2 isolates were evaluated as resistant to cefoperazone.

As a result of the ELISA test, 40 (11.42%) serum samples were evaluated as positive for *C. abortus*. But, *C. abortus* was not determined from 200 vaginal swab samples by PCR.

As a result of the analyses, no positive results were obtained for *C. abortus* using PCR, so it was not included in the analysis. However, a significant relationship was found between the RBPT and SAT tests used for serological diagnosis of Brucella spp. ($p<0.001$). A statistical analysis also found a significant correlation between the RBPT used for serological analysis of samples and the Bruce-Ladder method used for the identification of isolates ($p<0.001$). The same values were also found in the statistical analysis between SAT and Bruce-Ladder ($p<0.001$). Consequently, a significant correlation was found between the methods used for the detection of *Brucella* spp. (Table 5).

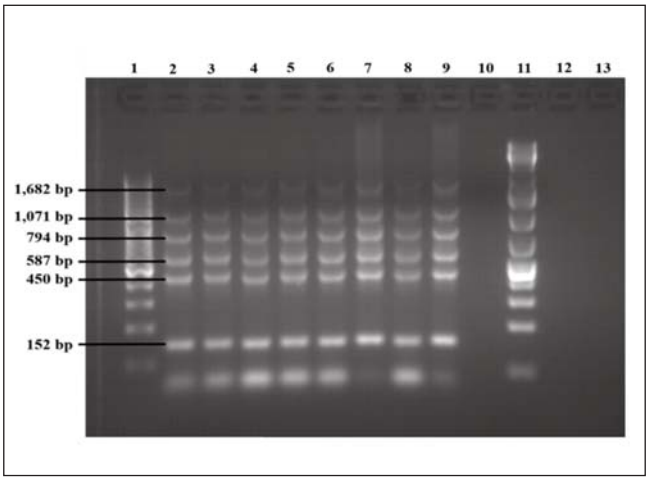

Figure 1 - Bruce-Ladder PCR image of *B. melitensis*. Columns 1 and 11: 100 bp DNA plus ladder, columns 2-9: *B. melitensis* (from vaginal swabs).

DISCUSSION

Ovine breeding, an important source of economy in Turkiye, holds great importance for the utilisation of pasture lands. Also, the ability to have more than one offspring in one litter provides a significant advantage. However, offspring losses associated with various reasons can lead to substantial losses for breeders and the national economy (16).

Some national and international studies have been carried out for the serological identification of brucellosis in aborted sheep. Abnaroodheleh et al., (1) reported positive results for brucellosis in 20 (19.8%) samples by RBPT and 18 (17.8%) samples by SAT. Karataş Yeni and Akça (17) reported positive results for brucellosis in 11.41% of the samples by RBPT and 10.05% by SAT. Tesfaye et al., (18) serologically examined 424 sheep and goat serum samples and reported that 50 (9.66%) of 424 animals were positive for *C. abortus*, while they found no seropositivity for *Brucella*. In 2816 serum samples, Zhang et al., (10) reported that seroprevalence of *C. abortus* and *Brucella* spp. was 8.38% and 0.11%, respectively.

In this study, SAT, RBPT and *C. abortus* were found to be 11.71%, 22.28% and 11.42%, respectively. When the results of the present study were compared with the results of other studies, SAT results were found to be higher than the values obtained by Karataş Yeni and Akça (17). RBPT results were evaluated as

Table 5 - Relationship between RBPT/SAT/Bruce-Ladder used for the diagnosis of *Brucella spp.*

	RBPT	**SAT**
Number of Samples	350	350
Positive	78 (22.28%)	41 (11.71%)
Negative	272 (77.71%)	309 (88.28%)
p-value	**0.0001***	
	RBPT	**Bruce-Ladder PCR**
Number of Samples	350	200
Positive	78 (22.28%)	9 (4.5%)
Negative	272 (77.71%)	191 (95.5%)
p-value	**0.00001***	
	SAT	**Bruce-Ladder PCR**
Number of Samples	350	200
Positive	41 (11.71%)	9 (4.5%)
Negative	309 (88.28%)	191 (95.5%)
p-value	**0.004***	

***p<0.05. RBPT:** Rose Bengal Plate Test; **SAT:** Standard Agglutination Test.

higher than the values obtained by Abnaroodheleh et al., (1), Karataş Yeni and Akça (17). When the results of the present study were evaluated, it was observed that the *C. abortus* rate obtained in the study was higher than the rate obtained by Zhang et al., (10) and Tesfaye et al., (18).

The differences between the results are attributed to the number of animals from which blood samples were taken, the different regions where the studies were conducted, and the raising conditions of the animals and the fact that in some studies, random sampling method was used to take blood serum samples from herds with a history of abortion to determine seroprevalence.

Among studies on the isolation and identification of *Brucella* agents, Sakmanoğlu et al., (19) reported that *B. melitensis* was identified in 32.07% (n=17) of 53 lamb stomach contents. Likewise, in a study conducted by Gülaydın et al., (20) in Van, they reported that they identified *B. melitensis* by RT-PCR in 7.96% (n=9) of 113 samples collected from sheep.

In the present study, the isolation rate of *Brucella* spp. and identification rate of *B. melitensis* were 4.5% (n=9). The differences between the data suggest that these differences may be due to differences in vaginal excretion periods of bacterial agents and sample sizes.

Brucella melitensis, B. abortus, and *B. suis* species are identified at the biotype level by four main tests: CO_2 demand, H_2S production, stain (thionine and basic fuchsin) sensitivity, and agglutination with monospecific A and M antisera. Several studies have been carried out in this context. When Khan et al., (21) examined 34 *Brucella* suspected isolates by AMOS PCR and Bruce-ladder PCR methods, they reported that they identified *B. melitensis* in 21 (61.76%) isolates and *B. abortus* in 8 (23.52%) isolates and found 21 strains as *B. melitensis* biotype 3 and 8 strains as *B. abortus* biotype 1 as a result of biotyping. Similarly, lhan et al., (22) reported that they identified *B. melitensis* biotype 3 in 41 *Brucella* isolates isolated from infected sheep (9 milk and 32 pharyngeal swabs of aborted foetuses).

Considering the results obtained in the present study, it was observed that the majority of *B. melitensis* isolates were identified as *B. melitensis* biotype 2. The results of other studies indicated that *B. melitensis* biotype 3 was identified in most of the isolates, but infections with all three biotypes have been reported around the world. Although the results of this study confirm the findings in Turkiye, it also draws attention to the abundance of biotype 2. Considering the difference between this condition and biotype 1, the vaccine biotype, there is a need for regional epidemiological studies to follow the epidemiologic traces of major strains.

In the present study, the antimicrobial susceptibilities of *B. melitensis* strains were investigated by the Kirby Bauer disk diffusion test. The findings of this study are compatible with those of the studies by Etiz et al., (23), and Dadar et al., (24). The results of the antibiotic susceptibility test, which showed no streptomycin resistance in the *B. melitensis* vaccine strain, rifampicin resistance in the RB51 vaccine strain, and resistance to tetracycline and doxycycline, one of the primary options in treatment, indicated no concern for the treatment of the disease.

The studies on *Chlamydia abortus* have generally been conducted serologically or at the *Chlamydia* spp. level. In this study, a seropositivity rate of 11.42% was determined by ELISA. The results of the present study are close to the results of the study conducted by Selim et al., (25) in 2021 (13.77%) and the study by Fayez et al., (26) (11.1%).

When the molecular studies on chlamydial abortions in Turkiye are examined, it is seen that they are limited in number, and the majority of them are based on conventional PCR. Aras et al., (27) reported *C. abortus* in 3% of aborted bovine foetus stomach content samples in Aksaray and Konya region and Kanat (28) stated that *C. abortus* was found in 15.40% of aborted sheep foetuses in Konya region.

Some international studies have been carried out to identify the presence of *C. abortus* by molecular methods, and reported positivity ranging from 0% to 87% (29, 30).

Following the extraction of 200 vaginal swab samples from aborted sheep, PCR analysis yielded no positivity for *C. abortus* in the present study. When comparing the reason behind why no results could be achieved in vaginal swab samples with other studies, it is thought that variables such as regional differences, differences in the study material, and the sampling period due to the intermittent excretion of the causative agent in vaginal secretions may be effective.

CONCLUSIONS

Consequently, it was concluded that *B. melitensis* and *C. abortus* may play a role in the abortion cases in the Siirt region. It is recommended to conduct further studies with a larger number of samples and animals to collect more effective data. Antibiotic use is not recommended due to the ineffectiveness of treatment against brucellosis infections in animals. However, as a result of antimicrobial susceptibility testing against *B. melitensis* isolates, it was determined that the agent was sensitive to doxycycline, tetracycline, gentamicin, streptomycin and ciprofloxacin. It is thought that the antimicrobial susceptibility results obtained may be important for public health.

Author's Contribution

Study concept and design: Muazzez YEŞILYURT, Aliye GÜLMEZ SAĞLAM. Funding acquisition: Aliye GÜLMEZ SAĞLAM. Collection of samples in the field: Muazzez YEŞİLYURT. Acquisition of laboratory data: Muazzez YEŞİLYURT, Aliye GÜLMEZ SAĞLAM Supervision: Aliye GÜLMEZ SAĞLAM. Writing-original draft preparation: Muazzez YEŞİLYURT, Aliye GÜLMEZ SAĞLAM. Writing-review and editing: Muazzez YEŞİLYURT, Aliye GÜLMEZ SAĞLAM.

Conflict of Interest

None of the authors have a conflict of interest to disclose.

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References

1. Abnaroodheleh, F., Emadi, A., and Dadar, M. 2021. Seroprevalence of brucellosis and chlamydiosis in sheep and goats with history of abortion in Iran. *Small Ruminant Research*., 202: 106459.
2. Al-Afifi, A. H., Almashhadany, D. A., Al-Azazi, A. S.. H., Khalaf, A. M., Odhah, M. N. A., and Naif A Al-Gabri, N. A. 2022. Prevalence of *Brucella* spp. in milk from aborted and non-aborted animals in Dhamar governorate, Yemen. *Italian Journal of Food Safety*., 11(4):10370. doi: 10.4081/ijfs.2022.10370.

3. Li, Z., Liu, P., Cao, X., Lou, Z., Zareba-Marchewka, K., Szymanska-Czerwinska, M., Niemczuk, K., Hu, B., Bai, X., and Zhou, J. 2018. First report of *Chlamydia abortus* in farmed fur animals. *BioMed Research International*. .
4. Malal, M. E., and Türkyılmaz, S. 2021. Identification and genotyping of *Chlamydia abortus* with MLVA from ruminant abortions in the Marmara region of Turkey. *The Thai Journal of Veterinary Medicine*., 51(1).
5. Gutierrez, J, Williams, E. J., O'Donovan, J., Brady, C., Proctor, A. F., Marques, P. X., Worrall, S., Nally, J. E., McElroy, M., Sammin, D. J., and Markey, B. K. 2011. Monitoring clinical outcomes, pathological changes and shedding of *Chlamydophila abortus* following experimental challenge of periparturient ewes utilizing the natural route of infection. *Veterinary Microbiology*., 147: 119-126.
6. Osman, W. A. 2013. Comparative evaluation of indirect ELISA, CF test and PCR for diagnosis of ovine enzootic abortion (Ovine chlamydophilosis). *Global Veterinaria*., 11(1): 65-70.
7. Hegazy, Y. M., Moawad, A., Osman, S., Ridler, A., and Guitian J. 2011. Ruminant brucellosis in the Kafr El Sheikh Governorate of the Nile Delta, Egypt: prevalence of a neglected zoonosis. *PLOS Neglected Tropical Diseases*., 5(1): e944, https://doi.org/10.1371/journal.pntd.0000944.
8. Selim, A., Attia, K., Ramadan, E., Hafez, Y. M., and Alamery Salman, A. 2019. Seroprevalence and molecular characterization of *Brucella* species in naturally infected cattle and sheep. *Preventive Veterinary Medicine*., 171.
9. Esmaeili, H., Bolourchi, M., Mokhber-Dezfouli, M. R., Farahani, R. K., and Teimourpour, A. 2021. Detection of *Chlamydia abortus* and risk factors for infection in small ruminants in Iran. *Small Ruminant Research*., 197: 106339. .
10. Zhang, H., Zhang, Z., Li, Y., Li, W., Jin, Y., Li, Z., Zhou, J., and Tong, D. 2023. Seroprevalence of Chlamydia abortus and Brucella spp. and risk factors for *Chlamydia abortus* in pigs from China. *Acta Tropica*., 248: 107050.
11. Büyük, F., and ahin, M. 2011. Investigation of *Brucella* species from various samples of aborted cattle in kars province (turkey) by cultural and molecular methods and epidemiological analysis of cases. *Kafkas Universitesi Veteriner Fakültesi Dergisi*., 17 (5): 809-816.
12. Sambrook, J. and Russell, D.W. 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press: 544-553.
13. Laroucau, K., Boumedine, K. S., and Rodolakis, A. 2001. Amplified fragment length polymorphism differentiation between the vaccine strain *Chlamydia psittaci* 1B and wild field strains. *Veterinary Record*., 149(11): 332-4.
14. Alton, G. G., Jones, L. M., Angus, R. D., and Verger, J. M. 1988. Techniques for the Brucellosis Laboratory. Institute National de la Vacherche Agronomique, (INRA) Paris. 1st Edition, pp. 63-129.
15. The European Committee on Antimicrobial Susceptibility Testing (EUCAST). 2022. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, http://www.eucast.org. 2022.
16. Bakır, G., and Mikail, N. 2019. Structural status of small livestock enterprises in Siirt province. *Ataturk University Faculty of Agriculture Journal*., 50(1): 66 -74.
17. Karataş Yeni, D., and Akça, D. 2021. Evaluation of the analytical efficiency of real-time pcr in the diagnosis of Brucellosis in cattle and sheep. *Kafkas Universitesi Veteriner Fakültesi Dergisi*., 27(4): 503-509. doi: 10.9775/kvfd.2021.25776.
18. Tesfaye, A., Sahele, M., Sori, T., Guyassa, C., and Garoma, A. 2020. Seroprevalence and associated risk factors for chlamydiosis, coxiellosis and brucellosis in sheep and goats in Borana pastoral area, southern Ethiopia. *BMC Veterinary Research*., 16: 145.
19. Sakmano lu, A., Uslu, A., Sayın, Z., Sanio lu Gölen, G., lban, A., Padron-Perez B., Karyeyen, Y., Gök, A., Tekindal, M.A., and Erganis, O. 2021. A one-year descriptive epidemiology of zoonotic abortifacient pathogen bacteria in farm animals in Turkey. *Comparative Immunology, Microbiology and Infectious*., 77: 101665.
20. Gülaydın, Ö., Öztürk, C., Ekin, . H., lhan, Z., and lhan, F. 2023. Investigation of selected bacterial agents causing sheep abortion in the Van Province by RT-PCR and histopathological methods. *Acta Veterinaria BRNO*., 92: 69-77.
21. Khan, A. U., Shell, W. S., Melzer, F., Sayour, A. E., Ramadan, E. S., Elschner, M. C., Moawad, A. A., Roesler, U., Neubauer, H., and El-Adawy, H. 2019. Identification, genotyping and antimicrobial susceptibility testing of *Brucella* spp. ısolated from livestock in Egypt. *Microorganisms*., 7: 603. doi:10.3390/microorganisms7120603.
22. lhan, Z., Solmaz, H., Ekin, and . H. 2013. In vitro antimicrobial susceptibility of *Brucella melitensis* ısolates from sheep in an area endemic for human brucellosis in Turkey. *Journal of Veterinary Medical Science*., 75(8): 1035–1040. doi: 10.1292/jvms.12-0163.
23. Etiz, P., Kibar, F., Ekeno lu, Y., and Yaman, A. 2015. Characterization of antibiotic susceptibility of *Brucella* spp. ısolates with E-Test Method. *Archıves of Clınıcal Mıcrobıology*., 6-1(1).
24. Dadar, M., Alamian, S., Brangsch, H., Elbadawy, M., Elkharsawi, A. R., Neubauer, H., and Wareth, G. 2023. Determination of virulence-associated genes and antimicrobial resistance profiles in *Brucella* isolates recovered from humans and animals in Iran using NGS technology. *Pathogens*., 12: 82. https://doi.org/10.3390/ pathogens12010082.
25. Selim, A., Manaa, E. A., Waheed, R. M., Alanzi, A. D. 2021. Seroprevalence, associated risk factors analysis and first molecular characterization of *Chlamydia abortus* among Egyptian sheep. *Comparative Immunology, Microbiology and Infectious Diseases*., 74: 101600.
26. Fayez, M., Elmoslemany, A., Alorabi, M., Alkafafy, M., Qasim, I., Al-Marri, T., and Elsohaby, I. 2021. Seroprevalence and risk factors associated with *Chlamydia abortus* infection in sheep and goats in Eastern Saudi Arabia. *Pathogen*., 10: 489.
27. Aras, Z., Sayın, Z., and Sanio lu Gölen, G. 2017. Investigation of the presence of *Chlamydophila abortus* in bovine abortions by PCR. *Eurasian Journal of Veterinary Sciences*., 33(2): 77-80.
28. Kanat, Ö. 2022. Molecular and histopathological investigation of Pestivirus, *Chlamydophila abortus* and *Listeria monocytogenes* infections in aborted sheep fetuses. *Hellenic Veterinary Medical Society Journal*., 73(1): 3889-3896. https://doi.org/10.12681/jhvms.26289.
29. Jimenez-Estrada, J. M., Escobedo-Guerra, M. R., Arteaga-Troncoso, G., Lopez-Hurtado, M., de Haro-Cruz, M., de Oca Jimenez, R. M., and GuerraInfante, F. M. 2008. Detection of *Chlamydophila abortus* in sheep (Ovis aries) in Mexico. *American Journal of Animal and Veterinary Sciences*., 3: 91-95.
30. Ababneh, H. S., Ababneh, M. M. K., Hananeh, W. M., Alsheyab, F. M., Jawasreh, K. M., and Ababneh, M. M. 2014. Molecular identification of chlamydial cause of abortion in small ruminants in Jordan. *Tropical Animal Health and Production*., http://dx.doi.org/10.1007/s11250-014-0654.