Investigation of selected bacterial agents causing sheep abortion in the Van Province by RT-PCR and histopathological methods

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Abstract

Abortion causes significant economic losses in the sheep industry. Determination of the aetiology is important in dealing with abortions. The present study was aimed to identify selected important bacterial pathogens in the abortion cases of sheep. A total of 113 samples (105 aborted sheep foetuses, 4 placentas, and 4 vaginal swab samples) from 85 different sheep flocks were examined by real-time PCR (RT-PCR) regarding *Chlamydia* (*C.*) spp., *C. abortus*, *Brucella* (*B.*) spp., *B. melitensis, Salmonella* (*S.*) spp., *S.* Abortusovis, *Coxiella* (*C.*) *burnetii, Listeria* (*L.*) spp., *L. monocytogenes,* and *Campylobacter* spp. All cases that were found to be positive for bacterial agents by RT-PCR, were examined pathologically. Tissue samples of foetuses that were found to be positive for *B. melitensis and L. monocytogenes* by RT-PCR were also investigated immunohistochemically. A total of 35 (30.9%) samples were found to be positive by RT-PCR, with 15 (42.8%), 9 (25.7%), 5 (14.2%), 4 (11.4%), 1 (2.8%), and 1 (2.8%) of them being identified as *C. abortus, B. melitensis, S.* Abortusovis, *C. burnetii, L. monocytogenes* and *Campylobacter* spp., respectively. The presence of the antigen was confirmed also immunohistochemically in the cases with *B. melitensis* and *L. monocytogenes*. As a consequence, *C. abortus* was found to cause the highest rate of sheep abortion cases, which should be taken into account when implementing control measures in epidemiological investigations.

Ovine, ruminants, reproduction, immunohistochemistry

Abortion is one of the most important reasons for infertility and they lead to significant financial losses in small ruminant livestock (Wu et al. 2014). The abortion rate of less than 2% in a flock is accepted as normal, however, a rate higher than 5% indicates the necessity of major precautions (Ay et al. 2017). Causes of abortion that can occur in each stage of pregnancy are classified as infectious or non-infectious causes (Brom et al. 2012). Non-infectious causes include nutritional disorders, sheltering, environmental circumstances, and misadministration of hormones and drugs (Kalender and Erdoğan 2014; Ay et al. 2017). Even though the diagnosis rate of abortion cases ranges between 5–50% worldwide, infectious agents were reported to be responsible for 90% of these cases (Ay et al. 2017). It has been reported that bacterial (e.g., brucellosis, campylobacteriosis, chlamydiosis, leptospirosis, listeriosis, salmonellosis, and Q-fever, etc.), viral (e.g., akabane virus, border disease, cache valley virus, caprine herpes virus and bluetongue virus, etc.), parasitic (e.g., neosporosis, sarcocystis, and toxoplasmosis, etc.) and fungal (e.g., aspergillosis and candidiasis, etc.) agents cause infectious abortion

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cases in small ruminants. On the other hand, the cases were noted to usually present a flock-based course (Milli 1998; Bastuji et al. 2006; Sharma et al. 2008; Kalender and Erdoğan 2014).

The identification of bacterial pathogens causing abortions presents major problems in the isolation of agents as well as lengthy procedure (Ay et al. 2017). Molecular diagnostic techniques such as PCR are suggested as an alternative diagnostic method for rapid identification of the aetiology (Ilhan et al. 2007; Saleh et al. 2013; Mahdavi et al. 2018). The present study was aimed to identify selected important bacterial pathogens in sheep abortion cases by real-time PCR (RT-PCR), and to examine the positive cases by histopathological and immunohistochemical methods.

Materials and Methods

Samples

The study was approved by the Animal Experiments Local Ethics Committee of Van Yüzüncü Yıl University by the Decision dated March 2, 2017 and numbered 2017/02.

The samples used in the study were collected from abortion cases in 85 different sheep flocks in the Van Province located in the Eastern Region of Türkiye during the lambing seasons of 2017–2018 and 2018–2019. A total of 101 stomach and 99 liver samples (95 of which belonged to the same foetus), 4 vaginal swabs, and 4 placenta samples taken from different sheep were used. The samples were taken approximately 2–18 h after abortion. They were transported with the aborted sheep foetuses to the laboratory of the Department of Microbiology, Faculty of Veterinary Medicine, Van Yüzüncü Yıl University, under cold chain conditions, the placenta samples in sterile sample containers and the vaginal swabs kept in a transport medium (L118598, LP Italiana SPA, Milan, Italy). Tissue samples were collected for pathologic and PCR investigation.

Method

Approximately 2–4 ml stomach content and 4–6 g liver samples obtained from the foetuses were taken into sterile plastic tubes. The liver and placenta samples were cut into small pieces using a sterilized clamp and lancet; then they were put into tubes containing 2 ml brain heart infusion broth (BHIB) (Merck, Darmstadt, Germany) and homogenized in the tissue lyser (LT 4241, Qiagen, Hilden, Germany). The homogenate was centrifuged at 2800 × *g* for 10 min and approximately 1.0–1.5 ml of the upper aqueous phase was transferred to sterile Eppendorf tubes. The vaginal swabs were transferred into tubes containing 5 ml BHIB and vortexed; then approximately 2–4 ml of the upper aqueous phase were transferred to sterile plastic tubes. The stomach content, liver, placenta, and swab samples were stored at −80 °C until RT-PCR analysis.

RT-PCR analysis

Extraction: Genomic DNA was obtained using a commercial kit (K0722, Thermo Fisher, Waltham, MA, USA). The extraction kit was used in accordance with the manufacturer's instructions.

Primers: In the study, genus-specific and species-specific primers were used for the investigation of *Chlamydia* (*C.)* spp., *C. abortus*, *Brucella* (*B.*) spp., *B. melitensis, Salmonella* (*S.*) spp., *S.* Abortusovis, *Coxiella* (*C.*) *burnetii, Listeria* (*L.*) spp., *L. monocytogenes* and *Campylobacter* spp. by RT-PCR (Table 1).

Amplification: Commercial mastermix (PN0047, A.B.T®, Ankara, Türkiye) 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 RT-PCR protocol was optimized according to the instructions of the manufacturer company that synthesized the primers (Table 1). In the optimization process, the initial denaturation stage at 94 °C for 10 min and the final extension stage at 72 °C for 10 min were carried out. The samples were primarily amplified with genus-specific primers for *Chlamydia* spp., *Brucella* spp., *Salmonella* spp., *Listeria* spp., and *Campylobacter* spp., following positive samples were amplified with primers specific for *C. abortus*, *B. melitensis, S.* Abortusovis and *L. monocytogenes.* As the positive control, DNA isolated from *B. melitensis* (16M strain), *Salmonella* Typhimurium (ATCC 14028), *L. monocytogenes* (ATCC 7644), *C. fetus* subsp. *fetus* (ATCC 33246), *C. abortus* (wild strain), and *C. burnetii* (wild strain) supplied by the Department of Microbiology, Faculty of Veterinary Medicine, Van Yüzüncü Yıl University was applied. Sterile DNase/RNase-free diethylpyrocarbonate (DEPC)-treated water (Applichem, Darmstadt, Germany) without DNA template was used as the negative control.

Histopathology and immunohistochemistry

Tissue samples were fixed in 10% formalin, processed routinely, embedded in paraffin, and sections stained with haematoxylin and eosin. Then the selected sections were stained with Brown-Brenn Gram stain, Periodic Acid Schiff (PAS), Machivello and Gimenez stains. Immunohistochemical (IHC) staining was performed using the avidin–biotin immunoperoxidase complex method. Rabbit antisera against *L. monocytogenes* (Listeria O antiserum poly, serotypes 1 and 4) (Difco, Detroit, USA) and *B. melitensis* (16M monospecific M antisera)

Table 1. Primers, bacteria, target genes, sequences, melting temperature (Tm), product lengths, and reference studies addressing the use of sources and primers used in this study.

Bacterium	Target (Gene)	Sequence (5'-3')	Amplification Protocol	Product Length (bp)	Reference
Chlamydia spp.	*23S rRNA*	**F:** GGGCTAGACACGTGAAACCTA			
		R:ACCGTAATGGGTAGGAGGGGT	94 °C - 60 s / 59 °C-60 s / 72 °C-60 s / 35 cycles	356	Nordentoft et al. 2011
C. abortus	*Pmp 90/91*	**F:** CTCACCATTGTCTCAGGTGGA			
		R: GGCAATCAGGTGCGACAATCT	94 °C-30 s / 56 °C-60 s / 72 °C-60 s / 35 cycles	821	Berri et al. 2009
Brucella spp.	*16S rRNA*	**F:** TCGAGCGCCCGCAAGGGG			
		R: AACCATAGTGTCTCCACTAA	94 °C-60 s / 53 °C-60 s / 72 °C-60 s / 35 cycles	905	Romero et al. 1995
B. melitensis	*IS711*	**F:** AAATCGCGTCCTTGCTGGTCTGA			
		R: TGCCGATCACTTAAGGGCCTTCAT	94 °C-60 s / 58 °C-60 s / 72 °C-60 s / 30 cycles	731	Bricker and Halling 1994
Salmonella spp.	*ompC*	**F:** ACCGCTAACGCTCGCCTGTAT			
		R: AGAGGTGGACGGGTTGCTGCCGTT	95 °C-30 s / 60 °C-45 s / 72 °C-60 s / 35 cycles	159	Kwang et al. 1996
S. Abortusovis	*IS200* element	**F:** CGATGAAAGCGTAAATAAGG			
		R: TTCTCTTGTCAGTCTCAAAC	94 °C-60 s / 48 °C-60 s / 72 °C-60 s / 30 cycles	900	Beuzon et al. 1997
C. burnetii	*IS1111a*	**F:** TATGTATCCACCGTAGCCAGT			
		R: CCCAACAACACCTCCTTATTC	94 °C-60 s / 58 °C-60 s / 72 °C-60 s / 35 cycles	687	Berri et al. 2009
Listeria spp.	*prs*	**F:** GCTGAAGAGATTGCGAAAGAAG			
		R: CAAAGAAACCTTGGATTTGCGG	94 °C - 30 s / 56 °C-45 s / 72 °C-30 s / 35 cycles	370	Doumith et al. 2004
L. monocytogenes	*listeriolysin*	**F:** GCATCTGCATTCAATAAAGA			
		R: TGTCACTGCATCTCCGTGGT	94 °C-30 s / 57 °C-45 s / 72 °C-30 s / 35 cycles	174	Deneer and Boychuk 1991
Campylobacter spp.	*C412*	**F:** GTTAAATAGTGTTCTTGGAG			
		R: CTAAGATATGGCTCTAACAA	94 °C-60 s / 56 °C-60 s / 72 °C-60 s / 35 cycles	816	Linton et al. 1996

(Pendik Veterinary Control and Research Institute, Istanbul, Türkiye) were used as the primary antibodies. For IHC, sections on adhesive slides were deparaffinized and dehydrated. During the antigen retrieval step, the tissue sections were heated in a microwave oven in 0.01 mol/l citric acid for 5 min and then cooled down for 20 min. Endogenous peroxidase was blocked by immersing the sections in 0.3% hydrogen peroxide in absolute methanol for 30 min. The sections were washed with phosphate-buffered saline (PBS; pH 7.2) and pretreated for 5 min with a protein blocker. All sections were incubated with the primary antibody for 60 min at room temperature. After rinsing with PBS, the sections were incubated for 20 min with a biotinylated goat anti-rabbit antibody at room temperature. After another PBS rinsing, the sections were treated with horseradish peroxidase-conjugated streptavidin for 20 min. After washing in PBS, DAB (Diaminobenzidine/Cell Signaling, 8090S) was used as a chromogen, while Mayer's haematoxylin was used for counterstaining (Sanderson et al. 2019).

The sections from encephalitic listeriosis with confirmed of *L. monocytogenes* and the sections obtained from aborted ovine foetuses found to be positive for *B. melitensis* were used as the positive control. The negative control replaced the primary antibody with PBS and was included for each slide run.

Results

RT-PCR results

In this study, 35 (30.9%) of 113 samples analyzed by RT-PCR were found positive for bacterial agents (Fig. 1). Of the RT-PCR positive samples, 15 (42.8%), 9 (25.7%), 5 (14.2%), 4 (11.4%), 1 (2.8%), and 1 (2.8%) were identified to be *C. abortus*, *B. melitensis, S.* Abortusovis, *C. burnetii*, *L. monocytogenes* and *Campylobacter* spp., respectively. All of the samples that resulted positive for *Chlamydia* spp., *Brucella* spp., *Salmonella* spp., and *Listeria* spp. were found positive for *C. abortus*, *B. melitensis*, *S.* Abortusovis, and *L. monocytogenes*, respectively. The distribution of agents identified by RT-PCR in the stomach, liver, vaginal swab and placenta samples is also shown in Table 2. None of the examined samples showed concurrent positivity for the tested bacterial agents.

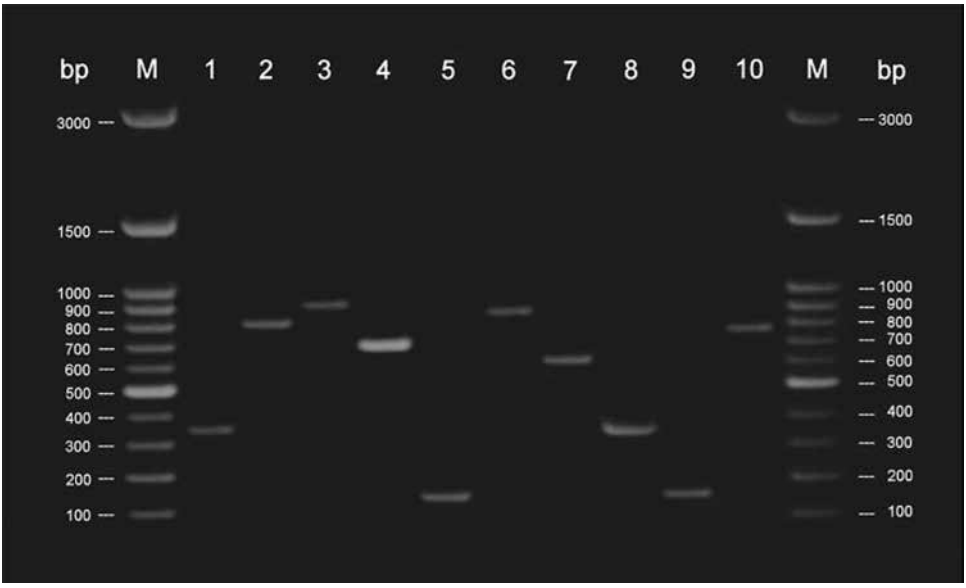

Fig. 1. Image of amplicons on agarose gel. M: 100 bp DNA ladder, 1: *Chlamydia* spp. positive sample, 2: *C. abortus* positive sample, 3: *Brucella* spp. positive sample, 4: *B. melitensis* positive sample, 5: *Salmonella* spp. positive sample, 6: *S. Abortusovis* positive sample, 7: *C. burnetii* positive sample, 8: *Listeria* spp. positive sample, 9: *L. monocytogenes* positive sample, 10: *Campylobacter* spp. positive sample

Table 2. The distribution of bacterial agents in foetal tissue and sheep samples.

Agent	Number of positive samples	Foetal tissue		Placenta	Vaginal Swab
		Stomach	Liver		
	3	-	+	*	*
	4	+	-	*	*
C. abortus	4	+	+	*	*
	2	+	-	*	*
	2	-	-	*	+
B. melitensis	3	+	-	*	*
	6	+	+	*	*
	2	+	-	*	*
S. Abortusovis	2	+	+	*	*
	1	-	-	+	*
C. burnetii	4	+	+	*	*
L. monocytogenes	1	-	+	*	*
Campylobacter spp.	1	-	-	*	+
Total	35	27	20	1	3

*No sample was taken; + positive finding

Pathology results

Macroscopically, few findings were observed in the aborted foetuses. While serosanguinous fluid within the body cavity, and multiple abscesses with a diameter of 1–2 mm were encountered in the liver of one aborted foetus that was identified to be *L. monocytogenes* positive by RT-PCR (Plate V, Fig. 2), no macroscopic change was noticed in the other organs. Subcutaneous oedema and serohaemorrhagic fluid within the body cavity were detected in the aborted foetuses found positive for *B. melitensis*. Grayish areas related to bronchopneumonia of the foetal atelectatic lung were found in two cases (Plate VI, Fig. 3).

All cases with positive RT-PCR were examined histopathologically. Tissue samples of foetuses that were found to be positive for *B. melitensis and L. monocytogenes* by RT-PCR were also investigated immunohistochemically. Histopathology examination of the foetal tissues positive for *C. abortus* by RT-PCR observed mononuclear cellular infiltration and areas of coagulative necrosis in the liver. Elementary body-like intracytoplasmic bacteria (inclusions), and vacuoles were determined in the cytoplasm of the hepatocytes by Macchiavello staining. The aborted foetuses with *S.* Abortusovis were observed with necrosis and mild mononuclear cellular infiltration in the liver. Multiple granulomatous hepatitis was identified in the foetus positive by RT-PCR for *C. burnetii* (Plate VI, Fig. 3A). Mononuclear cellular infiltrations composed of macrophages and lymphocytes were noticed being more intense particularly around the vessels. Multiple unstained foam-like vacuoles were remarkable in the macrophages. The agents were identified within the necrotic field in the centre of the granulomatous foci by Macchiavello staining (Fig. 3B). The necrotic foci were negative by PAS staining. Multiple abscess foci were detected in histopathological examination of the liver of the foetus found positive for *L. monocytogenes* by RT-PCR. Bacteria clusters were detected within the necrotic fields in the centre of these foci. The necrotic field was surrounded by neutrophils, leukocytes, and mononuclear cells. It was observed by Gram staining that the bacteria in these foci were blue-stained Gram positive bacteria and that these Gram positive agents were *L. monocytogenes* antigens in the immunohistochemical examination (Fig. 3C, D). The agents were identified in the cytoplasm of multiple macrophages by both immunoperoxidase and also Gram staining.

Bronchopneumonia was observed in two cases of *B. melitensis*-positive foetuses. The hepatocyte cordons were determined to be disorganized in the liver. In the portal field, mild-moderate mononuclear cellular infiltration and sporadic focal necrosis were encountered. Immunopositive staining was discovered in the *B. melitensis* positive cases in the immunohistochemical examination. *Brucella melitensis* antigens were located in the cytoplasm of macrophages, neutrophils, and/or extracellularly in the cellular debris in bronchi, bronchioles, and alveoli of the lung, extracellularly and/or intracellularly within the cytoplasm of macrophages in portal infiltrates and Kupffer cells of the liver (Plate VI, Fig. 3E, F).

Discussion

Abortion cases that cause significant economic losses in ruminants are multifactorial infections induced by various bacterial, viral, parasitic, and mycotic agents. Bacteria considered to be responsible for the infection include *Chlamydia* spp., *Brucella* spp., *Campylobacter* spp., *Salmonella* spp., *Coxiella* spp., *Listeria* spp., and *Leptospira* spp. (Szeredi et al. 2006; Quinn et al. 2011; Küçükayan et al. 2014; Sakmanoğlu et al. 2021).

The Van Province located in the Eastern Anatolian Region of Türkiye has been ranked at the top among the provinces with 2.708.012 sheep (TUIK 2019). Sheep rearing is one of the most important sources of income for the local community. Fertility is of critical importance in a healthily developing maintenance of the sheep rearing sector (Güner et al. 2016). Good veterinary practices for an appropriate and timely management of infections caused by bacterial pathogens are extremely important in sheep breeding. Detecting infected or vector animals is the first step in infection management (Büyük et al. 2020). In this study, a total of 113 aborted sheep samples were collected from the Van Province and its surroundings, and analysed for *Chlamydia* spp., *C. abortus*, *Brucella* spp., *B. melitensis, Salmonella* spp., *S.* Abortusovis, *C. burnetii, Listeria* spp., *L. monocytogenes,* and *Campylobacter* spp. by RT-PCR. All cases with positive RT-PCR were examined histopathologically. Tissue samples of foetuses that were found to be positive for *B. melitensis and L. monocytogenes* by RT-PCR were also investigated immunohistochemically. Of the RT-PCR-positive samples, 15 (42.8%), 9 (25.7%), 5 (14.2%), 4 (11.4%), 1 (2.8%), and 1 (2.8%) were identified to be *C. abortus*, *B. melitensis, S.* Abortusovis, *C. burnetii*, *L. monocytogenes* and *Campylobacter* spp., respectively.

Abortion cases caused by *Coxiella* and *Chlamydia* species are differentially diagnosed, usually by using Stamp, Giemsa, Gimenez, or Macchiavello staining techniques. In the present study, all the cases with positive RT-PCR were stained using the haematoxylin and eosin. The selected sections were stained Gram, Macchiavello, and Gimenez staining techniques to differentiate *L. monocytogenes*, *Brucella* spp., *Chlamydia* spp., and *Coxiella* spp. *Brucella* spp.-suspected sections stained with Gram stain were detected to be immunoreactive with the anti-*B. melitensis* antibody by immunohistochemistry, however, these sections were found to be negative for *Coxiella* spp. and *Chlamydi*a spp. using Macchiavello and Gimenez stains. Even when *Coxiella* spp. and *Chlamydia* spp. are stained with the same stains, *Coxiella* spp. are morphologically pleomorphic or thin rods-shaped, whereas *Chlamydial* particles are very small and round. Of those, elementary bodies are purple red-stained and have a size of 0.2–0.5 μm whereas reticular bodies are blue-stained and have a size of 0.9–1.0 μm (Schlafer and Foster 2016). The histopathological results obtained in the present study were consistent with the findings previously described in the cases infected with *C. abortus* and *C. burnetii* (Büyük et al. 2020; Dorsch et al. 2021). The histopathological examination revealed mononuclear cellular infiltration and areas of coagulative necrosis in the liver. Elementary body-like intracytoplasmic

bacteria (inclusions), and vacuoles were determined in the cytoplasm of the hepatocytes by Macchiavello staining compatible with *Chlamydia* spp. It has been reported that *C. burnetii* may cause bronchopneumonia by reaching the intestines and consequently the lungs when ingested and may lead to granulomatous hepatitis when transmitted via the haematogenous route (Agerholm 2013). Similarly, granulomatous necrotic foci were detected in the liver that was found to be positive for *C. burnetii* by PCR. Agents belonging to *Listeria* species cause critical infections in the animals including abortion. Şahin and Beytut (2006) have reported that diffuse coagulative necrosis was determined in the liver and lung tissues by the necropsy of 5 aborted foetuses taken from the sheep husbandry company including 120 head of sheep detected with abortion, and that *Listeria ivanovii* subsp. *ivanovii* was isolated in the samples. In the present study, multiple pale-coloured foci were encountered consistently with the abortive form of listeriosis in the foetal liver of a case in which *L. monocytogenes* was isolated. Histopathological examination of these foci revealed microabscesses composed of intense neutrophils, leukocytes, and multifocal necrotic fields. It was detected by the examination of the Gram positive bacterial clusters observed in the microabscesses using the immunoperoxidase method that the bacteria were *Listeria monocytogenes.* In the present study, the detection of *L. monocytogenes* in only 1 (0.8%) of the examined 113 samples suggested that this agent may have a low level of responsibility for sheep abortion cases observed in the Van Province and its surroundings.

Many studies have been carried out in Türkiye and also other regions of the world to identify the bacteria that cause abortions in sheep by applying the bacteriological culture, serological, and molecular techniques. In a study that examined the material of 463 aborted sheep foetuses sent to the Veterinary Control Central Research Institute from different provinces of Türkiye, *Brucella* spp. and *C. fetus* subsp. *fetus* were isolated from 139 (30.1%) and 6 (1.2%) of the samples, respectively (Küçükayan et al. 2014). In another study, a total of 90 aborted sheep foetuses were investigated by PCR in 2019 in different regions of Türkiye. It has been stated that 20 (22.2%), 17 (18.8%), 8 (8.8%), 4 (2.2%) and 4 (2.2%) samples were positive for *C. fetus, B. melitensis, Leptospira* spp., *B. abortus* and *C. burnetii,* respectively (Sakmanoğlu et al. 2021). It has been reported that *C. fetus* was detected in 68 (51.5%) of the samples in a study carried out in Iran that analysed vaginal swab samples taken from 132 aborted and 85 healthy sheep by the PCR method for *C. abortus*, *B. ovis*, *S. enterica*, *C. burnetii*, *C. fetus* subsp. *fetus* and *Yersinia enterocolitica* (Saleh et al. 2013). In a study that was carried out in Hungary, the authors examined the foetal membrane and stomach content samples taken from 246 aborted sheep and 75 aborted goats by conventional bacteriological methods; it has been reported that *C. abortus* was isolated in 113 (56%) and 13 (25%) of the sheep and goats, respectively. It has been noted that *C. jejuni*, *C. burnetii,* and *Leptospira* spp. were isolated in 1 (0.4%), 5 (2%), and 1 (0.4%) of the sheep samples, respectively, while *C. burnetii* and *L. monocytogenes* were isolated in 1 (0.4%) and 1 (0.4%) of the goat samples (Szeredi et al. 2006). In the present study, 35 (30.9%) of the cases were found to be positive by RT-PCR. Of the RT-PCR positive samples, 15 (42.8%), 9 (25.7%), 5 (14.2%), 4 (11.4%), 1 (2.8%), and 1 (2.8%) were identified to be *C. abortus*, *B. melitensis, S.* Abortusovis, *C. burnetii*, *L. monocytogenes* and *Campylobacter* spp., respectively. It has been noticed in the light of the studies related to the subject (Szeredi et al. 2006; Saleh et al. 2013; Küçükayan et al. 2014; Sakmanoğlu et al. 2021) that the bacterial agents considered to be responsible for the sheep abortion cases demonstrated significant differences with respect to both species and also rates. This condition may be associated also with the presence of some bacterial agents as endemic pathogens in certain geographical regions as well as it may be related to the application of different methods.

The main infection sources in abortions are aborted foetuses, placenta, and vaginal discharges of the aborted sheep. Bacteriological culture and molecular techniques such

as PCR methods can be used to detect the bacterial pathogens in samples taken freshly and maintained properly. However, conventional bacteriological culture methods of the agents with intracellular characteristics such as *Chlamydia* spp., *C. abortus,* and *C. burnetii* cannot be done and thus PCR-based techniques are used for the direct diagnosis of these agents. Rapid and reliable diagnoses of bacterial pathogens in infections are very important in preventing infection and thus reducing economic losses (Lorenz et al. 1998; Laroucau et al. 2002; Büyük et al. 2020). In this respect, PCR-based techniques have an important advantage and can identify the genus, species, and even subspecies of bacterial pathogens. In this research, we determined that the applied RT-PCR method was successful for the detection of some important bacterial pathogens in terms of both genus and species in aborted sheep samples.

Different samples have been used for the detection of bacterial pathogens in sheep abortion samples by bacteriological culture and PCR-based methods (Berri et al. 2002; Bastuji et al. 2006; Mahdavi et al. 2018; Büyük et al. 2020). In the present study, stomach content, liver, placenta, and vaginal swab samples were tested for the detection of bacterial pathogens by RT-PCR. Bacterial pathogens were detected in 26.7% of stomach content samples and 20.2% of liver samples. This indicates that stomach content analysis is more suitable for detection of the DNA of main bacterial pathogens compared to liver samples.

Prevention of the bacterial agents that cause abortions is extremely important for the sheep industry to be profitable. Hence, accurate diagnosis of the bacterial agents causing abortions is imperative. The findings of this study demonstrate that *C. abortus, B. melitensis*, *S.* Abortusovis*, C. burnetii, L. monocytogenes,* and *Campylobacter* spp. were responsible for 30.9% of the sheep abortions. *Chladmydia abortus* was found to be the most responsible bacterium in sheep abortions, which should be taken into account regarding the implementation of control measures in epidemiological investigations. In addition, the risk of the animal abortion transmitting the infection to humans should also be considered.

Conflict of Interest

There is no conflict of interest.

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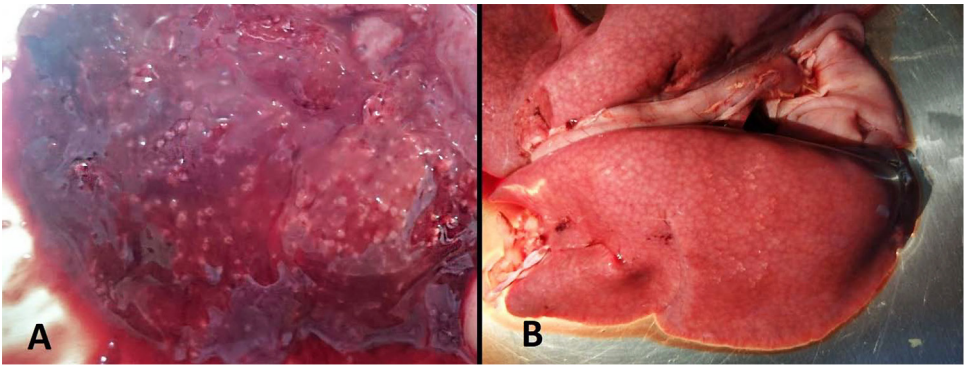

Fig. 2. A: Pintpoint multiple abscesses in the liver. *L. monocytogenes* positive aborted foetus; B: Bronchopneumonia in the lung. *B. melitensis* positive aborted foetus

Plate VI

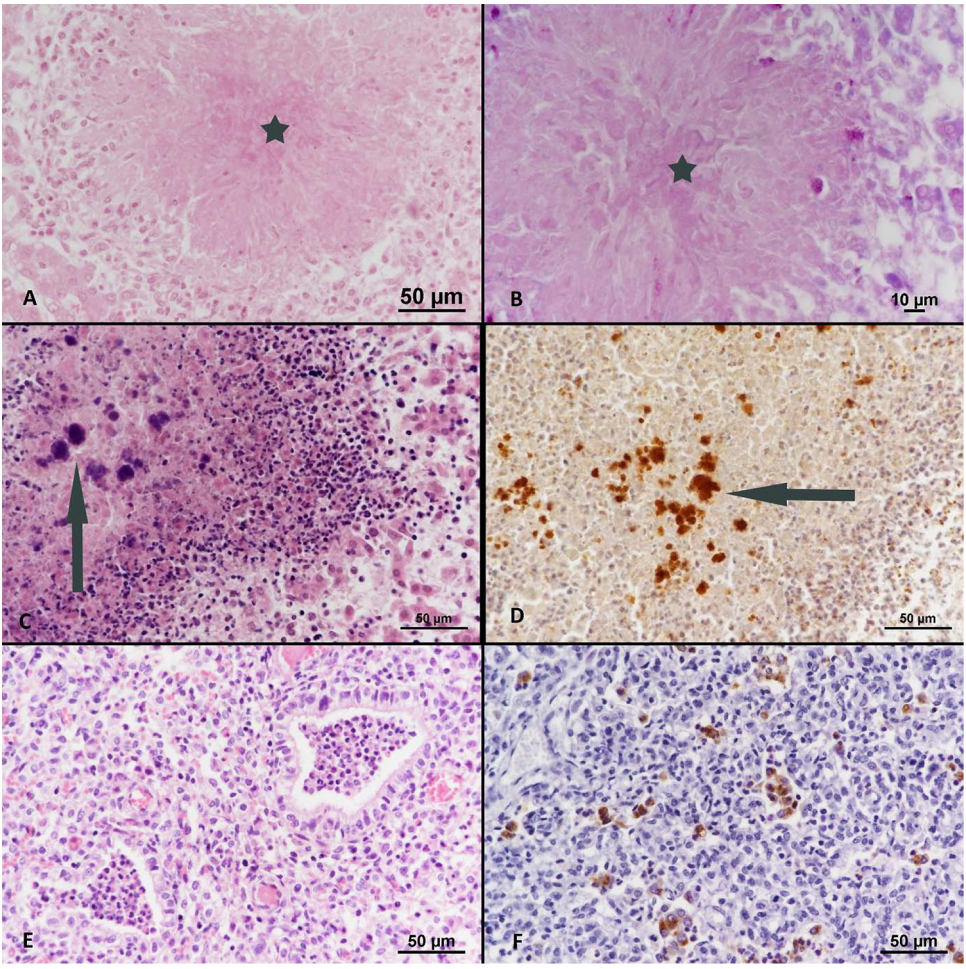

Fig. 3. A: Multiple granuloma in the liver of the fetus with *C. burnetii* positive by PCR. The granuloma was composed by necrotic center (★) surrounded by macrophages and lymphocytes, HE stain; B: *C. burnetii* positive liver. Purple-red stained agents (→) in the necrotic areas (★), Macchiavello stain; C: Foetal liver, L. monocytogenes positive by PCR. Neutrophils, leukocytes, and mononuclear cellular infiltration around the abscess with the necrotic centre. Blue-stained bacterial clusters in the necrotic foci (→) HE stain, D: *L. monocytogenes* positive bacterial foci located in the necrotic foci (→), DAB chromogen, immunoperoxidase stain; E: Lung, *B. melitensis* positive by PCR. Cellular exudate in the lumens of alveoli and bronchioles, bronchopneumonia, HE stain; F: *B. melitensis* antigens were detected in the cytoplasm of macrophage and neutrophils within the alveoli of the lung, DAB chromogen, immunoperoxidase stain