



RESEARCH ARTICLE

Investigation of *Listeria* and *Salmonella* Species and *Chlamydomphila abortus* in Aborted Sheep Fetuses Exhibiting Hepatitis Lesions by Immunohistochemical, Cultural and Molecular Methods

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ABSTRACT

Abortions in small ruminants represent a major concern in veterinary medicine due to their multifactorial etiology and the involvement of various zoonotic bacterial pathogens. This study aimed to investigate *Listeria* spp., *Salmonella* spp., and *Chlamydomphila abortus* in the livers of aborted sheep fetuses exhibiting hepatitis using histopathological, immunohistochemical (IHC), cultural, and molecular (PCR) methods. The study material consisted of liver samples from 296 aborted sheep fetuses in Kars Province and surrounding villages, between 2016 and 2023. Hepatitis was identified in 100 (33.78%) of the aborted fetuses. IHC analysis revealed positivity in 7 (7%) samples for *Listeria monocytogenes*, 13 (13%) samples for *Salmonella* spp., and 6 (6%) samples for *Chlamydomphila abortus*. Concurrent IHC positivity was detected in 3 samples for *Salmonella* spp. and *L. monocytogenes* and in 1 sample for *Salmonella* spp. and *C. abortus*. Cultural analysis revealed positivity in 4 (4%) samples for *Salmonella* spp. and in 4 (4%) samples for *L. monocytogenes*, while simultaneous culture positivity for both agents was observed in 1 (1%) sample. Direct PCR revealed that 11% (n= 11) of the samples were positive for *Salmonella* spp., 6% (n= 6) for *L. monocytogenes*, and 5% (n= 5) for *C. abortus*. Overall, *L. monocytogenes*, *Salmonella* spp., and *C. abortus* were identified as important causes of sheep abortions with hepatitis lesions in the Kars region, and immunohistochemistry proved to be a rapid and reliable diagnostic method with high concordance to culture and PCR.

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INTRODUCTION

The number of small ruminants in Türkiye is 58.45 million, with sheep occupying an important place, comprising approximately 46 million heads (TUIK, 2022). Similar to global livestock practices, the primary goals of Turkish livestock operations are to achieve high yields of meat, milk, leather, and wool with an additional aim to produce healthy offspring leading to increased profitability. Despite these goals, early embryonic death, abortion, fetal mummification, and the birth of abnormal offspring in ruminants result in significant economic losses (Chundekkad *et al.*, 2020). Abortions in sheep may occur

due to various infectious and non-infectious causes (Modise *et al.*, 2023). In most abortion cases, non-infectious causes remain undetermined because the abortion rate in ewes is low and therefore does not warrant detailed investigation. Consequently, infectious agents that can be rapidly diagnosed are of greater importance (Clune *et al.*, 2020; Esmaeili *et al.*, 2022). The primary infectious agents causing abortion are bacteria, including *Campylobacter* spp., *Brucella melitensis*, *Listeria* spp., *Salmonella* spp., *Coxiella burnetii*, and *Chlamydia abortus* (Chanton-Greutmann *et al.*, 2002; De Angelis *et al.*, 2022).

Listeriosis is a disease that affects humans and various animal species worldwide, including ruminants. *Listeria*

monocytogenes is the predominant agent of this zoonotic disease in ruminants, while *Listeria ivanovii* has rarely been reported in sheep abortions (Akca *et al.*, 2022). Abortion, meningoencephalitis, epticemia, and death are among the general clinical signs of *L. monocytogenes* infection (Haligur *et al.*, 2019). The disease is primarily transmitted through fetal membranes and maternal placentas of sick and asymptomatic animals, as well as through contaminated feed, water, or aborted materials (Barkallah *et al.*, 2016). Moreover, *Listeria* species are frequently present in improperly produced and/or stored silages (De Angelis *et al.*, 2022). In cases of listeriosis in sheep, abortions usually occur in the last stages of pregnancy (around the 12th week) (Barkallah *et al.*, 2014).

Salmonella enterica serovar *Abortusovis* (SAO) is a serovar of *Salmonella enterica* adapted to sheep. It causes infections in which abortion is the main symptom. In infected herds, the primary clinical sign is abortion, occurring during the last trimester of pregnancy in approximately 30-50% of pregnant ewes without any preceding clinical signs (Amagliani *et al.*, 2021). Unlike other *Salmonella* infections, *S. abortusovis* infection rarely results in gastrointestinal disease (Belloy *et al.*, 2009). The source and persistence of this agent in animals is due to feed and water contaminated with animal feces. No lesions specific to *S. abortusovis* infection are observed in the fetus. In cases of abortions caused by *Salmonella* and *Listeria* species, the bacteria multiply in the placenta and then infect the fetal liver, leading to septicemia and death in the fetus. Multifocal suppurative hepatitis may develop in the liver (Wray and Linklater, 2000).

Another microorganism that plays an important role in sheep abortions is *Chlamydophila abortus*. It is a zoonotic agent and causes enzootic abortion of ewes (EAE). This agent can cause serious systemic infections in animals and humans (Creelan *et al.*, 2000; Essing and Longbottom, 2015). In sheep infected with *C. abortus*, abortions usually occur during the last 2-3 weeks of pregnancy (Rocchi *et al.*, 2009). Pathological changes may be observed in the placenta, liver and the body cavities of the fetus. Microscopically, the placenta exhibits vasculitis and fibrinoid necrosis along with mononuclear cell infiltrations (Robert and Moeller, 2012; Kalender *et al.*, 2013).

Numerous studies conducted in different geographical regions and time periods in Türkiye have identified the etiology of abortions in sheep and emphasized their importance. *L. monocytogenes* and *L. ivanovii* have been reported as causative agents of sheep abortions (Akca *et al.*, 2022; Gulaydin *et al.*, 2023; Akar *et al.*, 2024). Similarly, *S. Abortusovis* has been identified as a cause of late-term abortions in sheep herds (Muz *et al.*, 1999; Gülaydin *et al.*, 2023). Studies have also reported *C. abortus* as the primary causative agent in enzootic sheep abortions (Büyük *et al.*, 2020; Malal and Turkyılmaz, 2021; Gülaydin *et al.*, 2023; Akpınar *et al.*, 2024). All these findings emphasize the need to investigate these bacterial agents in herds, as they have significant effects on productivity and public health. Therefore, this study aimed to investigate *Listeria* spp., *Salmonella* spp., and *Chlamydophila* spp., which are among the important bacterial agents that may cause abortions in sheep in the Kars province, using histopathological, immunohistochemical, cultural, and molecular methods.

MATERIALS AND METHODS

The study material consisted of sheep abortions observed in the city center and villages of Kars province, Türkiye, between 2016 and 2023. During the study period, 296 liver tissue samples from aborted sheep fetuses sent to the Department of Pathology, Faculty of Veterinary Medicine, Kafkas University, were examined. Based on anamnestic information provided by the animal owners, 180 fetuses (60.81%) were collected during the 4th and 5th months of gestation, while 116 fetuses (39.18%) were collected during the first trimester. A total of 150 samples (50.67%) were obtained from the aborted sheep vaccinated against *Brucella* spp. The lesion-containing portions of the liver tissue samples were fixed in 10% formalin for pathological examinations, while the remaining portions were kept at -20°C for the microbiological analysis.

Ethics committee report: Ethical approval for the study was obtained from the Animal Experiments Local Ethics Committee of the Kafkas University (Türkiye) (KAU-HADYEK/2021-050).

Histopathological examinations: For hematoxylin-eosin staining, tissue sections (4µm) were cut from paraffin blocks and passed through xylene baths for 10min to remove the paraffin. The sections were rehydrated by passing them through a series of alcohols of high to low concentrations and stained with Mayer's hematoxylin for 10min. The sections were rinsed with tap water, briefly dipped in 1% acid-alcohol, and washed again. Subsequently, the sections were immersed in ammonia water for 3min to obtain bright blue nuclear staining. The sections were then rinsed with distilled water for 2min, immersed in 96% ethanol, and counterstained with eosin for 5min. The sections were dehydrated by passing through 70% to 100% ethanol. The sections were cleaned twice with xylene for 10min and mounted with Entellan. Subsequently, the slides were examined using a light microscope (Olympus BX53) and photographed using the Cell^P software (Olympus Soft Imaging Solutions GmbH, version 3.4) (Dağ *et al.*, 2018).

Immunohistochemical examination: The immunohistochemical (IHC) staining of the section was conducted by the avidin-biotin peroxidase method. Following the deparaffinization and rehydration steps, the sections were incubated in 3% H₂O₂ for 20min. The sections were heated in citrate buffer (pH 6.0) in a microwave oven (800 W) for 10min for antigen retrieval. Non-specific staining was prevented by treating the sections with non-immune serum (Thermo Scientific Histostain IHC Kit, HRP, Broad Spectrum, REF: TP-125-HL) for 30min. The sections were incubated with primary antibodies, including anti-*Listeria monocytogenes* (NovusBio, NB100-65667, 1:100), anti-*Salmonella* spp. (NovusBio, NB600-1087, 1:100), and anti-*Chlamydia abortus* (Santa Cruz Biotechnology, sc-101593, 1:100) at 4°C for overnight. Subsequently, the sections were kept at room temperature for 3min with a biotinylated secondary antibody, followed by incubation with peroxidase-conjugated streptavidin, both provided in the Thermo Scientific Histostain IHC Kit (HRP, Broad Spectrum, REF: TP-125-HL). Color development was

achieved using an AEC chromogen solution (Thermo Fisher Scientific, REF: TA-125-HA). The sections were counterstained with Mayer's hematoxylin and mounted with ImmunoMount.

Microbiological analysis

Bacterial culture: For cultural analysis, *in vitro* culturable agents, *Listeria* spp. and *Salmonella* spp., were analyzed. For this purpose, homogenates obtained after homogenization of the liver samples with hepatitis were used.

Isolation of *Listeria* species from aborted fetal tissues was performed by modifying the method reported by McClain and Lee (1988). For this purpose, 25ml tissue homogenate was mixed with 225ml pre-enrichment liquid medium (Tryptic Soy broth (Merck, 1.05459)) and incubated at 30°C for 24 hours. Then, 10ml pre-enriched liquid medium was transferred to 90ml of Listeria Enrichment Broth (UVM formulation) (ThermoFisher, CM0863B) and incubated overnight at 30°C. Then, Listeria Selective Agar (LSA) (ThermoFisher, CM0856B) was inoculated from the enrichment broth and incubated at 30°C for 24 hours. The grey-black centered smooth colonies on LSA agar were selected for phenotypical confirmation as suspected *Listeria* spp. (Akca *et al.*, 2022).

Isolation of *Salmonella* species was performed according to the ISO 6579 standard. Tissue homogenates were incubated in a 1:10 peptone buffer at 37°C for 24 hours. Subsequently, 1 ml of this sample was transferred to Rappaport-Vassiliadis medium (ThermoFisher, CM0910B) and incubated at 42°C for 24 hours. Samples selectively enriched were added to Xylose lysine deoxycholate (XLD) (ThermoFisher, CM0469B) and Xylose lysine tergitol-4 (XLT-4) agar (ThermoFisher, CM1061B) and incubated at 37°C for 24-48 hours. Dark-centered pink colonies on XLD agar and black colonies on XLT-4 agar suspected as *Salmonella* spp. were selected for phenotypical confirmation (Hyatt and Weese, 2004).

Identification of *Listeria* spp. and *Salmonella* spp. suspected isolates were performed by phenotypic tests such as Gram staining, catalase, oxidase, motility, methyl red, and nitrate reduction tests for *Listeria* spp. and Gram staining, motility, H₂S production, lactose fermentation, oxidase, indole, methyl red, voges-proskauer and citrate for *Salmonella* spp. (TGW, 2025).

Molecular analysis

DNA extraction: The phenol/chloroform/isoamyl alcohol method was used in the extraction of total DNA from *Listeria* spp. and *Salmonella* spp. isolates obtained by cultural methods and total DNA from aborted fetal liver tissues (Sambrook *et al.*, 1989). Identified isolates and PCR-positive samples from the Kafkas University were used as positive controls in PCR analyses.

Quality and concentration of the DNA samples were evaluated on 1.5% agarose gel and a NanoDrop microvolume spectrophotometer (BioTek, USA). DNA samples with A260/280 ratios between 1.7 and 2.0 and DNA concentrations ranging between 20 and 75ng/μL were considered of sufficient purity and concentration for PCR amplification.

Direct PCR analysis: Aborted fetal liver samples were analyzed for the presence of *C. abortus*, an intracellular bacterium that cannot be cultured *in vitro*, by direct PCR. In addition, *Listeria* spp. and *Salmonella* spp. in fetal tissue samples were analyzed by direct PCR to compare with the cultural method.

Genus or species-specific PCR: Confirmation of the *Listeria* spp. and *Salmonella* spp. isolates was performed by genus or species-specific PCR methods.

Listeria species was investigated by PCR, as reported previously (Bubert *et al.*, 1999). For this purpose, primers MonoA (5'-CAAACGTGTAACACAGCTACT-3') (for *L. monocytogenes*), Iva1 (5'-CTACTCAAGCGCAAGCGGCAC-3') (for *L. ivanovii*), and Lis1B (5'-TTATACGCGACCGAAGCCAAC-3') (common) were used. The total reaction volume of PCR (20μl for each sample) consisted of 10 μl Hot Start Taq Master Mix (Qiagen, Hilden, Germany), 0.1μl each primer (10mm), 7.7μl PCR water, and 2μl template DNA. The PCR thermal cycle consists of an initial denaturation at 95°C for 5min, followed by 30 cycles including denaturation at 95°C for 15sec, primer binding at 58°C for 30 sec, synthesis at 72°C for 50sec, and a final elongation at 72°C for 10min.

Salmonella spp. was investigated by PCR, as reported previously (Rahn *et al.*, 1992). For this purpose, F (5'-GTGAAATTATCGCCACGTTCCGGCAA-3') and R (5'-TCATCGCACCGTCAAAGGAACC-3') primers were used. The total reaction volume of PCR (20μl for each sample) consisted of 10μl Hot Start Taq Master Mix (Qiagen, Hilden, Germany), 0.1μl each primer (10mm), 7.8μl PCR water, and 2μl template DNA. The PCR thermal cycle consists of an initial denaturation at 95°C for 5min, followed by 30 cycles including denaturation at 95°C for 15sec, primer binding at 50°C for 30sec, synthesis at 72°C for 50sec, and a final elongation at 72°C for 10min.

C. abortus was investigated by PCR, as reported previously (Crealan and McCullough, 2000). For this purpose, primers 8FP (5'-TGGTATTCTTGCCGATGA-3') and RP (5'-GATCGTAACTGCTTAATAAACCG-3') were used. The total reaction volume of PCR (20μl for each sample) consisted of 10μl Hot Start Taq Master Mix (Qiagen, Hilden, Germany), 0.1μl each primer (10mm), 7.8μl PCR water, and 2μl template DNA. The PCR thermal cycle consists of an initial denaturation at 95°C for 5min, followed by 40 cycles including denaturation at 94°C for 1min, primer binding at 50°C for 1min, synthesis at 72°C for 2min, and a final elongation at 72°C for 10min.

The PCR products were analyzed on a 1.5% agarose gel prepared with 1xTBE Buffer, stained with ethidium bromide, and visualized under a UV transilluminator. A 660bp product was considered as *L. monocytogenes*, a 1100bp product as *L. ivanovii*, a 284bp product as *Salmonella* spp., and a 479bp product as *C. abortus*.

Statistical analysis: The statistical analysis was carried out by interactive programs. Sensitivity, specificity, and diagnostic accuracy of the tests were determined using the MedCalc software (version 2024). The chi-square test was conducted using GraphPad software (version 2024).

RESULTS

Macroscopic findings: In the majority of aborted fetuses, subcutaneous edema and bloody-serous exudate in the abdomen and thoracic cavities were observed. In addition, pinhead-sized yellow-white foci were detected in the liver in some cases (Fig. 1).

Microscopic findings: Histopathological examination revealed that 100 (33.78%) of the 296 aborted lambs

exhibited hepatitis in the liver, with the inflammatory cell population being predominantly lymphohistiocytic in most cases. Cellular infiltrations were mainly observed in the portal areas. In addition to cell infiltration, hepatic hyperemia was detected in 20 (20%) of the 100 cases. Typhoid nodule formation was observed in 3 (3%) cases, characterized by central coagulative necrosis surrounded predominantly by histiocytes and lymphocytes, with fewer neutrophils. Furthermore, miliary abscess foci were identified in 4 (4%) of the cases (Fig. 2A-F).

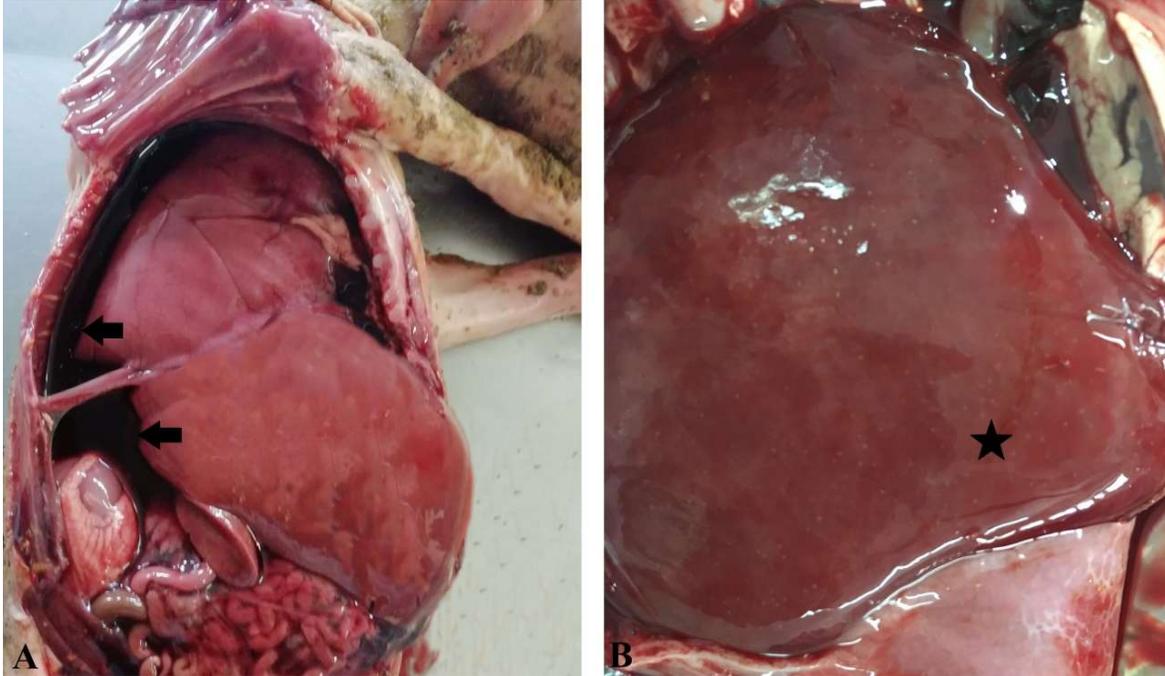


Fig. 1: Lamb aborted fetus, A: Bloody serous exudate accumulation in the abdominal and thoracic cavity (arrows), B: Miliary foci in the liver (asterisk).

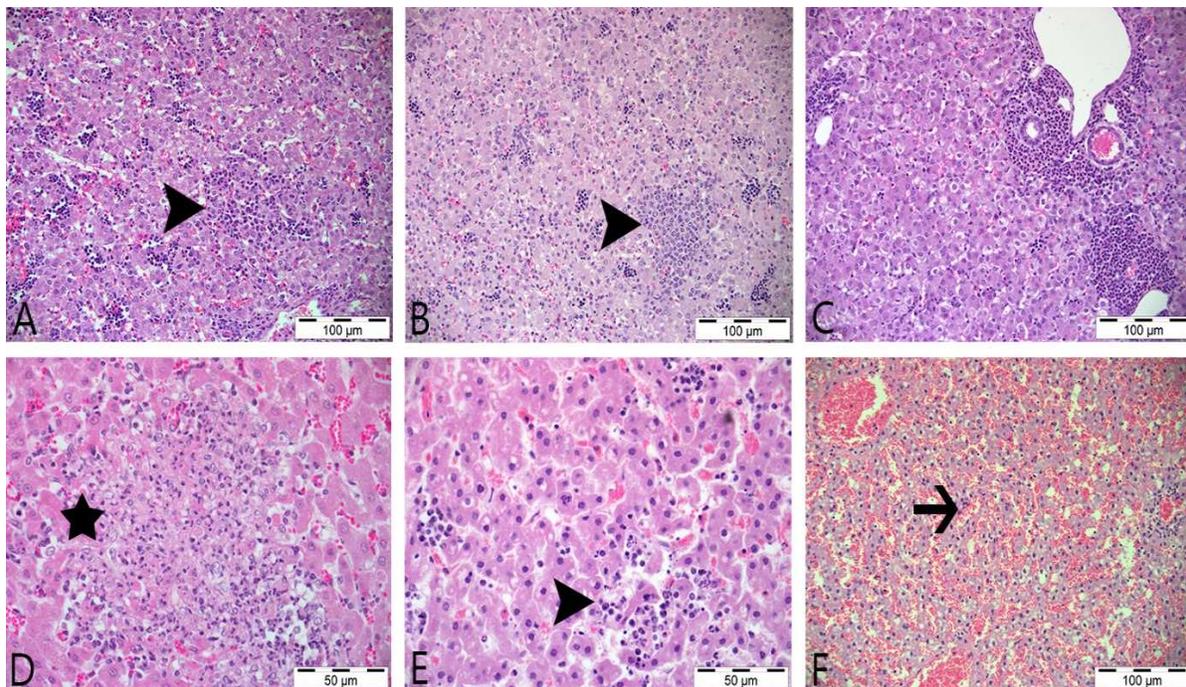


Fig. 2: Aborted lamb fetal liver tissue; A-B: Hepatitis table, lymphohistiocytic cell infiltration (arrowhead), 100µm. C: Portal triad (square), perivascular mononuclear cell infiltration, 100µm. D: Typhoid nodule (star), 50µm. E: Microabscess (arrowhead), 50µm. F: Hyperemia (arrow), 100µm. H&E.

Immunohistochemical (IHC) Findings: As a result of the immunohistochemical staining using primary antibodies against *L. monocytogenes*, *Salmonella* spp. and *C. abortus*, 7 (7%) of the 100 liver samples with hepatitis showed positive immunoreactivity for *L. monocytogenes*, 13 (13%) for *Salmonella* spp., and 6 (6%) for *C. abortus*. Among the 296 aborted cases, the positivity rates were 2.4% for *L. monocytogenes*, 4.3% for *Salmonella* spp. and 2% for *C. abortus*. Concurrent IHC positivity for *Salmonella* spp. and *L. monocytogenes* was detected in 3 (3%) samples, while

concurrent positivity for *Salmonella* spp. and *C. abortus* was detected in 1 (1%) sample. In immunohistochemical staining, *L. monocytogenes* positivity was detected granularly and intracytoplasmically, particularly in hepatocytes surrounding miliary abscesses (Fig. 3A-3B). *Salmonella* spp. positivity was detected in the cytoplasm of lymphohistiocytic cells and in surrounding hepatocytes within typhoid nodules (Fig. 3C-3D). *C. abortus* positivity was identified in the cytoplasm of hepatocytes (Fig. 3E-3F).

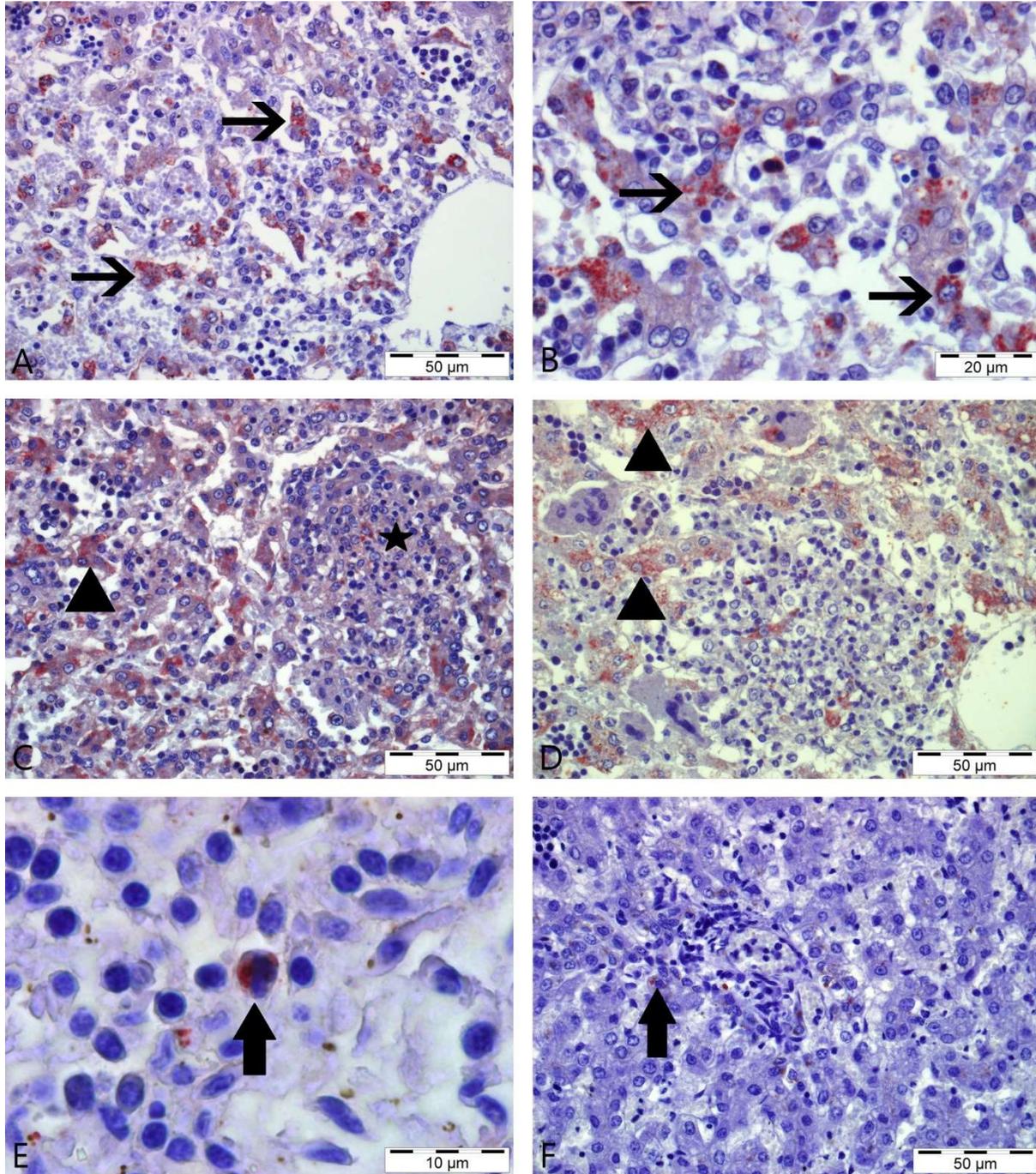


Fig. 3: Fetal lamb liver tissue; A-B: *Listeria* spp. IHC staining; granular form in the cytoplasm of hepatocytes. Positive immune reaction (thin arrows), 50μm. C-D: *Salmonella* spp. IHC staining; granular form in the cytoplasm of hepatocytes (arrowheads) and mononuclear cells (stars). 50μm. Positive immune reaction, E-F: *C. abortus* immunohistochemical staining; positive immunoreactivity in the cytoplasm of hepatocytes (thick arrows), IHC. E: 10μm, F: 50μm.

Microbiological analysis findings: As a result of the cultural analysis, 4 (4%) of the 100 samples were found to be positive for *Listeria* spp. and 4 (4%) were positive for *Salmonella* spp. Concurrent culture positivity for *Listeria* spp. and *Salmonella* spp. was observed in 1 (1%) sample. Genus-specific and species-specific PCR analyses of the isolates revealed that all *Listeria* isolates were identified as *L. monocytogenes* and all *Salmonella* isolates were identified as *Salmonella* spp. (Table 1, Fig. 4).

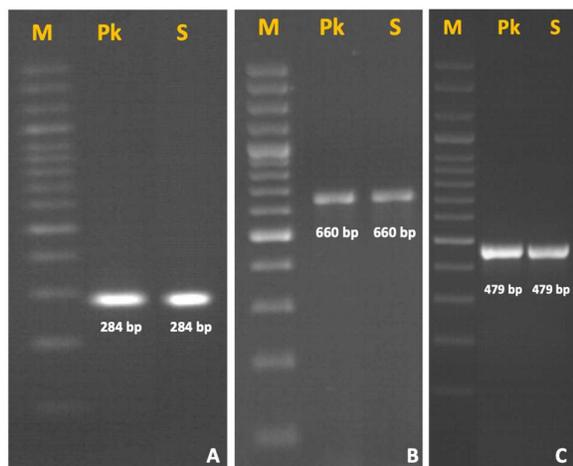


Fig. 4: Agarose gel (1.5%) electrophoresis image of PCR products. A: *Salmonella* spp., B: *L. monocytogenes*, C: *C. abortus*, M: Marker (Thermo Sci., SM0321), Pk: Positive control, S: Field isolate or sample.

Direct PCR analysis of the aborted fetal liver samples showed that 11 (11%) of the samples were positive for *Salmonella* spp., 6 (6%) for *L. monocytogenes*, and 5 (5%) for *C. abortus*. Concurrent positivity for *Salmonella* spp. and *L. monocytogenes* was detected in 3 (3%) samples by direct PCR, while concurrent positivity for *Salmonella* spp. and *C. abortus* was detected in 1 (1%) sample. All culture-positive samples for *Salmonella* spp. and *Listeria* spp. were also positive by direct PCR (Fig. 4).

No significant differences were found among the tests used to detect *Salmonella* spp., *L. monocytogenes*, and *C. abortus* in liver samples with hepatitis ($P > 0.05$). All samples that were positive in the cultural analysis were also found to be positive by direct PCR and IHC. Additionally,

all samples that were positive by direct PCR were also positive by IHC. The diagnostic accuracy of the IHC compared with direct PCR and cultural analysis was 98% and 91%, respectively, for the diagnosis of *Salmonella* spp. Almost a perfect agreement was observed between IHC and direct PCR, with a kappa value of 0.905, whereas a moderate agreement was observed between IHC and cultural analysis, with a kappa value of 0.436. For *L. monocytogenes*, the diagnostic accuracy of IHC compared with direct PCR and cultural analysis was 98% and 97%, respectively. Almost a perfect agreement was observed between IHC and direct PCR (with a kappa value of 0.918), whereas a substantial agreement was observed between IHC and cultural analysis, with a kappa value of 0.713. For *C. abortus*, the diagnostic accuracy of IHC compared with direct PCR was 99%. Almost a perfect agreement was observed between IHC and direct PCR, with a kappa value of 0.904 (Table 2).

Table 1: Results of analysis of liver samples that were positive by at least one of the different methods

Sample number	<i>Salmonella</i> spp.			<i>L. monocytogenes</i>			<i>C. abortus</i>	
	IHC	Culture	Direct PCR	IHC	Culture	Direct PCR	IHC	Direct PCR
15/13	+	-	+	-	-	-	-	-
17/13	-	-	-	+	+	+	-	-
15/17	-	-	-	+	-	-	-	-
39/17	++	-	+	++	-	+	-	-
43/17	++	+	+	++	+	+	-	-
37/18	-	-	-	++	-	+	-	-
64/18	-	-	-	-	-	-	+	+
214/18	-	-	-	++	+	+	-	-
01/19	+	-	-	-	-	-	-	-
34/19	+	-	+	-	-	-	-	-
72/19	+	-	+	-	-	-	-	-
143/19	+	-	+	-	-	-	-	-
342/19	-	-	-	-	-	-	+	+
33/20	-	-	-	-	-	-	+	-
34/20	-	-	-	-	-	-	+	+
45/20	++	-	+	-	-	-	-	-
53/20	++	-	+	+	+	+	-	-
61/20	++	+	+	-	-	-	-	-
192/20	++	+	+	-	-	-	++	+
250/20	-	-	-	-	-	-	+++	+
260/20	+++	+	+	-	-	-	-	-
15/21	+	-	-	-	-	-	-	-
TOTAL	13 (13%)	4 (4%)	11 (11%)	7 (7%)	4 (4%)	6 (6%)	6 (6%)	5 (5%)

- = No reaction, + = poor reaction, ++ = moderate reaction, +++ = strong reaction.

Table 2: Diagnostic values of the tests for the bacterial agents positivity

Test	Microbiological Methods		Analytic Diagnostic Values						
	Positive	Negative	Se (%) (%95 CI)	Sp (%) (%95 CI)	PPD (%) (%95 CI)	NPD (%) (%95 CI)	Diagnostic accuracy (%) (%95 CI)	Kappa (%95 CI) (SE)	
IHC	Direct PCR								
	<i>Salmonella</i> spp.								
	Positive	11	2	100	97.75	84.62	100	98	0.905
	Negative	0	87	(71.51-100)	(92.12-99.73)	(58.28-95.59)	(95.85-100)	(92.96-99.76)	(0.776-1)
	<i>L. monocytogenes</i>								
	Positive	6	1	100	98.94	85.71	100	98	0.918
	Negative	0	93	(54.07-100)	(94.21-99.97)	(46.06-97.68)	(96.11-100)	(92.96-99.76)	(0.758-1)
	<i>C. abortus</i>								
	Positive	5	1	100	98.95	83.33	100	99	0.904
	Negative	0	94	(47.82-100)	(94.27-99.97)	(41.58-97.23)	(96.15-100)	(94.55-99.97)	(0.717-1)
	Culture/PCR confirmation								
	<i>Salmonella</i> spp.								
Positive	4	9	100	90.62	30.77	100	91	0.436	
Negative	0	87	(39.76-100)	(82.95-95.62)	(19.26-45.29)	(95.85-100)	(83.60-95.80)	(0.146-0.726)	
<i>L. monocytogenes</i>									
Positive	4	3	100	96.88	54.17	100	97	0.713	
Negative	0	93	(39.76-100)	(91.14-99.35)	(30.45-80.24)	(96.11-100)	(91.48-99.38)	(0.406-1)	

DISCUSSION

Small ruminant farming is an economically important sector in Kars and serves as a primary source of livelihood, especially for nomadic and rural farmers (TUIK, 2022). Increased rate of infectious agents in sheep herds leads to significant economic losses and heightens the risk of spreading certain zoonoses. Although collaboration is carried out to enable the rapid and accurate identification of the causative agents in cases of abortions, the diagnostic process is generally long and complex (Borel *et al.*, 2014). In this process, where a definitive etiological diagnosis can be made in less than 50% of abortion cases, factors such as the wide variety or simultaneous occurrence of abortions, fetal autolysis, and the absence of specific prodromal symptoms further complicate the diagnostic process (Modise *et al.*, 2023). In the present study, the presence of *Salmonella* spp., *C. abortus*, and *L. monocytogenes* in sheep abortion cases in Kars was investigated using bacteriological isolation, PCR, histopathology, and immunohistochemistry. These findings confirm the role of these important bacterial agents in regional abortion cases and emphasize the value of combined diagnostic techniques for accurate identification, thereby justifying the need for multidisciplinary approaches.

Fetal hepatic lesions have consistently been reported as prominent histopathological findings in sheep abortions. For example, Buyuk *et al.* (2020) reported predominant mononuclear cell proliferation and increased Kupffer cell activation, along with a small number of neutrophils concentrated around the portal triad, in the histopathological evaluation of five aborted sheep fetuses with hepatitis in which *C. abortus* was identified as the etiological agent. Yaeger *et al.* (2021) reported hepatitis in only 5 (4%) of 119 liver tissues from aborted sheep fetuses, with microscopic features including central necrosis surrounded by mononuclear cell infiltration. Ali and Al-Bayati (2022) histopathologically examined 180 aborted lamb samples and described marked dilatation and congestion of the portal and central veins in the liver, mild periportal fibrosis, sinusoidal dilatation, hepatocyte necrosis in some areas, as well as pulmonary edema, emphysema, and significant peribronchial hemorrhage. Gülaydın *et al.* (2023) investigated 113 aborted sheep fetuses and observed serosanguinous fluid in most body cavities macroscopically, multiple 1-2mm abscesses in the liver in one case, and bronchopneumonia in the lungs in two cases, with no notable changes in other organs. Histopathology showed coagulative necrosis in the liver and mononuclear cell (macrophage and lymphocyte) infiltration, particularly around blood vessels. Consistent with these observations, the present study detected hepatitis in the livers of 100 (33.78%) out of 296 aborted sheep fetuses. The inflammatory infiltrates were predominantly lymphohistiocytic and concentrated mainly in the portal areas. Additionally, hyperemia, typhoid nodule formation, and miliary abscess foci in some cases further support the involvement of infectious agents in abortion pathogenesis.

Previous studies have identified *L. monocytogenes*, *S. Abortusovis*, *C. abortus*, and *T. gondii* as key abortion-causing agents in sheep (Agerholm *et al.*, 2006; Habrun *et al.*, 2006; Belloy *et al.*, 2009; Gutierrez *et al.*, 2012; De Angelis *et al.*, 2022). In some cases, the concurrent detection of multiple agents also highlights multiple etiologies in abortion cases. Agerholm *et al.* (2006)

investigated sheep abortions using immunohistochemistry, culture, and molecular methods, identifying *L. monocytogenes* as the frequently isolated agent and detecting dual infections in some cases. Habrun *et al.* (2006) investigated sheep herds with an abortion rate of nearly 40% in late pregnancy using culture and PCR methods and identified *S. Abortusovis* from 13 vaginal swabs and two fetuses. Belloy *et al.* (2009) investigated 24 fetuses, six vaginal discharges, and three dead sheep from aborted herds (27-91%) using culture and PCR methods, detecting *S. Abortusovis* in most cases. Gutierrez *et al.* (2012) detected *T. gondii* in 14% and *C. abortus* in 20% of the sheep aborted materials they examined using PCR, reporting a 6% prevalence of co-infection. De Angelis *et al.* (2022) identified *L. monocytogenes* in most sheep abortion cases using histopathology, culture, and PCR, while detecting concurrent positivity for *T. gondii* in some samples, emphasizing the value of multidisciplinary diagnosis. Deniz and Oruc (2023) detected *Brucella* spp. by culture and *C. abortus* by real-time PCR and immunohistochemistry in sheep abortion, concluding that no single method suffices and multidisciplinary approaches yield more reliable results. Gülaydın *et al.* (2023) examined 113 aborted fetal tissues from 85 different sheep herds using histopathology, immunohistochemistry, and RT-PCR, reporting the highest rates for *C. abortus*. High diagnostic consistency between the methods used in all of the aforementioned studies has also been emphasized.

In line with the literature, the present study's immunohistochemical analysis of 100 hepatitis-positive liver samples revealed *L. monocytogenes* positivity in 7 samples (7%), *Salmonella* spp. in 13 samples (13%), and *C. abortus* in 6 samples (6%). Across all 296 abortion cases, these rates were 2.4%, 4.3%, and 2%, respectively. Concurrent immunohistochemical positivity for *Salmonella* spp. with *L. monocytogenes* or *C. abortus* in some samples highlights the significance of mixed infections. *Salmonella* spp. was detected in 4 samples (4%), *Listeria* spp. was detected in 4 samples (4%), and concurrent culture positivity was detected in 1 sample (1%). Using PCR, *Salmonella* spp. was detected in 11 samples (11%), *L. monocytogenes* in 6 (6%), and *C. abortus* in 5 (5%), with concurrent *Salmonella* spp. and *L. monocytogenes* in 3 samples (3%) and *Salmonella* spp. and *C. abortus* in 1 sample (1%). All samples in which the causative agent was isolated were also detected as positive by direct PCR. The lower detection rates by culture reflect the challenge in isolating these fastidious microorganisms and indicate that bacteriological culture alone is generally insufficient for diagnosis. In contrast, the high PCR positivity rates demonstrate the superior sensitivity of molecular methods, and there is complete concordance between culture and PCR in positive cases. Overall, this study demonstrates that *Salmonella* spp., *L. monocytogenes*, and *C. abortus* play a significant role in sheep abortions in the region and highlights the reliability of combined diagnostic methods.

Conclusions: This study demonstrates that *Salmonella* spp., *L. monocytogenes*, and *C. abortus* are important etiological agents associated with abortion cases in sheep in the Kars region. The presence of characteristic macroscopic and histopathological liver lesions, along with immunohistochemical, microbiological, and molecular findings, confirms that these pathogens play a role either

alone or in co-infections. The high level of agreement observed between immunohistochemistry and direct PCR, especially when combined with either molecular or cultural methods, emphasizes the reliability of IHC as a valuable diagnostic tool. Overall, the findings indicate that no single diagnostic approach is sufficient for definitive diagnosis and emphasize the necessity of using complementary diagnostic methods for the accurate detection of abortion pathogens. Identifying the causative agents of abortion is critical for reducing reproductive losses, improving herd health management, and supporting disease control strategies with implications for both animal production and public health.

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