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Investigation of the Presence and Prevalence of Listeriosis in Clinical Samples in Van and its Region

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Abstract: Listeriosis is an infection that causes abortion in humans and various animals worldwide. The causative agent is spread by livestock faeces, especially ruminants, and has a zoonotic character, transmitted by ingesting contaminated silage and feed. In this study, it was aimed to investigate the presence of *Listeria* spp. in 120 samples (79 abortion material, 41 brain material) of sheep. For this purpose, the polymerase chain reaction (PCR) method was performed using specific oligonucleotide pairs for *Listeria* spp. all of the abortion materials from Van province and its districts were found to be negative. However, a total of 2 (4.87%) samples, one each from the Erciş and Gevaş districts, from sheep with clinical nervous symptoms were found positive. As a result, it was determined that *Listeria* spp. was sporadic in Van province. It was concluded that this situation may be due to the low use of silage in ovine breeding in Van province. It was thought that periodical studies should be carried out to determine the course of the disease in the region.

Keywords: *Listeria spp., PCR, Silage.*

Van'da Klinik Örneklerde Listeriosis'in Varlığının Araştırılması

Özet: Listeriozis, dünya genelinde insanlar ve çeşitli hayvanlarda abortusa neden olan bir enfeksiyondur. Etken özellikle ruminant gibi çiftlik hayvanlarının dışkılarıyla saçılarak, kontamine silaj ve yemlerin sindirim yoluyla alınmasıyla bulaşan zoonoz karakterli bir özelliğe sahiptir. Bu çalışmada koyunlara ait 120 örnek (79 abort materyali, 41 beyin materyali) kullanılarak *Listeria* spp. varlığının araştırılması hedeflendi. Bu amaçla *Listeria* spp. için spesifik oligonükleotid çiftlerinin kullanıldığı polimeraz zincir reaksiyonu (PZR) yöntemi kullanıldı. Yapılan değerlendirmede Van ili ve ilçelerine ait abort materyallerinin tamamı negatif bulundu. Ancak klinik olarak sinirsel semptom gösteren koyunlardan Erciş ve Gevaş ilçelerinden birer tane olmak üzere toplam 2 (%4.87) adet örnekte pozitiflik saptandı.

Sonuç olarak abort etkenleri arasında yer alan *Listeria* spp.'nin Van ilinde sporadik seyrettiği belirlendi. Bu durumun Van il genelinde küçükbaş hayvancılıkta silaj kullanımının oldukça düşük olmasından kaynaklanabileceği kanaatine varıldı. Hastalığın yöredeki seyri için periyodik çalışmaların yapılması gerektiği düşünüldü.

Anahtar Kelimeler: *Listeria spp., PZR, Silaj.*

Introduction

Listeriosis is a sporadic or endemic bacterial infection of humans and animals caused by *Listeria (L.) monocytogenes* (Orsi and Wiedmann, 2016). It is known that the disease is a significant public health problem because it is widespread and zoonotic throughout the world. *Listeria* spp. is a Gram-positive, facultative intracellular pathogen widely found in the environment. The genus *Listeria* has 27 species identified so far and has a broad host spectrum including soil, water, vegetation and animals (Carlin et al., 2021). Although there are many apathogenic species of *Listeria* spp., *L. monocytogenes* is one of the food and feed-borne pathogens with a zoonotic character, mainly transmitted from ruminants. Although many apathogenic species of *Listeria* spp. *L. monocytogenes* is a food and feed-borne pathogen with a zoonotic character, mainly transmitted from ruminants (Diriba et al., 2021). It can cause various clinical infections in both humans and ruminants, including meningoencephalitis, metritis, abortion, septicaemia, mastitis, gastroenteritis and conjunctivitis. Metritis can cause neonatal septicaemia, abortion and stillbirths, mainly in the last trimester of pregnancy (Orsi and Wiedmann, 2016). Farm animals, especially cattle, sheep and goats, are frequently affected (Roberts and Wiedmann, 2003). In addition, clinically healthy animals can excrete *L. monocytogenes* into the environment, and therefore, farms are also considered natural reservoirs (Nightingale et al., 2004; Rodriguez et al., 2021). It has been reported that the pathogen spreads to the environment as an asymptomatic carrier after oral ingestion. In addition, consuming contaminated silage in farms is considered one of the primary sources of ruminant listeriosis, and it has been reported that outbreaks may occur in farms (Aslantaş et al., 2023; García et al., 2016). The ability of *L. monocytogenes* to tolerate adverse conditions related to temperature, humidity and atmospheric oxygen makes it difficult to eradicate the pathogen on farms and in food processing plants. Due to the high morbidity and mortality of *L. monocytogenes*, economic losses occur in animal husbandry. In addition, *L. monocytogenes* can enter the food production chain and pose a potential health risk to humans (Steckler et al., 2018).

Detecting *Listeria* species using conventional diagnostic methods such as culture, immunohistochemistry, and serology can be time-consuming, complex, and sometimes inconclusive (Blumer et al., 2011; Leclercq et al., 2014). Considering their epidemic and zoonotic potential, there is a need for improved diagnostic methods for the detection of abortion agents to prevent transmission to both humans and animals and to limit their spread among animals. PCR method is one of the methods with high sensitivity and specificity in the diagnosis of pathogens (Barkallah et al., 2014; Goy et al., 2009).

Considering the potential of the PCR method for the etiological diagnosis of abortion in ruminants, this study aimed to investigate the presence of *Listeria* spp. in sheep with nervous symptoms and abortion cases by PCR method.

Material and Methods

Ethical consideration: Ethical approval of the study was obtained from Van Yüzüncü Yıl University Animal Researches Local Ethics Committee (Decision no: 2023/ 09-08), Van, Turkey.

Material: In this study, various samples taken from 79 cases of abortion and 41 cases with neural symptoms detected in sheep reared in 8 districts in Van and its region between 2020-2022 were used. The distribution of the districts sampled in the study is presented in Table 1. In addition, brain tissue taken from 41 sheep necropsies showing neural symptoms were included in the study. The samples were brought to Van Yüzüncü Yıl University, Faculty of Veterinary Medicine, Department of Microbiology laboratory under a cold chain.

Table 1. Locations of the samples analysed.

District	Number of Animals Sampled	
	Aborted fetuses and sheep Samples	Samples of Nervous Symptoms
Edremit	4	3
Erciş	0	8
Gevaş	0	5
Gürpınar	4	6
İpekyolu	15	3
Muradiye	3	3
Özalp	46	9
Tuşba	7	4
Total	79	41

Reference culture: The study used the *L. monocytogenes* (ATCC 7646) reference strain as a positive control.

Method: Tissue samples were cut into small pieces with sterile forceps and a scalpel in the laboratory. Two hundred milligrams each were transferred into tubes containing 20% glycerin Brain Heart Broth (Merck, 1.10493) and homogenized in a tissue lyser (Qiagen, Tissue Lyser). All samples were stored at -20°C until DNA isolation.

DNA isolation and amplification: Genomic DNAs of the previously homogenized samples were isolated using a commercial genomic DNA isolation kit (Hydra, Genomic DNA Purification Kit, HY-GDNA-100, İstanbul/ Türkiye). Genomic DNA isolation protocol was applied as recommended by the company.

In the amplification of the samples used in the study, the genus-specific *prs* gene region of *Listeria* spp. for genus-level identification and the *hly* gene region defined as a specific genetic marker for the molecular identification of *L. monocytogenes* at the species level were used as primers (Table 2).

Table 2. Oligonucleotide sequences used in the study.

Agent	Target Gene	Product size (bp)	Oligonucleotide sequence (5'-3')	Reference
Listeria spp.	*prs*	370	F: CTGAAGAGATTGCGAAAGAAG R: CAAAGAAACCTTGGATTTGCGG	(Doumith et al., 2004)
L. monocytogenes	*hyl*	456	F: GGGAAATCTGTCTCAGGTGATGT R: CGATGATTTGAACTTCATCTTTTGC	(Rodríguez-Lázaro et al., 2004)

For the preparation of the PCR mixture, 12.5 µl mastermix (Thermoscientific, 2X PCR Mastermix, K0171, Vilnius/Lithuania), 5 µl genomic DNA, 1.5 µl primers (F-R) and 4.5 µl PCR water (Bioshop, Canada) were used for a total reaction volume of 25 µl. In the amplification process for *Listeria* spp. using Thermal Cycler (Corbett Research, Qiagen GmbH, Sydney, Australia), the protocol of initial denaturation at 94 °C for 5 minutes, denaturation at 94 °C for 30 seconds, annealing at 55.5 °C for 45 seconds, extension at 72 °C for 45 seconds, final extension at 72 °C for 5 minutes for a total of 35 cycles was applied (Gülaydın et al., 2023).

In the amplification process for *L. monocytogenes*, after initial denaturation at 95 °C for 10 min, a 40-cycle protocol was applied as denaturation at 95 °C for 45 s, annealing at 63 °C for 45 s, and elongation at 72 °C for 45 s. Final elongation was performed at 72°C for 10 min.

DNA obtained from *the L. monocytogenes* (ATCC 7646) reference strain was used as a positive control, and deionized water was used as a negative control. The PCR amplicons were run on an agarose gel electrophoresis setup (Thermo Scientific, OwlR EasyCastTM B1, Ohio/ USA) using 2% agarose gel (Atlas, Ankara / Türkiye) and then analyzed under UV light in a Gel Imaging device (Genesis Spectronic®, USA).

Results

No positivity for *Listeria* spp. was detected in the 79 abortion samples examined in the study. However, in PCR analyses of brain samples taken from 41 sheep with neural symptoms, 2 (4.87%) positivities were detected, one each in the samples taken from Erciş and Gevaş districts (Figure 1).

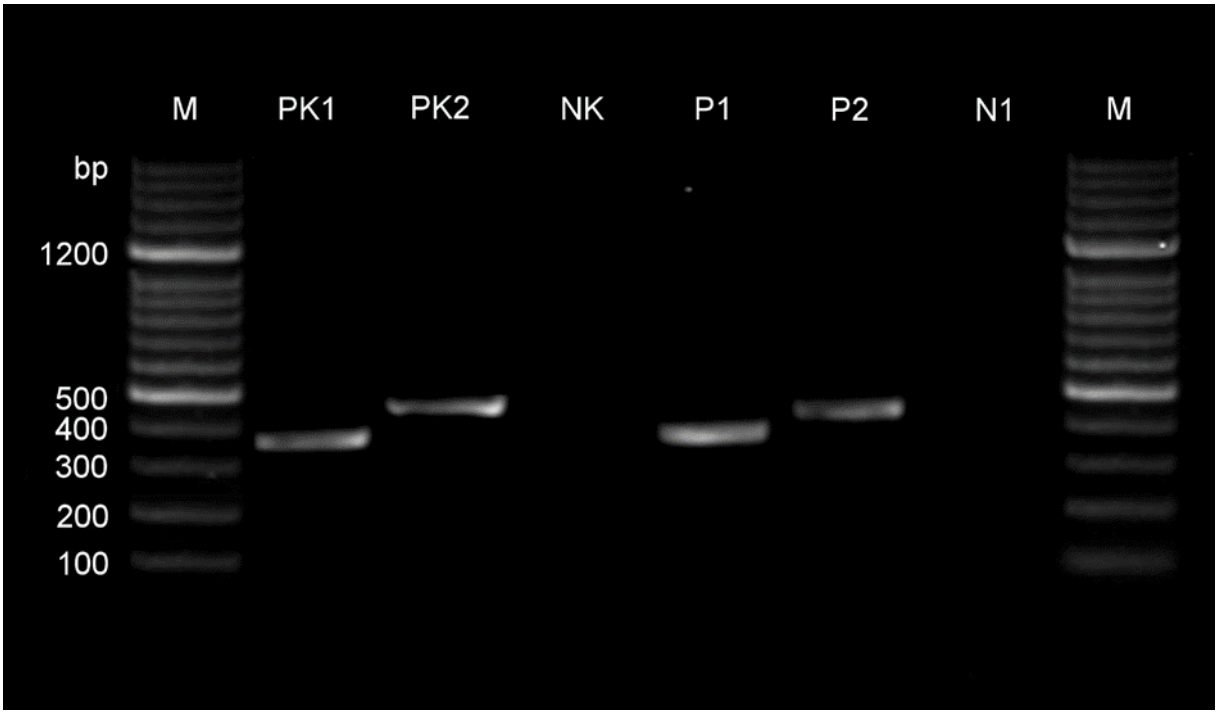

Figure 1. M: Ladder 100 bp marker (Vivantis); PK1: *Listeria* spp. Positive control; PK2: *L.monocytogenes* Positive control; NK: Negative control; P1: *Listeria* spp. Positive sample (370bp); P2: *L. monocytogenes* Positive sample (456bp); N1: Negative sample.

Discussion and Conclusion

Listeriosis is a highly prevalent zoonotic infectious disease reported in more than 40 animal species in different countries on six continents. It has become a significant health problem, especially in ruminants, and can be transmitted to humans (Karakurt et al., 2021). It was emphasized that *Listeria* is one of the bacteria that should not be overlooked among the factors causing abortion (Yeni et al., 2024). Studies on seroprevalence and detection of listeriosis in ruminants in Van and its region are very few. In this study, it was aimed to investigate the presence of

Listeria spp. in clinical and necropsy samples taken from sheep with abortion and nervous symptoms in Van and its region by molecular methods.

It was stated that neural symptoms due to listerial meningoencephalitis are only seen in adult ruminants, and the pathogenesis is not fully understood (Koopmans et al., 2023). It was emphasized that listeriosis cases are frequently associated with silage-fed animals, and the disease usually occurs in winter and early spring (Gorski et al., 2022). In a study conducted in Burdur province, the brain tissues of 15 sheep with nervous symptoms were examined by immunohistochemical method. All of them were positive for *L. monocytogenes*, and 12 of the animals examined were fed silage (Haligur et al., 2019). Similarly, in a study conducted in Kars province, it was reported that *L. monocytogenes* was isolated and identified in 12 of the samples in cultural analyses of brain and liver tissues taken from 18 sheep and cattle with clinical symptoms, and positive samples were also confirmed by immunofluorescent staining method (Karakurt et al., 2021).

In Türkiye, there is very limited literature on which listeriosis has been investigated by PCR in aborted fetuses. This study detected 2 (4.87%) positivity for *L. monocytogenes* in PCR analyses of brain samples taken from 41 sheep with neural symptoms. In a study conducted by (Gülaydın et al., 2023), on the etiological analysis of abortion cases in sheep in Van and its region by PCR method, it was reported that the presence of *L. monocytogenes* was detected in only 1 (0.77%) of 130 abortion samples. Similarly, it was reported that *L. monocytogenes* was isolated and identified in only 1 (0.91%) of the samples taken from 110 sheep and goats that were aborted in Elazığ and its region, and serological positivity was not detected (Muz et al., 1999). Similarly, it was reported that *Listeria* spp. could not be detected by PCR method in 179 cattle and sheep abortus materials collected from nine cities in different regions of Turkey (Yeni et al., 2024). In another study, aborted tissues from 7 different regions of Turkey were analysed by PCR method. However, L. monocytogenes could not be detected (Sakmanoğlu et al., 2021).

On the other hand, in a seroprevalence study conducted in Adana, blood sera of 557 aborted cattle were analysed, and 162 (40.9%) of the samples were found to be positive for *L. monocytogenes* (Yagci Yucel et al., 2014). In another study, 42.85% of blood serum samples taken from aborted cattle in 40 different facilities in İzmir, Kırıkkale, and Tokat provinces were serologically positive for *L. monocytogenes* (Yildiz et al., 2009).

In this study, all 79 abortus samples analyzed using PCR were negative for *Listeria* spp. The agent could not be detected in Van and its region, because the disease is associated with silage, and silage feeding is very limited in the area. This may be because the disease is widespread in the region due to the good level of silage feeding in the provinces where the studies were conducted, or the positivity may be high due to the possibility of cross-reactions in serological analyses. According to Bergeys Manual, it has been reported that the antigenic structures of *Listeria* strains *staphylococci* and *enterococci* and *Escherichia coli* bacteria can give cross-reactions due to antigenic proximity (Brenner et al., 2005). It was reported that silage feeding was applied in 7% of the fattening enterprises in Van province, 2% of which was in Erciş province, especially in cattle fattening (Budağ and Keçeci, 2013). Therefore, it is seen that listeriosis, which is reported among the causes of abortion, is sporadic in Elazığ region, similar to Van and its region.

Another reason for the region's low detection rate of listeriosis may be related to the method applied. It is thought that inhibitory substances, encountered mainly in the PCR method and caused by the lack of appropriate material, may be present in the samples used in this study, albeit to a lesser extent. In a related evaluation, it is claimed that IgG, lactoferrin, and hemoglobin, as well as anticoagulants such as heparin, inhibit PCR in blood samples used for PCR diagnosis of abortion cases. It has also been emphasized that protease activity in the blood may decrease PCR efficiency, and inhibitors in muscle tissues may inhibit all or part of Taq polymerase and cause false negative results (Kula and Gökpınar, 2018).

Inadequate sample collection is another factor contributing to the poor isolation rate of Listeria spp. In one study, it was stated that random sample collection in infections without adequate examination and clarification of the stage of the disease had a significant negative effect on the reliability of isolation and identification (Malik et al., 2002). Although this situation is not very likely for this study, it is more likely that the isolation rate is low due to the sporadic course of the disease.

In conclusion, it is essential to investigate the presence of zoonotic *Listeria* spp. in Van and its region to give direction to animal health and public health as well as disease control and prevention strategies. Although *Listeria* spp. was not detected in abortion samples in this study, the presence of the agent, which is frequently isolated from foodstuffs, should be routinely investigated and taken into consideration. This study demonstrated that listeriosis is still sporadic in Van and its region, and it was concluded that examination of the brain tissues of animals with neural symptoms would be beneficial.

Conflict of Interest

The authors declare that they have no conflict of interest or potential conflict of interest.

Ethical Approval

Ethical approval of the study was obtained from Van Yüzüncü Yıl University Animal Researches Local Ethics Committee (Decision no: 2023/ 09-08), Van, Turkey.

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