Listeria monocytogenes in Ruminants at an Abattoir: Prevalence, Virulence Characteristics, Serotypes and Antibiotic Resistance in Eastern Türkiye

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ABSTRACT

Listeria monocytogenes is a pathogenic bacteria that causes listeriosis in both animals and humans. The aim of this study was to determine at the abattoir the prevalence of *L. monocytogenes* in healthy cattle, sheep and goats. The serotype was also examined along with the antibiotic resistance and clonal relationships with virulence genes. The samples were examined for the isolation of *L. monocytogenes* according to Food and Drug Administration/Bacteriological Analytical Manual and International Organization for Standardization methods. The isolated species were identified via matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-MALDI-TOF MS and confirmed the isolates found using polymerase chain reaction, (PCR) after which they were serotyped with PCR and tested for antibiotic resistance against 16 antimicrobial agents using the disc diffusion method. The genetic similarity analysis (pulsed-field gel electrophoresis (PFGE) was performed. One abattoir located in Van state, Türkiye was selected and 600 samples were obtained by surface swabbing of 20 cattle, 80 sheep and 50 goat carcasses at two steps of slaughtering. Comparisons using a chi-square test of the prevalence of *L. monocytogenes* isolates from two visits, the four carcass surface parts, three animal species and two slaughter processes were made. Samples were evaluated for the presence of *L. monocytogenes* and 21 of the 150 carcasses (14%) were contaminated with *L. monocytogenes*. The highest prevalence of *L. monocytogenes* was found in cattle carcass (25%), followed by goat carcass (18%) and sheep carcass (8.75%). The most prevalent virulence factor genes *prs*, *iap*, *hlyA*, *inlA* and *plcA* were and detected in all isolates. The *inlB* identified in 15 (71.4%) isolates, followed by *prfA* 1 (4.7%). All of strains were encoded 1/4b (4d,4e) serotype gene. Resistance was observed most frequently to clindamycin (19.04%) and less frequently to erythromycin (9.5%), ampicillin (9.5%), penicillin G (9.5%), and trimethoprim-sulfamethoxazole (9.5%). Pulsed-field gel electrophoresis typing was used to identify a total of 21 *L. monocytogenes* isolates. Thirteen pulsotypes were identified from the isolates. *L. monocytogenes* was detected in the carcass of twenty-one animals, during slaughtering before washing (fifteen carcass), and after end washing (six carcasses). There were no significant differences in *L. monocytogenes* prevalence among three animal types, four carcass sites, two visits and two slaughter processes. The frequency of isolates are low, but the data suggest the potential public health risk that these carcasses represent as reservoirs of *L. monocytogenes* and cross-contamination occurs at different stages (animals, parts of carcass, stages of slaughtering). Therefore, for hygienic meat production, HACCP systems could be implimented effectively.

Keywords: Antimicrobial Resistance; *Listeria monocytogenes;* Serotype; PFGE; Virulence.

INTRODUCTION

Foodborne pathogens continue to pose significant threats to food safety and international trade. Worldwide, foodborne infections present a major public health concern (1). One thousand eight hundred seventy-six cases of listeriosis were recorded worldwide in 2020; this translates to an infection rate of 0.42 per 100,000 people in the European Union (2). After consuming pathogen-contaminated foods, severe health implications for listeriosis, ranging from an invasive to a non-invasive sickness, develop. People with cancer, diabetes, or acquired immunodeficiency syndrome (AIDS) are more likely to get invasive listeriosis. Newborns, the elderly and pregnant women are also more likely to get this type of listeriosis. In instances of invasive listeriosis, severe symptoms (gastroenteritis, meningitis, pneumonia, septicemia, and stillbirth/abortion) develop. In healthy individuals, noninvasive listeriosis and less severe symptoms (fever, diarrhea, headache, and muscular discomfort) are often noticed (3, 4).

An intracellular pathogen, *L. monocytogenes* has the ability to infect numerous animal species, including ruminants (cattle, sheep, and goats). It has been isolated mainly from the outer surfaces of cattle, sheep, goats, and poultry, although various reports have also detected its presence in muscle tissue, although at low proportions (5, 6). *L. monocytogenes* possesses an array of virulence factors contributing to its survival, host invasion, immune system evasion, and infectivity. Listeriolysin O (LLO, encoded by *hly*A), a virulence regulator (encoded by *prfA*), actin (encoded by a*ctA*), invasion-associated protein (encoded by *iap*), phosphatidylinositolphospholipase C (PI-PLC, encoded by *plcA*, *plcB*, and *plcC*), and a metalloprotease (encoded by *mp*l) are all components of *Listeria* pathogenic 1 (LIPI-1), while LIPI-2 contains the internalins (encoded by *inlA, inlC, and inlJ*) (7, 8).

For *L. monocytogenes* subtyping, antibodies that bind to the somatic (O) lipopolysaccharides and flagellin antigens on the bacterial outer membrane are used to identify the first level of subtyping. Thirteen serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7) have been found in *L. monocytogenes* that are categorized into four distinct lineages (I–IV) (9, 10). Lineage I and II (1/2a, 1/2b, and 4b) serotypes account for the vast majority of worldwide listeriosis cases. Most listeriosis epidemics are caused by contaminants 1/2a, 1/2b, 1/2c, and 4b in food or the food processing environment. When it comes to human listeriosis, genetic isolates from the lineage I strains are more frequently found, while those from the animal lineage III strains are more frequently found in animal specimens (11).

Zoonoses present the worrisome problem of transfer of antimicrobial-resistant organisms from animals to humans in an interconnected globalized world (12). *L. monocytogenes* has three mobile genetic elements for the emergence of antibiotic resistance: self-transferable plasmids, plasmids that can be mobilized and conjugative transposons that can be transferred. Efflux pumps have been reported to contribute to antimicrobial resistance in Listeria (13). The prevalence of antibiotic-resistant strains of *L. monocytogenes* has increased in recent years, particularly in the case of isolates collected from the food supply (14). Multidrug-resistant *L. monocytogenes*, notably in ready-to-eat foods, serves as a public health indicator, especially among those at high risk. In order to avoid an increase in medication resistance, it is critical to raise public awareness about the significance of food safety rules and the drugs administered to humans and animals (15).

Food animal and product contamination is a major concern since it is difficult to regulate and involves a wide range of elements. Animal handling activities, such as slaughtering and processing, as well as environmental elements such as fauna and water sources and animal dung disposal are examples of human factors (16).

There is a little data available with respect to the prevalence of *L. monocytogenes* in Türkiye. Since food-borne outbreaks of listeriosis have not reached epidemic levels in Türkiye yet, the disease hasn't been regularly recorded there (17). The aim of this study was to identify *L. monocytogenes* from randomly selected carcass samples of healthy ruminant animals (cow, sheep, goat) using matrix assisted laser desorption/ionization time-of-flight mass spectrometry-MALDI-TOF MS, to investigate the antibiotic resistance phenotype, virulence genes, serotypes of the isolates and to detect their genetic similarities using pulsed field gel electrophoresis (PFGE).

MATERIAL AND METHOD

Sample collection

In the period from November 2018 to December 2019, we collected carcass samples from 20 cattle, 80 sheep, and

50 goats at an abattoir in Van situated in the Eastern area of Türkiye. Sampling was performed in six-month periods (December: I; June: II) within one year. The animals sampled were randomly selected. The selected abattoir was visited twice to obtain 600 surface samples from 150 animals and carcasses at two stages of the slaughtering process (BW, before washing; and AW, after end washing). Over 600 samples of various areas of beef, sheep, and goat carcasses were gathered using swabs. The sampling region was thoroughly sampled for one minute using cotton swabs swabbed in both horizontal and vertical directions. For each beef, sheep and goat carcasses, four areas of 100 cm^2 (10 cm^2 x 10 cm^2) each located in the carcass locations (brisket, flank, hind leg and rectal) were swabbed. Sterile cotton swabs dipped in 10 ml of buffered peptone water were used to obtain carcass swabs before (n=300) and after (n=300) washing in accordance with techniques established by the Food and Drug Administration (FDA) (20) and the International Organization for Standardization (21). Firm and even pressure was applied as it was driven vertically (about 10 times) onto peripheral surfaces, and then the sampler was flipped and used to swab horizontally (roughly 10 times) and diagonally (approximately 10 times). The samples were transported immediately to the laboratory and stored at 4°C until processing.

Detection of *Listeria* spp. and *L. monocytogenes*

Each sample was tested for *Listeria* spp. and *L. monocytogenes* in accordance with FDA and ISO 11290-1 guidelines (22). *Listeria* spp. and *L. monocytogenes* were detected using the horizontal approach. Swab samples in buffered peptone water were homogenized 1:10 with half fraser broth (Lab M, Lancashire, UK) for 2 min using the Waring blender (New Hartford, CT, USA). The half fraser broth (Lab M, Lancashire, UK) was then incubated at 30°C for 24-26h. After incubation, 0.1 mL fluid was taken from each sample, inoculated into tubes containing 10 mL Fraser broth, and incubated at 37°C for 24-26h. After incubating, petri-dishes containing Oxford Agar (Himedia, Bombay) and Listeria Chromogenic Agar (Lab M, Lancashire,UK) were inoculated with a loopful (10 µl) fluid taken from each dilution. The inoculated petri-dishes were incubated at 37°C for 24-48h. After incubating, blackish green colonies were observed with a collapsed center, some of which had black-brown zones on the Oxford agar and blue colonies surrounded by an opaque halo on Listeria Chromogenic Agar (Lab M, Lancashire, UK). Following the incubation, five colonies suspected as *L. monocytogenes* were taken from each petri dish and inoculated into Tryptic Soy Agar-Yeast Extract (Himedia, M1214, Maharashtra, India) agar for purification and incubated at 30°C for 24h. All strains were kept in skim milk powder stocks at -80°C for further testing.

Matrix-Assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) identification of isolates

Biochemical characterization of all the isolates was performed using the MALDI-TOF MS identification system (bioMe´rieux, Marcy l'Etoile, France). Bacterial isolates were identified according to the manufacturer's recommendations. Cultures of the suspected isolates were inoculated in Blood Agar Base (Lab M, Lancashire, UK) and incubated at 37°C for 24 hours. A 1 µL matrix solution (saturated cyano-4-hydroxycinnamic acid solution in 50% acetonitrile and 2.5% trifluoroacetic acid solution) was pipetted into the wells of the VITEK MALDI-TOF MS, (Durham, NC 27712, USA) equipment, and the wells were kept at room temperature until the matrix solution had dried and the colonies had been analyzed. The slide was then put into the MALDI-TOF MS using the equipment cassette (23).

DNA isolation

Templates for the polymerase chain reaction (PCR) were prepared by a boiling method using procedure described by Kayode and Okoh *et al.* (24) with some modifications. In 5 mL of Tryptic Soy Broth (Merck, Germany), bacteria were cultured over night at 37°C. For five minutes, the mixture was centrifuged at 16,000 rpm. The cell pellet was re-suspended in 300 µL of sterile water and heated to 100°C after the supernatant had been decanted. The suspension was boiled for 10 minutes before being centrifuged at 16,000 rpm for 5 minutes. The supernatant served as a source of amplification's template. DNA concentration and purity were measured in a fluorometer (Invitrogen Qubit fluorometer, Turner BioSystems, Thermo Fischer Scientific, Waltham, Massachusetts, United States). Three microliters of the supernatant was used as as bacterial template DNA in the PCR assay. The DNA template was kept at -20°C pending usage.

Confirmation of *L. monocytogenes* with polymerase chain reaction (PCR)

All *L. monocytogenes* isolates identified using the MALDI-TOF MS method were confirmed by PCR using specific primers for lmo1030. Primer pairs 5' GCTTGTATTCACTTGGATTTGTCTGG-3'and 5'-ACCATCCGCATATCTCAGCCAACT-3' specific for lmo1030 (25) of *L. monocytogenes* was used by optimizing the different conditions. The PCR mixture was prepared as 1X PCR buffer (Thermo Scientific, Waltham, Massachusetts, United States), 1.5 mM $MgCl_2$ (Thermo Scientific), 0.2 mM dNTP mix (Thermo Scientific), 1.5 U Taq polymerase (Thermo Scientific), 1 µM of each primer, and 5 µL DNA in a total volume of 50 µL. The amplification of genes was performed with the first denaturation at 94°C for 3 min, followed by 35 cycles of DNA denaturation at 94°C for 15 seconds, at 50°C for 15 seconds, primer elongation at 72°C for 45 seconds, and the last elongation at 72°C for 8 minutes in a thermal cycler (Whatman Biometra GmbH, Niedersachsen, Germany). The amplicons obtained were run in 1.5% agarose at 80 V for electrophoresis. At the end of electrophoresis, genes were visualized on a UV transilluminator.

L. monocytogenes ATCC 7644 and *Listeria innocua* ATCC 33090 were used as the positive and negative control strain, respectively.

Genotypic characterization of virulence and serotype genes

All *L. monocytogenes* isolates were screened for the presence of virulence factor (VF) genes (*inlA*, *inlB*, *inlC*, *inlJ*, *hly*, *mpl*, *plcA*, *plcB*, *actA*, *prfA*, *iap*) and serotype genes (*lmoO737*, *lmo1118*, *ORF2819*, *ORF2110*) by PCR using the primers listed in Table 1. These methods by Sereno *et al.* (26) and Ramires *et al.* (27) were modified used for genotyping the *L. monocytogenes* isolates. *L. monocytogenes* ATCC 7644 (serotype 1/2c), Refik Saydam Culture Collection (RSKK) 472 (serotype 1/2b), RSKK 471 (serotype 1/2a) RSKK 475 (serotype 4b) were used as the positive control strain.

Antimicrobial susceptibility testing

To determine antibiotic resistance in the isolates gentamicin (CN), rifampin (RA), vancomycin (VA), clindamycin (CD), erythromycin (E), linezolid (LNZ), ampicillin (AM), benzylpenicillin (PenG), ciprofloxacin (CIP), quinupristin-dalfopristin (QD), trimethoprim-sulfamethoxazole (SXT), tetracycline (TE), meropenem (MEM), chloramphenicol (C) and teicoplanin (TEC) antibiotic discs (Oxoid, UK) were used (Table 2). Antibiotic resistance of *L. monocytogenes* isolates was investigated using disc diffusion method according to the rules of European Committee on Antimicrobial Susceptibility Testing (28). A classification system based on clinical breakpoint data from EUCAST documents was used to classify the widths of inhibition zones produced around discs of the antibiotics penicillin G, ampicillin, erythromycin, and sulfamethoxazole/trimethoprim. Due to the lack of standardization in *L. monocytogenes* susceptibility criteria, the critical values for *Staphylococcus spp.* and *Enterococcus spp*. described in the Clinical and Laboratory Standards Institute (29, 30) were used for the remaining antimicrobial agents according to the description of Andriyanov et al. (31). Multidrug resistant (MDR) isolates were interpretted according to the description of Magiorakos *et al.* (32) (*S.aureus* ATCC 25923 was used as quality control strain).

Pulsed-field gel electrophoresis analysis

Pulsed-field gel electrophoresis (PFGE) of ApaI (Thermo Fisher Scientific, Waltham, Massachusetts, USA)-digested genomic DNA samples of twenty-one *L. monocytogenes* isolates were carried out with a (CHEF) DR-III electrophoresis chamber (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the standard Centers for Disease Control and Prevention (CDC) PulseNet protocol (33). The TIFF images obtained by PFGE were analyzed with the BioNumerics version 7.6 software package (Applied Maths, Sint-Martens Latem, Belgium). PFGE profiles' relationships with one another were visualized using a dendrogram generated by clustering Dice similarity indices using the unweighted pair-group method with arithmetic mean (UPGMA). Isolates with Dice similarity index of ≥85% were classified into the same PFGE cluster. PFGE was done using *Salmonell*a Braenderup H9812 as the molecular weight marker.

Statistical analyses

The data on the mean, percentage, and frequency were analyzed using the SPSS ver. 26 (IBM Inc., Chicago, IL, USA). Comparisons of the prevalence of *L. monocytogenes* isolates from two visits, the four carcass surface parts (brisket, flank, hind leg and rectal), three animal species and two slaughter

Table 1. The oligonucleotide primers of virulence factors and serotype genes

Function of gene product	Target gene	Primer sequence (5'-3')	Fragment size (pb)	$T_{annealing}$ (°C)	References
Listeria genus	*prs*	F: GCTGAAGAGATTGCGAAAGAAG R: CAAAGAAACCTTGGATTTGCGG	370	55	(9)
Internalin A	*inlA*	F: ACGAGTAACGGGACAAATGC R: CCCGACAGTGGTGCTAGATT	800	60	(8)
Internalin B	*inlB*	F: TGGGAGAGTAACCCAACCAC R: GTTGACCTTCGATGGTTGCT	884	60	(8)
Internalin C	*inlC*	F: TGGGAGAGTAACCCAACCAC R: GTTGACCTTCGATGGTTGCT	471	60	(8)
Internalin J	*inlJ*	F: TGTAACCCCGCTTACACAGTT R: AGCGGCTTGGCAGTCTAATA	597	60	(8)
Listeriolisin O (LLO)	*hlyA*	F: GCAGTTGCAAGCCTTGGAGTGTGAA R: GCAACGTATCCTTCCAGAGTGATCG	456	60	(45)
Zinc metalloprotease	*mpl*	F: TTGTTCTGGAATTGAGGATG R: TTAAAAAGGAGCGGTGAAAT	502	60	(46)
Phosphatidylinositol phospholipase-C (PI-PLC)	*plcA*	F: CTCGGACCATTGTAGTCATCTT R: CACTTTCAGGCGTATTAGAAACGA	326	52	(47)
Phosphatidycholie phospholipase-C (PC-PLC)	*plcB*	F: GGGAAATTTGACACAGCGTT R: ATTTTCGGGTAGTCCGCTTT	261	60	(7)
Actin-polymerizing protein	*actA*	F: CCAAGCGAGGTAAATACGGGA R: GTCCGAAGCATTTACCTCTTC	650	60	(47)
Positive regulatory factor A	*prfA*	F: ACCAATGGGATCCACAAGA R: CAGCTGAGCTATGTGCGAT	467	60	(48)
Invasion-associated protein p60	*iap*	F: ACAAGCTGCACCTGTTGCAG R: TGACAGCGTGTGTAGTAGCA	131	60	(49)
Serovars 1/2a,1/2c,3a and 3c	*lmoO737*	F: AGGGCTTCAAGGACTTACCC R: ACGATTTCTGCTTGCCATTC	691	60	(9)
Serovars 1/2c and 3c	*lmo1118*	F: AGGGGTCTTAAATCCTGGAA R: CGGCTTGTTCGGCATACTTA	906	53	(9)
Serovars 1/2b,3b,4b,4d and 4e	*ORF2819*	F: AGCAAAATGCCAAAACTCGT R: CATCACTAAAGCCTCCCATTG	471	58	(9)
Serovars 4b,4d and 4e	*ORF2110*	F: AGTGGACAATTGATTGGTGAA R: CATCCATCCCTTACTTTGGAC	597	56	(9)

processes were made using a chi-square test (Fisher's exact). A P value <0.05 was considered statistically significant.

RESULTS

Isolation of *L. monocytogenes* from carcass samples

In this study, it was aimed to isolate and identify *L. monocytogenes* from 600 samples taken from 150 healthy animal carcasses in an abattoir. The overall prevalence of *L. monocytogenes* in cattle, sheep, and goat carcass surface samples from two visits to a slaughterhouse was found to be 3.5% (21). *L. monocytogenes* was most common in cattle (5/20, 25%) and goat (9/50 animals, 18%). A much lower prevalence was found in sheep (7/80 animals, 8.75%) (Table 3).

The relationship between the animal species and carcass sites, slaughter stages, and sampling visits for the presence of *L. monocytogenes* is presented in Table 4. According to the Table 4, *L. monocytogenes* contamination was not significantly different in animal species in comparison to carcass sites (P<0.05). The observed variation in *L. monocytogenes* concentration between animal species

Table 2. Zone diameter values of antibiotics used in this study

Antimicrobial class	Antimicrobials	Zone Diameter breakpoints		
		S ≥	I	R <
Penicillins	Ampicillin	16	–	16
Penicillins	Benzylpenicillin	13	–	13
Phenicols	Chloramphenicol	18	13-17	12
Fluoroquinolones	Ciprofloxacin	21	16-20	15
Lincosamides	Clindamycin	21	15-20	14
Aminoglycosides	Gentamicin	15	13-14	12
Macrolides	Erythromycin	25	–	25
Oxazolidinones	Linezolid	21	–	20
Carbapenems	Meropenem	26	–	26
Streptogramins	Quinupristin-dalfopristin	19	16-18	15
Ansamycins	Rifampin	20	17-19	16
Folate Pathway	Trimethoprim-sulfamethoxazole	29	–	29
Tetracyclines	Tetracycline	19	15-18	14
Lipoglycopeptides	Teicoplanin	14	11-13	10
Glycopeptides	Vancomycin	17	15-16	14

Table 3. Prevalence of *L. monocytogenes* in brisket, flank, hind leg, and rectal swab samples

Sample point	Month	n	Cattle								Sheep								Goat							
			brisket		flank		hind leg		rectal		brisket		flank		hind leg		rectal		brisket		flank		hind leg		rectal	
			n	Ps.	n	Ps.	n	Ps.	n	Ps.	n	Ps.	n	Ps.	n	Ps.	n	Ps.	n	Ps.	n	Ps.	n	Ps.	n	Ps.
BW	I	150	5	0	5	0	5	1	5	0	20	0	20	1	20	0	20	0	12	1	12	0	12	1	12	1
	II	150	5	1	5	1	5	0	5	1	20	0	20	1	20	1	20	1	13	1	13	1	13	1	13	1
AW	I	150	5	0	5	0	5	0	5	0	20	0	20	1	20	0	20	0	12	0	12	1	12	0	12	0
	II	150	5	0	5	0	5	1	5	0	20	1	20	1	20	0	20	0	13	0	13	0	13	0	13	1
Total		600	20	1	20	1	20	2	20	1	80	1	80	4	80	1	80	1	50	2	50	2	50	2	50	3

Ps: No of positive isolates *L. monocytogenes*, BW: before washing; AW: after end washing; I: December; II: June

and stage of the slaughter process was not significant ($P > 0.05$). From visit 1 in December to the second visit in June, 44.4% (goat), 28.6% (sheep), 20% (cattle) and 55.6% (goat), 71.4% (sheep), 80% (cattle) were found positive as *L. monocytogenes*, respectively. There was no significant difference in prevalence ($P < 0.05$) between the two sampling visits and animal species.

Virulence genes

The presence of virulence factor genes in the isolates is presented in Table 5. The most common virulence factor profiles, *prs*, *iap, hlyA, inlA* and *plcA* were found in all isolates (100%). Internalin B gene *inlB* was detected in fifteen (71.4%) One isolate (4.7%) was shown to contain the *prfA*-encoding gene. No isolates harbored *plcA*, *plcB*, *mpl, actA*, *inlC*, and *inlJ* genes.

Serotypes

By applying specific oligonucleotide primers for the detection of *L. monocytogenes* serogroups in carcass samples (Table 5), it was found that all (100%) isolates were positive for 4b, 4d or 4e serogroup, while none of the other serogroups tested (1/2a (3a), 1/2b (3b), 1/2c (3c) was detected among the strains.

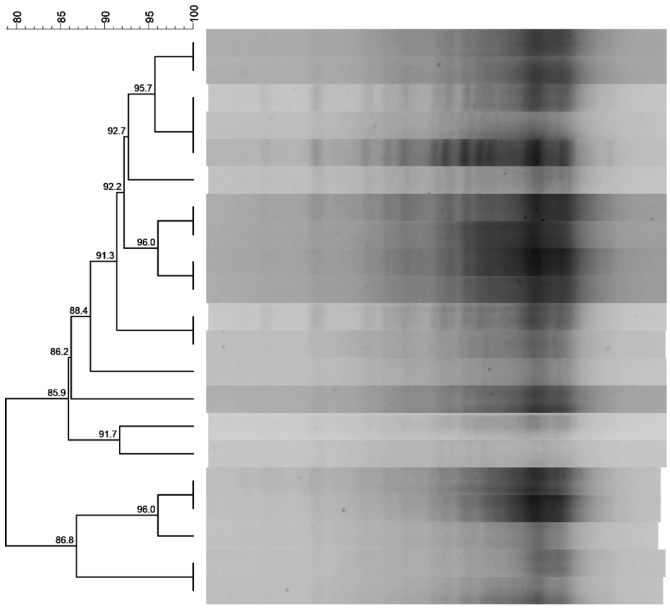
Table 4. The relationship between the animal species and carcass sites, slaughter stages, and sampling visits for the presence of *L. monocytogenes*

		Animal species						
		Goat		Sheep		Cattle		*p.
		n	%	n	%	n	%	
Carcass site	Brisket	2	22.2%	1	14.3%	1	20.0%	,758
	Flank	2	22.2%	4	57.1%	1	20.0%	
	Hind leg	2	22.2%	1	14.3%	2	40.0%	
	Rectal	3	33.3%	1	14.3%	1	20.0%	
Slaughter stage	Before washing	7	77.8%	4	57.1%	4	80.0%	,589
	After washing	2	22.2%	3	42.9%	1	20.0%	
Sampling visit	December	4	44.4%	2	28.6%	1	20.0%	,615
	June	5	55.6%	5	71.4%	4	80.0%	

*Chi-square test (Fisher's exact)

Table 5. Prevalence of virulence, and serotype genes in *L. monocytogenes* isolates

Strain	Positive isolates								
	Virulence genes							Serotype	
	prs	*iap*	*hly*	*inlA*	*inlB*	*plcA*	*prfA*	*ORF2819*	*ORF2110*
L–1	+	+	+	+	–	+	–	+	+
L–2	+	+	+	+	+	+	–	+	+
L–3	+	+	+	+	+	+	–	+	+
L–4	+	+	+	+	+	+	–	+	+
L–5	+	+	+	+	+	+	–	+	+
L–6	+	+	+	+	+	+	–	+	+
L–7	+	+	+	+	+	+	–	+	+
L–8	+	+	+	+	+	+	–	+	+
L–9	+	+	+	+	+	+	–	+	+
L–10	+	+	+	+	–	+	–	+	+
L–11	+	+	+	+	+	+	–	+	+
L–12	+	+	+	+	–	+	–	+	+
L–13	+	+	+	+	–	+	–	+	+
L–14	+	+	+	+	+	+	–	+	+
L–15	+	+	+	+	–	+	–	+	+
L–16	+	+	+	+	+	+	–	+	+
L–17	+	+	+	+	+	+	–	+	+
L–18	+	+	+	+	+	+	–	+	+
L–19	+	+	+	+	+	+	–	+	+
L–20	+	+	+	+	–	+	+	+	+
L–21	+	+	+	+	+	+	–	+	+



Strain	Animal species	Sample origin	Slaughter process	Sample date	Serotype
L20	Sheep	Brisket	AW	II	4b(4d,4e)
L21	Cattle	Rectal	BW	II	4b(4d,4e)
L3	Goat	Hind leg	BW	II	4b(4d,4e)
L7	Sheep	Flank	BW	II	4b(4d,4e)
L8	Sheep	Flank	BW	I	4b(4d,4e)
L4	Cattle	Hind leg	BW	I	4b(4d,4e)
L17	Cattle	Flank	BW	II	4b(4d,4e)
L18	Goat	Rectal	BW	I	4b(4d,4e)
L15	Goat	Flank	AW	I	4b(4d,4e)
L16	Cattle	Brisket	BW	II	4b(4d,4e)
L2	Cattle	Hind leg	AW	II	4b(4d,4e)
L9	Goat	Hind leg	BW	I	4b(4d,4e)
L6	Goat	Brisket	BW	I	4b(4d,4e)
L19	Goat	Rectal	AW	II	4b(4d,4e)
L1	Sheep	Hind leg	BW	II	4b(4d,4e)
L5	Goat	Brisket	BW	II	4b(4d,4e)
L12	Goat	Rectal	BW	II	4b(4d,4e)
L13	Sheep	Rectal	BW	II	4b(4d,4e)
L14	Goat	Flank	BW	II	4b(4d,4e)
L10	Sheep	Flank	AW	II	4b(4d,4e)
L11	Sheep	Flank	AW	I	4b(4d,4e)

Figure 1. *Apa*I-PFGE dendrogram based on DICE coefficient of similarity for *L. monocytogenes* strains isolated from different parts of cattle, sheep and goats at a slaughterhouse. Pulsotypes (isolates), P1 (L20, L21), P2 (L3, L7, L8), P3 (L4), P4 (L17, L18), P5 (L15, L16), P6 (L2, L9), P7 (L6), P8 (L19), P9 (L1), P10 (L5), P11 (L12, L13), P12 (L14), P13(L10, L11); BW, Before washing; AW, After end washing; I, December; II, June.

Phenotypic antibiotic resistance

The resistance patterns of *L. monocytogenes* towards the tested antimicrobial agents are presented in Table 6. Fourty-two percent of isolates (n=9/21) were resistant to at least one antimicrobial. Resistance to CD was the most common finding (19.4%), followed by resistance to E, (9.5%); AM, (9.5%); P, (9.5%) and SXT (9.5%). None of the isolates was resistant to C, CN, LNZ, MEM, RA, TE, TEC and VA. MDR isolates were seen in 9.5% of the isolates, mainly in L7 and L20.

PFGE analysis

PFGE analysis of 21 *L. monocytogenes* strains yielded 13 different pulsotypes (Figure 1). Seven of the pulsotypes were clusters while 6 were unique. Total clustering rate was found as 54%. According to similarity coefficient that is higher than 85%, two PFGE groups were identified. The largest group included 16 strains (L1, L2, L3, L4, L5, L6, L7, L8, L9, L15, L16, L17, L18, L19, L20, L21). Second PFGE group included 5 strains L10, L11, L12, L13 and L14.

DISCUSSION

Diseases that originate in animals or zoonoses, can be transmitted to humans in a number of different ways, including through direct touch, indirect environmental contact, or ingested food (34). Over four hundred twenty thousand people die every year in the world after eating contaminated food by foodborne bacteria, many of which are considered to be zoonotic, according to the World Health Organization (WHO) (35). Many foodborne pathogens, including *L. monocytogenes,* have their primary reservoirs in animals used for food production. Moreover, wild birds, fish, and shellfish, in addition to domestic animals including cattle, sheep, goats, horses and poultry, may become colonized by pathogenic strains of those bacteria (36).

The current research focuses especially on the variety of antimicrobial resistances, molecular lineages, virulence factors, and serogroups of *L. monocytogenes* isolated in abattoirs from cattle, sheep, and goats. We assessed potential spread of *L. monocytogenes* through cattle, sheep and goat in Van province in Eastern Türkiye. In this study, twenty-one (3.5%), of the 600 carcass surface samples tested were determined to be *L. monocytogenes*-contaminated (Table 3). Of the 21 isolates, 9 (18%) were from goats, 7 (8.75%) were from sheep and 6 (30%) were from cattle (Table 3). *L. monocytogenes* was isolated in cattle, sheep and goats from carcass swabs before washing (40%, 10%, 28%) and after end washing (10%, 7,5%, 8%) respectively. The incidence of *L. monocytogenes* contamination (8.75-30%) was reported for

Table 6. Antimicrobial susceptibility pattern of 21 *L. monocytogenes* isolated from examined cattle, sheep and goats

Antibiotics	Strains isolated from slaughterhouse																					Sensitivity of the total isolates			Antimicrobial class according to WHO*a*
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	S(%)	I(%)	R(%)	
AM	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	90.5	0	9.5	CI
C	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0	0	0	CI
CIP	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	95.3	4.7	0	CI
CD	I	R	I	I	I	I	I	I	R	S	I	I	I	R	I	I	I	S	R	S	I	14.2	66.6	19.04	CI
CN	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0	0	0	I
E	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	90.5	0	9.5	CI
LNZ	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0	0	0	CI
MEM	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0	0	0	CI
PenG	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	90.5	0	9.5	CI
QD	S	S	S	I	S	S	S	I	S	S	S	I	S	S	I	I	S	I	S	S	I	66.6	33.3	0	CI
RA	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0	0	0	CI
SXT	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	90.5	0	9.5	HI
TE	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0	0	0	CI
TEC	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0	0	0	HI
VA	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0	0	0	CI

a CI, critically important; I, important; HI, highly important (Angulo et al., 2009); ampicillin, AM; chloramphenicol, C; ciprofloxacin, CIP; clindamycin, CD; gentamicin, CN; erythromycin, E; linezolid, LNZ; meropenem, MEM; Benzylpenicillin, PenG; quinupristin-dalfopristin, QD; rifampin, RA; streptomycin, S; trimethoprim-sulfamethoxazole, SXT; tetracycline, TE; teicoplanin, TEC; vancomycin, VA.

cattle, sheep, and goat carcasses from an abattoir, adding to existing studies from various countries, including Iran (11), Qatar (37), Brazil (38) and Korea (39). Data available on the prevalence of *L. monocytogenes* from cattle, sheep and goat carcasses are diverse. Variations in the prevalence rates of *L. monocytogenes from* cattle, sheep and goat carcasses may reflect differences in geographic locations, hygienic conditions, husbandry practices, agro-climatic variations, sampling methods of detection, breeds and the age of animals. Possible contributors to the variation in outcomes includes the quality of the farms from which the animals are sourced and the health and disease status of the animals that are slaughtered.

L. monocytogenes is a major foodborne pathogen; however, there is a wide range of strains within the species, each with its own unique profile of virulence and pathogenicity. Some *L. monocytogenes* strains are naturally virulent and can cause substantial morbidity and mortality, while others are avirulent and cannot develop an infection within mammalian hosts (8). In the present study, all of twenty-one *L. monocytogenes* isolates (100%) were serovar (4b, 4d or 4e). Overall, twenty-one isolates tested positive for one or more of the VF genes. Among them, the prevalence of individual VF genes was 100% for *prs,* 100% for *iap,* 100% for *hlyA*, 100% for *inlA*, 100% for *plcA*, 71.4% for *inlB* and 4.7% for *prfA* (Table 5). Our findings are consistent with a study by Oh *et al.* (39) in Korea, where *actA, inlA, inlB, hlyA,* and *plcB* were observed in *L. monocytogenes* isolates, and is also similar to a study reported by Camargo *et al.* (40)and 62.3% of the isolates carried the bla CTX-M gene, among which CTX-M-1 was found to be the predominant ESBL types in chicken isolates, whereas CTX-M-15 was the commonest among BTM samples. Additionally, of 52 ESBL-EC isolates from chicken meat samples, 36.5%, 9.6%, and 7.7% harbored the bla TEM, bla CMY-2, and bla SHV-12 genes, respectively, compared with 28.5% of the bla TEM and 7.1% of the bla SHV-12 markers among BTM isolates. The fimH gene was present in 51 isolates of chicken and in 14 isolates of BTM samples, while other virulence genes iutA (n = 31 in Brazil, where *inlA, inlB, inlC, inlJ, plcA, hlyA, actA,* and *iap* were detected in all of *L. monocytogenes* isolates. In another study conducted by Iglesias *et al.* (38), *hlyA, prfA, plcA, plcB, actA, iap, mpl, inlA, inlB, inlC,* and *inlJ genes* was detected in all isolate of *L. mono-*

cytogenes. Food isolates are primarily composed of antigenic group 1/2 (1/2a, 1/2b, and 1/2c) strains, whereas 4b strains are responsible for more than half of all listeriosis cases globally. This data implies that isolates of serovar 4b are better suited to mammalian host tissues than bacteria of serogroup 1/2a (7). By applying specific oligonucleotide primers for the detection of *L. monocytogenes* serogroups in carcass samples (Table 5), it was indicated that all samples were positive for 4b (4d, 4e) serogroup while none of other serogroups tested (1/ 2a, 3a; 1/2c, 3c and 1/2b, 3b) was detected among the strains. In previous studies, the most frequent serotypes in *L. monocytogenes* from carcass samples were 1/2c or 3c (36); 4b, 4d or 4e (11); and 1/2a, and 1/2b (39). As a result, in this study, all of the isolates we obtained were virulent. Variation in the prevalence of virulence and serotype genes from *L. monocytogenes* reported in this and other studies may be the result of different sampling methods employed, types of samples, isolation procedures, the environmental conditions or geographical locations.

Although antimicrobials are life-saving medications, almost all bacteria that cause illnesses in humans are becoming more resistant, substantially compromising their efficacy (41). As a result of the use of antibiotics in food animals, microorganisms in these animals acquire antibiotic resistance genes that can be transmitted to humans (42). Food animals treated with antimicrobials are a major source of bacteria resistant to antimicrobials that can be transmitted to humans via food (41).

In this study, *L. monocytogenes* strains were tested for susceptibility to 15 antimicrobial drugs of veterinary and human health significance. *L. monocytogenes* strains were investigated in terms of the antimicrobial susceptibility. It was determined that a significant proportion of isolates were resistant to various drugs, particularly the lincosamides (CD), macrolides (E), penicillins (AM, PenG) and potentiated sulfonamide (SXT). The resistance patterns of *L. monocytogenes* to the tested antimicrobial agents are presented in Table 6. The resistance profiles of the *L. monocytogenes* isolates are as follows; clindamycin, 19.04%; erythromycin, 9.5%; ampicillin, 9.5%; penicillin G, 9.5% and trimethoprim-sulfamethoxazole 9.5%. Very high resistance to CD (71.8%), S (71.8%), AK (65.8%) and CXM (61.5%) was noted by Zhao *et al.* (43) in strains of *L. monocytogenes* isolated from ruminants in China. Oh *et al.* (39) found 93.3% resistance to P, 60% resistance to S, 26.7% resistance to AM and 20% resistance to TE in a group of 15 strains of *L. monocytogenes* isolated from abattoirs in Korea. Similarly, Lotfollahi *et al.* (11) demonstrated resistance to P and intermediate resistance to CD in *L. monocytogenes* isolated from sheep and goat in Iran. The results revealed the high rate of increase in multidrug resistance. These fluctuations may be expected to vary between the countries because of the differences in terms of the use of antibiotics. In *L. monocytogenes,* efflux pumps or the acquisition of genetic elements like plasmids and transposons can lead to enhanced tolerance or even resistance to antimicrobials (44).

Twenty-one *L. monocytogenes* at an abattoir were genotyped using PFGE (Figure 1). From all 21 *L. monocytogenes* isolates obtained from two visits, 13 pulsotypes (L1-L21) were identified. At 85% similarity, two primary patterns of *L. monocytogenes* isolates could be distinguished, and these were serotyped as 4b (4d, 4e). There were isolates in P2, P4, P5, P6, and P13 from several sampling sessions (I-II). On the same sampling trips, *L. monocytogenes* contamination was commonly seen and was represented by P1 and P11 isolates. Isolates from different animals during the different sampling visits and different slaughter processes possessed a significantly high similarity (100 %), such as L15 and L16 from goat flank and cattle brisket, L2 and L9 from cattle hind leg and goat hind leg, respectively, in cluster A. In Brazil, Iglesas *et al.* (38) and Camargo *et al.* (40) discovered *L. monocytogenes* in bovine carcasses before cold storage, as did Khen *et al.* (41) in Ireland. Unexpectedly, genetic profiles found in clusters I and II (L3-L7-L8; L2-L9; L10; L11; L15; 16; 17; 18; 20; 21) were identical across isolates derived from various dates (I-II) and animals (cows, sheep, and goats) (Figure 1). Cross-contamination or contamination from the same source at the abattoir could be responsible for this. It is possible, that *L. monocytogenes*, due to its psychotrophic characteristic, may multiply in the cold chamber during storage. Then their survival on cattle, sheep or goat carcass surface may allow trnasmision from carcasses to the abattoir environment and potentially to cattle, sheep, or goat carcasses based on the 28.5% (6/21 isolates) presence of *L. monocytogenes* in the carcass immediately before cooling.

In conclusion, *L. monocytogenes* isolates from an abattoir in Van, Türkiye, were studied as part of an epidemiological investigation. This study highlights the distribution, serovars, and genotypes of *L. monocytogenes* isolates and identified the presence of cross-contamination among carcasses in one abattoir. Although *L. monocytogenes* was isolated at low fre-

quencies along the meat production chain, the presence of the same clonal strain both before and after end washing from the different parts of animals indicated the presence of cross-contamination during slaughter operations. The relatively high prevalence of *L. monocytogenes* found in these animal species indicates a reservoir of potential *L. monocytogenes* infections for residents of this region.

In order to eliminate skin, feces, and carcass sources of contamination at various phases of animal rearing and slaughtering operations, the public health hazards associated with *L. monocytogenes* pathogens from food animals such as cattle, sheep, and goats need to be addressed at various levels. Appropriate abattoir design and internal controls are required to prevent microbial contamination, which influences consumer health and results in major public health issues. To preserve and enhance the hygienic conditions and meat quality provided by an abattoir, the degree and kind of microbial contamination must be monitored. Moreover, better understanding of the epidemiology and genetic structure of *L. monocytogenes* will also be useful in analyzing the animal origins. The findings obtained from these analyses provide additional information for developing control strategies.

CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest

ACKNOWLEDGEMENTS

I would like to thank Prof. Dr. Barş OTLU (Malatya Inonu University, Türkiye) for helping me with MALDI-TOF MS.

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