

The existence of *Listeria monocytogenes* in a cattle slaughterhouse and identification of serotypes by mPCR

Özgür ÇADIRCI, Ali GÜCÜKOĞLU, Göknuş TERZİ GÜLEL, Tolga UYANIK, Mustafa ALIŞARLI

Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Samsun, Turkey.

Summary: This study was carried out to investigate the presence of *Listeria monocytogenes* in samples obtained from a slaughterhouse in Samsun, Turkey. A total of 300 samples (25 samples per month) analyzed by Immunomagnetic Separation (IMS) based on conventional culture method. Then isolates were verified and serotyped by PCR using specific primers. According to the analysis, a total of 18 isolates were identified as *L. monocytogenes*. Distribution of these isolates were as; 5 isolates (serotype 1/2b or 3b) from 1 cattle carcass, 5 isolates (serotype 1/2b or 3b) from 1 knife, 2 isolates (serotype 4b or 4d, 4e) from 1 knife sharpener, 5 isolates (serotype 1/2a or 3a) from 1 floor sample, 1 isolate (serotype 1/2a or 3a) from 1 cowhide, respectively. As a result, carcasses are considered to pose a hazard to public health due to *L. monocytogenes* contamination. The development of slaughterhouse conditions and production technology, improving the education level of employees in food safety issues, prevention of contamination of foodborne pathogens to carcasses in slaughterhouses and implementation of effective sanitation methods should be provided.

Keywords: IMS, *Listeria monocytogenes*, serotyping, slaughterhouse.

Sığır kesimhanelerinde *Listeria monocytogenes* varlığı ve serotiplerinin mPCR tekniğiyle belirlenmesi

Özet: Samsun ilindeki mezbahada *Listeria monocytogenes* varlığının araştırılması amacıyla yapılan bu çalışma 12 aylık bir dönemi kapsamaktadır. Ayda bir kez olmak üzere her ay 25 örnek alınarak toplam 300 örnek *L. monocytogenes* varlığı bakımından analiz edildi. Örneklerden *L. monocytogenes* varlığının araştırılmasında Immunomagnetic Separation (IMS) bazlı klasik kültür tekniği, identifikasyonda biyokimyasal testler kullanıldı. İdentifiye edilen izolatlar PCR ile doğrulandı ve spesifik primerler ile serotiplendirildi. Yapılan biyokimyasal doğrulama testleri ve PCR analizleri sonucunda; 1 karkas örneğinden 5 izolat, 1 bıçak örneğinden 5 izolat; 1 bıçak bileyicisi örneğinden 2 izolat; 1 zemin örneğinden 5 izolat; 1 deri örneğinden 1 izolat olmak üzere toplam 18 izolat *L. monocytogenes* olarak belirlendi. Yapılan serotiplendirme sonucunda 1 karkas örneğinden elde edilen 5 izolat, 1 bıçak örneğinden elde edilen 5 izolat *L. monocytogenes* 1/2b (3b); 1 bıçak bileyicisi örneğinden elde edilen 2 izolat *L. monocytogenes* 4b (4d, 4e); 1 zemin örneğinden elde edilen 5 izolat ve 1 deri örneğinden elde edilen 1 izolat *L. monocytogenes* 1/2a (3a) olarak tespit edildi. Sonuç olarak karkaslardaki *L. monocytogenes* kontaminasyonunun halk sağlığı açısından tehlike oluşturabileceği değerlendirilmiştir. Mezbaşa şartlarının ve üretim teknolojisinin geliştirilmesi, çalışan personelin gıda güvenliği hususunda eğitim düzeyinin artırılması ve mezbahalarda etkin sanitasyon yöntemlerinin uygulanması ile gıda kaynaklı patojenlerin karkaslara bulaşmasının önlenmesi sağlanmalıdır.

Anahtar sözcükler: IMS, *Listeria monocytogenes*, mezbaşa, serotiplendirme.

Introduction

There are no pathogens on the carcass of healthy animals initially which have been slaughtered in compliance with hygiene rules. It is known that pathogenic factors are infecting carcass surfaces due to environmental contamination sources most of the times. Pathogens reproduce in carcass surfaces, and hence both infection and intoxication are formed. It is stated that, besides having *Listeria* species as the most common foodborne pathogen group in raw beef products, the existence of *L. monocytogenes* in processed meat products pose food safety risk (2, 3, 20, 30).

L. monocytogenes is a Gram-positive, intracellular bacterium and one of the most important food-borne

pathogen all over the world (21). It is especially influential with a mortality rate of 20%-30% among pregnant, newborns, elderly and immunosuppressed individuals. The disease is seen in two different forms known as invasive (especially pregnant uterus, central nervous system) and non-invasive (referred to as febrile listerial gastroenteritis) listeriosis. Although the incidence of listeriosis is lower than other foodborne pathogens, it is stated that rates of death from listeriosis are quite high (approximately 30%) (24).

Virulence of *L. monocytogenes* is dependent on virulence-associated genes such as *actA*, *hlyA*, *iap*, *inlA*, *inlC*, *mpl*, *plcA*, *plcB*, *opuCA*, *prfA*, and *clpC*. Listeriolysin O (LLO) which is coded by *hlyA* gene is the

most important virulence factor and allows the induction of “a pore-forming toxin” by *L. monocytogenes* serotypes (10, 21, 24).

There are 13 serotypes of *L. monocytogenes* and serotypes of 1/2a, 1/2b and 4b which are responsible for 98% of listeriosis cases in human beings. *L. monocytogenes* serotypes have diversity in terms of virulence. 4b serotype is the dominant cause in food-borne listeriosis outbreak (11).

In this research, i) isolation of *L. monocytogenes* from the floors, tools, hands of staff and beef carcasses, ii) identification of *L. monocytogenes* by IMS based conventional culture method and confirmation by PCR, iii) determination of *L. monocytogenes* serotypes by mPCR are aimed.

Materials and Methods

In this study, 300 samples were collected in a slaughterhouse between June 2012 and May 2013. Checkpoints where samples were taken: inner wall (2 samples), floor (2 samples), hands of staff (3 samples), knives (3 samples), knife sharpeners (3 samples), carcass hooks (3 samples), cowhide (3 samples), rectal swap (3 samples) and carcass surfaces (3 samples). Samples were periodically collected as 25 per month during one year and were brought to the laboratory under cold chain as soon as possible.

Wet-dry swab method was used in sample collection. While collecting samples from carcass surface, swab samples from 3 different areas (neck, legs and abdomen) were considered as a single sample (12).

IMS (immunomagnetic separation) based culture technique suggested by ISO 11290-1 (The International Standards Organization) (19) and Anon. (14) were used for *L. monocytogenes* isolation.

Swab samples were put in sterile tubes containing 10 ml Half Fraser Broth (Oxoid, CM0895; SR0166) and were incubated for 24 hours at 30°C. Following the pre-enrichment procedure, IMS process was conducted according to the manufacturer’s instructions.

50 µl Dynabeads-Listeria complex that was obtained from the IMS process was plated onto Listeria Selective Agar (Oxford Formulation, Oxoid, CM0856; SR0140) and petri dishes were incubated for 24-48 hours at 35°C. Five typical colonies (middle part collapsed dark brown or green color with a diameter of 2-3 mm surrounded by a black halo) were picked and plated onto TSA-YE (Tryptic Soy Agar - Yeast Extract, Fluka, 22091, Oxoid LP0021) in order to perform biochemical tests and then plaques were incubated for 24 hours at 30°C. As a result of gram stain, catalase, oxidase, mobility, indole, mobility, CAMP, β-hemolysis and rhamnase, xylose and mannitol fermentation tests, the isolates were identified as *L. monocytogenes*. *L. monocytogenes* ATCC 7644 (serotype 1/2c), *L. monocytogenes* RSKK 472 (serotype 1/2b), *L. monocytogenes* RSKK 471 (serotype 1/2a), *L. monocytogenes* RSKK 475 (serotype 4b), *S. aureus* ATCC 6923 and *R. equi* ATCC 3370 were used as positive control strains.

Confirmation of *Listeria monocytogenes* with PCR: In order to verify obtained isolates with PCR, *hlyA* primers designed by Bohnert et al. (9) and *iap* primer pairs designed by Köhler et al. (22) were used (Table 1).

Genomic DNA extraction: Genomic DNA extraction has been done in accordance with the method suggested by Amills et al. (4) by using Proteinaz K (Sigma, P2308) and Chelex 100 (Sigma C7901, Chelating Ion Exchange Resin).

Table 1. List of *L. monocytogenes* genes and primers used in the PCR assay (13).

Tablo 1. PCR analizinde kullanılan *L. monocytogenes* genleri ve primer dizilimleri (13).

Gen	Primer Sequence (5'-3')	PCR product (bp)	Reference
<i>hlyA</i>	PCRGO: GAATGTAACTTCGGCGCAATCAG PCRDO: GCCGTCGATGATTTGAACTTCATC	388	Bohnert et al. (9)
<i>iap</i>	IAP1: ACAAGCTGCACCTGTTGCAG IAP2: TGACAGCGTGTGTAGTAGCA	131	Köhler et al. (22)
<i>Imo0737</i>	F: AGGGCTTCAAGGACTTACCC R: ACGATTTCTGCTTGCCATTC	691	Doumith et al. (13)
<i>Imo1118</i>	F: AGGGGTCTTAAATCCTGGAA R: CGGCTTGTTCGGCATACTTA	906	Doumith et al. (13)
<i>ORF2819</i>	F: AGCAAAATGCCAAAACCTCGT R: CATCACTAAAGCCTCCCATTTG	471	Doumith et al. (13)
<i>ORF2110</i>	F: AGTGGACAATTGATTGGTGAA R: CATCCATCCCTTACTTTGGAC	597	Doumith et al. (13)

PCR amplification and electrophoresis: For *hlyA* gene, PCR mix was prepared as such; 1X PCR Buffer in total 50 µl volume, 1.5 mM MgCl₂, 0.1 mM dNTP, 0.5 U Taq-Polymerase (Sigma D4545), 1 µM from each primer and 5 µl template DNA. Amplification of *hlyA* gene was carried out in Thermal Cycler (Bio-Rad MJ mini Gradient CA - ABD) at 94°C with 5 minutes initial denaturation and 35 cycles, at 94°C with 30 seconds denaturation, at 65°C primer annealing for 45 seconds, at 72°C primer extension and at 72°C last extension (9).

For *iap* gene, PCR mix was prepared as such; 1X PCR Buffer in total 50 µl volume, 1.5 mM MgCl₂, 0.1 mM dNTP, 0.5 U Taq-Polymerase 1 µM and 5 µl DNA from each primer. Amplification of *iap* gene was programmed as 5 minutes initial denaturation at 94°C; 35 cycles of 30 seconds denaturation at 94°C, 45 seconds annealing at 55°C, 45 seconds extension at 72°C; 5 minutes of final extension at 72°C (22).

Amplicons were carried out in 2% agarose at 80 volt current for electrophoresis process (Bio-Rad PowerPac Basic Power Supply, Bio-Rad Wide Mini Sub-Cell GT Cell). *hlyA* and *iap* genes were visualized at UV-transilluminator at 388 bp and 131 bp, respectively.

Serotyping: In order to serotype *L. monocytogenes* isolates, primer sequences designed by Doumith et al. (13) were used (Table 1).

PCR amplification and electrophoresis: For serotyping, PCR mix was prepared as such: 1X PCR Buffer in total 50 µl volume, 1.5 mM MgCl₂, 0.1 mM dNTP, 0.5 U Taq-Polymerase, 1 µM from each primer and 5 µl template DNA. Serotyping conditions in Thermal Cycler were programmed as such: first denaturation at 94°C for 3 minutes; 35 cycles of denaturation at 94°C for

40 seconds, annealing at 53°C for 75 seconds, primer extension at 72°C for 45 seconds and final extension at 72°C for 5 minutes (13). Amplicons were carried out in 2% agarose at 80 volt current for electrophoresis process (Figure 2). For defining the current serotype the evaluation was as such: If an isolate was *Imo0737* positive: serotype 1/2a or 3a. If an isolate was both *Imo0737* and *Imo1118* positive: serotype 1/2c or 3c. If an isolate was *ORF2819* positive: serotype 1/2b or 3b. If an isolate was both *ORF2819* and *ORF2110* positive: serotype 4b or 4e, 4d.

Results

It was detected that, 5 of the samples (5/300, 1.6%) taken from slaughterhouse have been contaminated with *L. monocytogenes*. Amongst the samples contaminated with *L. monocytogenes*, one of them was obtained from cattle carcass (2.7%), one from blade (2.7%), one from blade sharpener (2.7%), one from floor (2.7%) and one from cowhide (2.7%). A total of 18 isolates (Table 2), which obtained from 5 positive samples, were determined to have *hlyA* and *iap* genes and confirmed as *L. monocytogenes* (Figure 1).

Three different serotypes (1/2a or 3a, 1/2b or 3b, and 4b or 4d, 4e) were obtained from 18 *L. monocytogenes* isolates. It has been detected that 10 of the isolates obtained from carcass and blade were *L. monocytogenes* 1/2b or 3b; 2 of the isolates obtained from blade sharpener were *L. monocytogenes* 4b or 4d, 4e; 6 of the isolates obtained from floor and skin sample were *L. monocytogenes* 1/2a or 3a. Serotypes of 1/2b and 1/2a were detected as dominant from the samples obtained from slaughterhouse whilst serotype 1/2c was not detected (Table 2).

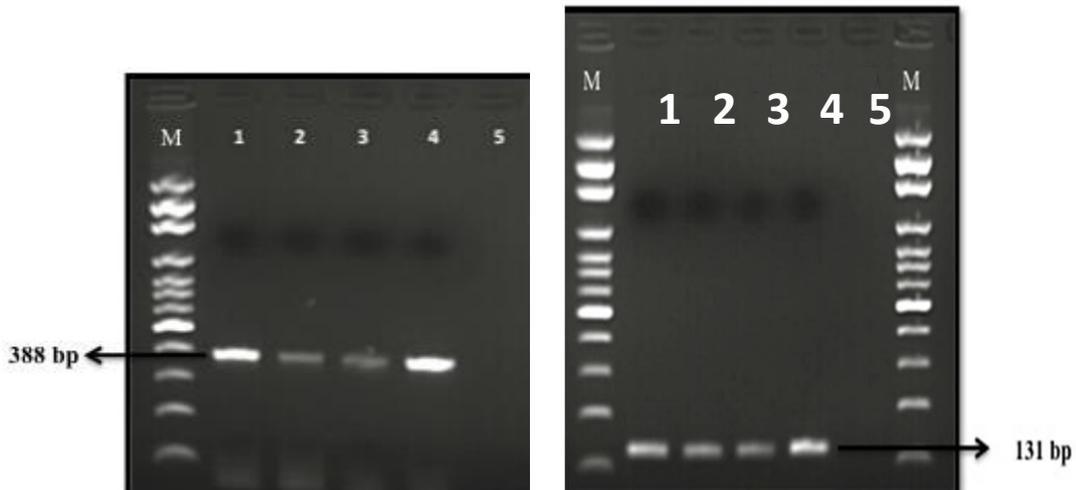


Figure 1. Electrophoresis image of *hlyA* (388 bp) and *iap* (131 bp) gene *L. monocytogenes* isolates by PCR. M: 100 bp DNA marker, lane 1: positive control for *hlyA* and *iap* gene (*L. monocytogenes* ATCC7644); lanes 2–4: *hlyA* and *iap* gene positive *L. monocytogenes* isolate; lane 5: negative control.

Şekil 1. *hlyA* (388 bp) ve *iap* (131 bp) genlerinin elektroforez görüntüsü. M: 100 bp DNA marker, sütun 1: *hlyA* ve *iap* genleri pozitif kontrol (*L. monocytogenes* ATCC7644); sütun 2–4: *hlyA* ve *iap* pozitif *L. monocytogenes* izolatları; sütun 5: negatif kontrol.

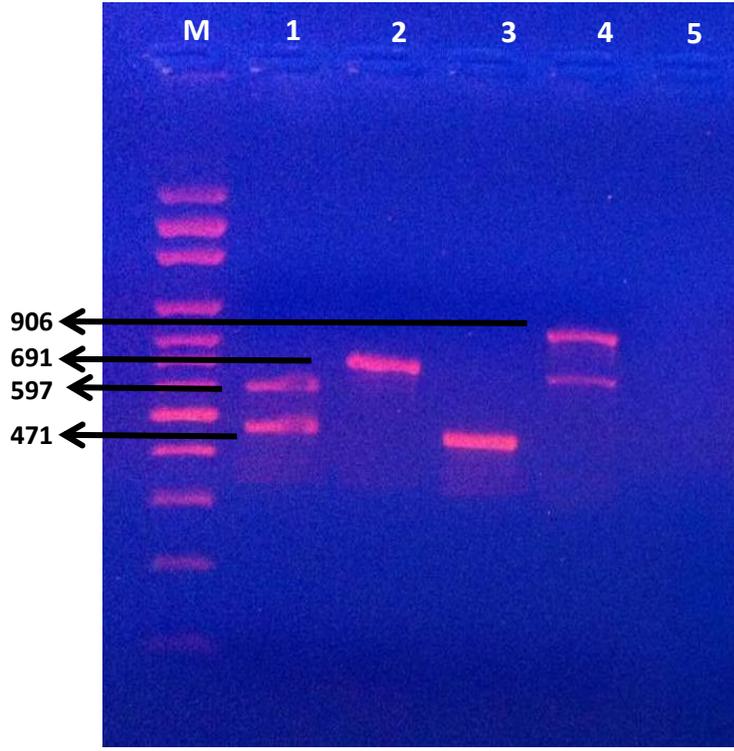


Figure 2. Electrophoresis image of positive and negative control. M: 100 bp DNA marker, lane 1: positive control of *L. monocytogenes* 4b (RSKK 475); lane 2: positive control of *L. monocytogenes* 1/2a (RSKK 471); lane 3: positive control of *L. monocytogenes* 1/2b (RSKK 472); lane 4: positive control of *L. monocytogenes* 1/2c (ATCC 7644); lane 5: negative control.

Şekil 2. Pozitif ve negatif kontrollerin elektroforez görüntüsü. M: 100 bp DNA marker, sütun 1: *L. monocytogenes* 4b pozitif kontrol (RSKK 475); sütun 2: *L. monocytogenes* 1/2a pozitif kontrol (RSKK 471); sütun 3: *L. monocytogenes* 1/2b pozitif kontrol (RSKK 472); sütun 4: *L. monocytogenes* 1/2c pozitif kontrol (ATCC 7644); sütun 5: negatif kontrol.

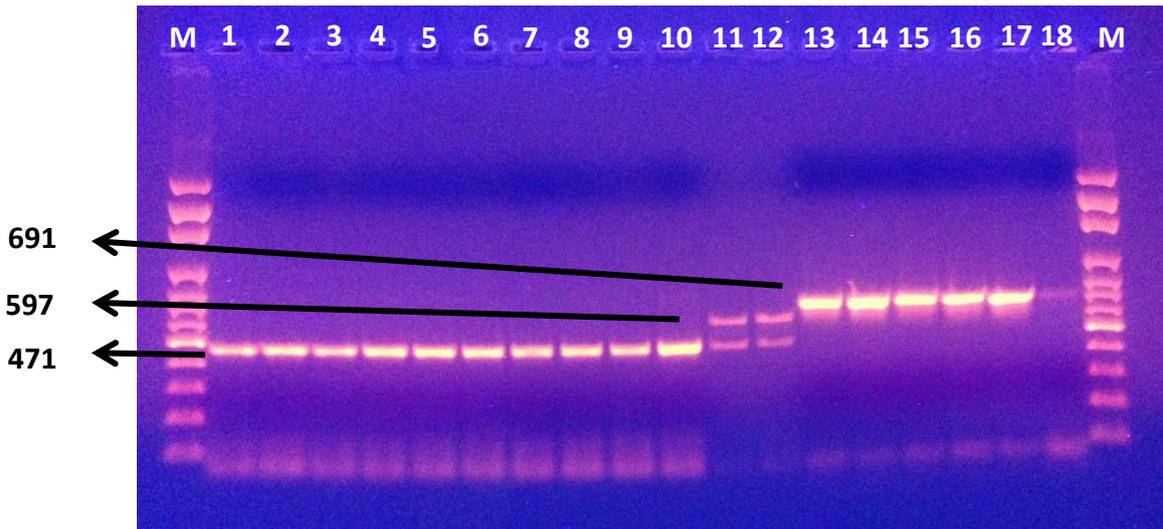


Figure 3. Serotype identification of *Listeria monocytogenes* isolates by multiplex PCR assay. M: 100 bp DNA marker, lanes 1-10: serotype 1/2b isolates; lanes 11-12: serotype 4b isolates; lanes 13-18: serotype 1/2a isolates.

Şekil 3. *Listeria monocytogenes* izolatlarının multiplex PZR ile serotiplerinin belirlenmesi. M: 100 bp DNA marker, sütun 1-10: serotip 1/2b; sütun 11-12: serotip 4b; sütun 13-18: serotip 1/2a.

Table 2. Occurrence of *L. monocytogenes* isolates and serotypes diversity in slaughterhouse samples.Tablo 2. Mezbaaha örneklerinde *L. monocytogenes* varlığı ve serotiplerinin dağılımı.

Sample (n=300)	<i>L. monocytogenes</i> positive samples	<i>L. monocytogenes</i> positive isolates	Serotype
Cattle carcass (n=36)	1	5	5 isolate 1/2b (3b)
Knife (n=36)	1	5	5 isolate 1/2b (3b)
Knife sharpener (n=36)	1	2	2 isolate 4b (4d, 4e)
Floor (n=24)	1	5	5 isolate 1/2a (3a)
Cattle skin (n=36)	1	1	1 isolate 1/2a (3a)
Inner wall (n=24)	-	-	-
Hands of staff (n=36)	-	-	-
Carcass hanging hook (n=36)	-	-	-
Rectal swap (n=36)	-	-	-

Discussion and Conclusion

From a total of 300 samples obtained from slaughterhouse, 5 of them (1.66%) were detected as *L. monocytogenes* positive. In various studies conducted in Turkey, the incidence of *L. monocytogenes* was reported to be between 1% and 5.17% in slaughterhouses (2, 20).

In this study, *L. monocytogenes* was isolated from cattle carcass, knife, knife sharpener, floor and cattle skin. During slaughtering, extraction of the cowhide and shredding, knives, knife sharpeners and also floors have been thought to be the primary contamination source of microorganism to cattle carcass. Since no microorganism have been isolated from staff hands, it can be suggested that contamination was not human originated.

In our study, 1 of 48 (2.08%) samples obtained from slaughterhouse wall and floor was detected *L. monocytogenes* positive. In contrast with the findings of our study, Yeşilyurt (32) detected the incidence of *L. monocytogenes* 4.2% (2/48) from the samples of slaughterhouse wall and floor.

In the study of Akkaya et al. (2), *L. monocytogenes* was not detected in the samples obtained from the wall of cold storage and the floor of meat transport vehicle. On the other hand, 10 of 100 (%10) samples obtained from slaughterhouse floor, 2 of 65 samples (3.07%) obtained from slaughterhouse inner wall, 1 of 65 samples (1.53%) obtained from cold storage floor and 1 of 20 (5%) samples obtained from the sides of the meat section of the transport vehicle were *L. monocytogenes* positive. In a study of Alişarlı et al. (3), *L. monocytogenes* was detected from slaughterhouse floor by the level of 6.7%. The findings of Akkaya et al. (2) and Alişarlı et al. (3) were consistent with the findings of our study. Similar to our findings, Sammarco et al. (29) reported that slaughterhouse surfaces and materials, equipments were the source of contamination.

On the other hand, in a study of Kahraman et al. (20), it has been stated that in 30 samples (6.97%) amongst 430

samples obtained from meat processing plants and 7 samples (7%) amongst 100 samples obtained from surfaces were *L. monocytogenes* positive. Researchers indicated that surfaces and equipments in food processing plants were the focal points for the microorganism.

In a study regarding the existence of *L. monocytogenes* at tools and materials used in slaughterhouse, the agent was detected in 10 of 120 (8.33%) samples obtained from cutting blades, choppers, shredding saws, hooks and cutting boards. Amongst 24 samples obtained from each tools and materials, the composition for positive *L. monocytogenes* was as such; 2 from cutting blades (8.33%), 2 from choppers (8.33%), 1 (4.16%) from meat saws, 3 from hooks (12.50%) and 2 from cutting boards (8.33%) (3). On the other hand, different from our study Akkaya et al. (2) detected *L. monocytogenes* from the samples collected from slaughterhouses (4 from shredding countertops (40%), 3 from knives (8.57%), 2 from choppers (20%), 6 from hooks (13.33%). Whilst the results of knife samples were very close, the other results were quite different. This may be because of sampling time, sampling technique, analysis method and the structure of the materials used. It was further considered that the lack of workers in implementation of hygiene rules was related with the sanitation quality of the slaughterhouses.

In a study of Kahraman et al. (20), *L. monocytogenes* was detected in 12 samples (9.23%) obtained from equipments. The percentages were also similar (10%, 8.8%, 11.9%) in the studies of Beumer and Giedfel (8), Alişarlı et al. (3) and Gudbjornsdottir et al. (17) respectively. On the other hand, Legnani et al. (23) reported the level of *L. monocytogenes* as 0.71% in their study.

Yeşilyurt (32) reported that he could not detect *L. monocytogenes* from any of the materials including 36 blades, 36 grinder and 72 hooks. One of 36 (2.78%) samples obtained from the hands of the workers and

gloves was declared to be detected *L. monocytogenes* positive. In the study of Akkaya et al. (2), 35 samples were obtained from the hands of the slaughterhouse workers and *L. monocytogenes* was detected as positive only in 1 sample (2.85%). Gudbjornsdottir et al. (17) also reported *L. monocytogenes* positive samples from the hands of workers from red meat and poultry plants. Alişarlı et al. (3), Aarnisalo et al. (1) and Kahraman et al. (20) could not detect *L. monocytogenes* from workers hands. The results of our study were similar to those by Alişarlı et al. (3), Aarnisalo et al. (1) and Kahraman et al. (20). Different from our findings, however, Yeşilyurt (32) and Akkaya et al. (2) had positive results. Considering those contradictory results, one may assume that results differ from each other due to lack of attention and hygiene of workers which plays a key role for food safety.

In this study, *L. monocytogenes* was detected in 1 of 36 samples (2.77%) obtained from cattle carcass surface. In parallel with our findings, the ratio was 6.8% in the study of Akkaya et al. (2) and 6.2% in the study of Alişarlı et al. (3). In the study of Fenlon et al. (16) in UK, *L. monocytogenes* was detected from 7% of carcasses. These data seem to support the findings of our study.

In a study of Barros et al. (7), *L. monocytogenes* was detected from 25.9% of bovine carcass samples whilst McNamara (26) detected only 4.1% from 2098 carcass samples. Vanderline et al. (31) reported the contamination level as 0.77% from 130 imported carcasses samples in Australia. Antoniollo et al. (5) identified *L. monocytogenes* with a level of 4.3% from 69 lamb carcass samples. In the study of Nicolas et al. (27) in France, *L. monocytogenes* was identified in 4 samples (20%) amongst 20 samples obtained from cattle carcass and 1 sample (14.28%) amongst 7 samples obtained from lamb carcass. It is seen that the results of the studies differ a lot. It is considered that the results of the studies for each country may differ because the terms of sanitation applied in slaughterhouses, different sampling techniques, method of analysis, hygiene conditions.

L. monocytogenes has 13 serotypes according to somatic (O) and flagella (H) antigenic structures. Serotype 4b, 1/2a, 1/2b and 1/c have been reported to cause 98% of foodborne listeriosis cases worldwide (21, 24). As a result of serotyping of isolates obtained in our study, we studied 18 isolates and detected that 55.6% was *L. monocytogenes* 1/2b, 33.3% was *L. monocytogenes* 1/2a and 11.1% was *L. monocytogenes* 4b. It was found in our study that 1/2b, 1/2a and 4b serotypes were dominant serotypes.

Hofer et al. (18) serotyped a total of 3122 *L. monocytogenes* isolates by using antiserum, 70 of which had milk product origin, that were obtained from different sources like food, human, animal and environment from Brazil between 1971-1997. In accordance with their

findings, it was reported that, amongst milk product derived isolates 58% were *L. monocytogenes* 1/2a, 26.6% were *L. monocytogenes* 4b, 14% were *L. monocytogenes* 1/2b and 1.4% were *L. monocytogenes* 1/2c. Within the total 3122 samples, *L. monocytogenes* 4b was the dominant serotype with the ratio of 45.4% which was followed by *L. monocytogenes* 1/2a as 20% and *L. monocytogenes* 1/2b as 20%. Makino et al. (25) also reported *L. monocytogenes* 1/2b was the dominant serotype from cheese samples (18).

In Turkey, Özkiraz (28) detected *L. monocytogenes* in 8 of 100 modified atmosphere packaged beef meat products sold in Samsun. It was detected that; from a total of 11 isolates, 3 of them were *L. monocytogenes* 1/2a, 4 of them were 1/2b, 1 was 1/2c and 3 were 4b. In studies on poultry, Erol and Şireli (15) isolated *L. monocytogenes* in frozen chicken carcasses that were offered for sale in Ankara with a method recommended by USDA/FSIS at the ratio of 30%. As a result of their serotyping, they detected *L. monocytogenes* 1/2a was the dominant serotype (73%) which followed by 1/2b, 1/2c and 4b respectively. Besides, Ayaz and Erol (6) reported that, as a result of serotyping *L. monocytogenes* isolates obtained from turkey meat, they detected 4b (51.4%) as dominant serotype which followed by 1/2a (27%) and 1/2b (21%) respectively. In our study, unlike the researchers, 1/2b was the most common serotype.

As a result, it was observed that *L. monocytogenes* could contaminate the carcasses during slaughtering because of slaughterhouse conditions, equipments and environment. Healthy meat can be obtained by transporting the cattles from farm to the slaughterhouse carefully, having necessary examinations and controls before slaughtering, having hygienic cutting in order to have both good flow of blood and animal welfare. Designing all departments in the slaughterhouse in accordance with hygiene rules and taking necessary measures in transition from clean areas to polluted areas, using tools, equipments and materials easy to clean and disinfect at all step, having well trained staff in food safety, applying quality management systems in slaughterhouses and having good hygiene practice procedures will help to prevent the contamination to carcasses.

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Address for correspondence:

Doç. Dr. Özgür ÇADIRCI
Ondokuz Mayıs University, Faculty of Veterinary Medicine,
Department of Food Hygiene and Technology,
Kurupelit, Samsun, Turkey.
Telephone: +90 362 3121919-2812
Fax: +90 362 4576622
e-mail: o.cadirci@omu.edu.tr