LWT - Food Science and Technology 113 (2019) 108363

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Characterization and subtyping of *Listeria monocytogenes* strains from butcher shops

Alejandra Londero[a,1], Magdalena Costa[a,*,1], Lucía Galli[a], Victoria Brusa[a], Luciano Linares[a], Mónica Prieto[b], Gerardo Leotta[a]

[a] IGEVET - Instituto de Genética Veterinaria, "Ing. Fernando N. Dulout", UNLP-CONICET LA PLATA, Facultad de Ciencias Veterinarias UNLP, 60 y 118 s/n, La Plata, Argentina

[b] Servicio Bacteriología Especial, Instituto Nacional de Enfermedades Infecciosas, ANLIS "Dr. Carlos G. Malbrán", Av. Vélez Sarsfield 563, Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina

ARTICLE INFO

Keywords:
Listeria monocytogenes
Ground beef
Butcher shop
PFGE
ERIC-PCR

ABSTRACT

We characterized *L. monocytogenes* strains isolated from ground beef (n = 40) and butcher shop environmental (n = 99) samples before and after implementing improvement actions. Strains were serotyped and subtyped by enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) and *Apa*I-pulsed-field gel electrophoresis (PFGE) to establish their epidemiological relationships, clarify the contamination dynamics and assess the impact of improvement measures. Serotype distribution was as follows: 1/2c (57.6%), 1/2b (18.7%), 4b (12.9%), 1/2a (6.5%) and 4a/4c (4.3%). Thirteen clones were simultaneously isolated in different butcher shops; 73.5% of establishments shared the same beef supplier. Cross-contamination was detected in *L. monocytogenes*-positive samples from 20% of butcher shops, mostly at the evaluation stage (91.7%). Up to five strains were isolated from each butcher shop at the evaluation (37%) and verification (22%) stages. Seven persistent clones were isolated over the three-year study period. Serotypes 4b and 1/2b significantly decreased during the evaluation stage. Although part of the contamination with *L. monocytogenes* was introduced by the beef supplier, the pathogen spread profusely in butcher shops, suggesting the relevance of keeping good hygiene control and management of the environment to prevent the spread of *L. monocytogenes* in butcher shops.

1. Introduction

Listeria monocytogenes is a foodborne pathogen with wide distribution in the environment and able to survive and proliferate under adverse environmental conditions. Infection of healthy individuals with *L. monocytogenes* normally leads to a self-limiting gastrointestinal infection with fever and diarrhea. Besides, it can cause spontaneous abortion in pregnant women as well as sepsis, encephalitis and meningitis in young (neonates), elderly and immunocompromised individuals. Although rare, listeriosis is a potentially fatal infection, having the highest hospitalization rates among foodborne diseases and an estimated 24% mortality rate (de Noordhout et al., 2014).

The high prevalence of *L. monocytogenes* in many food categories, such as ready-to-eat food, comprising dairy products, vegetables, meat and meat products, has been traditionally associated with cause of illness (Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017). Food contamination with *L. monocytogenes* may occur at any stage along the food chain through the environment, equipment, tools, employees, food products, customers or vendors (Alvarez-Ordonez, Leong, Hickey, Beaufort, & Jordan, 2015; Gomez et al., 2014; Wilks, Michels, & Keevil, 2006).

In ground beef, the prevalence of *L. monocytogenes* is high, ranging from 3.5 to 10.9% and reaching values as high as 52% (Rhoades, Duffy, & Koutsoumanis, 2009). This represents a risk to health, whether beef is consumed raw or used as raw material for the elaboration of ready-to-eat foods. Moreover, raw beef can be a vehicle to introduce the pathogen into the retail establishment or the consumer's kitchen, probably because *Listeria* can persist in the environment for long periods, and even low-level contamination may potentially spread subsequently to numerous foods (Hoelzer et al., 2011, 2014).

So far, data on *L. monocytogenes* levels in environmental sites of retail operations are scarce, and they are clearly necessary for a

* Corresponding author. IGEVET (UNLP-CONICET LA PLATA), Facultad de Ciencias Veterinarias UNLP, Av. 60 y 118, CC 296, La Plata (1900), Buenos Aires, Argentina.

E-mail address: mcosta@igevet.gob.ar (M. Costa).

[1] These two authors contributed equally to this work.

https://doi.org/10.1016/j.lwt.2019.108363
Received 29 May 2019; Received in revised form 1 July 2019; Accepted 5 July 2019
Available online 05 July 2019

comprehensive understanding of the contamination dynamics of this pathogen (Hoelzer et al., 2014). A practical knowledge of *L. monocytogenes* sources and food contamination routes can be useful to develop and apply effective control measures and well-designed food surveillance programs (Lakicevic & Nastasijevic, 2016).

We have previously demonstrated that the prevalence of *L. monocytogenes* in ground beef and environmental samples can be reduced by systematic monitoring and implementation of improvement actions for beef handling and food handler training (Leotta et al., 2016). In the present work, we deepened the research by characterizing 139 *L. monocytogenes* strains isolated from ground beef and the butcher shop environment to establish their epidemiological relationships, determine contamination routes and spread in retail establishments, and assess the effectiveness of improvement actions.

2. Materials and methods

2.1. Listeria monocytogenes strains

Listeria monocytogenes strains were isolated during the course of the pilot program called "Healthy Butcher Shops" conducted in the city of Berisso, Buenos Aires, Argentina (Leotta et al., 2016). A comprehensive risk assessment was performed in 86 butcher shops identified as B1 to B86. Ten abbatoirs (A–J) supplied beef to 70 of these butcher shops (Table 1). Abattoir B supplied most of the samples collected (56.9%).

Raw ground beef (n = 172) and environmental (n = 672) samples were analyzed for the presence of *L. monocytogenes*. Samples were collected during two sampling stages: evaluation (E) stage (2010–2011), after which improvement actions for butcher shops and consumers were implemented (Leotta et al., 2016), and verification (V) stage (2013), during which the impact of those actions was analyzed.

A total of 139 *L. monocytogenes* strains were isolated from raw ground beef (n = 40) and environmental (n = 99) samples. The latter included meat tables (n = 30), knives (n = 18), mincing machines (n = 27) and manipulator hands (n = 24).

2.2. Serotyping of L. monocytogenes isolates

Serotyping was performed with the Multiplex polymerase chain reaction (PCR) method described by Doumith, Buchrieser, Glaser, Jacquet, and Martin (2004), which allows the division of *L. monocytogenes* strains into four serotype groups: 1) 1/2a and 3a; 2) 1/2c and 3c; 3) 1/2b, 3b, and 7; 4) 4b, 4d, and 4e. Serological analysis was performed to confirm and specify the serotypes obtained, based on antibodies that specifically react with somatic (O) and flagellar (H) antigens and using a commercial *Listeria* antiserum (Denka Seiken, Tokyo, Japan) following the manufacturer's instructions. Strains that could not be serotyped by the PCR previously described were subjected to a second multiple PCR assay which is based on the amplification of the following target genes: prs, lmo0737, lmo1118, open reading frame 2110 (ORF2110), ORF2819, and prfA. This PCR assay classifies strains into five distinct molecular serogroups: IIa (serotypes 1/2a, 3a), IIb (1/2b, 3b, 7), IIc (1/2c, 3c), IVa (4a, 4c) and IVb (4b, 4 ab, 4d, 4e), and the variant profile of molecular serogroup IVb (IVb-v1) (Doumith et al., 2004; Huang et al., 2011). The control strains used for the PCR reactions were *L. monocytogenes* 1/2a CCBE105/04–380, *L. monocytogenes* 1/2b CCBE105/04–582, *L. monocytogenes* 1/2c CCBE105/04–754, *L. monocytogenes* 3a CCBE105/04–436, *L. monocytogenes* 3c CCBE105/04–412, *L. monocytogenes* 4a CCBE105/04–988, L. *monocytogenes* 4b CCBE105/04–868 and *L. innocua* CCBE105/04–90. These strains belong to the Special Bacteriology Culture Collection of the Argentinean National Institute of Infectious Diseases of the National Administration of Laboratories and Institutes of Health "Dr. Carlos Malbrán" (INEI-ANLIS, for its Spanish acronym) and were serotyped with conventional slide and tube O and H antigen agglutination (Denka Seiken Co, LTD, Japan) (Callejo et al., 2008).

The association between prevalence of serotype 1/2b and 4b strains and sampling stage (E and V) was determined by Chi-square test. Statistical analysis was performed with XLSTAT 2018.4, with significance set at $p < 0.05$.

Table 1
Abattoirs that supplied beef to the sampled butcher shops.

Abattoir	No. of butcher shops supplied	%
A	3	3.5
B	48	56.9
C	7	8.1
D	3	2.3
E	1	1.2
F	1	1.2
G	3	3.5
H	1	1.2
I	2	2.3
J	1	1.2
UK	16	18.6
Total	**86**	**100**

UK: Unknown.

2.3. Molecular subtyping

2.3.1. Enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR)

Genomic DNA was extracted with the Wizard Genomic DNA Purification kit (Promega, WI, USA). Primers ERIC 1R (5′-ATGTA AGCTCCTGGGGATTCAC-3′) and ERIC 2 (5′-AAGTAAGTGACTGGGGT GAGCG-3′) were used. Amplification reactions were performed in 25 μl of a solution containing 1 x Taq buffer with $(NH_4)_2SO_4$ (Thermo Scientific, MA, USA), 1 μM of each primer (IDT, Biodynamics, Argentina), 0.4 mM of each deoxynucleosidetriphosphate (PB-L, Argentina), 6 mM $MgCl_2$ (Thermo Scientific), 1.25 U DNA polymerase (Thermo Scientific) and 2 μl DNA extract. Amplifications were performed in a DNA thermal cycler (Life Express, Bioer, China) with the following temperature profiles: 1 cycle at 95 °C for 5 min; 35 cycles at 94 °C for 30 s, at 40 °C for 3 min, and at 72 °C for 2 min; and 1 cycle at 72 °C for 7 min. Finally, PCR products were separated by electrophoresis in a 2% agarose (Genebiotech, South Korea) gel in 1X TBE running buffer at 80 V for 3 h. Molecular weight marker 1 Kb Plus DNA Ladder (Thermo Scientific) was used as size standard.

2.3.2. Pulsed-field gel electrophoresis (PFGE)

For this analysis, the one-day standardized laboratory protocol for molecular subtyping of *L. monocytogenes* was employed (CDC, 2017). Restriction digestion of DNA in agarose plugs was carried out with *Apa*I (Thermo Scientific) enzyme during 18 h. MaestroGen slider imager (Maestrogen Inc., Nevada, USA) was used to obtain PFGE images of gels.

2.3.3. Image analysis

Tagged file format (TIFF) images obtained by ERIC-PCR and *Apa*I-PFGE were analyzed with BioNumerics version 6.6 software package (Applied Maths, Sint-Martens-Latem, Belgium) using the Dice coefficient and the unweighted pair group method with arithmetic mean (UPGMA) to generate dendrograms with 1.5% band matching tolerance. Two or more isolates with identical band pattern (100% similarity) were grouped in a cluster.

3. Results

Serotype distribution of *L. monocytogenes* strains isolated from ground beef and butcher shop environmental samples is shown in Table 2. The most prevalent serotype was 1/2c (57.6%), followed by 1/

Table 2
Serotypes of 139 *L. monocytogenes* strains isolated from ground beef and butcher shop environmental samples.

Sample	No. of isolates by serotype				
	1/2 a	1/2 b	1/2 c	4b	4a/4c
Ground beef	**5**	**8**	**21**	**3**	**3**
Environmental samples	**4**	**18**	**59**	**15**	**3**
Mincing machine	1	2	18	4	2
Manipulator hands	1	7	13	3	0
Meat table	2	5	17	5	1
Knife	0	4	11	3	0
Total	**9**	**26**	**80**	**18**	**6**

Table 3
Listeria monocytogenes serotype 1/2b and 4b isolates obtained from ground beef and environmental samples during the evaluation (E) and verification (V) stages after implementing improvement actions.

Samples	1/2 b		4b	
	Stage E	Stage V	Stage E	Stage V
Ground beef (n = 86)	7 (8.1)	1 (1.2)	2 (2.3)	1 (1.2)
Environmental samples (n = 336)	13 (3.9)	5 (1.5)	12 (3.6)	3 (0.9)
Total (n = 422)	20 (4.7)	6 (1.4)	14 (3.3)	4 (0.9)

The percentage of positive samples from the total number of samples analyzed is presented in parentheses.

2b (18.7%) and 4b (12.9%), whereas a lower proportion of strains corresponded to serotypes 1/2a (6.5%) and 4a/4c (4.3%). Serotype distribution was similar in ground beef and environmental samples.

Listeria monocytogenes serotype 1/2b and 4b-positive isolates recovered from ground beef and environmental samples during E and V are detailed in Table 3. Their prevalence decreased significantly from E to V (4b, 3.3 to 0.9%, $p = 0.0172$; 1/2b, 4.7 to 1.4%, $p = 0.0053$).

Of the total *L. monocytogenes* isolates analyzed by ERIC-PCR and *ApaI*-PFGE (n = 139), three strains (serotypes 1/2b, 4b and 1/2a) were excluded from the PFGE analysis due to DNA degradation. Cluster analysis of both typing methods yielded 18 clusters, but ERIC-PCR clusters included more strains. Therefore, a greater number of band patterns was obtained by *ApaI*-PFGE (n = 68) than ERIC-PCR (n = 54). There was scarce agreement between clusters obtained by the two subtyping methods. A great proportion of weak and diffuse bands of low molecular weight was obtained by ERIC-PCR, which difficult band assignation for group analysis. Since PFGE band patterns were clearer, PFGE results were considered more reliable and used for cluster analysis. The dendrogram obtained by *Apa*I-PFGE UPGMA is presented as Online Resource.

Sixty-eight *Apa*I-PFGE patterns with 11–28 bands and a similarity of at least 55.1% were obtained. Fifty strains showed unique *Apa*I-PFGE patterns and 86 isolates grouped in 18 clusters. Out of 18 *Apa*I-PFGE clusters, 13 grouped clones isolated from different butcher shops during the same sampling stage. Of 49 butcher shops involved, 36 (73.5%) were supplied by abattoir B.

Twenty percent of butcher shops with positive isolation (n = 12/60) had clones circulating inside the establishment. All cases except one (n = 11/12) occurred during the E sampling stage (see Online Resource). Clonal strains were isolated from ground beef and environmental samples in seven butcher shops during E: B2 (cluster I); B35,

Table 4
Cluster analysis of *Apa*I-PFGE gels.

Cluster	*ApaI*-PFGE pattern	Sampling stage	Serotype	Butcher shop	Abbatoir	Sample type	No of isolates
I	14	E	4b	2 (n = 2)	UK	B, K	2
II	16	E	4b	45, 58, 11	B	B, K, T	3
III	17	E	1/2b	84	B	K, T, H	3
				30, 2	UK		
IV	22	E & V	1/2b	16, 29, 77,	B	B, K, T, H	5
				22 (n = 2)	B and D		
V	23	E	1/2b	71 (n = 2)	A	K, T	2
VI	25	E & V	1/2b	43, 23	B	B, H, M	5
				68 (n = 2)	G		
				2	UK		
VII	26	E	1/2b	29, 14	B	K	2
VIII	29	E & V	1/2b	25, 34	UK	B, M	2
IX	31	V	4b	58, 63, 70	B	B, T, H	3
X	35	E	1/2c	58	B	H, M	2
				55	A		
XI	36	E & V	1/2c	23, 42	B	B, K, T	3
				86	C		
XII	39	E & V	1/2c	52, 58, 14, 17 (n = 4), 43, 63, 18, 4, 47, 7, 36 (n = 3), 76, 54, 10, 35	B	B, K, T, H, M	39
				(n = 2), 40, 47, 64	C		
				12, 86	J		
				80 (n = 2)	UK		
				33 (n = 2), 27, 32, 1 (n = 3), 56, 50 (n = 2)	A		
				55			
XIII	43	E & V	1/2c	38, 16, 42	B	B, K, H	4
				68	G		
XIV	45	E	1/2a	45 (n = 2)	B	B, M	2
XV	48	E	1/2c	42 (n = 2)	B	B, K	2
XVI	53	V	1/2c	59, 51	B	B	2
XVII	56	V	1/2c	65	B	B, M	2
				68	G		
XVIII	59	E & V	1/2c	64, 17, 4	B	B, M	3

Sampling stage: E, evaluation; V, verification.
Sample type: B, ground beef; K, knife; T, meat table; H, manipulator hands; M, mincing machine.
UK: Unknown.

Table 5
Number of butcher shops with more than one *Listeria monocytogenes* strain during the same sampling stage.

	Butcher shops			
Evaluation stage	14 (2)	4 (3)	4 (4)	–
Verification stage	9 (2)	3 (3)	–	1 (5)

Between parenthesis, number of different strains isolated per butcher shop.

B36, B50 and B1 (cluster XII); B45 (cluster XIV); and B42 (cluster XV). No clonal strains were isolated during V in any butcher shop. Besides, clones were isolated from different environmental samples in four butcher shops during E, namely, B22 (cluster IV), B71 (cluster V) and B33 and B80 (cluster XII), and one butcher shop during V (B17; cluster XII) (Table 4).

In addition, seven clones (clusters IV, VI, VIII, XI, XII, XIII and XVIII) were isolated during both sampling stages from different butcher shops, indicating persistence throughout the three years of study. Finally, the same clone was isolated during both sampling stages in two butcher shops (B36 and B68; cluster XII) (see Online Resource).

On the other hand, up to five different clones were isolated in the same butcher shop. From the 59 *L. monocytogenes*-positive butcher shops, 22 (37.3%) and 13 (22.0%) were contaminated with more than one clone during E and V, respectively (Table 5).

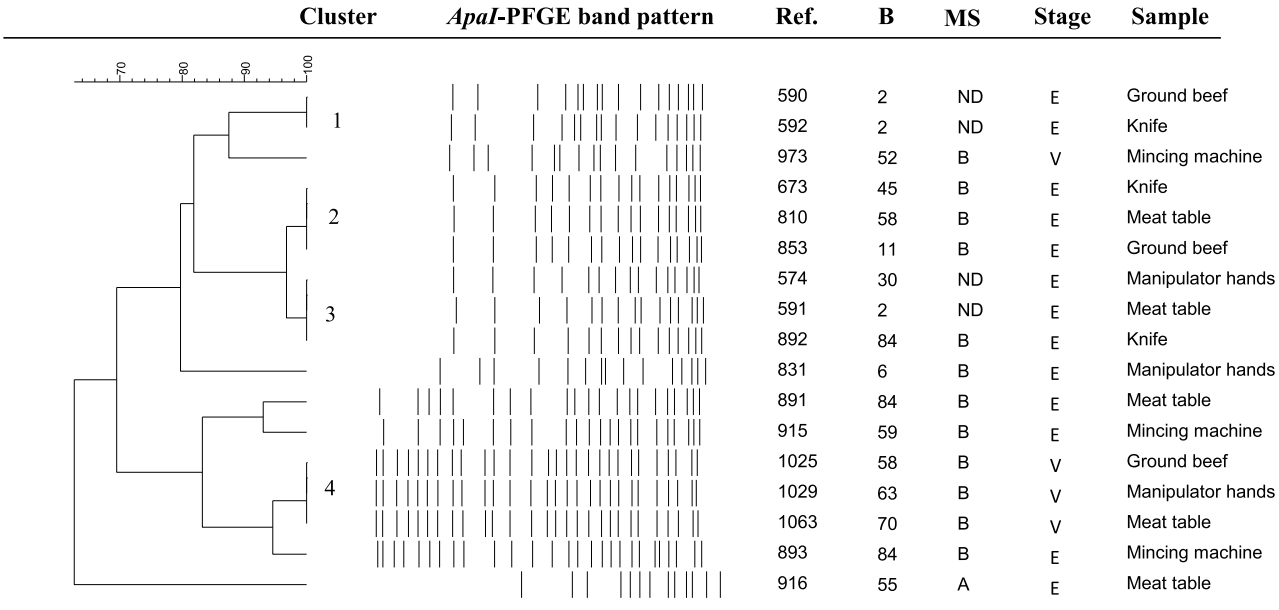
An independent *ApaI*-PFGE cluster analysis was performed for serotypes 4b (Fig. 1) and 1/2b (Fig. 2). From 18 *L. monocytogenes* serotype 4b strains isolated, 10 patterns including four clusters were obtained in the *ApaI*-PFGE dendrogram (Fig. 1). One strain was excluded from the PFGE analysis due to DNA degradation. Cluster 1 grouped two strains from ground beef and knife samples of the same butcher shop isolated during E. In two opportunities, clusters included isolates recovered from three different butcher shops during E: cluster 2 (B45, B58, and B11) and cluster 3 (B30, B2, and B84). Cluster 4 grouped isolates from three different butcher shops during V (B58, B63, and B70). Butcher shops from which the same clones were isolated shared the same beef supplier (abattoir B) in all cases in which the origin of beef could be determined (cluster 2 and 4). No persistent clones were found. Three *L. monocytogenes* strains were isolated from B2 and other three from B84 during E.

From 26 *L. monocytogenes* serotype 1/2b strains isolated, 11 unique patterns and four clusters (α, β, γ and δ) were obtained in the *ApaI*-PFGE dendrogram (Fig. 2). Clusters α and β included isolates recovered from meat table and knife samples from the same butcher shop (B22 and B71, respectively) during E. Cluster γ grouped clones isolated from the same butcher shop (B68) during E and V. Also, the clone of cluster α was isolated from four different butcher shops, all of which shared the same beef supplier (abattoir B). Clones of clusters α and δ also shared the same provider (abattoir B). The clone of cluster γ was isolated from four butcher shops, of which only two shared provider (B43 and B68, abattoir B). During E, two serotype 1/2b strains were isolated form B29 and three from B15.

4. Discussion

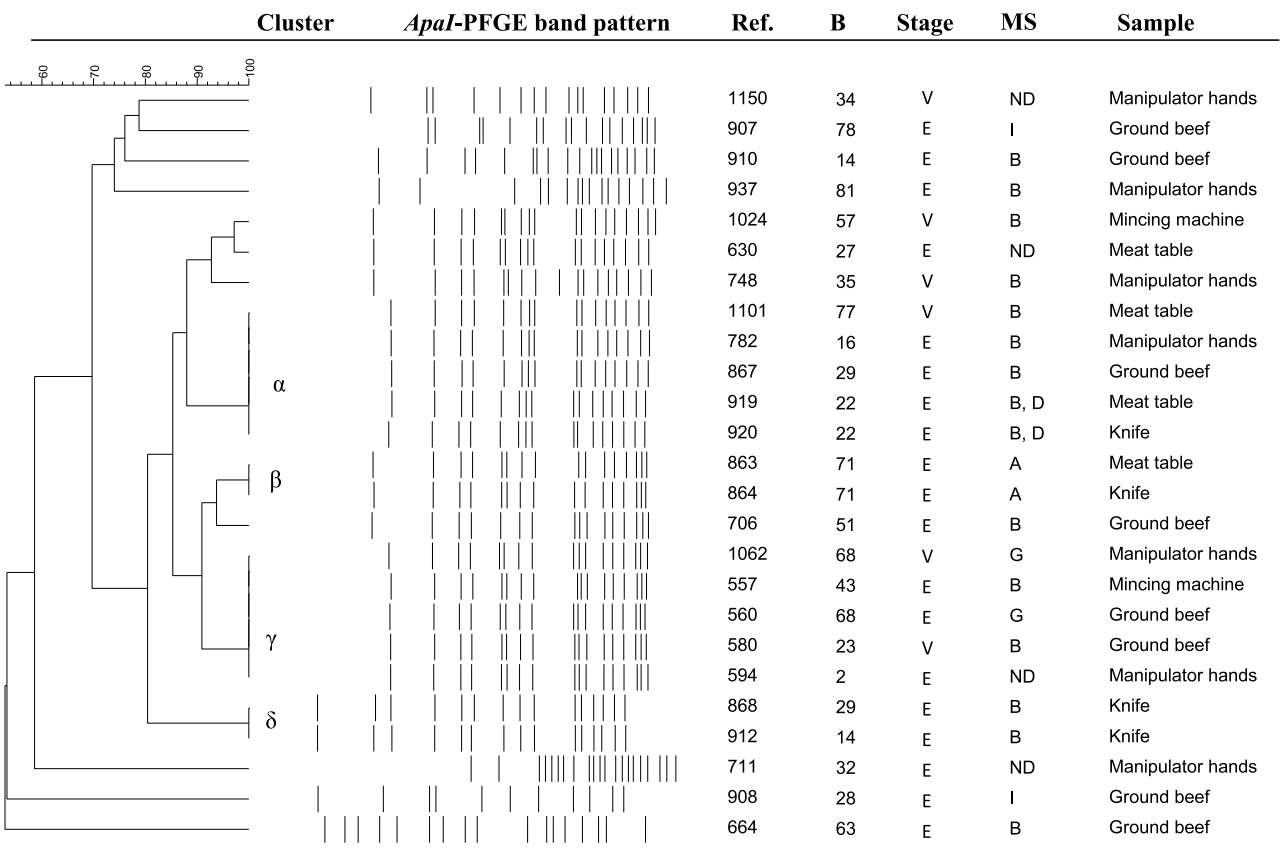
In a previous study, we reported a statistically significant reduction in the prevalence of *L. monocytogenes* in ground beef (from 46.5% to 15.1%), meat tables (from 32.1% to 11.9%), knives (from 23.8% to 10.7%), mincing machines (32.1%–13.1%) and manipulator hands (from 28.6% to 7.1%) after the implementation of improvement actions in butcher shops (Leotta et al., 2016). On this basis, in the present work we characterized 139 *L. monocytogenes* isolates obtained from ground beef and environmental samples during the evaluation and verification stages after applying improvement actions to clarify the contamination dynamics and assess the impact of improvement measures.

Results revealed that the most prevalent serotype was 1/2c, followed by a high proportion of 1/2b and 4b serotypes over the rest. Thus far, serotype distribution in ground beef and the retail environment had not been documented in Argentina. In other countries, evidence from the period 1971–1994 indicated that 1/2a, 1/2b and 1/2c were the most prevalent serotypes in meat (Jay, 1996). This is in line with recently published studies (Gianfranceschi, Gattuso, Tartaro, & Aureli, 2003) reporting that 1/2c and 1/2a were prevalent in meat products and meat production plants (n = 306) from Italy. Additionally, Kramarenko et al. (2013) described that 1/2a (77%) and 1/2c (18%) were the most frequent *L. monocytogenes* serotypes in raw meat from Estonia, whereas serotype distribution was 1/2a (36.8%), 1/2c



Ref.: isolate refence, B: Butcher shop, MS: meat supplier, ND: not determined, E: evaluation stage, V: verification stage.

Fig. 1. Dendrogram representing genetic relationships of 17 L. monocytogenes serotype 4b isolates based on ApaI-PFGE profiles.



Ref.: isolate refence, B: Butcher shop, MS: meat supplier, ND: not determined, E: evaluation stage, V: verification stage.

Fig. 2. Dendrogram representing genetic relationships of 25 L. monocytogenes serotype 1/2b isolates based on ApaI-PFGE profiles.

(34.0%), 1/2b (17.9%) and 4b (11.3%) in 106 *L. monocytogenes* isolates collected from surfaces of meat processing plants and meat products from Spain (Martin et al., 2014). In Chile, Montero et al. (2015) revealed some associations between particular foods and serotypes, such as raw meat and serotype 1/2c. In relation to the previously cited reports, we found a lower prevalence of 1/2a and higher prevalence of 1/2b and 4b serotypes. Our results are in accordance with studies performed in Brazil, one describing the prevalence of serotype 1/2c over serotypes 1/2b and 4b in 143 *L. monocytogenes* strains isolated from ground beef in Sao Paulo (Ristori et al., 2014), and the other serotype distribution in beef cut isolates (1/2c, 50.6%; 1/2b, 24.0%; 4b, 17.7%; 1/2a, 7.6%) (Camargo, Woodward, & Nero, 2016).

Listeria monocytogenes strains differ in their epidemic potential and in their ability to cause disease. Serotypes 4b, 1/2a and 1/2b account for more than 95% of human listeriosis cases around the world (Kathariou, 2002). In Argentina, strains of serotypes 4b and 1/2b are responsible for 42.0 and 49.0% of the documented cases of invasive and perinatal listeriosis, respectively (RSA-CONICET, 2017). Considering the relevance of serotypes 4b and 1/2b as disease cause in Argentina, strains of those serotypes were analyzed separately (see Results section). The number of strains of both serotypes significantly decreased ($p < 0.05$) after the implementation of improvement actions.

Listeria monocytogenes can be introduced in beef and the butcher shop environment during processing at the abattoir, storage, transporting or through raw materials. In the present study, cluster analysis of *ApaI*-PFGE profiles detected 13 clones circulating simultaneously in different butcher shops. Abattoir B was the beef supplier of 73.5% of butcher shops in this situation, suggesting that at least part of *L. monocytogenes* contamination in retail shops was introduced with beef.

We also detected a remarkable spread of the pathogen inside butcher shops. In 20% of establishments positive for *L. monocytogenes*, more than one clone was found in different samples, indicating cross-contamination. Thus, foodstuffs free of *L. monocytogenes* could result contaminated when exposed to the retail environment. In line with this observation, Kanuganti, Wesley, Reddy, Mckean, and Hurd (2002) reported a lower prevalence of *L. monocytogenes* in carcasses (2.4%) and cow intestines (9.3%) than in ground beef obtained from these animals (50.2%). Kurpas, Wieczorek, and Osek (2018) detected isolates of *L. monocytogenes* in ground beef from negative carcasses, suggesting that contamination of meat occurred after processing as an effect of cross-contamination. In our study, cross-contamination was between ground beef and environmental samples and between different environmental samples. Whereas clonal strains were isolated from knife and meat tables in the same butcher shop (B22, B71) during E, no strains were isolated in those butcher shops during V. Based on this result, we hypothesized that the intersection between the blade and the blade handle and the wooden meat table were plausible surfaces for *L. monocytogenes* biofilm formation. The implementation of improvement actions, including the replacement of wooden for Teflon meat tables and the application of sanitation standard operating procedures (SSOP), may have contributed to preventing pathogen colonization of these surfaces. Moreover, 91.7% of cases of cross-contamination occurred during E, while no cross-contamination with serotypes 1/2b and 4b occurred during V. Therefore, our results clearly demonstrate the positive effect of improvement actions to reduce the spread of *L. monocytogenes* inside butcher shops.

Numerous studies have shown that *L. monocytogenes* distributed in food processing environments can persist in environmental niches for months or even years (Ferreira, Wiedmann, Teixeira, & Stasiewicz, 2014; Hoelzer et al., 2014). In our study, seven persistent clones were detected throughout the three years of study. We also found two cases in which a clone was isolated in the same butcher shop in both sampling

stages. Such persistence was probably due to the survival and growth of those strains in niches within the food environment, or to their repeated reintroduction from an external source over time. Also, failure in the application of improvement actions could have contributed to *L. monocytogenes* persistence in the butcher environment.

Our study also revealed the simultaneous presence of different *L. monocytogenes* strains in the same butcher shop, evidencing a high diversity of the pathogen at the retail environment. Interestingly, while serotype 1/2b and 4b strains were relatively scarce (23.2 and 13.9%, respectively), more than one clone from those serotypes was found circulating in some butcher shops. Despite the explanation for this observation remains to be elucidated, possibly serotype 4b and 1/2b strains of *L. monocytogenes* colonized niches and diversified in the environment of butcher shops.

In conclusion, our results show that although part of the contamination with *L. monocytogenes* was introduced through the beef supplier, the pathogen profusely spread inside butcher shops. We conspicuously demonstrated the relevance of environmental control and management by using good hygiene practices and manufacturing procedures to prevent the spread of *L. monocytogenes* in butcher shops.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Acknowledgments

The authors thank A. Di Maggio for manuscript correction and editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2019.108363.

Conflicts of interest

This manuscript had not been submitted for consideration in any other journal. All authors have read and approved the manuscript and all are aware of its submission to LWT-Journal of Food Science and Technology, and declare no conflict of interests.

References

Alvarez-Ordonez, A., Leong, D., Hickey, B., Beaufort, A., & Jordan, K. (2015). The challenge of challenge testing to monitor *Listeria monocytogenes* growth on ready-to-eat foods in Europe by following the European Commission (2014) Technical Guidance document. *Food Research International, 75*, 233–243. https://doi.org/10.1016/j.foodres.2015.06.004.

Buchanan, R. L., Gorris, L. G. M., Hayman, M. M., Jackson, T. C., & Whiting, R. C. (2017). A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control, 75*, 1–13. https://doi.org/10.1016/j.foodcont.2016.12.016.

Callejo, R., Prieto, M., Martinez, C., Aguerre, L., Rocca, M. F., Martinez, G., et al. (2008). Estudio mediante PCR múltiple de serotipos de *Listeria monocytogenes* aislados en Argentina. *Revista Argentina de Microbiología, 40*, 89–92.

Camargo, A. C., Woodward, J. J., & Nero, L. A. (2016). The continuous challenge of characterizing the foodborne pathogen *Listeria monocytogenes*. *Foodborne Pathogens and Disease, 13*(8), 405–416. https://doi.org/10.1089/fpd.2015.2115.

CDC (2017). *Standard operating procedure for PulseNet PFGE of Listeria monocytogenes.* https://www.cdc.gov/pulsenet/pdf/listeria-pfge-protocol-508c.pdf July 2017.

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., & Martin, P. (2004). Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *Journal of Clinical Microbiology, 42*(8), 3819–3822. https://doi.org/10.1128/JCM.42.8.3819-3822.2004.

Ferreira, V., Wiedmann, M., Teixeira, P., & Stasiewicz, M. J. (2014). *Listeria monocytogenes* persistence in food-associated environments: Epidemiology, strain characteristics, and implications for public health. *Journal of Food Protection, 77*(1), 150–170. https://doi.org/10.4315/0362-028X.JFP-13-150.

Gianfranceschi, M., Gattuso, A., Tartaro, S., & Aureli, P. (2003). Incidence of *Listeria monocytogenes* in food and environmental samples in Italy between 1990 and 1999: Serotype distribution in food, environmental and clinical samples. *European Journal of Epidemiology, 18*, 1001–1006.

Gomez, D., Azon, E., Marco, N., Carraminana, J. J., Rota, C., Arino, A., et al. (2014). Antimicrobial resistance of *Listeria monocytogenes* and *Listeria innocua* from meat products and meat-processing environment. *Food Microbiology, 42*, 61–65. https://doi.org/10.1016/j.fm.2014.02.017.

Hoelzer, K., Pouillot, R., Van Doren, J. M., & Dennis, S. (2014). Reduction of *Listeria monocytogenes* contamination on produce – a quantitative analysis of common liquid fresh produce wash compounds. *Food Control, 46*, 430–440. https://doi.org/10.1016/j.foodcont.2014.06.011.

Hoelzer, K., Sauders, B. D., Sanchez, M. D., Olsen, P. T., Pickett, M. M., Mangione, K. J., ... Oliver, H. F. (2011). Prevalence, distribution, and diversity of *Listeria monocytogenes* in retail environments, focusing on small establishments and establishments with a history of failed inspections. *Journal of Food Protection, 74*(7), 1083–1095. https://doi.org/10.4315/0362-028X.JFP-10-567.

Huang, B., Fang, N., Dimovski, K., Wang, X., Hogg, G., & Bates, J. (2011). Observation of a new pattern in serogroup-related PCR typing of *Listeria monocytogenes* 4b isolates. *Journal of Clinical Microbiology, 49*(1), 426–429. https://doi.org/10.1128/JCM.01207-10.

Jay, J. M. (1996). Prevalence of *Listeria* spp. in meat and poultry products. *Food Control, 7*(4/5), 209–214.

Kanuganti, S. R., Wesley, I. V., Reddy, P. G., Mckean, J., & Hurd, H. S. (2002). Detection of *Listeria monocytogenes* in pigs and pork†. *Journal of Food Protection, 65*(9), 1470–1474.

Kathariou, S. (2002). *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. *Journal of Food Protection, 65*(11), 1811–1829.

Kramarenko, T., Roasto, M., Meremäe, K., Kuningas, M., Põltsama, P., & Elias, T. (2013). *Listeria monocytogenes* prevalence and serotype diversity in various foods. *Food Control, 30*(1), 24–29. https://doi.org/10.1016/j.foodcont.2012.06.047.

Kurpas, M., Wieczorek, K., & Osek, J. (2018). Ready-to-eat meat products as a source of *Listeria monocytogenes*. *Journal of Veterinary Research, 62*(1), 49–55. https://doi.org/10.1515/jvetres-2018-0007.

Lakicevic, B., & Nastasijevic, I. (2016). *Listeria monocytogenes* in retail establishments: Contamination routes and control strategies. *Food Reviews International, 33*(3), 247–269. https://doi.org/10.1080/87559129.2016.1175017.

Leotta, G. A., Brusa, V., Galli, L., Adriani, C., Linares, L., Etcheverria, A., ... Signorini, M. (2016). Comprehensive evaluation and implementation of improvement actions in butcher shops. *PLoS One, 11*(9), e0162635. https://doi.org/10.1371/journal.pone.0162635.

Martin, B., Perich, A., Gomez, D., Yanguela, J., Rodriguez, A., Garriga, M., et al. (2014). Diversity and distribution of *Listeria monocytogenes* in meat processing plants. *Food Microbiology, 44*, 119–127. https://doi.org/10.1016/j.fm.2014.05.014.

Montero, D., Bodero, M., Riveros, G., Lapierre, L., Gaggero, A., Vidal, R. M., et al. (2015). Molecular epidemiology and genetic diversity of *Listeria monocytogenes* isolates from a wide variety of ready-to-eat foods and their relationship to clinical strains from listeriosis outbreaks in Chile. *Frontiers in Microbiology, 6*, 384. https://doi.org/10.3389/fmicb.2015.00384.

de Noordhout, C. M., Devleesschauwer, B., Angulo, F. J., Verbeke, G., Haagsma, J., Kirk, M., ... Speybroeck, N. (2014). The global burden of listeriosis: A systematic review and meta-analysis. *The Lancet Infectious Diseases, 14*(11), 1073–1082. https://doi.org/10.1016/s1473-3099(14)70870-9.

Rhoades, J. R., Duffy, G., & Koutsoumanis, K. (2009). Prevalence and concentration of verocytotoxigenic *Escherichia coli, Salmonella enterica* and *Listeria monocytogenes* in the beef production chain: A review. [Review]. *Food Microbiology, 26*(4), 357–376. https://doi.org/10.1016/j.fm.2008.10.012.

Ristori, C. A., Rowlands, R. E., Martins, C. G., Barbosa, M. L., Yoshida, J. T., & Franco, B. D. (2014). Prevalence and populations of *Listeria monocytogenes* in meat products retailed in Sao Paulo, Brazil. *Foodborne Pathogens and Disease, 11*(12), 969–973. https://doi.org/10.1089/fpd.2014.1809.

RSA-CONICET (2017). *Evaluación de riesgos de Listeria monocytogenes en chacinados embutidos secos y salazones crudas*.

Wilks, S. A., Michels, H. T., & Keevil, C. W. (2006). Survival of *Listeria monocytogenes* scott a on metal surfaces: Implications for cross-contamination. *International Journal of Food Microbiology, 111*(2), 93–98. https://doi.org/10.1016/j.ijfoodmicro.2006.04.037.