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Research Note

Analysis of *Listeria monocytogenes* Strain Distribution in a Pork Slaughter and Cutting Plant in the Province of Quebec

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ABSTRACT

Following the 2008 Canadian listeriosis outbreak associated with ready-to-eat (RTE) meat products, regulations on the presence of *Listeria monocytogenes* in RTE food production facilities were modified by Health Canada, confirming the need to control this pathogen, not only in the final product but also in the plant environment. Information on the occurrence of this microorganism during the early steps of production, such as the slaughtering process and in the cutting area, is scarce in Canada. In this study, we sampled different production steps in a slaughtering and cutting plant in the province of Quebec over a 2-year period. The lairage pens, representative areas of the slaughter line, and cutting zones were targeted after their respective cleaning procedures. A total of 874 samples were analyzed for the presence of *L. monocytogenes*. Characterization was done by first genoserogrouping the isolates using multiplex PCR and then using a pulsed-field gel electrophoresis approach. *L. monocytogenes* was detected throughout all production stages. The 108 positive samples found were analyzed further, and we established that there were 4 different serogroups, with serogroup IIb being the most prevalent. The results of pulsed-field gel electrophoresis analysis showed a significant decrease in the diversity of strains from the first areas of the plant to the cutting room (10 pulsotypes in 13 positive samples in lairage and 9 in 86 positive samples in cutting) and also showed the overrepresentation of a single predominant strain in the cutting room environment (type 1, representing 96.1% of the isolates). Biofilm formation analysis of the strains cannot exclusively explain the transitions we observed. A strong genotypic similarity between strains isolated in the early production areas and some strains in the cutting room was shown. These results support the need for better surveillance of *L. monocytogenes* prior to RTE food production in order to design control strategies that are better adapted from a public health perspective.

Listeria monocytogenes is a major concern in public health because listeriosis, the foodborne disease it causes, is associated with high mortality rates in susceptible populations, such as the elderly and the immunosuppressed. Listeriosis is chiefly caused by the consumption of contaminated food, particularly ready-to-eat (RTE) meat products, and is therefore of great concern for the food industry *(10, 14)*. *L. monocytogenes* has frequently been isolated from pork products *(1, 6, 34, 37),* and outbreaks have regularly been associated with the consumption of contaminated pork *(12, 15, 40)*. In 2008, a listeriosis outbreak associated with RTE processed meat products occurred across Canada, affecting 57 people and causing 22 deaths *(52)*. The strain associated with this outbreak was of serovar 1/2a (serogroup IIa). This serovar was also linked to a cheese-associated outbreak that occurred in the same year in Quebec *(21)*. We now know that this serovar 1/2a (serogroup IIa) has been widely prevalent in human cases in Canada over the last 20 years and that related strains could be considered a new epidemic clone *(32)*. These events forced Health Canada to reevaluate their regulations on the presence of *Listeria* in RTE food production and encouraged it to impose controls not only over finished products but also in the processing environment. There is, however, neither regulation nor surveillance of this pathogen in the prior production steps, and limited knowledge is available on the presence and persistence of *Listeria* in slaughtering and cutting plants. More information on this subject is vital to evaluate the impact of these steps on the introduction of *L. monocytogenes* into plants in which further processing occurs.

L. monocytogenes is ubiquitous in the environment, and its routes of introduction into food production facilities are not known precisely. Ambiguous reports are available on the possible introduction of this pathogen into plants by live

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animals *(5, 30, 53)*. *L. monocytogenes* has been found in the feces, intestines, and tonsils of healthy pigs on farms at levels ranging from 0 to 61% *(16, 30, 47, 53)*. Contaminated pigs could therefore be responsible for the contamination of the environment in lairage pens or during the slaughtering and processing operations *(4, 25)*. Differences in husbandry practices, such as the use of wet feeding, which is common in Europe, as well as the use of various isolation protocols, could possibly be responsible for the differences in prevalence in the studies *(5, 7, 53)*. Some authors have suggested that *L. monocytogenes* contamination levels upon plant entry are amplified in the chilling and cutting zone *(47)*. In this environment, strains can become resident and very hard to eradicate through washing and disinfection. They can even persist over long periods, ranging from a few months to 12 years *(8, 38, 39, 47–49)*. The hypothesis that these persistent strains are better adapted to the plant environment and could be better biofilm producers was challenged in a recent review *(8)*. Because of the presence of these persisting strains within the chilling and cutting environment, the levels of contamination of raw pork meat have been shown to increase significantly after these steps *(20, 30, 35, 41)*.

Once within the RTE processing plant, the presence of these contaminated products increases the risk of environmental cross contamination of the products by *L. monocytogenes (9, 45)*. Control during the RTE production steps is considered crucial, as reflected by the Health Canada control policies *(2)*. In the current policies, *L. monocytogenes* strains are considered equally in terms of their risk levels. However, recent investigations suggested that not all *L. monocytogenes* strains have the same pathogenic potential. In fact, it has been shown that the presence of a premature stop codon (PMSC) in the internalin A (InlA)–coding gene (occurring as frequently as in 35% of the strains originating from food) *(33)* reduces invasiveness *(29, 51)*. However, the association between resident strains and PMSC is still controversial *(26)*.

There is currently little information on the presence, circulation, and types of strains in the environment of pork slaughterhouses in Canada. In this study, we first described the presence of *L. monocytogenes* in different areas in the pork production continuum in a slaughter and cutting plant in Quebec, Canada. We also studied the distribution of different strains in the different production areas of this food plant and the strains' biofilm formation ability in connection with their recurrence in the plant. We also assessed their virulence through the detection of a PMSC in the *inlA* gene sequences.

MATERIALS AND METHODS

Sampling. Samples were collected during a 2-year survey in a Quebec pork slaughterhouse and cutting plant that produces a significant portion of the province's pork production (25%). The first sampling began in 2009 and was conducted approximately every 2 weeks over the summer and part of the fall in the lairage pens immediately after the pigs had left. The samples were collected by swabbing 100 cm^2 of the floor surface 10 times in 6 different pens with sterile, moistened cotton swabs. Six hundred samples were collected in total over 9 visits.

The residual *L. monocytogenes* contamination for the slaughter and cutting process steps was assessed in the winter of 2011 via three visits to the plant over 3 months. Samples were taken in areas representative of the process steps after their respective sanitation procedures were done. Samples from equipment and environmental surfaces at the slaughtering steps (pre- and postevisceration and chilling) and cutting rooms were collected using swabs premoistened with physiological buffer, and neutralizing broth was added immediately after sampling. These samples covered a maximum surface of 900 cm^2. A total of 274 samples were collected from 72 sites during these three visits.

***L. monocytogenes* detection.** *L. monocytogenes* detection was conducted on 874 samples using a method based on the Health Canada MFHPB-30 standard technique. The first enrichment was conducted in University of Vermont medium 1 (UVM-1; Lab M, Heywood, United Kingdom) broth for 24 h at 30°C, and the second in Fraser broth (Lab M, United Kingdom) for 48 h at 37°C. Both broths were inoculated onto agar *Listeria* Ottavani and Agosti (ALOA; AES Chemunex, Bruz, France) and incubated for 48 h at 37°C. Identification of a maximum of three typical isolates was conducted by using the Christie-Atkins-Munch-Petersen (CAMP) test and by evaluating hemolysis, motility in semi-solid agar, and carbohydrate (xylose, rhamnose, and mannitol) use in broths (Difco, BD, Sparks, MD).

The genus and species of each of the isolates was confirmed by amplification of the *prs* and *prfA* genes, respectively, and the genoserogrouping of the isolates was done using a multiplex PCR protocol amplifying four fragments (Table 1) as described by Kerouanton et al. in 2010 *(31)*.

PFGE genotyping. Pulsed-field gel electrophoresis (PFGE) was done according to the Centers for Disease Control PulseNet protocol for *L. monocytogenes (23)*. Enzymes *Asc*I and *Apa*I were used to cleave the bacterial DNA. Migration was achieved using the contour-clamped homogeneous electric field technique. Patterns were compared using BioNumerics software (version 6.5, Applied Maths, Kortrijk, Belgium). Similarity between the patterns was determined based on band positions by using the Dice correlation with a position tolerance of 1% *(18)*. The strains were clustered using the unweighted pair group method with arithmetic means, and a dendrogram was then constructed *(18)*. For each different combined *Apa*I and *Asc*I PFGE profile highlighted, a pulsotype number was attributed. One strain of each pulsotype was randomly selected for further biofilm and sequencing analysis.

Biofilm formation. The biofilm formation of the different *L. monocytogenes* strains was evaluated using the same technique described by Djordjevic et al. *(13)*. The strains were grown overnight in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE). The cultures were diluted 1/100 in TSBYE 1/20, and 100-μl amounts were grown in 96-well round-bottom culture plates for 40 h at 35°C. Each well was delicately washed with sterile saline to remove planktonic cells. Biofilm was then colored using crystal violet at 1%. The plates were washed with sterile saline, crystal violet was solubilized with 95% ethanol, and the absorbance was read at 550 nm using a plate reader. A minimum of 15 measures from two technical replicates was done for a representative isolate of each pulsotype. Biofilm production was standardized using *L. monocytogenes* ATCC 15313 biomass formation.

InlA sequencing. The *inlA* gene of a representative strain of each pulsotype was sequenced after overlapping amplification as described by Ragon et al. *(42)*, using the Sanger method at the

TABLE 1. *Genes used for serogrouping* L. monocytogenes *and the serotypes associated with each combination*

Serogroup	*L. monocytogenes* serovar(s)	Gene: *lmo0737*	*orf2819*[a]	*lmo118*	*orf2110*
IIa	1/2a, 3a, 1/2c	+	−	−	−
IIb	1/2b, 3b	−	+	−	−
IIc	1/2a, 3a, 1/2c	+	−	+	−
IVa	4c	−	−	−	−
IVb	4ab, 4b, 4e	−	+	−	+

[a] *orf,* open reading frame.

Centre d'Innovation Génome Québec (3730xl DNA Analyzer, Applied Biosystems, Foster City, CA). Sequences were aligned and screened for mutations causing a PMSC by using ClustalX 2.1 software.

LSPQ strain comparison. The patterns of each pulsotype were compared with those of the Laboratoire de Santé Publique du Québec (LSPQ) database, which contains data on strains from food and environmental sources, as well as from a human surveillance program conducted since 2001.

Statistical analysis. A Fisher's exact test was used to compare the contamination in the different plant areas. All analyses were conducted using SPSS 20 (licensed to Université de Montréal) with an alpha risk value of 0.05. The diversity of the strains in the different plant zones was evaluated using Simpson's index of diversity *(27).*

RESULTS

Lairage pens. *L. monocytogenes* was detected in 10 out of 600 samples (1.7%). These positive samples were detected in 6 of the 60 pens sampled and on only three of our visits (Table 2). All positive pens were found to be contaminated on only one visit, with the exception of one pen that was found to be positive on the seventh and ninth visits. Additionally, only one sample was found to be positive for each of the positive pens, with the exception of one pen on the seventh visit where four samples were found to be positive. Each incident where there was detection of *L. monocytogenes* was interspersed with visits that produced only negative samples. Three isolates were serogrouped and genotyped per positive sample for a total of thirty isolates. Among the 10 positive samples, 7 presented strains from the IIa serogroup, 1 from serogroup IIb, 1 from serogroup IVb, and 1 showed a mixed population of IVb and IIa (Table 2). Ten different pulsotypes were shown to be present in the 10 positive samples; 7 pulsotypes were associated with serogroup IIa, 1 with IIb, and 2 with IVb. Two samples had mixed populations of two different genotypes, and one of these had two different serogroups. One of the pulsotypes was present on two of the visits (pulsotype 12) but was detected in two different pens (Table 2).

Slaughtering and evisceration areas. In the precutting room, 39.4% of the sites and 19.1% of the samples were positive for *L. monocytogenes* (Table 3). It was also found in 66.7% of the sites sampled and 33.3% of the samples taken from the slaughtering area. In the evisceration section, 36% of the sites and 18.1% of the samples were positive, and in the chilling zone, 40% of the sites and 13.3% of the total samples were positive. Positive samples were found on the equipment (splitting saws, viscera tray, etc.) and within the environment. Strains from the slaughtering, evisceration, and chilling areas were mostly from serogroup IIc (53.8%), which was absent from the lairage pens. The majority of these strains were related to pulsotype 16. This pulsotype was found on three consecutive visits. Strains of serogroups IIb (38.5%) and IIa (7.7%) were also found (Tables 3 and 4 and Fig. 1). For this section of the plant, a total of eight different pulsotypes were detected, and all of them except pulsotype 16 were only found sporadically (Table 4).

TABLE 2. L. monocytogenes *detection, serotypes, and pulsotypes in the lairage pen environment*

Visit	Sampled pens: No. positive	Sampled pens: Total no.	Samples: No. positive	Samples: Total no.	Serogroup(s) (pulsotype[s])
1	0	4	0	40	—[a]
2	0	4	0	40	—
3	0	4	0	40	—
4	0	12	0	120	—
5	1	8	1	80	IIa (12)
6	0	4	0	40	—
7	2	4	5	40	IIa (3, 19, 22); IIb (7); IVb (4, 24)
8	0	8	0	80	—
9	3	12	4	120	IIa (2, 11, 12)
Total	6	60	10	600	—

[a] —, no serogrouping and pulsotyping results are available for this visit.

TABLE 3. L. monocytogenes *detection in the slaughtering, evisceration, chilling, and cutting steps in all three visits combined*

	Sampling sites			Samples			
Sampling area	No. positive	Total no.	%	No. positive	Total no.	%	Pulsotypes
Precutting room							
Slaughtering	2	3	66.7	3	9	33.3	5, 16, 17
Evisceration	9	25	36	8	44	18.1	1, 8, 9, 10, 16
Chilling	2	5	40	2	15	13.3	16, 18
Total	13	33	39.4	13	68	19.1	
Cutting room							
Equipment	7	14	50	18	41	43.9	1, 11, 15
Environment	6	6	100	20	30	66.7	1, 6, 15
Conveyor	15	19	78.9	48	126	38.1	1, 5, 11, 13, 14, 16
Total	28	39	71.8	86	206	41.7	

Cutting area. In the cutting room, 71.8% of the sites that were sampled and 41.7% of the individual samples were positive for *L. monocytogenes* (Table 3). Positive sampled sites included conveyer surfaces in contact with the products (78.9% of the sites and 38.1% of the samples) and the equipment (50% of the sites and 43.9% of the samples) (Table 3). Most of the 86 strains found in this part of the plant were from the IIb serogroup (86%), highlighted by pulsotypes 1 and 5. Strains of serogroups IIa (9.3%), IIc (3.5%), and IVb (1.2%) were also found (Tables 3 and 4 and Fig. 1). In the cutting room, a total of 10 different pulsotypes were found. Pulsotype 1 accounted for 83.1% of all our isolates in this section of the plant. Pulsotypes 1, 5, 11, and 13 were present on two or more visits. Pulsotypes 1, 5, and 16 were found both in the cutting and the slaughtering zones, and pulsotype 6, which was previously found in the lairage pens, was also present in the cutting room (Tables 2 and 4).

Our results showed an increase in the proportion of *L. monocytogenes*-positive samples in the lairage pens and cutting room (Fisher's exact test, $P < 0.05$). We also observed a reduction in diversity and a decrease of the Simpson index, which was 0.99 in the lairage pens, 0.81 in the slaughter and evisceration area, and 0.31 in the cutting rooms. This reduction of diversity coincided with the emergence of pulsotypes 16 and 1 in the slaughtering and evisceration areas and the emergence of a different strain (pulsotype 1) in the cutting room, respectively. However, some highly genetically similar strains were present in both the lairage pens and the cutting rooms.

Strain characterization. Some differences in biofilm formation ability were observed according to strain pulsotypes. Strains of pulsotypes 1 and 5 (which were those most frequently found in the cutting area and were of serogroup IIb) presented average biofilm-forming ability. However, strains of pulsotypes 16, 17 (serogroup IIc), 19, and 22 (serogroup IIa) showed higher values for biofilm production (between 20 and 60% higher than the control strain) (Fig. 2).

Of the 21 strains for which *inlA* was sequenced, 6 presented a mutation causing a PMSC, on amino acid 606 in the strains selected to represent pulsotypes 1, 5, and 9 (serogroup IIb) and at position 700 in the strains representing pulsotypes 13, 14, and 15 (serogroup IIa). A deletion of three amino acids was also detected between position 738 and 740 in the pulsotype 6 strain.

LSPQ strain comparison. Eleven of the pulsotypes that were detected in this study have never been described by the LSPQ either in human cases or in food surveillance (pulsotypes 2, 3, 4, 8, 10, 11, 12, 13, 16, 18, and 23). Four were previously found in food surveillance (pulsotypes 1, 5, 15, and 17), and six were previously found in human cases, with four being found sporadically (pulsotypes 7, 9, 19, and 22) and two frequently (pulsotypes 6 and 14). Pulsotype 6, the type most commonly found over the past 12 years, and pulsotype 22 (serogroup IIa), which had a PFGE identical to that of a strain that caused a large outbreak linked with cheese in 2008, were both isolated in lairage pens in this study *(21)*.

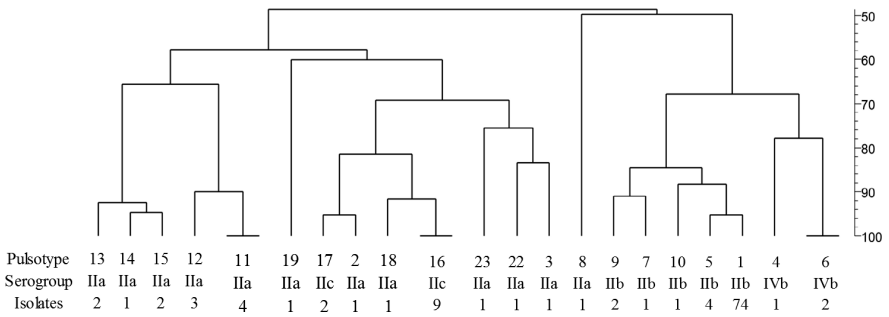

FIGURE 1. *Dendrogram of the combined* Asc*I and* Apa*I pulsotypes in relation to their serogroups. Pulsotype gives the code of the combined* Apa*I*-Asc*I profile, Serogroup the serogroup associated with each profile, and Isolates the number of isolates detected for each profile.*

TABLE 4. L. monocytogenes *serogroups and pulsotypes in the slaughtering, evisceration, chilling, and cutting areas*

	Visit 1		Visit 2		Visit 3		Total	
Sampling area, serogroup	No. of isolates	Pulsotype(s)	No. of isolates	Pulsotype(s)	No. of isolates	Pulsotype(s)	No. of isolates	% of total
Precutting room								
IIa	1	18	0	—[a]	0	—	1	7.7
IIb	3	**1**,[b] 8, 10	2	**5**, 9	0	—	5	38.5
IIc	3	**16**	1	**16**	3	**16**, **17**	7	53.8
IVb	0	—	0	—	0	0	0	0
Total	7		3		3		13	100
Cutting room								
IIa	5	**11**, **13**, 15	3	**11**, **13**, 14	0	—	8	9.3
IIb	28	**1**, **5**	25	**1**, **5**	21	**1**, **5**	74	86
IIc	1	**17**	1	**16**	1	**16**	3	3.5
IVb	0	—	0	—	1	6	1	1.2
Total	37		29		23		86	100

[a] —, no pulsotyping results are available for this serogroup on this visit.
[b] Pulsotypes found on more than one visit are in boldface.

DISCUSSION

The primary objective of this study was to describe the distribution of *L. monocytogenes* in different areas in the pork production continuum in a slaughter and cutting plant in Quebec, Canada. In the lairage pens, an environment with a larger quantity of fecal matter, *L. monocytogenes* was found at low frequency. Live pigs have been shown to introduce *L. monocytogenes* into the plant *(25)*. Pigs can excrete this pathogen in their feces *(7, 43)* and can contaminate the environment during the waiting period. Low contamination rates of the pigs at the farm could explain the low contamination levels found in this area of the slaughterhouse. This is supported by other studies, which report fecal contamination levels ranging from 0 to 16% at slaughterhouses *(16, 17, 19, 28, 50)*. Furthermore, the pathogen was found only in three of nine visits. This supports the hypothesis that only a small portion of the batches of pigs entering slaughtering were excreting *L. monocytogenes,* in accordance with the reported low farm contamination level. Furthermore, the high strain diversity (high Simpson's index value) in the area where we obtained seven different pulsotypes from three different serogroups in the five samples collected from two different pens on the seventh visit seems to show that a variety of strains enter the plant. Our observations could be a result of the infrequent entry of batches of excreting pigs in the plant.

Most of the strains collected were of serogroup IIa, which includes serovar 1/2a, among others. As mentioned above, this serovar is the one most frequently associated with sporadic occurrences and outbreaks in human cases in Canada *(32)*. Strains from serogroup IVb, which includes serovar 4b, were also found but at a low level. One of the pulsotypes of this serogroup (pulsotype 6) was commonly

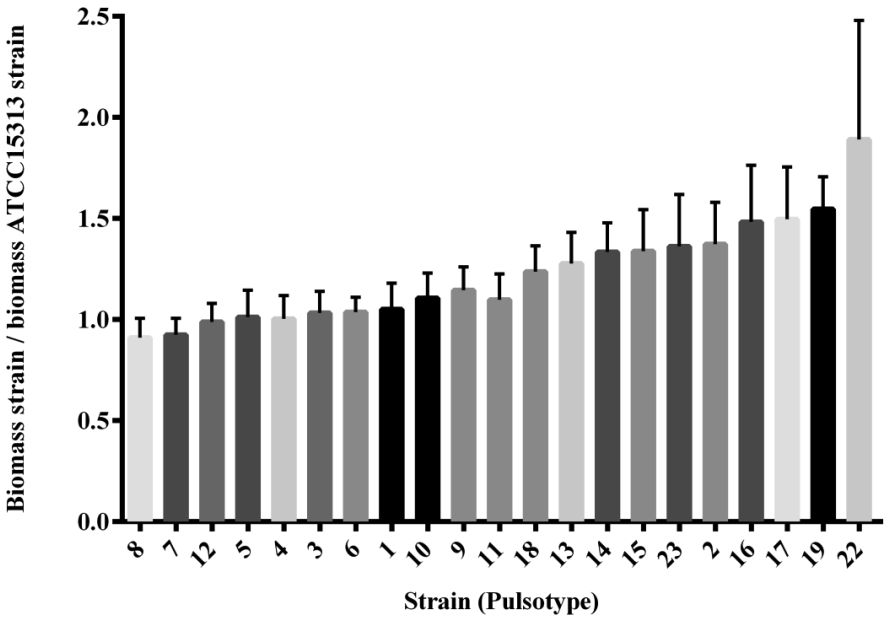

FIGURE 2. *Biofilm-forming ability of the* L. monocytogenes *strains according to their pulsotype in comparison with* L. monocytogenes *ATCC 15313 as a standard. Error bars show standard deviations.*

found in human cases by the LSPQ surveillance over the last 5 years. Serovar 4b, which is included in serogroup IVb, is already known to include highly virulent strains and is the serovar most frequently associated with large human outbreaks *(44, 46)*. In our study, the strains of serogroup IVb presented a 3-amino-acid deletion in the *inlA* sequence that has been linked with highly invasive strains *(33)*. The presence of strains of this serogroup in the feces of live pigs has already been described, supporting the evidence that live pigs may introduce these strains into meat processing plants *(7, 53)*. Despite the low contamination level found in lairage pens, a variety of pulsotypes was found. However, none of the pulsotypes could be detected on consecutive visits. It seems, therefore, that there were no persisting strains in the lairage environment. These results suggest that, under the conditions studied, pigs could be responsible for the regular introduction of new transient *L. monocytogenes* strains in the lairage pens, explaining the large diversity of pulsotypes we observed. These results are consistent with those of Hellstrom et al. *(25),* who showed that *L. monocytogenes* contamination of a plant could originate from the farm. Furthermore, our results suggest that some of these strains presented highly pathogenic properties. These findings regarding the lairage areas prompted us to assess contamination during the subsequent steps of production over a second series of visits.

In the areas that were involved in the precutting steps of the process, such as slaughtering, evisceration, and chilling, *L. monocytogenes* was detected in the environment and on the equipment even after sanitation procedures. It is well known that during the carcass prechilling steps, the environment and the equipment can be contaminated following contact with fecal matter or tonsils, which are frequently contaminated by *L. monocytogenes (4, 22)*. In our sampling of the evisceration area of the plant, we found the splitting saws to be contaminated on two different visits. These saws could be responsible for cross contamination of the carcasses, as shown by Autio et al. *(4)*. In our study, the contamination levels in the chilling room environment were found to be almost identical to the contamination in the previous areas. These findings contrast with the existing data in the literature, which reported chilling as an important amplification step *(47)*.

Most of the strains that were identified were associated with serogroup IIc and pulsotype 16, followed by serogroups IIb and IIa. Since the pulsotype 16 strain was not detected in the preceding step and it was found to be present at this stage in high levels, it appears that this strain has been selected by this environment. It is reasonable to conclude, therefore, that it has emerged as a major strain and that it is not being eliminated by the cleaning procedures implemented. This pulsotype was found six times on three different visits and was present on the splitting saws on two different visits, which could indicate that this strain is persistent on the evisceration line. Our results indicate that this strain (pulsotype 16) produces more biofilm in vitro than the average strain found in the plant. This could explain its persistence in this environment. Since it seems that the splitting saw is difficult to clean and disinfect, it is important for processors to adapt their cleaning and disinfection protocols and put more emphasis on this area. None of the strains found in this environment are known by the LSPQ to cause human infections.

On the three different visits to the cutting room, we found that 41.7% of the sampled sites remained positive after sanitation. These results seem high compared with those of other studies, which showed levels ranging between 9 and 20% *(9, 24, 38)*. However, it is important to note that our samples were taken on surfaces that we considered to be at risk of contamination even after washing and disinfection, given organic residue accumulation or because they were hard to clean given the design of the plant. Furthermore, the cutting room has been described in many studies as an important step for the amplification of *L. monocytogenes* contamination, as its temperature and the constant presence of organic matter are favorable to the growth of psychrophilic organisms. This can explain the much higher level of contamination found in this part of the plant compared with the levels in the slaughtering, evisceration, and lairage pen areas *(36, 47, 49)*. A total of 10 different pulsotypes were found in this area of the plant. Two of these pulsotypes (pulsotypes 1 and 5) were found on each of the three sampling visits. Since these two pulsotypes were found on numerous sampling sites, over multiple visits, and after washing and disinfection, they appear to be routinely present in the cutting room environment and could therefore be qualified as persistent. Pulsotype 1 accounted for 83.1% of all the *L. monocytogenes* strains found in the cutting room. This result seems to be in accordance with previous observations of the existence of major strains in the plant environment that are very well adapted to the conditions of meat processing plants *(22, 38)*. In this case, it seems that there is a change in the major persisting strain between the slaughter area, where the main pulsotype is 16, and the cutting area, where pulsotype 1 is the most frequent. We would have expected the persisting strains (pulsotypes 1 and 5) to have an advantage in biofilm production (better attachment capacities on surfaces such as stainless steel and/or better growth capacity at 4°C) *(3, 9, 54)*. However, in the biofilm production experiments that we conducted in vitro, the biofilm formation of the strains of these pulsotypes was not significantly higher than the average. The presence of strains of this pulsotype on a large variety of surfaces (even after cleaning and disinfection) seems to contradict the idea that the persistence is only caused by the presence of harboring sites that have a protective effect because they are hard to reach or difficult to clean and disinfect *(8)*. It is interesting to note that the major strain (pulsotype 1) belongs to serogroup IIa, which includes serovar 1/2b. This serovar is part of lineage I, which has been shown to be overrepresented in human cases of listeriosis and has been linked to 15% of incidents in Canada (in third place behind strains of serovars 1/2a and 4b) *(11)*. However, we detected a PMSC in this major strain; this mutation has been shown to affect the invasiveness of *L. monocytogenes (51)*. These results are consistent with those of the LSPQ, which only described this strain sporadically in food surveillance. The presence of this potentially noninvasive strain in high levels in the cutting room could hide the presence of more virulent

strains and also shows the need to include strain characterization in the *Listeria* surveillance. It is also interesting to note that there was a residual presence of strains of serogroup IVb in the cutting area. These strains (pulsotype 6) bear a 3-amino-acid deletion. As previously explained, this deletion has been described in highly invasive *L. monocytogenes* 4b strains *(33)*. This is consistent with our findings, since pulsotype 6 was commonly found by the LSPQ in human surveillance cases.

Using PFGE analysis, we also showed the presence of strains that were found both in the slaughtering and the cutting environment and other strains possibly in the lairage pens and the cutting room. These results indicate that pathogenic strains entering the plant via the lairage pens could circulate through the plant and enter the cutting area, where the contamination could be amplified by selective environmental conditions, such as low temperature. It is interesting to note that strains isolated in the cutting room environment in the 2011 winter were found in the same plant years before in the 2009 lairage pen sampling. This could indicate a recurring introduction of this strain into the plant which is reaching the cutting room, even if it is in a small proportion compared with the other, more overrepresented pulsotypes.

In conclusion, we have shown that *L. monocytogenes* is present in all parts of the slaughtering and cutting plant studied in Quebec, with low levels of pathogenic strain contamination in the lairage pens. These strains may be introduced by shedding pigs and then amplified by the emergence of environmentally adapted strains in the slaughtering and cutting room areas even after washing and disinfection. We have also shown that a variation in recurrent strains occurs over the different steps of processing in the plant. The presence of different dominant strains in the slaughtering and slicing areas of the plant was illustrated via our successive visits. The meat produced in this environment is a raw ingredient for the RTE industry, where *L. monocytogenes* control is very important. Better control of *L. monocytogenes* in the slaughtering and cutting areas could reduce the risk of contamination in the subsequent steps of production.

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