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Listeria Prevalence and *Listeria monocytogenes* Serovar Diversity at Cull Cow and Bull Processing Plants in the United States†

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ABSTRACT

Listeria monocytogenes, the causative agent of epidemic and sporadic listeriosis, is routinely isolated from many sources, including cattle, yet information on the prevalence of *Listeria* in beef processing plants in the United States is minimal. From July 2005 through April 2006, four commercial cow and bull processing plants were sampled in the United States to determine the prevalence of *Listeria* and the serovar diversity of *L. monocytogenes.* Samples were collected during the summer, fall, winter, and spring. *Listeria* prevalence on hides was consistently higher during cooler weather (28 to 92% of samples) than during warmer weather (6 and 77% of samples). The *Listeria* prevalence data collected from preevisceration carcass ranged from undetectable in some warm season samples to as high as 71% during cooler weather. *Listeria* on postintervention carcasses in the chill cooler was normally undetectable, with the exception of summer and spring samples from one plant where >19% of the carcasses were positive for *Listeria.* On hides, *L. monocytogenes* serovar 1/2a was the predominant serovar observed, with serovars 1/2b and 4b present 2.5 times less often and serovar 1/2c not detected on any hides sampled. *L. monocytogenes* serovars 1/2a, 1/2c, and 4b were found on postintervention carcasses. This prevalence study demonstrates that *Listeria* species are more prevalent on hides during the winter and spring and that interventions being used in cow and bull processing plants appear to be effective in reducing or eliminating *Listeria* contamination on carcasses.

The intracellular pathogen *Listeria* is present throughout the environment and is represented by a number of species, including *L. innocua, L. seeligeri, L. welshimeri, L. grayi, L. ivanovii,* and *L. monocytogenes. L. monocytogenes* is of most significant concern to human health because it is the causative agent of epidemic and sporadic listeriosis *(8, 13, 17).* In pregnant women, newborns (younger than 1 year of age), elderly people (>65 years old), and immunocompromised individuals, the consequences from contracting listeriosis can be particularly severe, with mortality rates approaching 30% in individuals who become infected *(8). L. monocytogenes* has 13 known serovars, but four of them (1/2a, 1/2b, 1/2c, and 4b) are responsible for over 95% of reported human listeriosis cases, and major outbreaks of listeriosis have been caused by *L. monocytogenes* 4b *(5, 7, 12, 13).*

Listeria is routinely isolated from numerous animal sources, including cattle *(15),* and a clear relationship between food source and disease was established in 1981 when an outbreak of listeriosis was linked to contaminated coleslaw *(8, 18).* The presence of *Listeria* in fresh beef that is to be cooked has been considered inconsequential, and because of this attitude, information on the prevalence of *Listeria* in beef processing plants in the United States is minimal *(15). Listeria* has, however, been considered a problem in ready-to-eat (RTE) foods, and recently the producers of RTE products have started to require suppliers to certify raw materials as free of *L. monocytogenes.*

In spite of efforts to eradicate *L. monocytogenes* from RTE foods, contamination continues to occur *(2, 9, 16, 19).* In a survey of raw and RTE meats and poultry products at retail markets in Canada, *L. monocytogenes* was found in 52% of raw ground beef tested *(2). L. monocytogenes* also was found in 3.5% of the ground beef samples tested from retail outlets in the state of Washington *(16).* These two data sets clearly show the wide range of *L. monocytogenes* contamination in retail ground beef products. One source for this contamination may be from the beef carcasses themselves. In a study published in 2004, the prevalence of *L. monocytogenes* on beef carcasses ranged from 0.8 to 18.7% on hides and 0.0 to 1.1% on postintervention carcasses *(15).* These data suggest that *L. monocytogenes* is found at low levels on carcasses, but these data were collected during warm weather months when a psychrophilic organism like *L. monocytogenes* is not expected to be present at high levels. Furthermore, this survey evaluated only plants dealing with commercially fed beef and not with plants dealing with culled cows and bulls, which are major sources of ground product.

In this report, data are presented on the prevalence of

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† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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TABLE 1. *Variation in the prevalence of* Listeria *species on hides, preevisceration carcasses, and postintervention carcasses among seasons at four U.S. cull cow and bull processing plants*[a]

	Listeria prevalence (%) on:											
	Hides				Preevisceration carcasses				Postintervention carcasses			
Plant	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring
A	72.1	77.4	92.1	80.0	23.7	32.1	71.1	47.4	28.9	1.1	0.0	19.5
B	64.7	61.1	80.0	80.0	60.0	22.6	44.0	41.6	1.1	5.8	0.0	0.0
C	13.7	6.8	37.6	28.4	2.6	0.0	9.5	4.7	0.0	0.0	0.0	0.0
D	6.3	14.2	69.5	70.0	0.0	7.4	23.7	36.8	1.7	0.0	0.0	0.0

[a] Total prevalence of all known *Listeria* species: *L. innocua, L. seeligeri, L. welshimeri, L. grayi, L. ivanovii,* and *L. monocytogenes.* Data are the percentage of positive samples collected on 2 days. Prevalences that differ by 10.6% or more are significantly different ($P < 0.05$) for all comparisons.

Listeria species and the diversity of *L. monocytogenes* serovars on hides, preevisceration carcasses, and postintervention carcasses of culled cows and bulls presented for slaughter. The samples were collected during each of four seasons from July 2005 through April 2006 in the United States. Pulsed-field gel electrophoresis (PFGE) was used to compare isolates found on postintervention carcasses in the chill cooler.

MATERIALS AND METHODS

Hide and carcass sample collection. Swab samples were collected from hides (1,000-cm^2 area), preevisceration carcasses (after hide removal and before preevisceration wash; 8,000 cm^2), and postintervention carcasses (8,000-cm^2 area) by swabbing the surfaces with sterile sponges prewetted with 20 ml of buffered peptone water. The samples were collected from four commercial cow and bull slaughter plants in the United States during each of four seasons (summer, fall, winter, and spring) from July 2005 through April 2006. For each season, 95 samples of each type were collected per day (2 days of sampling) from four different plants. Samples were transported at ~2°C in insulated coolers with ice blocks and were received at the U.S. Meat Animal Research Center within 24 h of collection for analyses of *Listeria* species.

Sample processing. Sponge samples were enriched in Trypticase soy broth and incubated at 25°C for 2 h and then at 42°C for 6 h and then at 4°C for ≥4 h before the secondary enrichment as described by Guerini et al. *(10).* All samples were incubated at the prescribed temperatures in a Precision Scientific model 818 incubator (Thermo Electron Corp., Milford, Mass.). After the primary enrichment, a 100-μl aliquot from each sample was subjected to a secondary enrichment at a 1:30 ratio in Fraser broth (Becton Dickinson, Sparks, Md.) at 35°C for 40 h *(10).* Samples were then plated onto *Listeria* CHROMagar (DRG International, Mountainside, N.J.) and incubated at 37°C for 24 h. Colonies with a blue phenotype representing *L. innocua, L. seeligeri, L. welshimeri,* or *L. grayi* or blue colonies with a halo representing *L. ivanovii* and *L. monocytogenes* were confirmed to be *Listeria* spp. or a *L. monocytogenes*–specific serovar by multiplex PCR *(4).*

PFGE analysis. *L. monocytogenes* serovar 1/2c isolate fingerprints were generated and analyzed in this study based on PFGE separation of *Asc*I-digested genomic DNA used by members of PulseNet (Centers for Disease Control and Prevention, Atlanta, Ga.). Pulsed-field gel–certified agarose (SeaKem Gold Agarose) was obtained from Cambrex Bio Science Rockland Inc. (Rockland, Maine), Tris-borate-EDTA running buffer and proteinase K were purchased from Sigma (St. Louis, Mo.), and *Asc*I was purchased from New England Biolabs (Beverly, Mass.). *Salmonella* serotype Braenderup strain H9812 was used as a control and for standardization of gels *(11).* Banding patterns were analyzed and comparisons were made using Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium) employing the Dice similarity coefficient in conjunction with the unweighted pair group method using arithmetic averages for clustering. Isolates were grouped into types that likely had the same origin based on fingerprint pattern similarities. Types were defined strictly as isolates that grouped together and had identical banding patterns.

Statistical analysis. The least significant difference ($P < 0.05$) in prevalence percentage was calculated using PEPI software (version 2; USD, Inc., Stone Mountain, Ga.) *(6).* Prevalences that differed by 10.6% or more were considered significantly different for all comparisons.

RESULTS AND DISCUSSION

Listeria on hides was consistently more prevalent in cooler weather (Table 1). An analysis of the average temperature of the 14 days preceding each sampling trip revealed that plants C and D had temperatures approximately 6°C higher than those at the other two plants (data not shown). In plants in cooler areas (plants A and B), the prevalence of total *Listeria* species on hides was 61 to 92% for all seasons. At plants C and D, the prevalence of *Listeria* species on hides was significantly lower in the summer and fall seasons (6 to 14%). During the winter months, the prevalence of *Listeria* on hides increased significantly to approximately 70% at plant D and 38% at plant C as compared with ~14% in the summer months. The increased prevalence of *Listeria* on hides was maintained at plant D during the spring sampling period, but the prevalence at plant C decreased by ~25% as compared with summer. *Listeria* species are capable of growing over a wide range of temperatures (−7 to as high as 45°C) *(7, 14),* and the higher prevalence associated with the cooler weather is consistent with the ability of *Listeria* species to grow at lower temperatures. Because cattle presented for slaughter at the individual plants sampled in this study came from many different geographic locations, it was not possible to document regional differences in the prevalence of *Listeria* species.

TABLE 2. *Variation in the prevalence of* L. monocytogenes *on hides, preevisceration carcasses, and postintervention carcasses among seasons at four U.S. cull cow and bull processing plants*[a]

	L. monocytogenes prevalence (%) on:											
	Hides				Preevisceration carcasses				Postintervention carcasses			
Plant	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring
A	14.2	7.4	41.6	47.9	3.2	3.2	23.2	19.5	25.3	0.5	0.0	18.9
B	8.4	8.4	11.6	22.6	2.1	1.6	4.2	8.9	0.5	0.0	0.0	0.0
C	0.5	1.1	6.3	0.5	0.5	0.0	0.5	0.5	0.0	0.0	0.0	0.0
D	0.0	4.2	16.8	21.6	0.0	1.1	3.2	5.3	0.0	0.0	0.0	0.0

[a] Data are the percentage of positive samples collected on 2 days. Prevalences that differ by 10.6% or more are significantly different ($P < 0.05$) for all comparisons.

The data collected from preevisceration carcass samples revealed lower levels of *Listeria* compared with those on hide samples (Table 1). In general, the prevalence on preevisceration carcasses was significantly higher ($P < 0.05$) in the winter and spring sampling periods than in the summer and fall, with the exception of plant B in the summer. At plant B, in the summer the overall prevalence of *Listeria* on preevisceration carcasses was 60%, whereas the prevalence in fall, winter, and spring ranged from 22 to 44%. The increase in *Listeria* species on preevisceration carcasses during the cooler months (winter and spring), similar to that found for prevalence on hides, was not surprising because hides are the chief source of carcass contamination *(1).*

The prevalence of *Listeria* species in samples from postintervention carcasses in the chill cooler was 0 to 6%, with the exception of summer and spring samples from plant A. In those samples, ~29 and ~20% of the carcasses were positive in the summer and spring, respectively. Because the study was initiated in the summer of 2005 and completed in the spring of 2006, a reasonable amount of time elapsed between these two sampling periods, yet a similar spike in *L. monocytogenes* in the cooler was found. Approximately 87% of the *Listeria* species on postintervention carcasses from this summer sampling period belonged to two *L. monocytogenes* serovars (1/2c and 4b) and in the spring, ~97% of the positive samples belonged to *L. monocytogenes* serovar 1/2c (Table 2). These isolates were analyzed using PFGE to evaluate the possibility of a common source.

The prevalence of *L. monocytogenes* isolates was generally low in the summer and fall but increased in the winter and spring (Table 2). Samples collected from postintervention carcasses in the chill cooler from all seasons had less than 1% prevalence for *L. monocytogenes* with the exception of the plant A summer and spring sampling periods (Table 2). Using multiplex PCR, we identified the four serovars (1/2a, 1/2b, 1/2c, and 4b) that account for >95% of

TABLE 3. *Variation in the serovars of* L. monocytogenes *isolated from swab samples collected from hides, preevisceration carcasses, and postintervention carcasses at different seasons at four U.S. cull cow and bull processing plants*[a]

		No. of *L. monocytogenes* isolates from:											
		Hides				Preevisceration carcasses				Postintervention carcasses			
Serovar	Plant	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring
1/2a	A	21	6	53	80	5	1	23	34	3	1	0	0
	B	6	1	4	23	0	1	0	7	0	0	0	0
	C	0	0	2	1	1	0	1	1	0	0	0	0
	D	0	8	24	23	0	0	1	3	0	0	0	0
1/2b	A	4	2	11	10	1	1	4	2	0	0	0	0
	B	9	2	10	11	3	2	6	8	0	0	0	0
	C	0	1	0	0	0	0	0	0	0	0	0	0
	D	0	0	8	16	0	0	4	7	0	0	0	0
1/2c	A	0	0	0	0	0	1	1	0	28	0	0	36
	B	0	0	0	0	0	0	0	0	1	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0
4b	A	2	6	25	4	0	3	18	1	19	0	0	0
	B	1	13	8	15	1	0	2	4	0	0	0	0
	C	1	1	10	0	0	0	0	0	0	0	0	0
	D	0	0	4	6	0	2	1	0	0	0	0	0

[a] In some cases, isolates of multiple serovars may have been recovered from a single sample.

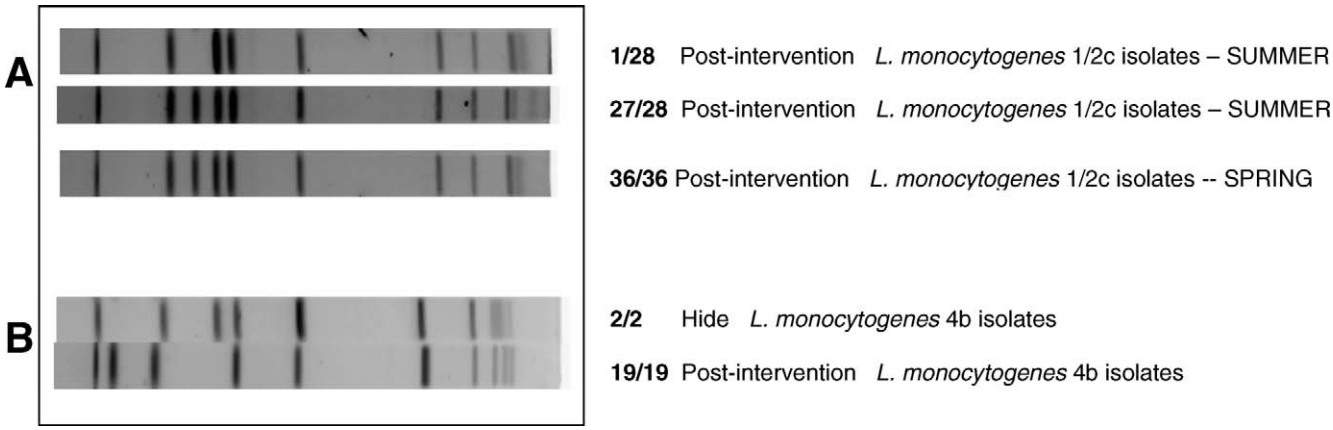

FIGURE 1. *Pulsed-field gel electrophoresis (PFGE) comparison of* L. monocytogenes *isolates. (A) Representative PFGE pulsotypes from* L. monocytogenes *serovar 1/2c isolates recovered from the chill cooler in summer 2005 and spring 2006 at plant A. (B) Representative PFGE pulsotypes from* L. monocytogenes *serovar 4b isolates recovered from the hide (2 isolates) and postintervention carcasses (19 isolates) during summer 2005 at plant A.*

the *L. monocytogenes* isolates recovered from foods and infected humans *(3, 5, 7, 12, 13).* Serovar 1/2a was the predominant serovar on hides, with a prevalence 2.5 times that of any of the other three serovars (Table 3). The prevalence of serovars 1/2b and 4b on hides was the same (Table 3). Serovar 1/2c was not detected on hides at any plant and was recovered only sporadically from carcasses, yet it was detected on postintervention carcasses in the chill cooler in plant A (28 positive carcasses) in the summer (36 positive carcasses) and the spring (Table 3), indicating a possible in-cooler source for this pathogen. From 3,040 samples collected during this project, 28 samples (0.92%) contained more than one *L. monocytogenes* serovar. In the winter and spring sampling periods when the prevalence of *Listeria* species was higher, 26 (1.7%) of 1,520 samples had multiple *L. monocytogenes* serovars, and from 1 hide sample collected during the winter, 3 serovars were recovered.

In addition to prevalence and serovar data, PFGE analysis of the 64 postintervention chill cooler 1/2c isolates from plant A was performed to determine whether a common source may have contributed to the presence of this pathogen on carcasses in the chill cooler. No hides or preevisceration carcasses sampled during the summer or fall contained detectable *L. monocytogenes* 1/2c, so the source of contamination is unknown. PFGE analysis of the 64 isolates collected during these two seasons (28 summer isolates and 38 spring isolates) revealed that 63 of the 64 isolates were identical and 1 sample from the summer sampling period was unique, differing by only one band (Fig. 1A). This finding suggests that the *L. monocytogenes* serovar 1/2c isolate present on postintervention carcasses from the chill cooler in the summer and spring seasons originated from a common in-cooler source. Chill coolers have high humidity and cool temperatures, both of which are optimal environmental conditions for growth of *Listeria* species *(7, 14).* The 19 *L. monocytogenes* 4b isolates recovered from postintervention carcasses at plant A during the summer season were compared with the only two 4b isolates recovered from hides during the same sampling period (Fig. 1B). However, the *L. monocytogenes* 4b isolates from hides did not match those found on carcasses, suggesting that the 4b isolates found on postintervention carcasses came from a different source.

In this study, *Listeria* species were more prevalent on hides of cattle presented for slaughter at plants in cooler climates and in the winter and spring seasons. More comprehensive information on the prevalence of *Listeria* generally and of *L. monocytogenes* specifically in the postharvest beef processing chain has come from a study of large plants processing commercially fed beef *(15).* The present study provides a snapshot of *Listeria* prevalence from sampling in the summer, fall, winter, and spring months at commercial cull cow and bull processing plants in the United States, and differences not found in this study may be attributable to multiple factors, including processing plant, weather, and sources of cattle presented for slaughter on the sampling days. Interventions being used in cow and bull processing plants appear to be effective in practically eliminating *Listeria* species from carcasses. The two exceptions are potentially attributable to in-cooler contamination, based on the lack of matching isolates from hides and preevisceration carcasses during those sampling periods. The cooler provides an environment that encourages growth of *Listeria,* and additional monitoring of chill coolers may be necessary to further reduce *L. monocytogenes* in the food supply.

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