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Research Note

Listeria monocytogenes Contamination Pattern in Pig Slaughterhouses

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ABSTRACT

Ten low-capacity slaughterhouses were examined for *Listeria* by collecting a total of 373 samples, of which 50, 250, and 73 were taken from carcasses, pluck sets, and the slaughterhouse environment, respectively. Six slaughterhouses and 9% of all samples were positive for *Listeria monocytogenes*. Of the samples taken from pluck sets, 9% were positive for *L. monocytogenes*, the highest prevalence occurring in tongue and tonsil samples, at 14% and 12%, respectively. Six of 50 (12%) carcasses were contaminated with *L. monocytogenes*. In the slaughterhouse environment, *L. monocytogenes* was detected in two, one, one, and one sample originating from the saws, drain, door, and table, respectively. Carcasses were contaminated with *L. monocytogenes* in those two slaughterhouses, where the mechanical saws, used for both brisket and back splitting, were also positive for *L. monocytogenes*. A total of 58 *L. monocytogenes* isolates were characterized by pulsed-field gel electrophoresis typing. The isolates were divided into 18 pulsotypes, 15 of which were detected in pluck sets. In two slaughterhouses, where the carcasses were contaminated with *L. monocytogenes*, the same pulsotypes were also recovered from splitting saws. In addition, identical pulsotypes were recovered from pluck sets. Our findings indicate that *L. monocytogenes* of tongue and tonsil origin may contaminate the slaughtering equipment that may in turn spread the pathogen to carcasses. Thus, it is of the utmost importance to follow good manufacturing practices and to have efficient cleaning and disinfection procedures to prevent equipment being contaminated with *L. monocytogenes*.

Listeria monocytogenes has been the causative agent of several foodborne listeriosis outbreaks involving milk and dairy products, vegetables, salads, meat products, and fishery products (10). Thus, the prevention of *L. monocytogenes* contamination in food products is of major importance. In order to be able to prevent contamination of food items by the bacterium, the contamination routes and the initial sources of *L. monocytogenes* contamination must be identified.

Listeria has been recovered from slaughterhouse environments, and the carcasses, tonsils, and feces of animals (6, 11, 14, 18, 19, 21). It has been suggested that *Listeria* detected in carcasses may have a nonfecal origin and that a possible contamination source may be the equipment (17). However, the initial source and contamination sites of *Listeria* during pig slaughtering remain obscure. To better understand *Listeria* contamination, it is important to determine the similarities and dissimilarities of isolates recovered from different origins. Genotypic typing methods have been used to track contaminants in food-processing facilities (2, 8, 16). By comparing the isolates of different origins, it is possible to trace the contamination routes and establish the initial source of plant contamination.

This study was set up to establish the prevalence and possible contamination routes of *L. monocytogenes* in pig

slaughterhouses. Contamination sites were identified by sampling the environment, pluck sets, and carcasses of 10 low-capacity slaughterhouses and by typing isolates using pulsed-field gel electrophoresis (PFGE) (4, 5, 25), which is regarded as the gold standard for typing.

MATERIALS AND METHODS

Sampling. In low-capacity slaughterhouses, the maximum amount of slaughtering is 20 animal units/week and 1,000 animal units/year (9). Pigs' body weights of >100 kg are counted as 0.2 and other as 0.15 animal units. There are 57 low-capacity pig slaughterhouses in Finland. Ten geographically representative slaughterhouses, having no apparent association with each other, were examined for *Listeria* spp. A total of 373 samples were collected, of which 50, 250, and 73 samples were from carcasses, pluck sets, and slaughterhouse environment, respectively. The carcasses and pluck sets (tongue, tonsil, heart, liver, kidney) were sampled by swabbing with 7.5-cm by 7.5-cm sterile gauze-pads premoistened in sterile 0.1% peptone water. The backs of carcasses were sampled after splitting (Fig. 1), and pluck sets were sampled by swabbing the entire surface of offals hanging from hooks. The slaughterhouse environment, equipment, and employee aprons were sampled by sponge (Technical Service Consultants Ltd., Heywood, Lancashire, UK) sampling technique (7, 20), and drains were examined by collecting 25 ml of drain water. The environmental samples in slaughterhouses 2, 4, 5, 7, and 8 were obtained prior to slaughtering and in slaughterhouses 1, 3, 6, 9, and 10 after slaughtering. The slaughterhouses to be sampled before or after slaughtering were chosen at random.

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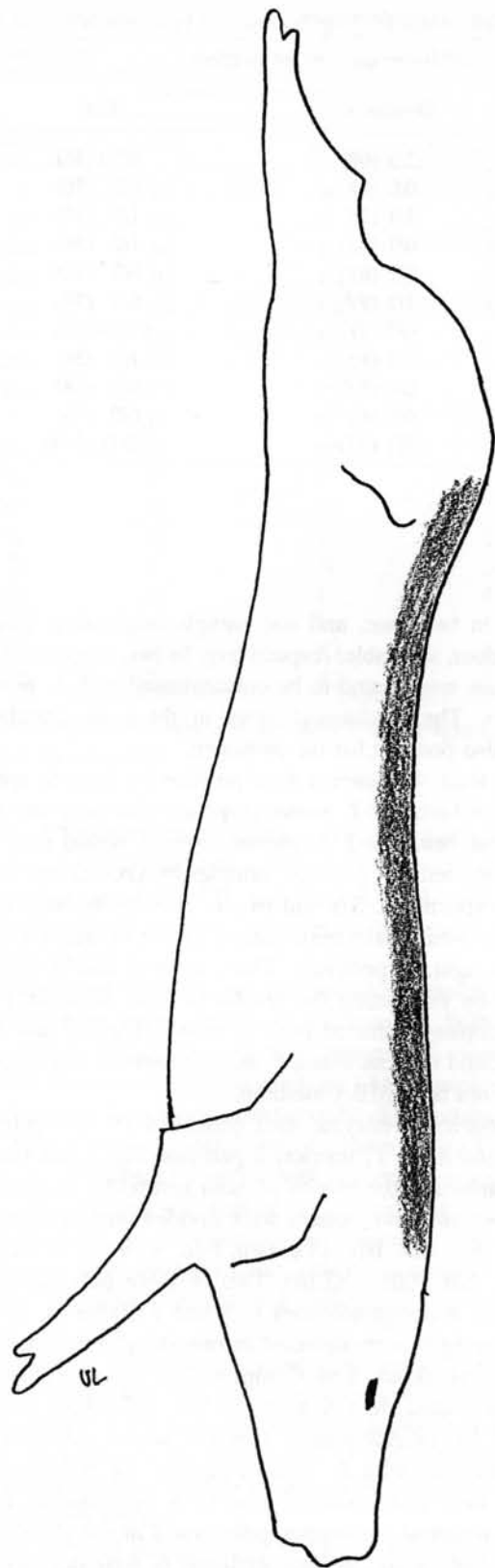


FIGURE 1. Sampled areas on pork carcasses (indicated in black).

Bacteriological analysis. Examination for *L. monocytogenes* was carried out using a one-step enrichment method according to the Nordic Committee on Food Analysis (1), with the addition of LMBA agar plate (13) (trypticase soy agar base [Difco, Detroit, Mich.], 10 g/liter lithium chloride, 10 mg/liter polymyxine B sulfate [Sigma Chemicals, St. Louis, Mo.], 20 mg/liter ceftazidime

TABLE 1. Prevalence of *Listeria* spp. and *L. monocytogenes* in pluck sets, carcasses and environment of 10 slaughterhouses

Source	No. of samples	No. of positive samples (%)	
		<i>Listeria</i> spp. ^a	<i>L. monocytogenes</i>
Pluck sets			
Tongues	50	8 (16)	7 (14)
Tonsils	50	6 (12)	6 (12)
Hearts	50	3 (6)	3 (6)
Livers	50	3 (6)	3 (6)
Kidneys	50	4 (8)	3 (6)
Carcasses	50	6 (12)	6 (12)
Environment			
Drains	10	4 (40)	1 (10)
Aprons	10	1 (10)	0
Tables	10	1 (10)	1 (10)
Knives	10	0	0
Doors	10	1 (10)	1 (10)
Saws	10	3 (30)	2 (20)
Others	13	1 (10)	0
Total	373	41 (11)	33 (9)

^a Includes *L. monocytogenes*.

[Abtek Biologicals Ltd, Liverpool, England], 5% sterile defibrinated sheep blood). Each sample was incubated in *Listeria* enrichment broth (Oxoid, Basingstoke, UK) at 30°C for 48 h. Enrichment broth was plated on Oxford agar (Oxoid) and on LMBA agar plates. The plates were incubated at 37°C for 48 h. Five suspected colonies from both Oxford agar and LMBA agar were streaked on blood agar. The β -hemolytic colonies were identified by Gram staining, catalase reaction, motility microscopically at 25°C, and further identified using API *Listeria* (BioMérieux SA, Marcy l'Etoile, France). When no β -hemolytic colonies were observed, the nonhemolytic colonies were further identified as described above. Two *L. monocytogenes* isolates from each positive sample, one from each selective agar, were maintained frozen in Protect bacterial preservation tubes (Technical Service Consultants) at -70°C prior to serotyping and PFGE typing.

In situ DNA isolation and PFGE. A total of 58 *L. monocytogenes* isolates were characterized by PFGE. In situ DNA isolation and PFGE were performed as described by Autio et al. (2). *Apal* and *Ascl* (New England Biolabs, Beverly, Mass.) were used for restriction endonuclease digestion. The samples were electrophoresed as described by Autio et al. (2). The pulse times for *Apal* and *Ascl* ramped from 1 s to 35 s for 18 h. Low range PFG markers (New England Biolabs) were used for fragment size determination. The gels were stained with ethidium bromide and were digitally photographed with an Alpha Imager 2000 documentation system (Alpha Innotech, San Leandro, Calif.). The genotypes were considered closely related when a three-fragment difference was not exceeded (23).

Serotyping. One strain from each pulsotype was serotyped with commercial antisera (Denka Seiken, Tokyo, Japan) according to manufacturer instructions.

RESULTS

Listeria spp. was detected in 11% of samples (Table 1). *Listeria* species recovered were *L. monocytogenes* and

TABLE 2. Prevalence of *Listeria* spp. and *L. monocytogenes* in 10 low-capacity slaughterhouses

Slaughterhouse	No. of samples positive for <i>L. monocytogenes</i> / <i>Listeria</i> spp. ^a (no. of samples)			
	Carcasses	Pluck sets	Environment	Total
1	2/2 (5)	5/5 (25)	2/3 (6) ^b	9/10 (36)
2	0/0 (5)	0/0 (25)	0/0 (6) ^c	0/0 (36)
3	0/0 (5)	0/0 (25)	1/1 (7) ^b	1/1 (37)
4	0/0 (5)	1/1 (25)	0/1 (8) ^c	1/2 (38)
5	0/0 (5)	0/0 (25)	0/2 (8) ^c	0/2 (38)
6	4/4 (5)	1/1 (25)	1/1 (9) ^b	6/6 (39)
7	0/0 (5)	15/16 (25)	0/2 (7) ^c	15/18 (37)
8	0/0 (5)	0/0 (25)	1/1 (8) ^c	1/1 (38)
9	0/0 (5)	0/1 (25)	0/0 (8) ^b	0/1 (38)
10	0/0 (5)	0/0 (25)	0/0 (6) ^b	0/0 (36)
Total	6/6 (50)	22/24 (250)	5/11 (73)	33/41 (373)

^a Includes *L. monocytogenes*.

^b Samples taken after slaughtering.

^c Samples taken prior to slaughtering.

Listeria innocua. *L. monocytogenes* was detected in 9% of samples. Altogether 10% of pluck set samples were positive for *Listeria* spp. and 9% for *L. monocytogenes*. The highest prevalence of *L. monocytogenes* was detected in tongue and tonsil samples, at 14% and 12%, respectively, whereas contamination of hearts, kidneys, and livers was 6%. Six of 50 (12%) carcasses were contaminated with *L. monocytogenes*.

Listeria was detected in 8 of 10 slaughterhouses (Table 2). The incidence of *Listeria* spp. and *L. monocytogenes* varied from 0 to 49% and from 0 to 40%, respectively. In five slaughterhouses, *Listeria* spp. was detected in pluck sets, with slaughterhouse 7 having a particularly high level (64%) of contamination.

In the slaughterhouse environment, *Listeria* spp. and *L. monocytogenes* were detected in 15% and 7% of samples, respectively. *L. monocytogenes* was detected in one of five slaughterhouses where environmental samples had been collected prior to slaughtering. The sampling site was the drain. In slaughterhouses where environmental samples were taken after slaughtering, *L. monocytogenes* was de-

tected in two, one, and one sample originating from the saws, door, and table, respectively. In two slaughterhouses, carcasses were found to be contaminated with *L. monocytogenes*. The mechanical saws in these slaughterhouses were also positive for the pathogen.

In total, 41 samples were positive for *Listeria* spp., 33 of which harbored *L. monocytogenes*. Had only one selective agar been used, *L. monocytogenes* would only have been detected in 27 and 31 samples by Oxford and LMBA agar, respectively. Six and two *L. monocytogenes*-positive samples would have been missed by use of only Oxford or LMBA agar, respectively. Thus, optimal results were obtained by combining the results of both selective media. Six samples contained both *L. monocytogenes* and *L. innocua*, and in these cases, *L. monocytogenes* was recovered only from the LMBA medium.

Restriction enzyme *AscI* generated 16 restriction patterns, and *ApaI*, 17 restriction patterns (Fig. 2 and Table 3). By combining the results of both restriction enzymes, 58 *L. monocytogenes* isolates were divided into 18 pulsotypes (I, II, IIIa, IIIb, IIIc, IVa, IVb, IVc, V, VI, VII, VIII, IX, X, XI, XII, XIIIa, XIIIb). Two or more pulsotypes were detected in slaughterhouses 1, 3, and 7 (Table 4). At most, 10 pulsotypes were detected in one slaughterhouse, and in a single pluck set, a maximum of five different pulsotypes was recovered. In five samples, two pulsotypes were detected. In slaughterhouses 1 and 6, where carcasses were contaminated with *L. monocytogenes*, the pulsotypes detected were also recovered from saws. Furthermore, in both cases, identical pulsotypes, pulsotype I in slaughterhouse 1 and pulsotype IIIc in slaughterhouse 6, were detected from pluck sets. One strain from each pulsotype was serotyped. The strains from pulsotypes II, IIIa, IIIb, IIIc, IVa, IVb, IVc, V, VI, VII, VIII, IX, X, XI, XII, XIIIa, XIIIb were serotype 1/2a, and from pulsotype I, serotype 1/2c.

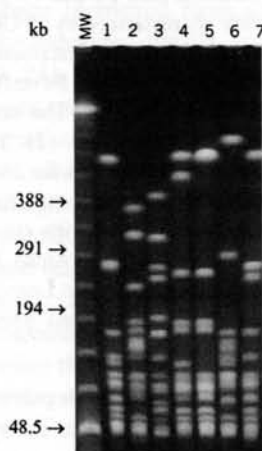


FIGURE 2. *ApaI* digests of *L. monocytogenes* strains. Lanes: MW, low range PFGE marker; 1, pulsotype VI; 2, pulsotype XII; 3, pulsotype X; 4, pulsotype XIIIa; 5, pulsotype XIIIb; 6, pulsotype VII; 7, pulsotype VIII.

DISCUSSION

Eight slaughterhouses were contaminated with *Listeria*, with only two slaughterhouses being *Listeria* negative. This

TABLE 3. *Pulsotypes of L. monocytogenes isolates detected in slaughterhouses*

Pulsotype	Restriction pattern ^a		No. of isolates
	AscI	ApaI	
I	A1	A	8
II	A2	B	6
IIIa	B1	C1	2
IIIb	B2	C4	1
IIIc	B2	C1	12
IVa	C1	C2	1
IVb	C1	C3	2
IVc	C2	C5	1
V	D	D	1
VI	E	E1	5
VII	F	F	1
VIII	G	E2	3
IX	H	G	2
X	I	H	1
XI	J1	I	6
XII	J2	J	1
XIIIa	K1	K1	3
XIIIb	K2	K2	2

^a Restriction patterns share a letter when a three-fragment difference is not exceeded.

result is in agreement with findings in high-capacity slaughterhouses by Gill and Jones (12), Sammarco et al. (19), Saide-Albornoz et al. (18), and Korsak et al. (14), all of whom describe a variable occurrence of *Listeria* among slaughterhouses.

Differences between selective media in detection of *L. monocytogenes* were present. In six samples, both *L. innocua* and *L. monocytogenes* were detected, *L. monocytogenes* having been recovered from the LMBA medium. On LMBA media, *L. monocytogenes* can be distinguished from other *Listeria* spp. by detection of β -hemolysis. The best result was obtained by using both selective media. This finding is consistent with that of other researchers (26), who recommend use of multiple plating. However, even better results may have been obtained with the combined use of multiple enrichment media and more than one selective plating agar (22).

A total of 18 pulsotypes were identified by PFGE typing. In five samples, two pulsotypes were detected from a single sample. This is in agreement with previous studies (2, 15) and stresses the importance of characterization of several isolates from a single sample in epidemiological and contamination studies.

No identical pulsotypes were detected among slaughterhouses. However, closely related strains were recovered from slaughterhouses 1, 3, and 6, as well as from slaughterhouses 3 and 7, having no apparent association with each other. Carcasses in two slaughterhouses were contaminated with *L. monocytogenes*. In these slaughterhouses, the saws used for splitting the carcasses were also contaminated with the pathogen. Moreover, the isolates from the saws and the carcasses were determined by PFGE typing to have identical restriction patterns. This suggests that the saws may

TABLE 4. *Distribution of L. monocytogenes pulsotypes in pig slaughterhouses*

Slaughterhouse	Pulsotypes (no. of isolates)		
	Pluck sets	Carcasses	Environment
1	I (2) II (6) IIIa (2)	I (3)	I (3)
3			IVa (1) IIIb (1)
4	IX (2)		
6	IIIc (2)	IIIc (8)	IIIc (2)
7	IVb (2) IVc (1) VI (5) VII (1) VIII (3) X (1) XI (6) XII (1) XIIIa (3) XIIIb (2)		
8			V (1)

be the contamination site of the carcasses in these slaughterhouses. This result concurs with the study of Nesbakken et al. (17) that proposes slaughterhouse equipment as a possible source of carcass contamination.

However, the initial origin of *Listeria* contamination has been unclear. Although *Listeria* may be present in pig feces, *Listeria* detected in carcasses has been speculated to have a nonfecal origin (18), and that animal strains do not easily contaminate meat and are replaced by other better adapted strains (3). Bunčić (6) showed pigs to be carriers more often of *L. monocytogenes* (45%) in the tonsillar tissue than excretors of the bacteria in feces. Unfortunately, Bunčić (6) neither examined the carcasses nor used any typing methods to investigate the role of tonsils as a possible source of carcass contamination. In our study, 14% of tongues and 12% of tonsils were found to harbor *L. monocytogenes*. The saws used for splitting carcasses were also used as brisket saws, and therefore, it is possible that the origin of *Listeria* on the saws was the tongues and tonsils. Moreover, the pulsotypes detected on the saws after slaughtering and on the carcasses were also found in the pluck sets. Fifteen pulsotypes were detected in pluck sets, and at most, five pulsotypes were detected in a single pluck set. Therefore, it is possible that a wide range of *L. monocytogenes* strains initially enter the plant with the animals and only a few of them have characteristics enabling them to survive, adapt to, and colonize in the processing environment. This is supported by earlier findings of dominant *L. monocytogenes* clones in food-processing environments, strains that have proven to be able to persist for several years (16, 24).

Pluck sets in slaughterhouse 7 were found to be contaminated heavily with *Listeria*. In this slaughterhouse, the pluck sets had been rinsed with tap water, which might have increased dissemination of *Listeria* from the tonsils and

tongues through the pluck set. However, the pluck sets were also rinsed in slaughterhouse 9, but only one pluck set sample was *Listeria* positive. This may be due to differences in the occurrence of *L. monocytogenes* carrier pigs.

It is pertinent to ask whether the contamination results we obtained in low-capacity pig slaughterhouses would also be valid in high-capacity slaughterhouses. The prevalence and diversity results of *L. monocytogenes* in pluck sets can be generalized because, in many cases, pigs from the same farm are slaughtered both in low- and high-capacity slaughterhouses. When pig tonsils are removed together with the pluck set (tongue, esophagus, trachea, lungs, heart, diaphragm, kidneys, and liver), *Listeria* may spread from the tonsils and tongue to the remainder of the pluck set and carcass. Mechanical equipment in abattoirs may become contaminated either directly or indirectly with *Listeria* originating from tonsils and tongues. Therefore, it is essential to follow good manufacturing practices and to utilize efficient cleaning and disinfection procedures to prevent equipment contamination. In addition, more research must be conducted in high-capacity slaughterhouses to establish the role of tonsils and tongues in *Listeria* contamination.

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