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Research Note

Prevalence and Genetic Diversity of *Listeria monocytogenes* in the Tonsils of Pigs

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ABSTRACT

This study was set up to establish the prevalence of *Listeria monocytogenes* in the tonsils of sows and fattening pigs from five Finnish slaughterhouses and to evaluate the genetic similarity of *L. monocytogenes* strains isolated from the tonsils. A total of 271 pig tonsils (132 tonsils from fattening pigs and 139 from sows) from five different slaughterhouses in various parts of Finland were studied from June 1999 to March 2000. Overall, 14 and 4% of pig tonsils harbored *L. monocytogenes* and *Listeria innocua*, respectively. The prevalence of *L. monocytogenes* in tonsils of fattening pigs (22%) was significantly higher than in sows (6%). The isolates (n = 38) recovered from tonsils showed a wide genetic diversity by means of 24 different pulsed-field gel electrophoresis (PFGE) types presented by the strains. Moreover, in numerical analyses of restriction patterns, no association was found between the clustering of strains and the slaughterhouses, and strains showing a similar PFGE type were recovered from pigs of different slaughterhouses. The high prevalence of *L. monocytogenes* showing various PFGE types in the tonsils of pigs could indicate a potential source of contamination of pluck sets, carcasses, and the slaughterhouse environment and of subsequent processing steps.

Listeria monocytogenes is a foodborne pathogen that causes the disease listeriosis in a well-defined risk population. Various food products have been associated with both epidemics and sporadic cases of listeriosis. Three outbreaks—in 1992, 1993, and 2000—have been linked to consumption of the pork products rillettes and jellied tongue *(8, 11, 12)*. Therefore, prevention of pork product contamination with *L. monocytogenes* is of major importance.

L. monocytogenes has been recovered from slaughterhouse environments, carcasses, tonsils, and feces of pigs *(7, 10, 14, 17, 18)*. It has been suggested that *Listeria* detected in carcasses might have a nonfecal origin and that equipment could be a possible contamination source *(15)*. Moreover, in low-capacity slaughterhouses, where the maximum amount of slaughtering is 100 pigs per week and 5,000 pigs per year, with each pig weighing over 100 kg *(9)*, it has been suggested that *L. monocytogenes* originating from pork tonsil, even though rejected after inspection, and tongue might contaminate the slaughtering equipment. Equipment could in turn spread the pathogen to carcasses *(4)*, and in this way, carcasses can introduce the tonsil-originating pathogen into pork product processing plants. Noting this, there is high value in the study of the role and genetic diversity of *L. monocytogenes* in the tonsils of pigs.

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In this study, the prevalence of *L. monocytogenes* in the tonsils of the sows and fattening pigs from five Finnish slaughterhouses was established. The genetic similarity levels of *L. monocytogenes* strains isolated from tonsils was determined by the characterization of the strains with pulsed-field gel electrophoresis (PFGE) typing.

MATERIALS AND METHODS

Samples. A total of 271 pig tonsils, including 132 tonsils from fattening pigs and 139 from sows, from five different slaughterhouses in various parts of Finland were studied from June 1999 to March 2000. In 2002, the average size of pig herds was about 140 fattening pigs, each weighing over 50 kg. The average age of slaughtered fattening pigs is 6 months, and weight is 100 to 125 kg. In slaughterhouses involved in this study, line speeds varied from 80 to 180 fattening pigs per hour and from 400 to 800 per working day. A maximum of 300 sows were slaughtered in every slaughterhouse every month. The number of different herds slaughtered each day varied from 5 to 50. The tonsils were cut out, frozen immediately after evisceration, and examined within 2 months after removal. A 10-g sample of tonsil tissue without surface sterilization was homogenized in 90 ml of half-Fraser broth (Oxoid, Basingstoke, UK) for 1 min in a stomacher blender.

Isolation of *L. monocytogenes*. The isolation of *L. monocytogenes* was carried out according to the ISO method *(1)* with the use of PALCAM (Oxoid), Oxford (Oxoid), and LMBA (trypticase soy agar base [Difco, Sparks, Md.], 10 g/liter lithium chloride, 10 mg/liter polymyxine B sulfate [Sigma Chemicals, St. Louis, Mo.], 20 mg/liter ceftazidime [Abtek Biologicals Ltd., Liverpool, UK], 5% sterile defibrinated sheep blood) selective plates.

TABLE 1. *The prevalence of* L. monocytogenes *and* L. innocua *in tonsils of fattening pigs and sows in five slaughterhouses*

	No. of positive samples/total no. of samples (%)					
	Fattening pigs		Sows		Total	
Slaughterhouse	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*	*L. innocua*
A	7/29 (24)	0/29 (0)	0/36 (0)	1/36 (3)	7/65 (11)	1/65 (2)
B	11/29 (38)	1/29 (3)	4/31 (13)	0/31 (0)	15/60 (25)	1/60 (2)
C	2/30 (7)	3/30 (10)	0/30 (0)	2/30 (7)	2/60 (3)	5/60 (8)
D	1/14 (7)	0/14 (0)	3/11 (27)	1/11 (9)	4/25 (16)	1/25 (4)
E	8/30 (27)	1/30 (3)	2/31 (6)	3/31 (10)	10/61 (16)	4/61 (7)
Total	29/132 (22)	5/132 (4)	9/139 (6)	7/139 (5)	38/271 (14)	12/271 (4)

In situ DNA isolation and PFGE. In situ DNA isolation and PFGE was performed as described by Autio et al. *(2, 3)* with the use of Pronase (Roche Diagnostics GmbH, Mannheim, Germany) instead of proteinase K.

PFGE pattern analysis. *Asc*I macrorestriction patterns were analyzed by BioNumerics software (Applied Maths, Sint-Martens-Platen, Kortrijk, Belgium). The similarities among restriction patterns on the basis of band position was expressed as a Dice coefficient correlation. The position tolerance was optimal when set at 1.1% for the total length of the pattern with no increase. The clustering and construction of the dendrogram was performed by the unweighted pair group method with the use of arithmetic averages (UPGMA).

Statistical analysis. For the statistical analysis, the chi-square test was used.

RESULTS

The *Listeria* species recovered from pig tonsils were *L. monocytogenes* and *L. innocua.* Overall, 14 and 4% of pig tonsils harbored *L. monocytogenes* and *L. innocua,* respectively (Table 1). The prevalence of *L. monocytogenes* in the tonsils of fattening pigs (22%) was significantly higher than in sows (6%) ($P < 0.001$). The overall prevalences of *L. monocytogenes* among pigs from different slaughterhouses varied from 3 to 25%.

The characterization of 38 *L. monocytogenes* isolates yielded 24 PFGE types (Table 2 and Fig. 1). The different PFGE types in slaughterhouses number from two to eight. Strains showing PFGE types 6, 8, and 13 were found in pigs of different slaughterhouses. In slaughterhouse B, strains possessing PFGE types 6 and 9 were found both from fattening pigs and from sows. The numerical analysis of macrorestriction patterns is shown in Figure 1.

In the isolation of *L. monocytogenes,* three selective agar plates were used (Table 3). Of the 38 positive samples, 37 were detected both by LMBA and PALCAM, but only 31 positive samples were recovered with Oxford. All *L. monocytogenes*–positive samples were detected after both the primary and secondary enrichment steps when both LMBA and PALCAM agars were used.

DISCUSSION

Both fattening pigs and sows in Finland harbor *L. monocytogenes* in their tonsils after slaughtering. A total of 14% of the pigs were found to be tonsillar carriers, which is in agreement with our earlier study showing a 12% carrier rate in pigs at low-capacity slaughterhouses *(4).* An even higher proportion (45%) of tonsillar carriers was reported by Bunčić *(7)* in pigs in Yugoslavia, whereas Kanuganti et al. *(13)* showed a 7% carrier rate in pigs in the United States.

In our study, fattening pigs (22%) were shown to have a significantly higher carrier rate than sows (6%). To our knowledge, this is the first time that differences in *L. monocytogenes* tonsillar carrier rates in different age groups of pigs have been reported. It is possible that sows are naturally more resistant to the bacteria than young animals. Differences were also found in the carrier rate among different slaughterhouses, which could be a result of geographical differences in the prevalence of infected herds. Additionally, differences in the carrier rate, both in different age groups and among different slaughterhouses, might be affected by the husbandry and feeding practices associated with pigs *(7, 20).* Skovgaard and Nørrung *(20)* demonstrated that the prevalence of feces containing *L. monocytogenes* in specific pathogen-free herds might be low compared with

TABLE 2. *Distribution of* L. monocytogenes *PFGE types in tonsils of fattening pigs and sows in five slaughterhouses*[a]

Slaughterhouse	No. of PFGE types	Fattening pigs		Sows	
		No. of isolates	PFGE types	No. of isolates	PFGE types
A	7	7	1, 2, 3, 4, 7, 8, 24	0	
B	7	11	6 (6), 9, 11, 12, 13, 14	4	6, 9 (2), 10
C	2	2	13, 15	0	
D	3	1	16	3	5 (2), 8
E	8	8	6, 17 (2), 20 (2), 21, 22, 27	2	18, 19
Total	24	29		9	

[a] When more than one isolate belonged to one PFGE type, the number of isolates is presented in parentheses.

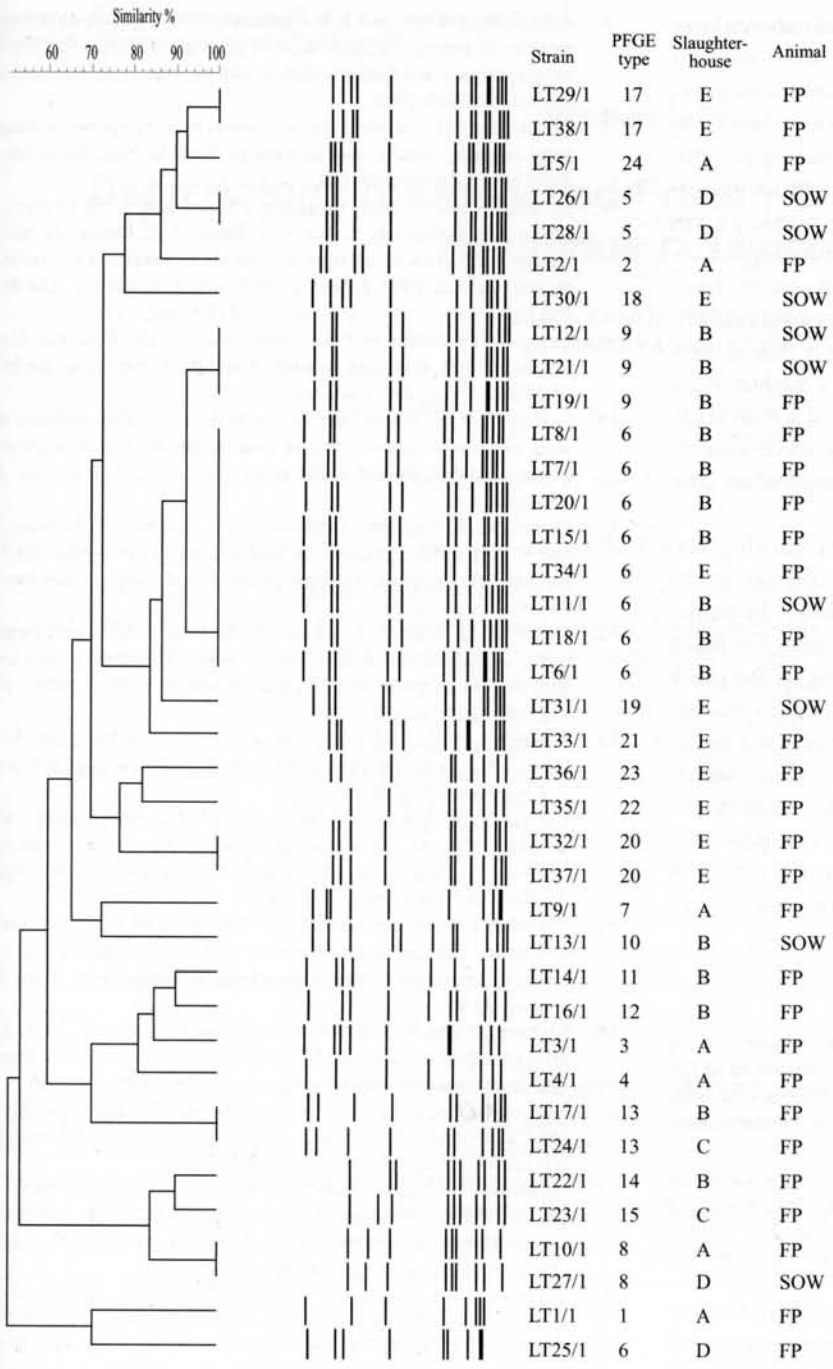

FIGURE 1. *Dendrogram of PFGE patterns of* L. monocytogenes *strains isolated in tonsils of pigs. A similarity analysis was performed with the Dice coefficient, and clustering was performed by UPGMA. FP, fattening pig.*

others. It has also been shown that the type of feed significantly affects the contamination rates in pigpens by *L. monocytogenes (5).* Belœil et al. *(5)* noticed that both wet feed pens and wet feed harbored *L. monocytogenes,* whereas dry feed pens and dry feed were free of the bacteria. Further research is needed to establish the factors effecting the healthy hosting of *L. monocytogenes* in pigs.

TABLE 3. Listeria monocytogenes*–positive samples recovered from different selective plates*

	No. of *L. monocytogenes*–positive samples			
	PALCAM	Oxford	LMBA	Total
Primary enrichment	37	31	35	38
Secondary enrichment	37	31	34	38
Total	37	31	37	38

In the isolation of *L. monocytogenes,* the use of PALCAM or LMBA plates resulted in the detection of 37 positive samples out of 38, whereas only 31 positive samples were recovered with Oxford. All *L. monocytogenes*–positive samples were detected after both the primary and secondary enrichment steps when both LMBA and PALCAM agar were used. Our finding is in agreement with the results of those who recommend the use of multiple plating media *(19, 21).*

A total of 24 PFGE types were presented by 38 *L. monocytogenes* strains. The number of PFGE types is great when compared, for example, with the number of PFGE

types presented by strains of *Yersinia pseudotuberculosis,* another foodborne pathogen isolated from the tonsils of pigs *(16).* These data suggest that the *L. monocytogenes* strains recovered from the tonsils of pigs show wide diversity by the number of different macrorestriction patterns obtained. In the numerical analysis of the restriction patterns, the strains divided into two known genomic groups *(6).* Moreover, no association was found between the clustering of strains and the slaughterhouses, and strains showing a similar PFGE type were recovered from pigs of different slaughterhouses. These results indicate that diverse *L. monocytogenes* strains are entering the slaughterhouse along with the pigs. Also, findings suggest that the diversity of *L. monocytogenes* strains in the housing environments of pigs is wide. These facts must be considered when contamination studies are performed.

A high prevalence of *L. monocytogenes* showing various PFGE types in the tonsils of pigs is a potential source of *Listeria* contamination in the slaughterhouse. In particular, when pig tonsils are removed together with the pluck set, the pathogen can spread from the tonsils to the pluck set and carcass. Moreover, *L. monocytogenes* originating from the tonsils can either directly or indirectly contaminate slaughterhouse equipment in abattoirs and consequently cause carcass contamination. Further research is needed to compare the strains isolated from the tonsils of pigs with the strains recovered from meat products in order to evaluate the role of tonsils as an initial source of pork product contamination.

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