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Prevalence of *Listeria monocytogenes* in Broilers at the Abattoir, Processing Plant, and Retail Level

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ABSTRACT

The environment and products from two broiler abattoirs and processing plants and raw broiler pieces at the retail level were sampled for *Listeria monocytogenes* in order to evaluate the contamination level of the broiler carcasses and products. Sampling started in the slaughtering process and finished with raw broiler meat or ready-to-eat cooked product. Sampling sites positive for *L. monocytogenes* at the broiler abattoir were the air chiller, the skin-removing machine, and the conveyor belt leading to the packaging area. The *L. monocytogenes* contamination rate varied from 1 to 19% between the two plants studied. Furthermore, 62% (38 of 61) of the raw broiler pieces, bought from retail stores, were positive for *L. monocytogenes*. Altogether, 136 *L. monocytogenes* isolates were obtained for serotyping and pulsed-field gel electrophoresis (PFGE) characterization performed with two rare-cutting enzymes (*Apa*I and *Asc*I). Altogether three serotypes (1/2a, 1/2c, and 4b) and 14 different PFGE types were obtained using information provided from both *Apa*I and *Asc*I patterns for discrimination basis. The two broiler abattoirs studied did not share the same PFGE types. However, the same PFGE types found in the raw broiler pieces at the retail level were also found in the broiler abattoirs where the broilers had been slaughtered.

Listeria monocytogenes is a ubiquitous environmental bacterium and an opportunistic pathogen with the potential to cause human listeriosis. In severe cases, listeriosis may lead to death. Outbreaks have been associated with the consumption of coleslaw *(36)*, soft cheeses *(22, 26)*, milk *(16, 20)*, butter *(28)*, fish products *(19)*, and meat and poultry products *(1, 14, 24, 25, 37, 40)*.

L. monocytogenes have been isolated from raw poultry in many countries. Pini and Gilbert *(33)* found that 60% of raw chickens in the United Kingdom were contaminated with *L. monocytogenes*. A survey of retail shops in the United States showed that 23% of fresh poultry carcasses were contaminated with *L. monocytogenes (4)*. In Norway, 61% of 90 chicken carcass samples were positive for the bacterium *(35)*. Loncarevic et al. *(27)* reported 0 to 64% prevalence of *L. monocytogenes* in raw broiler meat. In Belgium and France, 10 to 15% of broiler chicken carcasses were positive for *L. monocytogenes (39)*. In a Spanish study, 64% of the poultry samples were found positive for hemolytic *Listeria (21)*. Raw broilers are cooked before consumption, but there is a risk of cross-contamination to other foodstuffs in the consumer kitchen. *L. monocytogenes* is able to grow and form biofilms on various food-processing surfaces, enhancing survival *(8)*. Improper cleaning and disinfection of machines in poultry abattoirs may lead to contamination of the poultry carcasses. The contamination of poultry probably occurs in the poultry abattoir during the processing *(32)*.

The majority of *L. monocytogenes* isolated from food or human clinical cases belong to serotypes 1/2a, 1/2b, and 4b *(34)*. Serotyping can be used as a preliminary typing method, but DNA-based methods are needed to obtain more discriminative typing results. Pulsed-field gel electrophoresis (PFGE) is a useful method for the fine structure comparison and molecular typing of *L. monocytogenes (9)*. DNA-based characterization methods have also been found suitable for contamination studies in food processing *(3, 5, 6, 17, 18)*. Ojeniyi et al. *(32)* used serotyping, phagetyping, ribotyping, and PFGE in a study of Danish poultry abattoirs. In their study, PFGE was the most discriminating of these typing methods.

The focus of this study was to evaluate the level of *L. monocytogenes* contamination at two broiler abattoirs and broiler meat processing plants and at retail level in raw broiler meat. Furthermore, we aimed to reveal where in the processing line *L. monocytogenes* contamination of the broiler carcasses occurs. These contaminating steps need to be recognized in order to be able to direct special cleaning and disinfection attention to them. For this purpose and to obtain results on the biodiversity of *L. monocytogenes,* the isolates from different broiler abattoirs and from the raw broiler pieces at retail level were characterized with PFGE in order to clarify the contamination with *L. monocytogenes*.

MATERIALS AND METHODS

Sampling procedure at the broiler abattoirs and product plants. Sampling was performed at two broiler abattoirs and at two associated broiler processing plants that were located separately from the abattoirs. Sites were sampled three times at either 1- or 2-week intervals in 1996, resulting in 498 samples. Some

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TABLE 1. *Sampling for* L. monocytogenes *at two broiler abattoirs in 1996*

Sampling site/sample type (total/pooled analyses)	Visit to broiler abattoir X[a]			Visit to broiler abattoir Y[a]		
	1	2	3	1	2	3
Crate washer/organic material (30/6)	5/1	5/1	5/1	5/1	5/1	5/1
Defeathering machine/organic material (30/6)	5/1	5/1	5/1	5/1	5/1	5/1
Opening machine/swab (15/3)	5/1	ND[b]	ND	5/1	5/1	ND
Neck skin/skin (80/16)	10/2	20/4	20/4	10/2	10/2	10/2
Neck skin trimmer/swab (30/5)	5/1	5/1	ND	5/1	5/1	5/1
Floor drain at the abattoir/swab (13/5)	5/1	5/1	ND	1/1	1/1	1/1
Water-chilling tank/water (5/4)	1/1	2/2	2/1	ND	ND	ND
Air chiller/swab (31/7)	5/1	5/1	5/1	6/2	5/1 LM	5/1 LM
Skin-removing machine/skin (80/16)	10/2	20/4	20/4 LM[c]	10/2	10/2 LM	10/2
Automatic cutting machine/broiler meat (30/6)	5/1	5/1	5/1	5/1	5/1	5/1
Conveyor belt/swab (30/6)	5/1	5/1	5/1	5/1	5/1	5/1 LM
Gloves from packaging worker/rinsing water (6/2)	3/1	ND	ND	3/1	ND	ND
Floor drain in the packaging area/swab (7/2)	5/1	ND	ND	2/1	ND	ND
Packaged raw broiler/skin and meat (30/18)	5/5	5/5	5/5	5/1	5/1 LM	5/1
Total	89/27	88/23	87/26	82/19	81/17	71/15

[a] No. of samples/pooled analyses.
[b] ND, not done.
[c] LM, one analysis was found positive for *L. monocytogenes.*

of the samples were pooled together, and a total of 127 analyses for *L. monocytogenes* were done. The slaughtering process was assessed throughout, starting with dirt from the transportation crates and concluding with the ready products, either raw broiler pieces from the abattoirs or cooked ready-to-eat broiler products from the broiler product plants (Tables 1 and 2). Sampling was performed during working hours in the late evening, before cleaning and disinfection took place.

Samples from the abattoir processing environment were taken with gauze sponges moistened with *Listeria* enrichment broth (Oxoid, Basingstoke, UK). The area sampled was 20 by 20 cm. When possible, organic material was included in the sample. The gauze sponges were placed into sterile plastic bags. Water samples were collected in sterile plastic jars. Neck skin samples were taken with sterile scissors and put into a sterile bag. Gloves from workers at the packaging area were rinsed with sterile water into a sterile plastic bag. Before analysis, the packaged raw or processed broiler products were kept at 4°C until the sell-by-date in order to reveal the prevalence of *Listeria* contamination in them before cooking and consumption. Small pieces were cut with sterile scissors from the surface to make a 25-g sample. All samples were kept refrigerated at 4°C until analysis was performed.

Sampling of raw broiler pieces at retail level. Altogether, 61 raw broiler piece samples were bought in 1997 and 1998 from retail stores (Table 3). The samples of 1997 had been sold unpackaged, and the samples from 1998 had been packaged under modified atmosphere containing 80% N_2 and 20% CO_2 at the broiler abattoir. Analysis for *L. monocytogenes* was started on the purchase day for samples that had been sold unpackaged or on the sell-by-date for packaged samples. Each sample was placed into a sterile plastic bag. Fifty milliliters of peptone (0.1%)-saline (0.85%) was added, and the broiler part in the plastic bag was macerated by hand. Twenty-five milliliters of this peptone-saline was used for the enrichment procedure.

Isolation of *L. monocytogenes*. The isolation procedure was started within 24 h. A 25-g (or 25-ml of water or peptone-saline) sample was mixed with 225 ml *Listeria* enrichment broth (Oxoid, Basingstoke, Hampshire, UK), except for the environmental gauze sponges that were not weighed. The isolation method of the Nordic Committee on Food Analysis was followed using the one-stage enrichment procedure *(31)*. The *Listeria* enrichment broth was incubated at 30°C for 48 h. After incubation, enrichment broth was streaked with a sterile cotton tip on Oxford plates (Oxoid)

TABLE 2. *Sampling for* L. monocytogenes *at two broiler product plants in 1996*

Sample	Visit to broiler product plant X[a]			Visit to broiler product plant Y[a]			Total
	1	2	3	1	2	3	
Macerated raw broiler mass	5/1	ND[b]	5/1	10/2	10/2 LM[c]	10/2 LM	40/8
Swab from slicer	5/1	ND	5/1	ND	5/1	5/1	15/3
Packaged ready-to-eat broiler product	5/5	ND	5/5	5/1	5/1	5/1	25/13
Raw ground broiler meat	ND	1/1	ND	ND	ND	ND	1/1
Total	15/7	1/1	15/7	15/3	20/4	20/4	81/25

[a] No. of samples/pooled analyses.
[b] ND, not done.
[c] LM, one analysis was found positive for *L. monocytogenes.*

TABLE 3. *Prevalence of* L. monocytogenes *in broiler piece samples at retail level in 1997 to 1998 slaughtered at different broiler abattoirs*

	No. of positive samples/total no. of samples slaughtered at broiler abattoir			
Broiler part sample	X	Y	Z	Total
Leg	5/7[a]	12/13	2/8	68% (19/28)
Drumstick	0/1	3/4	1/1	67% (4/6)
Breast	0/1	11/17	2/7	52% (13/25)
Wings	ND[b]	1/2	ND	50% (1/2)
Total	56% (5/9)	78% (28/36)	31% (5/16)	62% (38/61)

[a] Packaged in MA (modified atmosphere).
[b] ND, not done.

and incubated for 48 h at 37°C. One to 10 typical dark esculin-positive colonies were streaked on horse blood agar plates, and incubated overnight at 37°C, for the detection of hemolysis. All hemolytic colonies were checked for motility at 25°C by growing them overnight in brain heart infusion broth (Oxoid), catalase production, and were Gram stained. API-*Listeria* kits (Bio-Meriéux, Rhone, France) were used for *Listeria* species identification.

Chromosomal DNA analysis by PFGE. Pure cultures were grown on horse blood agars for 24 h at 37°C. A single colony was inoculated into 5 ml of brain heart infusion broth (Oxoid) and incubated overnight at 37°C. DNA isolation was performed as described by Maslow et al. *(29),* with the modifications described by Björkroth et al. *(7).* Briefly, cells from overnight brain heart infusion broth (Oxoid) cultures were mixed with an equal volume of 2% (wt/vol) low melting temperature agarose (InCert agarose; FMC Bioproducts, Rockland, Maine). Instead of using insert molds, GelSyringe dispensers (New England Biolabs, Beverly, Mass.) were used according to the manufacturer's instructions. In order to obtain complete cell lysis 10 U/ml mutanolysin (Sigma, St. Louis, Mo.) were added to the lysing solution. Inactivation of proteinase K (Sigma) and restriction endonuclease digestion of the agarose-embedded DNA was performed as described by New England Biolabs.

Two rare-cutting restriction enzymes, *Apa*I and *Asc*I (New England Biolabs), previously found suitable for *L. monocytogenes* characterization, were used for cleaving the DNA *(9–13, 23, 30).* The samples were electrophoresed through 1.0% (wt/vol) agarose gel (SeaKem Gold; FMC Bioproducts) in 0.5× TBE (45 mM Tris, 4.5 mM boric acid, pH 8.3, and 1 mM sodium EDTA) at 200 V at 10°C, using a Gene Navigator system with a hexagonal electrode (Pharmacia, Uppsala, Sweden). *Apa*I and *Asc*I restriction fragments were resolved with pulse times ramping linearly from 0.5 to 29.5 s over 20 h. Midrange PFG marker I and lambda ladder PFG marker (New England Biolabs) were used as fragment size markers.

Interpretation of the PFGE results. A letter was assigned chronologically for every restriction enzyme pattern for both restriction enzymes. PFGE types were obtained combining the two restriction enzyme patterns to one unique PFGE type. Types were considered closely related in the presence of at most a three-band difference (one genetic event), as described by Tenover et al. *(38).* This relationship was indicated in the type nomenclature by a shared Roman numeral.

Serotyping. Serotyping was performed using commercial *Listeria* antisera according to the instructions given by the manufacturer *(2).* However, for determination of the flagellar H-antigens A, B, C, and D, the strains were incubated at 26°C, instead of 30°C, in 0.2% brain heart infusion-agar tubes in order to increase motility. One isolate representing each PFGE type was serotyped, as it has been shown that isolates representing the same PFGE types are representatives of the same serotype *(9, 11, 30).*

RESULTS

***L. monocytogenes* at two broiler abattoirs and product plants.** One percent (1 of 91) of the samples from broiler abattoir X and product plant X associated with it were positive for *L. monocytogenes* (Tables 1 and 2). The positive sample originated from the broiler abattoir. Two isolates were obtained for typing from the positive sample. From broiler abattoir Y and the associated product plant Y, 11% (7 of 62) of the samples were positive. Five positive samples were found at the broiler abattoir, and from these samples 16 isolates were obtained for typing (1 to 5 isolates/sample). Two positive samples were obtained from the broiler product plant Y. Five to six isolates were obtained from each of these samples. At broiler abattoir Y, *L. monocytogenes* were isolated from the air chiller, from the skin removing machine, and from the conveyor belt in the packaging area of raw broiler meat. Altogether, 37 *L. monocytogenes* isolates were obtained from the eight positive samples. Two isolates were from abattoir X, 24 from abattoir Y, and 11 from broiler product plant Y.

***L. monocytogenes* in broiler piece samples from retail stores.** Of the raw broiler piece samples bought from retail stores, 62% (38 of 61) were positive for *L. monocytogenes* (Table 3). The broilers had been slaughtered at three different broiler abattoirs (X, Y, and Z). Of broiler pieces from broilers slaughtered at abattoirs X, Y, and Z, 56% (5 of 9), 78% (27 of 36), and 31% (5 of 16), respectively, were positive for *L. monocytogenes.* Altogether, 100 *L. monocytogenes* isolates were obtained for further typing, representing 1 to 3 isolates from each positive sample.

PFGE-typing and serotyping results. *Apa*I and *Asc*I generated 12 different patterns (A to L) and 13 (A to M) patterns, respectively, when one-band differences were taken into consideration. Combining these patterns, 14 distinct PFGE types were obtained. Three different serotypes were obtained: 1/2a (10 PFGE types), 1/2c (3 PFGE types), and 4b (1 PFGE type) (Table 4).

TABLE 4. *Division of* L. monocytogenes *isolates into different types, and the distribution at different broiler abattoirs, broiler product plants, and in broiler meat slaughtered at different broiler abattoirs*

Type	Serotype	Restriction enzyme profile		No. of isolates from broiler abattoir		No. of isolates from broiler product plant		No. of isolates from broiler meat slaughtered at broiler abattoir			
		ApaI	*AscI*	X (1996)	Y (1996)	X (1996)	Y (1996)	X (1998)	Y (1996)	Y (1997)	Z (1996)
Ia	1/2a	A	A	2	0	0	0	6	0	0	0
Ib	1/2a	K	K	0	0	0	0	7	0	0	0
Ic	1/2a	K	L	0	0	0	0	4	0	0	0
Id	1/2a	L	M	0	0	0	0	3	0	0	0
II	1/2a	E	E	0	1	0	0	0	0	0	0
III	1/2a	F	F	0	0	0	1	0	0	0	0
IVa	1/2a	G	G	0	0	0	0	0	0	0	5
IVb	1/2a	I	I	0	0	0	0	0	0	2	0
V	1/2a	H	H	0	0	0	0	0	0	2	0
VI	1/2a	J	J	0	0	0	0	0	0	1	0
VIIa	1/2c	C	C	0	4	0	7	0	8	4	0
VIIb	1/2c	D	D	0	7	0	1	0	0	49	3
VIIc	1/2c	C	D	0	0	0	0	0	0	5	0
VIII	4b	B	B	0	4	0	2	0	0	8	0

Isolates from broiler abattoirs were of five different PFGE types (Table 4). Both isolates from the single positive sample from poultry abattoir X were of PFGE-type Ia. PFGE-type Ia was still detected 2 years later in raw broiler purchased from retail stores and originating from broiler abattoir X. Three closely related types (Ib, Ic, and Id) could be found in 1998 as well. Four different PFGE types (II, VIIa, VIIb, and VIII) were determined among the isolates from abattoir Y (Table 5). Isolates of PFGE-type VIIb were obtained from the air chiller and from the conveyor belt to the packaging area. Isolates representing four different *L. monocytogenes* PFGE types (III, VIIa, VIIb, and VIII) were found in two macerated raw broilermass samples from broiler product plant Y. Three PFGE types shared the same type as the isolates taken from the air chiller, the skin-removing machine, and the conveyor belt from the associated broiler abattoir Y. In 1996, type VIIa was identified in raw broiler pieces originating from broiler abattoir Y. In 1997, this same type was found in raw broiler pieces, together with other types (IVb, V, VIIb, VIIc, and VIII). In raw broiler pieces originating from broiler abattoir Z, an abattoir not included in this study, two different types were located (IVa and VIIb).

The single isolate of PFGE-type III was the only glucose-1-phosphate-positive isolate in the API-*Listeria* test, and it had been isolated from a macerated broiler mass sample. One isolate, located in a raw broiler piece, was rhamnose negative. This isolate was of PFGE-type VIIc that was shared with four other isolates. Three different PFGE types were found in one macerated raw broiler mass sample, and two different PFGE types were detected in six raw broiler piece samples in 1997 and in three raw broiler samples in 1998. This stresses the importance of typing more than just one isolate from each sample.

DISCUSSION

One percent of samples analyzed from broiler abattoir and product plant X were positive compared to 11% in broiler abattoir and product plant Y. This result is similar to a Danish study in which positive samples for *L. monocytogenes* varied between 0.3 and 18.7% in seven Danish poultry abattoirs *(32)*. *L. monocytogenes* contamination of broiler carcasses in the broiler abattoirs probably occurred during or after the chilling step in the skin-removing machine, because no positive samples were found prior to these steps. This is similar to the findings of Clouser et al. *(15),* who concluded that *L. monocytogenes* contamination of turkey carcasses probably occurs during chilling. In the present study, the skin-removing machine was a positive sampling site at both broiler abattoirs, indicating an important contamination point of *L. monocytogenes.* Raw broiler meat for broiler products may act as a source of broiler product and broiler plant contamination, because *L. monocytogenes* could be found in the macerated raw broiler mass.

Even though the contamination level in the broiler abattoirs was low (1 to 11%), the prevalence of *L. monocytogenes* in raw broilers at retail level was 62%. This could be due to cross-contamination at retail level, because the

TABLE 5. *Distribution of PFGE types in broiler abattoir Y and broiler product plant Y[a] at* L. monocytogenes*-positive sampling sites*

Sample	PFGE types found at visit:		
	1	2	3
Air chiller	ND[b]	VIII	VIIb
Skin-removing machine	ND	VIIa	ND
Conveyor belt to the packaging area	ND	ND	II, VIIb
Packaged raw broiler	ND	VIIa	ND
Macerated raw broiler mass[a]	ND	VIIa	III, VIIa, VIIb, VIII

[a] Sample taken from boiler product plant Y.
[b] ND, not detected.

majority of the samples were sold unpackaged. Of the broiler leg samples, 68% (19 of 28) were positive for *L. monocytogenes,* with the corresponding figure for broiler breast samples at only 52% (13 of 25). This is possibly because legs have more skin on their surfaces than breasts, and *L. monocytogenes* contamination from the contaminated abattoir environment occurs at the surface of the broilers.

The isolates from the skin-removing machine in broiler abattoir X and from raw broiler at retail level 2 years later harbored the same PFGE type (Ia). Furthermore, types closely related to type Ia (Ib to Id) were found in raw broiler meat in 1998. This indicates that type Ia may have existed at the broiler abattoir for at least 2 years and continued to be found in contaminated raw broiler. In our earlier study at an ice cream plant, we found that one *L. monocytogenes* type had survived at least 7 years, with closely related types evolving, probably by mutation, without persisting in the plant environment *(30).*

Five different types, none of them PFGE-type Ia, were found in broiler abattoir Y. In 1996, only one of these types (VIIa) was identified in raw broiler meat. In 1997, three (VIIa, VIIb, and VIII) of the earlier five types determined at broiler abattoir Y could still be found in raw broiler meat. Type VIIb was the dominant finding in raw broiler meat from broiler abattoir Y in 1997. It is interesting to note that this type had been located in samples from the air chiller in 1997, indicating an important contaminating step in the slaughtering process.

In broiler parts slaughtered at broiler abattoir Z, which was not included in the study, two different types were identified. Type IVa was unique to these samples, but type VIIb was also found in broiler samples from broiler abattoir Y. It is possible that cross-contamination at retail level had occurred, because these broiler part samples were kept unpackaged on display at retail level. Another possibility is that both broiler abattoirs may harbor this same type in the processing line.

In this study, three different serotypes were detected (1/2a, 1/2c, and 4b). One isolate representing each PFGE type was serotyped. The predicted serotype distribution, based on these results is as follows: 65% of all isolates were of serotype 1/2c (88 of 136), 25% (34 of 136), of serotype 1/2a, and 10% (14 of 136) of serotype 4b.

This differs from the serotype distribution found in poultry in another study, where serotypes 1/2b (64%), 1/2c (18%), 3b (6%), and incomplete (12%) were observed *(4).* In Denmark, the researchers found 98% to be of serotype 1 and only 2% were of serotype 4 (32). Unfortunately, the researchers did not complete serotyping for the H-antigens, making closer comparison difficult. Of the 61 raw broiler parts, 54 were sold unpackaged at retail level, which could also explain the higher contamination rate. It is likely that cross-contamination among broiler pieces may have occurred.

This study shows that there may be an association between finding a higher rate of *L. monocytogenes*-positive samples from the broiler abattoir equipment and processing environment and the prevalence of *L. monocytogenes* in raw broiler meat at retail level. In broiler abattoirs, special cleaning attention should be given at important identified contamination steps, such as the air chiller, the skin-removing machine, and the conveyor belts. It appears that different broiler abattoirs have their own *L. monocytogenes* strains that may contaminate the broiler carcasses during processing. Special attention to hygiene is needed especially at the retail level to prevent cross-contamination of unpackaged broiler meat with *L. monocytogenes* and to temperature control during transport and handling. Furthermore, raw broiler meat in consumer kitchens should be cooked thoroughly and handled as a potential source of *L. monocytogenes* in order to avoid potential illness and cross-contamination to other food items.

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