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## Research Note

# Prevalence and Genetic Characterization of *Listeria monocytogenes* in Retail Broiler Meat in Estonia

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## ABSTRACT

The prevalence and genetic diversity of *Listeria monocytogenes* in raw broiler legs at the retail level in Estonia were studied. A total of 240 raw broiler legs (120 from Estonia and 120 of foreign origin, which had been imported to Estonia from Denmark, Finland, Hungary, Sweden, and the United States) from 12 retail stores in the two largest cities in Estonia (Tallinn and Tartu) were investigated from January to December 2002. Of these, 70% were positive for *L. monocytogenes*. The prevalence of *L. monocytogenes* in broiler legs of Estonian origin (88%) was significantly higher than in broiler legs of foreign origin (53%) ( $P < 0.001$ ). Altogether, 169 (106 Estonian and 63 imported) *L. monocytogenes* isolates were characterized by pulsed-field gel electrophoresis (PFGE) typing after treatment with the restriction enzyme *AscI*. The isolates showed a wide genetic diversity, with 35 different PFGE types obtained. Of these, 11 PFGE types came only from isolates of broiler legs of Estonian origin, 4 of Danish origin, 2 of Finnish origin, and 4 of Hungarian origin. Fourteen PFGE types came from isolates of broiler legs that originated from various countries. The strains that shared the same PFGE types from isolates of Estonian origin were recovered from broiler legs that came from different stores over the course of several months. Seventy-one *L. monocytogenes* isolates, including all PFGE types, were serotyped, and three serotypes (1/2a, 1/2b, and 4b) were obtained. Serotype 1/2a accounted for 96% of the isolates.

The consumption of foods contaminated with *Listeria monocytogenes* can result in listeriosis, an uncommon but potentially fatal disease. Listeriosis can be life threatening to elderly persons, persons with weakened immune systems, and women who are pregnant. Poultry products have been associated with listeriosis (7–10, 14, 15, 22).

Healthy birds may shed *L. monocytogenes* in fecal material asymptotically (23). However, poultry meat becomes contaminated during slaughter and processing (17, 18, 20). Contamination rates for *L. monocytogenes* in raw poultry products have ranged from 10 to 62% (3, 6, 12, 17, 19, 21, 24–26). The prevention of poultry product contamination with *L. monocytogenes* is therefore of major importance.

Within the past few years, consumption of poultry meat has increased in Estonia and presently stands at about 22 kg per capita (2). To our knowledge, no data exist about the prevalence of *L. monocytogenes* in poultry products. The goal of this study was thus to determine the prevalence of *L. monocytogenes* in raw broiler legs of Estonian and foreign origin sold on the Estonian retail market. To obtain information on the diversity of *L. monocytogenes* isolates, genotyping with pulsed-field gel electrophoresis (PFGE) was performed.

## MATERIALS AND METHODS

**Samples.** A total of 240 raw broiler legs (120 from Estonia and 120 of foreign origin) from 12 retail stores (supermarkets) in the two largest cities (Tallinn and Tartu) of Estonia were studied from January to December 2002. All samples of Estonian origin were from one of the country's main producers of poultry products, where broiler chickens were reared for meat and slaughtered after 6 or 7 weeks. Of these, 104 were obtained from stores that sold only products of the main producer, and 16 were obtained from stores that also sold poultry products from other countries. Of the samples of foreign origin, 60, 18, 21, 12, and 9 were imported from Denmark, Finland, Hungary, Sweden, and the United States, respectively. Products of Estonian origin were fresh, and those of foreign origin were frozen. All broiler legs had been stored unpackaged on the store counter (1 to 5°C). Each sampled broiler leg was placed in a separate sterile plastic bag. During transportation to the laboratory, the samples were kept cool in portable insulated boxes by ice packs and were stored at 4°C until analysis.

**Isolation of *L. monocytogenes*.** Microbiological analyses for *L. monocytogenes* were started within 24 h of sample collection. One hundred milliliters of peptone (0.1%)–saline (0.85%) solution was added to the whole broiler leg in the plastic bag, and the broiler leg was massaged by hand for 1 min. Twenty-five milliliters of this peptone–saline solution was used for the enrichment procedure. The isolation of *L. monocytogenes* was carried out by a two-step enrichment method according to the recommendations of the International Organization for Standardization, with the use of half-Fraser and Fraser broth (Oxoid, Basingstoke, Hampshire, UK) (1). Both enrichment broths were plated on PALCAM agar

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TABLE 1. Prevalence of *Listeria monocytogenes* in raw broiler legs in retail stores (A–L) in Estonia

City or store	No. of positive samples/total no. of samples (%) originating from different countries <sup>a</sup>						
	DK	EE	FI	HU	SE	US	Total
<b>Tallinn</b>							
A	NS <sup>b</sup>	30/38 (79)	NS	NS	NS	NS	30/38 (79)
B	NS	10/12 (83)	NS	NS	NS	NS	10/12 (83)
C	NS	2/2 (100)	NS	NS	NS	NS	2/2 (100)
D	NS	17/19 (90)	NS	NS	NS	NS	17/19 (90)
E	22/49 (45)	10/10 (100)	NS	NS	10/12 (83)	2/9 (22)	44/80 (55)
F	NS	1/3 (33)	10/18 (56)	NS	NS	NS	11/21 (52)
G	NS	NS	NS	16/21 (76)	NS	NS	16/21 (76)
Subtotal	22/49 (45)	70/84 (83)	10/18 (56)	16/21 (76)	10/12 (83)	2/9 (22)	130/193 (67)
<b>Tartu</b>							
H	NS	4/4 (100)	NS	NS	NS	NS	4/4 (100)
I	3/11 (27)	3/3 (100)	NS	NS	NS	NS	6/14 (43)
J	NS	6/6 (100)	NS	NS	NS	NS	6/6 (100)
K	NS	19/19 (100)	NS	NS	NS	NS	19/19 (100)
L	NS	4/4 (100)	NS	NS	NS	NS	4/4 (100)
Subtotal	3/11 (27)	36/36 (100)	NS	NS	NS	NS	39/47 (83)
Total	25/60 (42)	106/120 (88)	10/18 (56)	16/21 (76)	10/12 (83)	2/9 (22)	169/240 (70)

<sup>a</sup> Country: DK, Denmark; EE, Estonia; FI, Finland; HU, Hungary; SE, Sweden; US, United States.

<sup>b</sup> NS, no samples available.

(Oxoid) and *L. monocytogenes* blood agar (Lab M, Bury, Lancashire, UK), as suggested by Johansson (13). Five typical colonies from each selective plate were streaked on blood agar, and five beta-hemolytic colonies were confirmed by catalase reaction, Gram staining, and biochemical identification with the API *Listeria* test (bioMérieux, Marcy-l'Etoile, France).

**In situ DNA isolation and PFGE.** Altogether, 169 *L. monocytogenes* isolates were obtained for PFGE typing and represented one isolate from each positive sample. Cultures for DNA isolation were grown overnight in Trypticase soy broth (Difco, Becton Dickinson, Sparks, Md.) at 37°C. In situ DNA was isolated and digested with the restriction enzyme *AscI* (New England Biolabs, Beverly, Mass.) in agarose plugs and was then characterized by PFGE as described by Autio et al. (4) with the use of pronase (Roche Diagnostics GmbH, Mannheim, Germany) instead of proteinase K.

**PFGE pattern analysis.** Numerical analysis of *AscI* macrorestriction patterns was performed by the computer software program BioNumerics 3.5 (Applied Maths, Sint-Martens-Latem, Belgium). Similarity analysis was carried out by use of the Dice coefficient (position tolerance, 1.0%). The clustering and construction of the dendrogram were performed by the unweighted pair-group method with arithmetic averages.

**Serotyping.** Serotyping was performed with commercial *Listeria* antisera according to the instructions given by the manufacturer (Denka Seiken, Tokyo, Japan), with some modifications. For detection of the O-antigen, the cells were cultured on Trypticase soy agar (TSA; Difco, Becton Dickinson) plates. Detection of the flagellar H-antigens (A, B, C, and D) was performed at 25°C in TSA tubes.

**Statistical analysis.** The prevalence data were analyzed statistically by the chi-square test.

## RESULTS

Of the raw broiler legs purchased from retail stores in Estonia, 70% were positive for *L. monocytogenes* (Table 1). The prevalence in broiler legs of Estonian origin varied from 33 to 100% and, in legs of foreign origin, from 22 to 83% from various stores. The prevalence of *L. monocytogenes* in broiler legs of Estonian origin (88%) was significantly higher than in broiler legs of foreign origin (53%) ( $P < 0.001$ ). Of the broiler legs bought from stores selling only products of the predominant Estonian poultry meat plant, 89% were positive for *L. monocytogenes*. The broiler legs of Estonian origin purchased in Tartu had a significantly higher contamination level than those purchased in Tallinn, 100 and 83%, respectively ( $P < 0.05$ ).

The characterization of *L. monocytogenes* isolates recovered from broiler legs of Estonian ( $n = 106$ ) and foreign ( $n = 63$ ) origin yielded 22 and 24 PFGE types, respectively. Combining these PFGE types, 35 different types were obtained (Fig. 1). Of these PFGE types, 11 (2, 5, 6, 7, 14, 22, 25, 27, 28, 30, and 34) came only from isolates of broiler legs of Estonian origin, 4 (16, 20, 23, and 31) of Danish origin, 2 (15 and 32) of Finnish origin, and 4 (9, 10, 13, and 35) of Hungarian origin. Fourteen (1, 3, 4, 8, 11, 12, 17, 18, 19, 21, 24, 26, 29, and 33) PFGE types came from isolates that originated from more than one country. PFGE types 4, 21, and 33 were predominant, accounting for 13, 15, and 10% of the isolates, respectively. The isolates of these three PFGE types were recovered from broiler legs of Danish, Estonian, Hungarian, and U.S. origin.

In some cases, the same PFGE types were recovered from broiler legs that had originated from different countries but that had been obtained from the same stores (Table

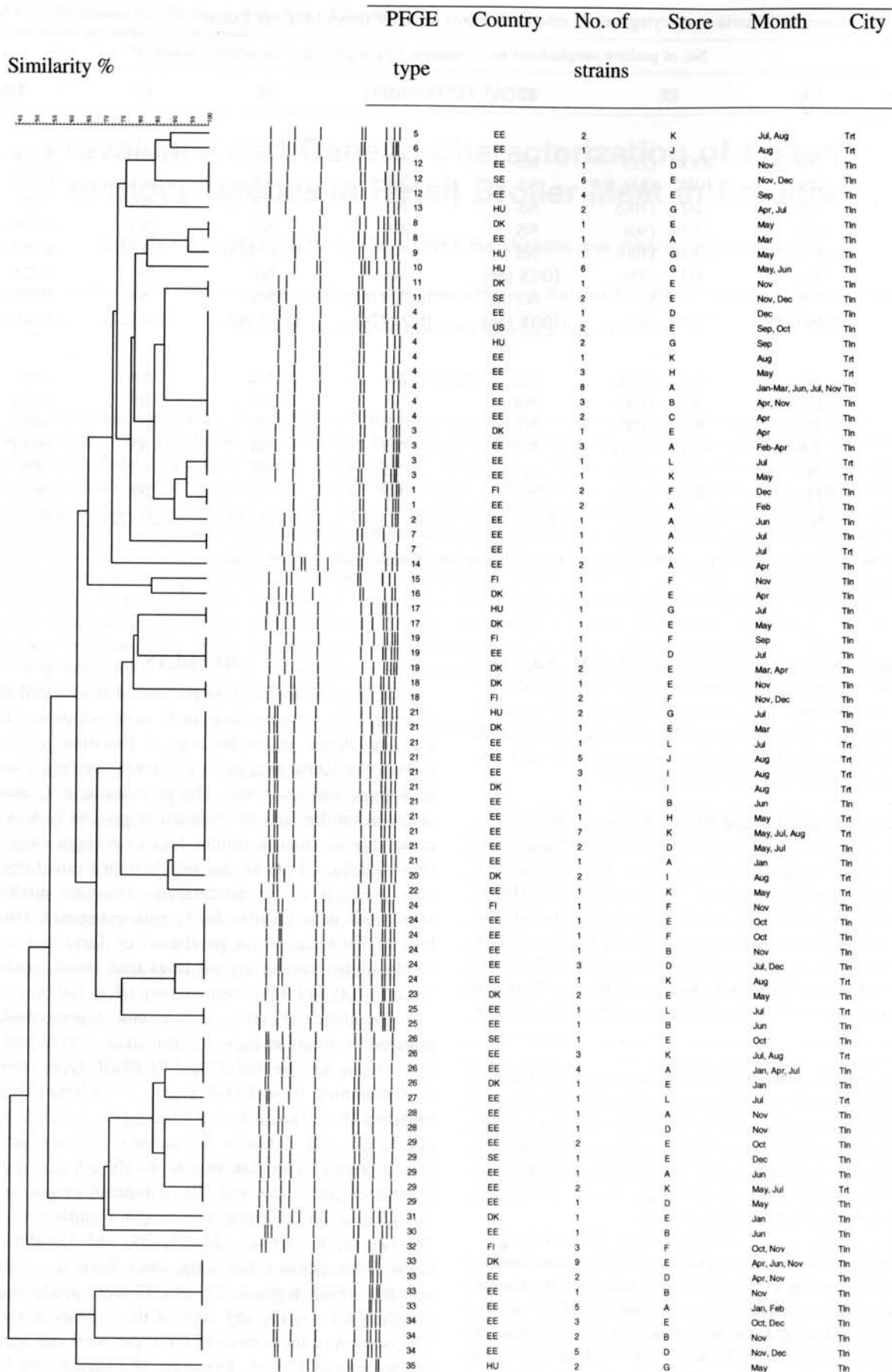


FIGURE 1. Dendrogram of defined PFGE patterns of *Listeria monocytogenes* strains isolated from raw broiler legs in retail stores in Estonia. Similarity analysis was performed by use of the Dice coefficient, and clustering was performed by the unweighted pair-group method with arithmetic averages (position tolerance, 1.0%). Country: DK, Denmark; EE, Estonia; FI, Finland; HU, Hungary; SE, Sweden; US, United States. City: Tln, Tallinn; Trt, Tartu.

TABLE 2. Distributions of *Listeria monocytogenes* PFGE types and serotypes in raw broiler legs in retail stores (A–L)

Store	No. of PFGE types	PFGE types originating from different countries <sup>a</sup>						No. of serotyped isolates		
		DK	EE	FI	HU	SE	US	1/2a	1/2b	4b
A	12		1, 2, 3, 4, 7, 8, 14, 21, 26, 28, 29, 33					14		
B	7		4, 21, 24, 25, 30, 33, 34					5		
C	1		4					1		
D	9		4, 12, 19, 21, 24, 28, 29, 33, 34					6		
E	17	3, 8, 11, 16, 17, 18, 19, 21, 23, 26, 31, 33	12, 24, 29, 34			11, 12, 26, 29	4	20	2	
F	6		24	1, 15, 18, 19, 24, 32				5	2	
G	7				4, 9, 10, 13, 17, 21, 35			5	1	1
H	2		4, 21							
I	2	20, 21	21					1		
J	2		6, 21					1		
K	9		3, 4, 5, 7, 21, 22, 24, 26, 29					6		
L	4		3, 21, 25, 27					1		
Total	35							65	5	1

<sup>a</sup> Country: DK, Denmark; EE, Estonia; FI, Finland; HU, Hungary; SE, Sweden; US, United States.

2). In store E, strains of PFGE types 12 and 29 were found in broiler legs of Estonian and Swedish origin, and strains of PFGE types 11 and 26 were found in legs of Danish and Swedish origin. Isolates of PFGE type 24 were detected in broiler legs of both Estonian and Finnish origin that were obtained from store F. Strains of PFGE type 21 were detected in store I in broiler legs of both Estonian and Danish origin.

In several cases, the same PFGE types were detected in samples of Estonian origin that came from different stores over the course of several months (Fig. 1). The strains of PFGE type 4 were obtained from broiler legs that came from various stores during 10 different months and from store A during six different months. Isolates of PFGE types 3, 21, and 24 were detected in broiler legs from various stores during five different months.

One to eight representative isolates from each PFGE type were selected for serotyping, which resulted in a total of 71 isolates. Three different serotypes were obtained: 1/2a, 1/2b, and 4b (Table 2). All broiler legs of Estonian origin and most broiler legs of foreign origin had serotype 1/2a. Isolates of serotype 1/2b were of Danish, Finnish, and Hungarian origin, and isolates of serotype 4b were of Hungarian origin.

## DISCUSSION

Raw broiler legs showed a high level of contamination with *L. monocytogenes* (70%). The prevalence of *L. mon-*

*ocytogenes* in broiler legs of Estonian origin in general and in broiler legs obtained from stores selling only products of the predominant Estonian poultry meat plant (stores A, B, C, D, H, J, K, and L) was higher (88 and 89%, respectively) than that reported by Genigeorgis et al. (11) (16%) or Miettinen et al. (17) (68%) in broiler legs. Furthermore, the broiler legs of Estonian origin bought in Tartu were all (100%) contaminated by *L. monocytogenes*. To our knowledge, this is the first time that such a high prevalence of *L. monocytogenes* in raw broiler legs has been reported. The high prevalence in broiler legs at the Estonian retail level could be because of contamination that may have occurred during processing at the plant. However, cross-contamination of retail poultry products cannot be excluded, because products were sold unpackaged.

Thirty-five PFGE types were presented by 169 *L. monocytogenes* strains. These data suggest that the *L. monocytogenes* strains recovered from the broiler legs showed wide genetic diversity. The PFGE types recovered from the broiler legs of Estonian (2, 5, 6, 7, 14, 22, 25, 27, 28, 30, and 34) and Hungarian origin (9, 10, 13, and 35) were obtained from stores that sold only products from these countries and were possibly associated with the producing country. Strains that shared the same PFGE types (14 PFGE types) were identified among isolates of broiler legs that originated from different countries. For example, the strains of PFGE types 1, 3, 4, 8, 12, 19, 21, 24, 26, 29, and 33 were common in broiler legs of both Estonian and foreign



origin. Three predominant PFGE types (4, 21, and 33), possessed by 38% of all strains, were recovered from broiler legs of Danish, Estonian, Hungarian, and U.S. origin. The strains of PFGE types 11, 17, and 18 were of Danish, Finnish, Hungarian, and Swedish origin.

Because the broiler legs had been sold unpackaged and from the same counter, one of the reasons for the same PFGE types could be cross-contamination in the stores that sold products that had originated from various countries. In stores E, F, and I, the same PFGE types (12, 21, 24, and 29) were detected in broiler legs of both Estonian and foreign origin (Table 2). In store E, the strains from broiler legs of Danish and Swedish origin shared the same PFGE types (11 and 26).

Recovery of strains that shared the same PFGE types (3, 4, 21, and 24) from different stores obtained during the course of several months suggests a wide temporal distribution of many of the *L. monocytogenes* strains isolated in broiler legs. Because the broiler legs of Estonian origin came from one processing plant, the PFGE types are likely associated with contamination during processing (17, 18, 20). The occurrence of the same PFGE types (3, 4, 21, and 24) in broiler legs of Estonian origin during the course of several months indicates that these strains are persistent (16). Furthermore, *L. monocytogenes* from broiler legs can contaminate retail counters and cause cross-contamination of other raw foods if hygienic procedures are inadequate. This emphasizes the need for strict hygienic conditions during processing and at the retail level to prevent cross-contamination.

Serotype 1/2a was predominant in Estonian poultry products. The same serotype was also predominant in raw chicken from Portugal (12). In the United States and Spain, serotype 1/2b (5, 26) and, in Finland, serotype 1/2c (17) have been the most common serotypes found in poultry meat.

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