Epidemiol. Infect. (2014), **142**, 2251–2260. © Cambridge University Press 2014
doi:10.1017/S0950268813003385

Human isolates of *Listeria monocytogenes* in Sweden during half a century (1958–2010)

G. LOPEZ-VALLADARES[1], W. THAM[1]*, V. SINGH PARIHAR[1], S. HELMERSSON[2], B. ANDERSSON[3], S. IVARSSON[4], C. JOHANSSON[4], H. RINGBERG[5], I. TJERNBERG[3], B. HENRIQUES-NORMARK[4] AND M.-L. DANIELSSON-THAM[1]

[1] *Department of Restaurant and Culinary Arts, Örebro University, Grythyttan, Sweden*
[2] *Department of Environmental Assessment, SLU, Uppsala, Sweden*
[3] *Department of Bacteriology, Malmö University Hospital, Malmö, Sweden*
[4] *The Swedish Institute for Infectious Disease Control and MTC, Karolinska Institutet, Solna, Sweden*
[5] *Regional Centre for Communicable Disease Control and Prevention, Malmö, Sweden*

Received 22 April 2013; Final revision 19 November 2013; Accepted 11 December 2013; first published online 20 January 2014

SUMMARY

Isolates of *Listeria monocytogenes* ($n=932$) isolated in Sweden during 1958–2010 from human patients with invasive listeriosis were characterized by serotyping and pulsed-field gel electrophoresis (PFGE) (*Asc*I). Of the 932 isolates, 183 different PFGE types were identified, of which 83 were each represented by only one isolate. In all, 483 serovar 1/2a isolates were distributed over 114 PFGE types; 90 serovar 1/2b isolates gave 32 PFGE types; 21 serovar 1/2c isolates gave nine PFGE types; three serovar 3b isolates gave one PFGE type; and, 335 serovar 4b isolates gave 31 PFGE types. During the 1980s in Sweden, several serovar 4b cases were associated with the consumption of European raw soft cheese. However, as cheese-production hygiene has improved, the number of 4b cases has decreased. Since 1996, serovar 1/2a has been the dominant *L. monocytogenes* serovar in human listeriosis in Sweden. Therefore, based on current serovars and PFGE types, an association between human cases of listeriosis and the consumption of vacuum-packed gravad and cold-smoked salmon is suggested.

Key words: Cheese, *Listeria monocytogenes*, PFGE, serotyping, salmon.

INTRODUCTION

Listeria monocytogenes is a pathogen associated with food and is mainly transmitted through ready-to-eat food such as soft cheese, vacuum-packed gravad and cold-smoked salmon, salads, raw milk, and uncooked meat. The clinical picture consists of septicaemia and/or meningitis, and abortion in pregnant women. Listeriosis primarily affects pregnant women, newborns, and individuals with weakened immune systems. The overall case-fatality rate from invasive listeriosis is from 20% to >50% [1].

In Sweden, the first isolation of *L. monocytogenes* was from rabbits in 1910 [2], and in 1960, human listeriosis became a notifiable disease [3]. Sweden is divided into 21 counties and surveillance is based on these administrative regional units (Fig. 1). During 2009, Sweden had the second highest number of cases reported ($n=73$), and had the second highest number

* Author for correspondence: Dr W. Tham, Department of Restaurant and Culinary Arts, Örebro University, Grythyttan, Sweden.
(Email: Listeria@telia.com)

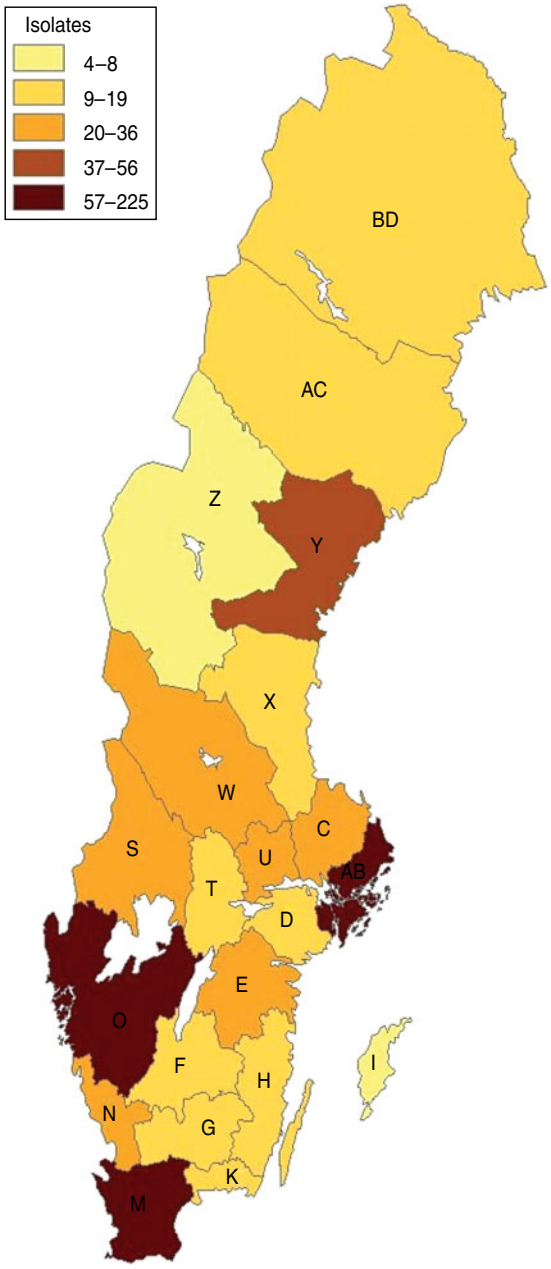

Fig. 1 [*colour online*]. Number of isolates collected from the different counties in Sweden. *Götaland region*: Skåne (M); Blekinge (K); Halland (N); Västra Götaland (O); Gotland (I); Kronoberg (G); Kalmar (H); Jönköping (F); Östergötland (E). *Svealand region*: Örebro (T); Södermanland (D); Västmanland (U); Uppsala (C); Stockholm (AB); Värmland (S); Dalarna (W). *Norrland region*: Gävleborg (X); Västernorrland (Y); Jämtland (Z); Västerbotten (AC); Norrbotten (BD). For four isolates there was no information.

of reported cases within the EU (0·78 cases/100 000). During 2013, Sweden had the highest number of cases ($n=93$) since 1960, i.e. 0·97 cases/100 000 people [4]. In developed countries where listeriosis is documented, the incidence rate is around 0·3/100 000 population [5]. Scandinavian countries have consistently higher rates than other countries, which is possibly due to the consumption of smoked fish [5].

L. monocytogenes isolates from human patients in Sweden suffering from invasive listeriosis during 1986–2007 have been characterized through serotyping and pulsed-field gel electrophoresis (PFGE) [6]. The aim of the present study was to continue the characterization of human *L. monocytogenes* isolates for the periods before (1958–1985) and after (2008–2010). A second aim was to relate genotypic results to epidemiological information, and to identify possible clustering of *L. monocytogenes* genotypes in time, season, location, age, or gender. In the present paper, results from the entire 53-year period (1958–2010) are presented and discussed.

MATERIALS AND METHODS

L. monocytogenes isolates

Isolates of *L. monocytogenes* ($n=932$) from patients with invasive listeriosis were received from clinical laboratories throughout Sweden and represented 64% of the 1460 human listeriosis cases reported during the entire 53-year period: a mother–child pair was considered as one case. The overall case-fatality rate was estimated as 30%. Isolates were from blood ($n=531$), cerebrospinal fluid ($n=137$) or other sources ($n=68$) of sporadic cases, and from one known outbreak in 1994/1995 involving eight patients [7]. Data regarding source was lacking for 196 isolates and duplicate isolates from patients were disregarded. The isolates were frozen at −75 °C in 80% brain heart infusion (Merck, Germany) and 20% v/v glycerol until analysis by serotyping and PFGE.

Serotyping

All *L. monocytogenes* isolates were serotyped with *Listeria* O antiserum types I/II, I, IV, V/VI, VI, VII, VIII, IX and H antiserum A, AB, C, D (Mast Diagnostics, UK) according to the manufacturer's instructions, with some modifications [6].

PFGE

Each isolate was restricted with the *Asc*I enzyme and subsequently subjected to PFGE, according to Graves & Swaminathan [8] with some modifications [6]. Gels with profiles were visualized by short-wave ultraviolet

Table 1. *Distribution of* L. monocytogenes *isolates in Sweden by serovar, PFGE type and region during 1958–2010*

Region	Population 2011* *n* (%)	Isolates (*n*)	Serovars					PFGE types (*n*)	Most common PFGE type					
			1/2a	1/2b	1/2c	3b	4b		1/2a:4A	1/2a:4B	1/2a:9B	4b:3	4b:6	4b:5
Götaland	4 542 263(47·9)	526	255	58	13	3	197	141	30	27	17	44	46	39
Svealand	3 787 796(39·9)	294	154	26	6	0	108	99	14	10	11	18	20	18
Norrland	1 152 796(12·2)	108	73	6	2	0	27	47	14	3	8	5	1	6
No data		4	1	0	0	0	3	4	0	0	0	0	0	1
Total	9 482 855(100·0)	932	483	90	21	3	335	183†	58	40	36	67	67	64

PFGE, Pulsed-field gel electrophoresis.
* Statistics Sweden [54].
† Total number of PFGE types identified.

(312 nm) light and photographed with a Polaroid camera. The pictures were analysed visually and the DNA restricted fragments were sized against lambda ladder PFG marker no. 340 S (New England BioLabs Inc., USA). Image analysis software was not used for comparison.

PFGE types were established based on the number and distribution of bands in each profile. The profiles were considered distinguishable if there was a difference of one band or more. Each unique PFGE strain was designated in a specific way. For example, serovar, *Asc*I profile, and variant; thus, the designation 1/2a:4A indicated the strain belonged to serovar 1/2a, *Asc*I profile 4, and variant A.

RESULTS AND DISCUSSION

Results from a previous characterization of human Swedish *L. monocytogenes* isolates from 1986 to 2007 by Parihar *et al*. [6], are included in the present study. Thus, the present paper covers the characterization of *L. monocytogenes* isolates from 932 human patients with invasive listeriosis in Sweden during the period 1958–2010.

Characterization

Of the 932 human *L. monocytogenes* isolates, 183 different PFGE types were identified, of which 83 PFGE types were each represented by only one isolate. The number of isolates, serovars, number of PFGE types, and the six most common PFGE types of *L. monocytogenes* isolates per region during 1958–2010 are presented in Table 1. The distribution of *L. monocytogenes* isolates, serovars, and the six most common PFGE types per year (1958–2010) are presented in Table 2. Thus, 483 serovar 1/2a isolates were distributed in 114 PFGE types (an average of 4·24 isolates per PFGE type); 90 serovar 1/2b isolates gave 32 PFGE types (2·81 isolates per PFGE type); 21 serovar 1/2c isolates gave nine PFGE types (2·33 isolates per PFGE type); three serovar 3b isolates gave one PFGE type; and 335 serovar 4b isolates gave 31 PFGE types (10·80 isolates per PFGE type) (Table 3).

The period studied (1958–2010) was divided into three periods: 1958–1971, when three serovars 1/2a, 1/2b, and 4b were equally common; 1972–1995, when serovar 4b was the main *L. monocytogenes* serovar in human listeriosis; and 1996–2010, when serovar 1/2a was the main *L. monocytogenes* serovar

Table 2. *Serovars, PFGE types and the six most common PFGE types of* L. monocytogenes *isolates in Sweden, per year, during 1958–2010*

Year	Isolates (*n*)	Serovars					PFGE types (*n*)	Most common PFGE types					
		1/2a	1/2b	1/2c	4b	3b		1/2a:4A	1/2a:4B	1/2a:9B	4b:3	4b:6	4b:5
1958	5	2	1	1	1		4						
1959	1	1					1						
1960	0												
1961	0												
1962	3		2	1			3						
1963	5	3	2				4						
1964	0												
1965	3		1		2		3						1
1966	2		1		1		2						1
1967	3	1	1		1		3						
1968	1	1					1						
1969	4	2			2		4						1
1970	3		2		1		3						
1971	4	2			2		3					2	
1972	9	3			6		4					1	5
1973	6	1	1		4		4					2	
1974	15	1			14		9					7	1
1975	15				15		4					6	7
1976	13				13		4				3	8	1
1977	16	1	1		14		8				5	4	2
1978	9		3	1	5		7						1
1979	11				11		5				5	3	1
1980	11	1	5	1	4		9				1		1
1981	13	5			8		9				2		3
1982	12	3	1		8		7	1			6		1
1983	11	1	1		9		5				3	3	3
1984	7	3	0		4		2					4	
1985	16	6	3	2	5		14				1		3
1986	2	1			1		2						1
1987	33	9		1	23		11	3			9		7
1988	41	24	3	1	13		22	8	1		3	1	4
1989	23	10	1	1	11		14		1		3		6
1990	27	11	1	1	14		10	6			3	6	
1991	17	5	1	1	10		14				1	1	1
1992	23	9	2		12		16	1			4		2
1993	24	17		1	6		15	1	2	1		3	3
1994	28	11	4	1	12		17	2			2	1	
1995	21	9			12		13	1	1		4	4	
1996	20	14			6		13	1	2		2		
1997	16	8	2		6		13		1	1		1	
1998	21	12	4		5		16	3	1	1	1		
1999	22	11	4	1	6		15	2	1				1
2000	42	27	8	1	6		24	5			1		
2001	46	27	7	1	10	1	25	2	7	3	2		4
2002	38	26	9	1	2		26	2	2				1
2003	48	40	4		4		27	1	3	2	1	1	
2004	28	17	1	2	8		21	1	2	2	1		1
2005	17	10	5		2		15	1		1			
2006	24	16	1	2	5		17	3		3	1		
2007	29	25	0	0	4		21	1	1	3	1		
2008	32	20	2	0	10		23		2	6	1	2	1
2009	49	40	2	0	7		27	2	7	5		2	
2010	63	47	4	0	10	2	28	11	6	8	1	5	
Total	932	483	90	21	335	3		58	40	36	67	67	64

PFGE, Pulsed-field gel electrophoresis.

Table 3. *Serovar and PFGE type distribution of* L. monocytogenes *isolates in Sweden during 1958–2010*

Serovar	Isolates *n* (%)	PFGE types	Average*
1/2a	483 (51·8)	114	4·24
1/2b	90 (9·7)	32	2·81
1/2c	21 (2·3)	9	2·33
3b	3 (0·3)	1	3·00
4b	335 (35·9)	31	10·80
Total	932 (100·0)	187†	

PFGE, Pulsed-field gel electrophoresis.
* Average number of isolates per PFGE type.
† 183 PFGE types were identified. Four PFGE types are shared by serovars 1/2a and 1/2c.

(Table 4). As the present study was retrospective, the data about the sources were not supported by Swedish epidemiological investigations of outbreaks or case-control studies of sporadic cases. However, food sampling has been conducted several times in Sweden during different periods [9–13].

L. monocytogenes isolates from 1958 to 1971

From 1958 to 1971, serovars 1/2a, 1/2b, and 4b were equally common, although based on a small number of available human isolates ($n = 34$). During a possible outbreak of listeriosis in Uppsala at the beginning of the 1960s, bacteriological investigations on eggs and poultry failed to identify *Listeria* bacteria [14]; however, this investigation was conducted two decades before the foodborne route for *L. monocytogenes* was established.

L. monocytogenes isolates from 1972 to 1995

Epidemic cheese-borne strains

Of the available human isolates ($n = 403$) from 1972 to 1995, serovar 4b was shared by 234 (58·1%) and serovar 1/2a was only shared by 131 isolates (32·5%) (Table 4). During the 1980s, large outbreaks and sporadic cases of foodborne listeriosis due to closely related strains sharing serovar 4b were reported from both the USA and Europe [15]. An indistinguishable PFGE type was identified in a Swiss soft cheese outbreak between 1983 and 1987 [16], a Danish outbreak in 1985–1987 [17] and again in 1989–1990 [18], the latter probably due to blue mould cheese and, in California in 1985 due to fresh cheese [19]. One of the most common PFGE types during 1972–1995 in Sweden, shared the same PFGE type (4b:6) as the epidemic cheese-borne strains from Switzerland, Denmark, and USA [15, 20, 21].

Raw milk soft cheeses and L. monocytogenes *serovars*

One hypothesis was a majority of human *L. monocytogenes* serovar 4b isolates in Sweden, during the period 1972–1995, came from soft raw milk cheeses imported from European countries. In 1987, Beckers *et al.* [22] reported nine (65%) of 14 French soft raw milk cheeses purchased in The Netherlands were positive for *L. monocytogenes*. Eppert *et al.* [23] found 15 (46·9%) of 32 French soft raw milk cheeses sampled on the German market between 1992 and 1994 were positive for *L. monocytogenes*. Of 30 French soft raw milk cheeses purchased during 1989–1993 in Sweden, 13 (43·3%) were positive for *L. monocytogenes* [9].

In 1995, the first documented outbreak of listeriosis in France, which involved 36 patients, was linked to soft raw milk cheese, Brie de Meaux: the epidemic strain belonged to serovar 4b [24–26]. In 1997, another 14 people became ill after consuming the soft raw milk cheese Livarot, Pont l'Évêque (serovar 4b) [25, 27] and in 1999, three more cases were due to the soft raw milk cheese Epoisses (serovar 4b) [26]. These cheese brands were available on the Swedish market during 1989–1993 [9]. In Belgium in 1997, a case of listeriosis in a 73-year-old immunocompromised man was associated with the consumption of a French Camembert cheese bought a few days before he became ill: the isolates from both patient and cheese shared serovar 1/2a [28]. In 1998, de Valk *et al.* [29] stated 'soft cheese may account for a substantial proportion of sporadic listeriosis', and in a case-control study in metropolitan France during 1997, 49% of the sporadic cases of listeriosis studied could be attributed to eating soft cheese [29].

A majority of the decrease in *L. monocytogenes* in ready-to-eat foods, including cheese, in France during the 1990s is probably related to stricter control measures implemented during food production [30]. In cheese production, milking hygiene has improved and cows with *L. monocytogenes* mastitis are not used for milk production: several further precautionary measures have been implemented in raw milk cheese preparation [25, 26, 31]. The number of samples from dairy products contaminated at $\geqslant 100$ c.f.u./g decreased by 41% between 1993/1994 and 1995/1996

Table 4. *Serovar and PFGE type distribution of* L. monocytogenes *isolates in Sweden, by period, during 1958–2010*

	1958–1971			1972–1995			1996–2010		
Serovar	Isolates *n* (%)	PFGE types (*n*)	Average*	Isolates *n* (%)	PFGE types (*n*)	Average*	Isolates *n* (%)	PFGE types (*n*)	Average*
1/2a	12 (35·30)	11	1·1	131 (32·5)	51	2·6	340 (68·7)	87	3·1
1/2b	10 (29·4)	7	1·4	27 (6·7)	16	1·7	53 (10·7)	21	2·5
1/2c	2 (5·9)	2	1·0	11 (2·7)	7	1·6	8 (1·6)	4	2·0
3b	0 (0)	0	0	0 (0)	0	0	3 (0·6)	1	3·0
4b	10 (29·4)	6	1·6	234 (58·1)	21	11·1	91 (18·4)	25	3·6
Total	34 (100·0)			403 (100·0)			495 (100·0)		

PFGE, Pulsed-field gel electrophoresis.
* Average number of isolates per PFGE type.

[27]. As ready-to-eat products became less contaminated, the number of listeriosis cases in France decreased by 68% between 1987 and 1997 [27]. In France, the annual number of listeriosis cases per million population, fell from 5·2 in 1995 to 3·8 in 1996 [27]. Since 1996, serovar 4b has been reduced to the second or third most common serovar in human clinical cases in Sweden.

L. monocytogenes isolates from 1996 to 2010

Cold-smoked and gravad fish and L. monocytogenes *serovar 1/2a*

There has been a shift in human listeriosis in Sweden and *L. monocytogenes* serovar 1/2a isolates are now more frequent than serovar 4b. In Finland, serovar 1/2a isolates became dominant in 1991 [32], but 1/2a was also the major serovar in Sweden in 1988 and 1993. In Canada, a predominant *L. monocytogenes* serovar 1/2a clone caused human listeriosis cases and outbreaks during 1988–2010 [33].

Serovar 1/2a is the serovar regularly found in cold-smoked and gravad salmon purchased in Sweden and, *L. monocytogenes* has been found in gravad and cold-smoked fish samples. In a recent study 14% of each group were positive for the presence of *L. monocytogenes*, and the National Food Administration of Sweden concluded these products 'constitute the main problem' [34]. A European Union (EU)-wide survey on *L. monocytogenes* during 2010 and 2011 aimed to estimate the prevalence of *L. monocytogenes* in hot- or cold-smoked or gravad fish within the EU. Packaged (not frozen) hot- or cold-smoked or gravad fish (3053 batches) from 3632 retail outlets in 26 EU member states and one non-EU country were sampled, both on arrival at the laboratory and at the end of their shelf-life. Across the entire EU, the prevalence in fish samples was 10·4% at the time of sampling and 10·3% at the end of shelf-life. The proportion of samples with a *L. monocytogenes* count exceeding 100 c.f.u./g was 1% at the time of sampling and 1·7% at the end of shelf-life [35].

Gravad and cold-smoked salmons are vacuum-packed ready-to-eat products with a lengthy best-before date of 4–5 weeks: therefore, they constitute an optimal environment for the facultative anaerobic, psychrotrophic organism *Listeria*. The consumption of gravad and cold-smoked salmons increased markedly in Sweden during the 1990s [36]. In 1996 in Sweden, 20·7% of gravad and 11·5% of cold-smoked vacuum-packed fish purchased, especially salmon, harboured *L. monocytogenes* [10]. In the UK, a survey of retail cold-smoked fish found 236/1344 (17·4%) products were positive for *L. monocytogenes*, all samples had a level of <100 c.f.u./g [37]. The current PFGE types found in human cases of invasive listeriosis in Sweden are frequently encountered in vacuum-packed cold-smoked and gravad salmon/rainbow trout [11–13]. In Spain, a cluster of *L. monocytogenes* isolates in smoked salmon share pulsotype 1, a type recently isolated from clinical cases in Spain [38]. In Brazil, 41% of salmon samples in a gravlax salmon processing line were positive for *L. monocytogenes* [39].

A decrease or stabilization in the number of human isolates sharing serovar 4b and an increasing number of serovar 1/2a isolates have also been identified in other countries, e.g. Italy [40] and Switzerland [41].

L. monocytogenes *serovar 4b*

In Sweden, the decrease in the number of listeriosis cases caused by serovar 4b strains since 1996 might

Table 5. *Distribution of* L. monocytogenes *isolates in Sweden by gender and age during 1958–2010*

		Age (years)										
	Gender *n** (%)	1–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–99	No data
Female	354 (42·6)	4	6	14	23	30	40	73	87	65	10	1
Male	449 (54·1)	6	5	4	11	18	58	120	115	98	12	2
No data	27 (3·3)	0	0	0	0	0	1	1	0	0	0	25
Total	830 (100·0)	10	11	18	34	48	99	194	202	163	22	28

* Pregnant and newborns are excluded.

Table 6. *Serovar distribution of* L. monocytogenes *isolates in pregnant-associated cases and the elderly in Sweden, by decade, during 1958–2010*

			Serovars				Serovars		
Period	Isolates *n* (%)	Pregnant/child (*n*)	1/2a	1/2b	4b	Elderly ⩾60 years (*n*)	1/2a	1/2b	4b
1958–1969	27 (3·0)	7	0	2	4	3	1	2	0
1970–1979	101 (10·8)	33	2	1	30	34	3	4	26
1980–1989	169 (18·1)	22	5	1	16	85	33	8	38
1990–1999	219 (23·5)	22	6	1	15	145	80	8	52
2000–2010	416 (44·6)	18	11	2	5	314	233	33	43
Total	932 (100·0)	102	24	7	70	581	350	55	159

be due to precautionary measures taken during cheese production in France and other European countries. In a study from May to December 1999, only four (4·4%) of 91 French soft raw milk cheeses (red smear) were positive for *L. monocytogenes* [42]. Although this represented an improvement, the overall results from analyses of 329 European soft, semi-soft, and hard red-smear cheeses made of raw or pasteurized milk were not encouraging, as 6·4% were positive for *L. monocytogenes*. Thus, the authors concluded that red-smear cheeses should still be regarded as a considerable public health risk. A EU-wide survey in 2010 and 2011 sampled 3452 cheeses at the end of their shelf-life from retail outlets in 26 EU member states and one non-EU country. The prevalence across the entire EU was only 0·47%. The EU-level proportion of cheese samples with a *L. monocytogenes* count exceeding 100 c.f.u./g was 0·06% at the end of shelf-life [35]. During the 1990s, other food products were carriers of *L. monocytogenes* serovar 4b and caused outbreaks in France, such as pork tongue in jelly in 1992 [43] and rillettes in 1993 [44]. However, to our knowledge, those products were not generally imported into Sweden. The National Food Administration of Sweden requested two large national samplings of food products for analysis of *L. monocytogenes*; the first in 2001 [45, 46] and the second in 2010 [34]. Products such as pig tongue in aspic and rillettes were not tested by the National Food Administration nor sampled by the different Health Boards in these studies, as they are not popular foods and not generally available from retail outlets.

Historical, seasonal, and geographical variation, 1958–2010

In the collection of 932 human *L. monocytogenes* isolates from 1958 to 2010, the majority (29·5%) of serovar 1/2a isolates were isolated during October–January, with a peak in October, whereas, most (32·6%) of the serovar 4b and 1/2b isolates were isolated during June–October, with a peak in August. In the USA, more serovar 1/2a isolates are found in water during autumn and winter, whereas, serovar 4b are more often isolated during summer [47].

In the north of Sweden (Fig. 1), i.e. Jämtland (Z), Västerbotten (AC) and Norrbotten (BD), cases of listeriosis are registered almost entirely during January–March, and in the south of Sweden, i.e. Skåne (M), the majority of cases are registered during summer and autumn (Fig. 1). Of the most common PFGE types, 1/2a:4A was distributed across 17 counties

and 4b:5 was distributed across 16 counties. Conversely, PFGE types 4b:6, indistinguishable from the epidemic cheese-borne strains in Switzerland, Denmark, and USA (1983–1990), and PFGE types 4b:3 and 1/2a:4b were concentrated in Götaland (south region of Sweden), possibly due to its proximity to the European continent. In Norrland (north region of Sweden), PFGE type 4b:6 was reported in only one county, type 4b:3 was reported in three counties and 1/2a:4B in two counties (Table 1).

Some PFGE types are present over long periods, such as 1/2b:75A (1970–2010) and 4b:5 (1965–2008), whereas, others appear during limited periods, such as 1/2a:53 (1985–1995) and 1/2a:11B (1990–1994). The two currently dominant PFGE types in Sweden, 1/2a:4A and the closely related 1/2a:4B, were almost non-existent during the 1960s and 1970s (Table 2). Lukinmaa *et al.* [32] suggest a PFGE closely related to the Swedish type 1/2a:4A was common in sporadic cases in Finland from 1994 onwards. Similarly, Clark *et al.* [48] state isolates of one unique type were not seen before 2002 in Canada, but have been detected in each subsequent year. Emerging PFGE types could reflect a change in dietary habits [32].

Relationship between PFGE type and serovar

Generally, all *L. monocytogenes* isolates belonging to one PFGE type share the same serovar. However, isolates within four PFGE types were distributed in either serovar 1/2a or 1/2c, i.e. 1/2:12A (six 1/2a, nine 1/2c isolates), 1/2:12B (four 1/2a, two 1/2c), 1/2:93 (two 1/2a, two 1/2c), and PFGE type 1/2:105 (one 1/2a, one 1/2c). Although correlation between 'pulsotypes and serotypes' is reported [40], few *L. monocytogenes* isolates with indistinguishable PFGE profiles displaying different serovars (1/2a and 3a [32], 3b and 1/2b [49]) have been identified.

Age and gender, 1958–2010

Women dominated the 20–49 years age group, even though pregnant women were excluded as they are more sensitive to *L. monocytogenes* [50]: in the age range 50–89 years, the majority (59·4%) of cases were male (Table 5). Predominance of males has also been reported by Schlech [1]. Pregnancy-associated cases decreased over time (Table 6) similarly to some other countries, where decreased or constant numbers have been reported [51]. The decrease in Sweden is possibly due to dietary recommendations from the National Food Administration which has provided advice on food to pregnant women since the early 1990s. According to the dietary advice, pregnant women should avoid gravad and smoked fish at the end of its shelf-life, cheeses made from unpasteurized milk, and even some soft cheeses made from pasteurized milk, and sliced cold cuts and cold foods at the end of their shelf-life. The preponderance of serovar 4b in pregnancy-associated isolates is in agreement with other studies [51–53]. However, there was an increase in listeriosis in Sweden in the older age group ($\geqslant$60 years) which constituted 11·1% of all cases reported during 1958–1969; 33·7% in 1970–1979; 50·3% in 1980–1989; 66·2% in 1990–1999; and 75·5% in 2000–2010 (Table 6). Even in other European countries there is an increased incidence of listeriosis in the elderly population aged >60 years [51].

CONCLUSION

Between 1972 and 1995, human listeriosis in Sweden was mainly caused by serovar 4b, possibly due to the consumption of raw soft cheese imported from European countries. The number of cases caused by serovar 4b has decreased due to improved cheese-production hygiene, but since 1996, serovar 1/2a has been the dominant serovar. Based on the serovars and the PFGE types identified, an association between human cases of listeriosis and the consumption of vacuum-packed gravad and cold-smoked salmon is suggested.

ACKNOWLEDGEMENTS

This study was financially supported by the Research Foundation of Ivar and Elsa Sandberg, to whom we express our gratitude.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Schlech III WF.** Foodborne listeriosis. *Clinical and Infectious Disease* 2000; **31**: 770–775.
2. **Hülphers G.** Liver necrosis in rabbit caused by a hitherto unknown bacterium. XV International Symposium on Problems of Listeriosis, Uppsala, Sweden, 12–15 September, 2004. Abstract 147A.

3. **Larsson S, Cronberg S, Winblad S.** Clinical aspects on 64 cases of juvenile and adult listeriosis in Sweden. *Acta Medica Scandinavica* 1978; **204**: 503–508.
4. **Folhälsomyndigheten.** (http://folkhalsomyndigheten.se/amnesomraden/statistik-och-undersokningar/sjukdomsstatistik/listeriainfektion/), 2013.
5. **Todd ECD, Notermans S.** Surveillance of listeriosis and its causative pathogen, *Listeria monocytogenes*. *Food Control* 2011; **22**: 1484–1490.
6. **Parihar VS, *et al.*** Characterization of human invasive isolates of *Listeria monocytogenes* in Sweden 1986–2007. *Foodborne Pathogens and Disease* 2008; **5**: 755–761.
7. **Ericsson H, *et al.*** An outbreak of listeriosis suspected to have been caused by rainbow trout. *Journal of Clinical Microbiology* 1997; **11**: 2904–2907.
8. **Graves LM, Swaminathan B.** PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. *International Journal Food Microbiology* 2001; **65**: 55–62.
9. **Loncarevic S, Danielsson-Tham M-L, Tham W.** Occurrence of *Listeria monocytogenes* in soft and semi-soft cheeses in retail outlets in Sweden. *International Journal of Food Microbiology* 1995; **26**: 245–250.
10. **Loncarevic S, Tham W, Danielsson-Tham M-L.** Prevalence of *Listeria monocytogenes* and other Listeria species in smoked and gravad fish. *Acta Veterinaria Scandinavica* 1996; **37**: 13–18.
11. **Mandorf E.** *Listeria monocytogenes* in vacuum-packed salmon. (degree project), pp. 33 [in Swedish]. Faculty of Veterinary Medicine, SLU, Uppsala, 2003. (http://www.uppsatser.se/uppsats/003533496a/).
12. **Peiris WIP, *et al.*** Gravad (gravlax) and cold-smoked salmon, still a potential source of listeriosis. *Journal of Foodservice* 2009; **20**: 15–20.
13. **Kannius S, Karlsson J.** Microbiological analysis of three risk products (degree project), MÅ1607 [in Swedish]. School of Hospitality, Culinary Arts and Meal Sciece, Örebro University, 2010 (http://oru.diva-portal.org/smash/record.jsf?pid=diva2:352352).
14. **Ekelund H, *et al.*** Listeria infection in the foetus and the new-born: a clinical, pathological and epidemiological study. *Acta Paediatrica* 1962; **51**: 698–711.
15. **Kathariou S.** *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. *Journal of Food Protection* 2002; **65**: 1811–1829.
16. **Bille J, Glauser MP.** Listeriosis in Switzerland [in German]. *Bullettin des Bundesamtes für Gesundheitswesen* 1988; **3**: 28–29.
17. **Samuelsson S, *et al.*** Human listeriosis in Denmark 1981–1987 including an outbreak November 1985–March 1987. *Journal of Infection* 1990; **20**: 251–259.
18. **Jensen A, Frederiksen W, Gerner-Smidt P.** Risk factors for listeriosis in Denmark 1989–1990. *Scandinavian Journal of Infectious Disease* 1994; **26**: 171–178.
19. **Linnan MJ, *et al.*** Epidemic listeriosis associated with Mexican-style cheese. *New England Journal of Medicine* 1988; **319**: 823–828.
20. **Buchrieser C, *et al.*** Pulsed-field gel electrophoresis applied for comparing *Listeria monocytogenes* strains involved in outbreaks. *Canadian Journal of Microbiology* 1993; **39**: 395–401.
21. **Ericsson H, *et al.*** Division into five groups by REA of the most frequently isolated phagovar of *Listeria monocytogenes* in Sweden 1976–1985. *Medical Microbiology Letters* 1996; **5**: 145–155.
22. **Beckers HJ, Soentoro PSS, Delfgou-van Asch EHM.** The occurrence of *Listeria monocytogenes* in soft cheeses and raw milk and its resistance to heat. *International Journal of Food Microbiology* 1987; **4**: 249–256.
23. **Eppert I, *et al.*** Listeria and coliforms in 'real' and 'wrongly declared' raw milk soft cheeses [in German]. *Archiv für Lebensmittelhygiene* 1995; **46**: 85–88.
24. **Goulet V, *et al.*** Listeriosis from consumption of raw-milk cheese. *Lancet* 1995; **345**: 1581–1582.
25. **de Valk H, Vaillant V, Goulet V.** Epidemiology of human listeriosis in France [in French]. *Bulletin de l'Académie Nationale de Médecine* 2000; **184**: 267–274.
26. **De Buyser M-L, *et al.*** Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. *International Journal of Food Microbiology* 2001; **67**: 1–17.
27. **Jacquet Ch, *et al.*** Human listeriosis in France in 1997 [in French]. *Bulletin Épidémiologique Hebdomadaire* 1998; **33**: 142–143.
28. **Gilot P, *et al.*** Sporadic case of listeriosis associated with the consumption of a *Listeria monocytogenes*-contaminated 'Camembert' cheese. *Journal of Infection* 1997; **35**: 195–197.
29. **de Valk HM, *et al.*** Risk factors for sporadic listeriosis in France. Programme and abstracts – XIII International Symposium on Problems of Listeriosis, Halifax, 28 June–2 July 1998. Abstract no. 22.
30. **Goulet V, *et al.*** Effect of prevention measures on incidence of human listeriosis, France, 1987–1997. *Emerging Infectious Diseases* 2001; **7**: 983–989.
31. **Sanaa M, Coroller L, Cerf O.** Risk assessment of listeriosis linked to the consumption of two soft cheeses made from raw milk: Camembert of Normandy and Brie of Meaux. *Risk Analysis* 2004; **24**: 389–399.
32. **Lukinmaa S, *et al.*** *Listeria monocytogenes* isolates from invasive infections: variation of sero- and genotypes during an 11-year period in Finland. *Journal of Clinical Microbiology* 2003; **41**: 1694–1700.
33. **Knabel SJ, *et al.*** Sequence typing confirms that a predominant *Listeria monocytogenes* clone caused human listeriosis cases and outbreaks in Canada from 1988–2010. *Journal of Clinical Microbiology* 2012; **50**: 1748–1751.
34. **Thisted Lambertz S, *et al.*** Prevalence and level of *Listeria monocytogenes* in ready-to-eat foods in Sweden 2010. *International Journal of Food Microbiology* 2012; **160**: 24–31.
35. **European Food Safety Authority.** Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods in the EU, 2010–2011, Part A: *Listeria monocytogenes* prevalence estimates. *EFSA Journal* 2013; **11**(6): 3241, 75 pp.

36. **Loncarevic S.** *Listeria monocytogenes* with special reference too food products and human listeriosis. Acta Universitatis Agriculturae Suecia. Veterinaria 31 (doctoral thesis), Uppsala, Sweden 1998.
37. **ACMSF (Advisory Committee on the Microbiological Safety of Food).** *Ad Hoc* Group on Vulnerable Groups. Report on the increased incidence of listeriosis in the UK. Published by Food Standards Agency July 2009. FSA/1439/0709, 86 pp.
38. **Garrido V, *et al.*** Surveillance of listeriosis in Navarre, Spain, 1995–2005 – epidemiological patterns and characterisation of clinical and food isolates. *Eurosurveillance* 2008; **13**: 1–6.
39. **Cruz CD, *et al.*** Epidemiological survey of *Listeria monocytogenes* in a gravlax salmon processing line. *Brazilian Journal of Microbiology* 2008; **39**: 375–383.
40. **Gianfranceschi MV, *et al.*** Distribution of serotypes and pulsotypes of *Listeria monocytogenes* from human, food and environmental isolates (Italy 2002–2005). *Food Microbiology* 2009; **26**: 520–526.
41. **Pak SI, *et al.*** Risk factors for *L. monocytogenes* contamination of dairy products in Switzerland, 1990–1999. *Preventive Journal of Veterinary Medicine* 2002; **53**: 55–65.
42. **Rudolf M, Scherer S.** High incidence of *Listeria monocytogenes* in European red smear cheese. *International Journal of Food Microbiology* 2001; **61**: 91–98.
43. **Jacquet C, *et al.*** Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992. *Applied Environmental Microbiology* 1995; **61**: 2242–2246.
44. **Goulet V, *et al.*** Listeriosis outbreak associated with the consumption of rillettes in France in 1993. *Journal of Infectious Disease* 1998; **177**: 155–160.
45. **Rosengren Å.** Information for municipalities 2001–11–06: National Project 2001, Listeria monocytogenes in refrigerated ready to eat food. Swedish National Food Administration, reg. 1987/00, 2001, pp. 11.
46. **Rosengren Å, Lindblad M.** National Project 2001. *Listeria monocytogenes* in refrigerated ready to eat food. Swedish National Food Administration. Report 13, 2003, pp. 37.
47. **Orsi RH, den Bakker HC, Wiedmann M.** *Listeria monocytogenes* lineages: genomics, evolution, ecology, and phenotypic characteristics. *International Journal of Medical Microbiology* 2011; **301**: 79–96.
48. **Clark CG, *et al.*** Surveillance for *Listeria monocytogenes* and listeriosis, 1995–2004. *Epidemiology and Infection* 2010; **138**: 559–572.
49. **Revazishvili T, *et al.*** Comparative analysis of multilocus sequence typing and pulsed-field gel electrophoresis for characterizing *Listeria monocytogenes* strains isolated from environmental and clinical sources. *Journal of Clinical Microbiology* 2004; **42**: 276–285.
50. **Okike IO, Lamont RF, Heath PT.** Do we really need to worry about Listeria in newborn infants? *Pediatric Infectious Disease Journal* 2013; **32**: 405–406.
51. **Goulet V, *et al.*** Increasing incidence of listeriosis in France and other European countries. *Emerging Infectious Diseases* 2008; **14**: 734–740.
52. **McLauchlin J.** Distribution of serovars of *Listeria monocytogenes* isolated from different categories of patients with listeriosis. *European Journal of Clinical Microbiology and Infectious Disease* 1990; **9**: 210–213.
53. **Doorduyn Y, *et al.*** Invasive *Listeria monocytogenes* infections in the Netherlands, 1995–2003. *European Journal of Clinical Microbiology and Infectious Diseases* 2006; **25**: 433–442.
54. **Statistics Sweden.** (www.scb.se), 2013.