DIVERSITY OF *LISTERIA MONOCYTOGENES* ISOLATED FROM HUMANS, FOOD, AND ENVIRONMENTAL SOURCES IN NORWAY

Olav Rosef[1,2], Halvdan Klæboe[1], Algimantas Paulauskas[2], Daiva Ambrasienė[2]
[1]*ATP-Innovation, 3800 Bø i Telemark, Norway. Tel. +4791714454, E-mail: orosef@online.no*
[2]*Vytautas Magnus University, Department of Biology*
Vileikos str. 8, Kaunas LT-44404; Lithuania
Tel. +370 37 32 79 02; Fax. +370 37 32 79 16; E-mail: a.paulauskas@gmf.vdu.lt

Abstract. A total of 325 *Listeria monocytogenes* isolates from human, food and environmental sources were ribotyped with the Qualicon Automated Riboprinter Microbial Characterization System, using the enzyme E*co*R1. The human isolates (n=137) represented all isolates from clinical cases in Norway from 1992 to 2005. The food and environmental isolates (n=188) were collected from different food related sources in Norway in the period 1989–2002. A total of 37 ribotypes were differentiated. Most common ribotypes (i.e., ribotypes represented by >5 isolates) were isolated from human as well as food and environmental sources. The exceptions were ribotypes DUP-1062D and 1058A, which were found only among food and environmental samples (14 and 5 times, respectively), and DUP-1042A, which was identified only among human clinical isolates. DUP-1030A, the most frequent ribotype among the human isolates, as well as ribotypes DUP-1042B, DUP-1042C, DUP-1049B and DUP-1062B were identified from both foods, environmental and human sources. DUP-1039C (n=54) and DUP-1045B (n=27) were frequently isolated from patients, food and environmental samples.

The isolates were classified into lineages based on ribotyping results. The lineage I strain DUP-1038B was isolated every year from 1992 to 2005 from the human clinical samples. Out of 137 listeriosis cases, 76 (55.6%) were caused by lineage II strains. We found a considerable overlap between ribotypes, lineages and isolation sources. It does not seem possible to establish food strain specific regulations for *L. monocytogenes* based on ribotyping.

Keywords: *Listeria monocytogenes*, human isolates, environmental isolates, food safety, ribotyping, lineages.

LISTERIA MONOCYTOGENES, IŠSKIRTŲ IŠ LIGONIŲ, MAISTO IR APLINKOS ŠALTINIŲ, ĮVAIROVĖ NORVEGIJOJE

Olav Rosef[1,2], Halvdan Klæboe[1], Algimantas Paulauskas[2], Daiva Ambrasienė[2]
[1]*3800 Bø i Telemark, Norway.*
[1]*ATP-Innovation,, N-3800 Bø Telemarkas, Norvegija; tel. +479 171 4454; el. paštas: orosef@online.no*
[2]*Biologijos katedra, Vytauto Didžiojo universitetas, Vileikos g. 8, Kaunas LT-44404*
tel. +370 37 32 79 02; faks. +370 37 32 79 16; el. paštas: a.paulauskas@gmf.vdu.lt

Santrauka. 325 *Listeria monocytogenes* izoliatai iš žmogaus, maisto ir gamtinės aplinkos šaltinių išanalizuoti automatine „DuPont Qualicon RiboPrinter®“ sistema, naudojant E*co*R1 fermentus. Visi žmogaus izoliatai (n=137) paimti 1992–2005 metais iš ligonių. Maisto ir gamtinės aplinkos izoliatai surinkti Norvegijoje 1989–2002 metais iš skirtingų su maistu susijusių šaltinių. Iš viso identifikuotos 37 *L. monocytogenes* padermės. Dažniausiai aptinkamos padermės išskirtos tiek iš žmogaus, tiek ir iš maisto bei aplinkos. Išimtis – padermės DUP-1062D ir 1058A, rastos maiste ir aplinkoje (atitinkamai 14 ir 5 atvejai), o DUP-1042A aptikta tik ligonių izoliatuose. DUP-1030A daržniausiai randama tarp žmogaus izoliatų, o DUP-1042B, DUP-1042C, DUP-1049B ir DUP-1062B – visuose šaltiniuose. Iš ligonių, maisto ir aplinkos išskirtų izoliatų dažniausios padermės buvo DUP-1039C (n=54) ir DUP-1045B (n=27). Izoliatai pagal gautus rezultatus suskirstyti į dvi gimininges linijas. I linijos padermė DUP-1038B iš ligonių išskirta kiekvienais 1992–2005 metais. Iš 137 listeriozės atvejų – 76 (55,6 proc.) priklausė II linijos sukėlėjams. Nustatyta ryški priklausomybė tarp padermių, gimininių linijų ir išskyrimo šaltinių.

Raktažodžiai: *Listeria monocytogenes*, izoliatai iš žmogaus ir aplinkos, giminingumas, prietaisas „RiboPrinter®“, maisto sauga.

Introduction. *Listeria. monocytogenes* infections have been responsible for the highest hospitalisation rates (91%) amongst known food-borne pathogens and have been linked to sporadic episodes and large outbreaks of human illness worldwide (Jemmi and Stephan 2006). Listeriosis affects primarily pregnant women, older adults and persons with weakened immune systems (e.g., due to diabetes, organ transplants, cancer, AIDS) (McLauchlin et al. 2004). *L. monocytogenes* may cause invasive disease such as bacteremia, meningitis, and severe prenatal infections (Gellin and Broome 1989). Infections during pregnancy may lead to premature delivery, infection of the newborn, or even stillbirth (Lindemann 1990). Listeriosis is a significant public health concern because of a mortality rate of about 30% (Cossart 1998). It is estimated to 255 deaths (19% of the listeriosis cases) in USA in 2011 (www.cdc.gov/foodborneburden/surveillance-systems).

The incidence of human listeriosis in Norway is at a low and fairly stable level with between 15 and 30 cases per year reported in the last 10 years (http://www.msis.no/). An outbreak, which involved six reported cases traced to contaminated, vacuum packed

cold cuts from a Norwegian meat producer, occurred in 1992 (Lassen and Caougant 1992). In 2005, a hospital outbreak with three cases was reported, probably caused by cold cuts, as the same strain of *L. monocytogenes* was isolated from both the patients and on a slicing machine in the hospital kitchen (Hofshagen et al. 2005). In 2007, 50 persons were diagnosed with listeriosis in Norway. The relatively high number of cases in this year was caused by a hospital outbreak in Oslo, Norway, where 17 patients became ill after consumption of contaminated soft cheese. Five of the patients died (http://www.fhi.no/).

L. monocytogenes is regularly isolated from meat, poultry and fish processing plants (Heir et al. 2004, Nesbakken et al. 1996, Rørvik et al. 2003, Senczek et al. 2000. Klæboe et al. 2005), and is a pathogen of major concern to the industry. It is more resistant than most foodborne pathogens to heat, salt, nitrite and acidity (Doyle et al. 2001), and can survive in industrial environments for years (Hoffman et al. 2003, Rocourt et al. 2001). It may enter the processing environment through a wide range of sources, and the production environment represents the most important source for contamination of the final product (Autio et al. 1999, Rørvik et al. 1997). This represents a serious problem because of the ability of this pathogen to survive and grow at refrigeration temperature (Farber and Peterkin 1991).

L. monocytogenes has emerged as the leading cause of food recalls due to microbiological concerns. The economic impact may be considerable. As an example, *L monocytogenes* contamination led a US poultry producer to recall 27.4 million pounds of fresh and frozen poultry products in 2002 (CDC 2002). The European Commission has a limit of 100 cfu/g *L. monocytogenes* throughout the shelf-life for RTE- foods able to support growth of the bacterium. This recommendation is based on epidemiological data indicating that *L. monocytogenes* represents a very low risk for all population groups when the concentration is below 100 cfu/g (Chen et al. 2003).

L. monocytogenes is a highly diverse species. It has been divided into at least four different genetic lineages, which have been suggested to differ in their pathogenic potential. Lineage I strains have been linked to the majority of human listeriosis outbreaks worldwide (Heir et al. 2004, Jeffers et al. 2001, Norton et al. 2001b, Sauders et al. 2006, Ward et al. 2004, Wiedmann et al. 1997). Lineage II strains are more common among food isolates than clinical cases (Gray et al. 2004, Orsi et al. 2011), suggesting that these isolates may be better adapted to non-host environments. Lineage III and IV strains are predominantly isolated from animal clinical cases and are only rarely isolated from environmental and food samples or human clinical cases (Jeffers et al. 2001, Roberts et al. 2006, Tsai et al. 2011, den Bakker et al. 2012). *L. monocytogenes* lineages thus may have adapted to different host and non-host associated ecological niches. Numerous methods can classify *L. monocytogenes* into the three lineages. Ribotyping is standardized and reliable and lineage classification through ribotyping correlates well with more time-consuming methods e.g. pulsed field gel electrophoresis (PFGE) (Brosch et al. 1994), *act*A sequencing (Zhou and Jiao 2005), or sequence data for *sig*B (Moorhead et al. 2003) *fla*A, *iap* and *hly* genes (Rasmussen et al. 1995).

With the automated riboprinting procedure, human clinical strains can easily be compared with food and environmental isolates. The aims of this study were to compare *L. monocytogenes* ribotypes from environmental, food and human clinical cases in order to explore possible associations between food sources and human listeriosis cases and evaluate the possibility to exclude certain *L. monocytogenes* ribotypes from industry regulations.

Material and methods

Bacterial strains. The human clinical listeriosis isolates (n=137) represented all collected in Norway between 1992 and 2005 by The National Institute of Public Health. From fish, poultry, meat and environmental sources, a total of 90, 26, 43 and 29 isolates were ribotyped, respectively. The meat isolates (n=40) were obtained from Animalia, Oslo, Norway, and were isolated from samples collected in four processing plants (sample years 1989 – 1993 and 1998 – 2002). Other food and environmental isolates (n=148) were supplied by Norwegian School of Veterinary Science. The isolates represented were obtained from 84 processing plants or environmental sites. For 55 of these sources only one isolate was analysed, from the remaining sources from two to 12 isolates were examined. When more than one isolate were examined, they differed in serotype, ET-REA-type, as determined earlier by multilocus enzyme electrophoresis and microrestriction enzyme analysis (Rørvik et al. 2000), sample type and/or date of isolation. From fish, poultry, meat and environmental sources, a total of 90, 26, 43 and 29 isolates were ribotyped, respectively. All *L. monocytogenes* isolates were maintained at - 80°C.

Ribotyping. All isolates were characterized by automated ribotyping, and classification to lineage, based on the ribotypes, was performed as previously reported. Ribotype patterns were obtained with the Automated Qualicon Riboprinter® Microbial Characterization System, following the manufacturer's instructions (Qualicon Inc., Wilmington, Del.). Cells were grown overnight on blood agar plates at 37°C and colony picks were transferred to a lysis buffer where the cells were inactivated by incubation for 10 minutes at 90°C and placed in the Riboprinter®. The Riboprinter® carried out restriction digest of chromosomal DNA, separated the restriction fragments by agarose gel electrophoresis, transferred the fragments to a nylon membrane, probed the membrane with a chemiluminescent ribosomal probe and recorded the image produced. Restriction enzyme E*co*R1 was used on all isolates. Each E*co*R1 pattern was compared to a library of ribotype patterns supplied by Qualicon (the DUP-ID library) and placed in groups defined by pattern similarity > 0.85% (i.e. *L. monocytogenes* DUP-1025). Each ribotype pattern was also independently compared to all other isolate patterns generated, and these isolate-to-isolate comparisons were used to define "ribogroups." The ribogroups generated by this internal comparison differs from the groupings generated from comparison with the DuPont ID pattern library because the criteria for estab-

lishing ribogroups are more stringent. The similarity threshold for an isolate joining a ribogroup is an adaptive value between 0.90 and 0.96, depending of the size of the ribogroup. If one DUP-ID contained more than one ribogroup, the DUP-ID was divided into subgroups with an additional alphabetical letter (i.e. DUP-1025B). A ribotype not recognized by the DuPont database was automatically given a name according to the machine-, batch-, and lane-number of the actual gel (i.e. 181-88-S-1).

Clustering of ribotypes. Images generated by the Riboprinter® were saved in TIFF format and transferred to the GelCompar® II software (Applied Maths, Sint-Martens-Laten, Belgium) for computer analysis. One typical isolate was selected to represent each ribotype in the cluster analysis. Similarity between the ribotypes was determined by Pearson correlation using default settings for optimization (1.56%) and position tolerance (1.00%). Manual adjustments were done after visual inspection. The dendrogram was generated by the unweighted pair group method with arithmetic averages (UPGMA).

Serotyping: *L .monocytogenes* isolates were serotyped by Bacto-Listeria-O antisera serotype 1 and 4 (Difco Laboratories, Detroit, Mi., USA)

Statistical analysis: Association between source and lineage was compared and analysed statistically by means of Pearson`s $\chi 2$ test using the statistical package STATISTICA for Windows 5.5.

Results

Ribotypes were determined for a total of 325 *L. monocytogenes* isolates. The 26 DUP-ID's identified by the Riboprinter®'s software were classified into 37 "modified DUP-ID's" after visual evaluation; for the rest of this manuscript, we will refer to these 37 ribotypes. Of the 37 ribotypes, 22 different ribotypes were found among the human clinical isolates (n=137) and 33 ribotypes were found among the food and environmental isolates (n=188) (Table 1). Eight ribotypes were represented by >10 isolates (14-58 isolates per ribotype), while the remaining twenty-nine ribotypes represented between 1 and 7 isolates. DUP-1039C and DUP-1030A were found from every source investigated and were the most common ribotype, representing 58 (18%) and 54 (17%) of the total isolates, respectively (Table 1). Six isolates representing four ribotypes were not recognized by the DuPont database.

The five ribotypes most prevalent (n>10) among the human isolates represented 90 out of 137 isolates (Table 2). DUP-1030A was the most frequently isolated ribotype (n=31) followed by DUP-1038B (n=22) and DUP-1042B and DUP-1042C (n=12), respectively.

A total of 57 (41.6%) of the human isolates, were assigned to lineage I while76 (55.6%) were assigned to lineage II. Human isolates were associated with lineage I (χ^2 =17.53, p< 0.0001). Among the food isolates, 36 were classified into lineage I and 119 into lineage II. Eight of the isolates could not be classified into lineage (Table 1). Food and environmental isolates were associated with lineage II (χ^2 =15.29, p= 0.0001).

The ribotypes could be divided into three main clusters (Fig 1). Cluster 1 and 3 consisted of lineage I isolates, while cluster 2 was lineage II isolates. One exception, DUP-1062D which is a lineage II strain, was assigned in the lineage I-cluster 1. This strain (n=14) was isolated from ready to eat food, raw food and production environment but were not found among the human isolates. Isolates that were not assigned to any lineage were evenly spread among the 3 clusters. DUP-1035 belonging to lineage II isolated one time from ready to eat food could not be clustered

A total of 66% of the human listeriosis cases from 1992–2005 were caused by only five ribotypes. Clusters (≥4 isolates) of certain ribotypes were seen in five different years (Table 2). One of them represents a reported outbreak (1992), while the others have not been recognized as outbreaks.

The 8 most common ribotypes, except of DUP-1062D, which were exclusively isolated from food sources (n=14), were found among human as well as food and environmental isolates. These represented 81% of the isolates (n=325).There was a considerable overlap between the ribotypes and sources. There was a significant correlation between ribotypes lineage I from patients and meat production environment and ready to eat food and between fish, ready to eat, fish production environment, meat production environment and meat ready to eat. No clear correlation between ribotypes from patients and raw food was observed (Table 3).

The distribution of serotypes is shown in Figure 1. Serotype 4 was only found among lineage I isolates.

Discussion

We compared ribotypes among human clinical isolates to those observed in different foods and in the environment to evaluate the hypothesis that some *L. monocytogenes* ribotypes may be adapted to specific niches. Isolates from Norwegian foods and food processing environment and human clinical cases showed great diversity. The 325 samples were divided into 37 different ribotypes as shown in Table 1. In spite of this diversity, most human infections in Norway in the last years were caused by only a few ribotypes and 66% of the human listeriosis cases were caused by only five ribotypes as shown (Table 2). This agrees with the findings done by Gray et al. (2004), who describe the eight most common ribotypes from their survey on listeriosis isolates. Four of these ribotypes (DUP-1038B, DUP-1039C, DUP-1042B and DUP-1042C) are among the most prevalent ribotypes in the present material (Table 2). Clusters (≥4 isolates) of certain ribotypes were seen in five different years DUP 1042C and represented a reported outbreak (1992), while the others have not been recognized as outbreaks (Table 2). A range of the ribotypes (17) were identified from both patients and food sources (Table 1, Fig 1), but since no dietary history of the patients was available, it remains unknown whether these foods have been sources for human illness. We, however, found significant correlation between strains from food products, production environment and patients (Table 3). This may be the reason for the high number of infected patients with lineage II strains.

Table 1. **Distribution of the 325 *L. monocytogenes* strains isolated from patients, food sources and the environment.** DUP-ID refers to the DuPont Microbial Database. Patterns were visually evaluated and assigned into specific subtypes within a given DUP ID (e.g. DUP-1042A, 1042B) if a given DUP-ID included multiple distinct ribotype patterns. R= raw food, PE = production environment, RTE = ready to eat food, OF = other food, E = environment

DUP-ID	Lineage	Patients	Fish			Chicken		Meat			Etc.		Total
		1992–2005	R	PE	RTE	R	RTE	R	PE	RTE	OF	E	
1025B	I	1											1
1027B	I	4			1								5
1038B	I	22	1	2	4	1			2	3		1	36
1042A	I	6									1		7
1042B	I	12	2	1		2			1	1			19
1042C	I	12		2	3	2			2	2		1	24
1044E	I				1								1
1051D	I			1	1								2
1052A	I	2											2
1035	II				1								1
1041	II	1							1				2
1047	II											1	1
1050	II	1						1		1			3
1030A	II	31		1	9	6		2	4	4		1	58
1030B	II	1				1					1		3
1039C	II	14	2	4	6	8	1	3	1	5	1	9	54
1039D	II	5		1								1	7
1039E	II	2											2
1045A	II	2		1	2	1							6
1045B	II	7	1	4	7				1	1		6	27
1046A	II	1										2	3
1048A	II											1	1
1048B	II	1				1							2
1048D	II				1								1
1049B	II	3	1		1			1				1	7
1053B	II			1	2					1			4
1053C	II		2	1									3
1058A	II			1	3							1	5
1058C	II				1				1				2
1062B	II	7	2	1	3							1	14
1062D	II			4	5	1		2	1	1			14
19157	**					1							1
16635B	**				1								1
116-239-S-2*	**			1									1
116-931-S-1*	**	1											1
181-84-S-3*	**				1								1
181-88-S-1*	**	1					1			1			3
Total		137	11	26	53	24	2	9	14	20	3	26	325

*=Not recognized by the DuPont identification database
**=Not classified into lineage

Multiple lines of evidence support the conclusion that the ribotypes DUP-1038B and DUP-1042B are widely distributed and have a high likelihood of causing human disease (Gray et al. 2004, Jeffers et al. 2001, Sauders et al. 2006). Norton et al. (2001b) found that these two ribotypes represented almost 35% of their human isolates, and were seven times more likely to be isolated from human clinical samples than from smoked fish industry. These ribotypes were found in cluster 3 and cluster 1, respectively (Fig 1). In the present study we found the same ribotypes in 36 out of 137 human clinical isolates and 21 out of 188 food and environmental isolates. The most prevalent ribotype (DUP-1030A) was frequently identified from both food, environmental, and human clinical samples. This agrees with the findings by Gray et al. (2004). The second most common ribotype (DUP-1039C) was found from 23% of the food and environment samples and from 10% of the human clinical samples. A previous study done in the Nordic countries concluded that this ribotype was the most common ribotype found in the food industry, being present in 10 of the 12 food processing plants investigated (Suihko et al. 2002). In a survey

done in America, DUP-1039C was isolated from 48.9% of the samples (Norton et al. 2001a). This shows that some *L. monocytogenes* ribotypes are globally distributed with a high prevalence. On the other hand, Sauders et al. (2004) found DUP-1042C to be exclusively isolated from foods, while in the present study this ribotype was found in 12 out of the human isolates (n=137) and 12 out of 188 food and environmental isolates (Table 1). In a survey done by Gray et al. (2004), DUP-1062A was the most common ribotype found among the food isolates. This ribotype was not isolated at all from the 188 food and environmental samples in the present study, nor among 226 smoked salmon isolates ribotyped in another survey done earlier in Norway (Klæboe et al. 2010). In another survey done in Norway, a rare ribotype (DUP-1023C) was dominating in two salmon processing plants (Klæboe et al. 2006) with 50 of 110 isolates. This is an uncommon ribotype found only sporadically elsewhere; supporting certain subtypes may be largely plant and/or location specific.

Fig. 1. **Eco**R1 **patterns and clustering of 37 different *L. monocytogenes* ribotypes.** DUP-ID numbers refer to the DuPont Identification Database; patterns were visually evaluated and assigned into specific subtypes within a given DUP ID (e.g.,.DUP-1042A, 1042B). RTE= ready to eat food, R= raw food, PE = production environment, E = environment; * indicates that lineage of a given ribotype is not known.

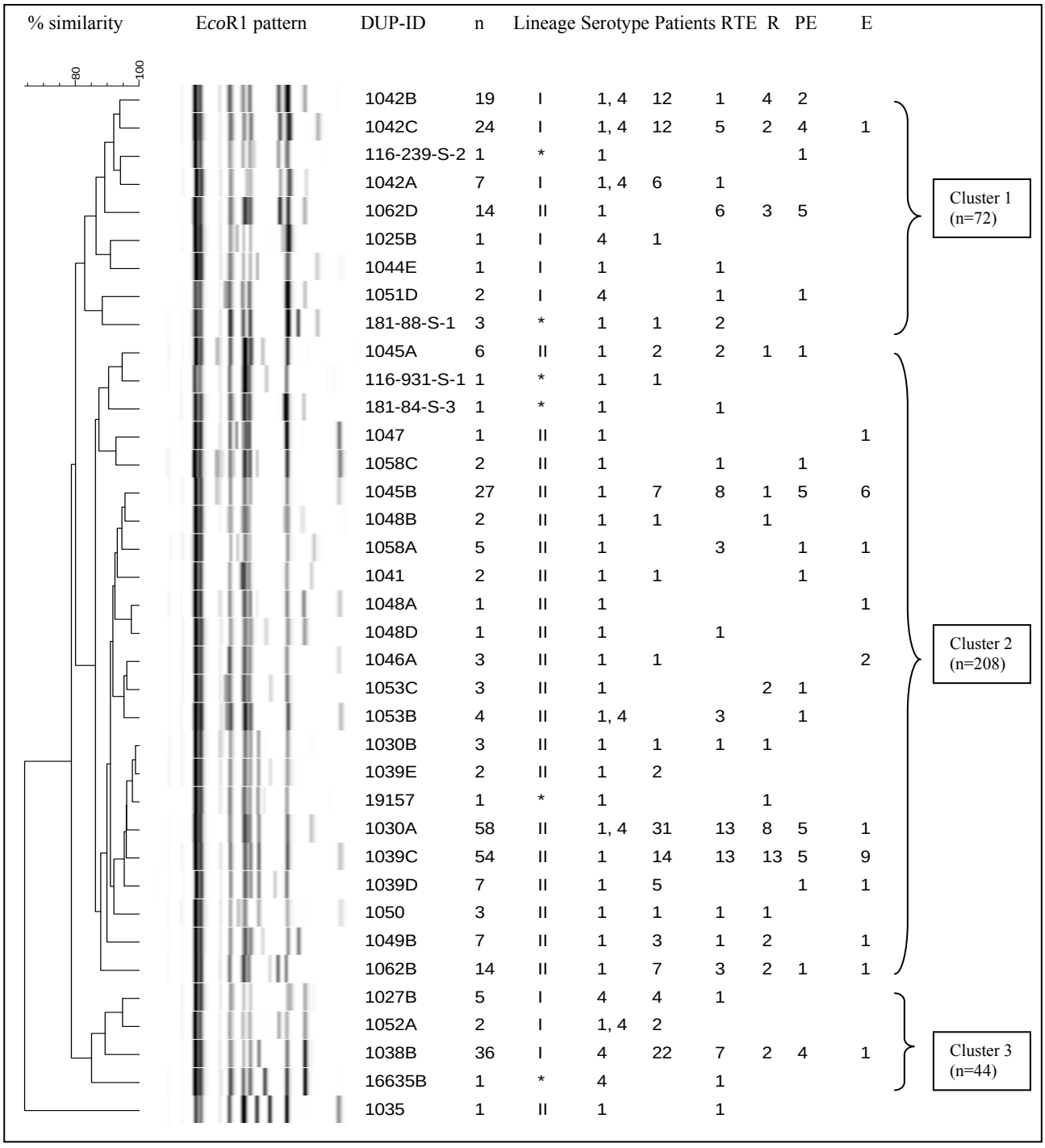

Table 2. **The five most prevalent *L. monocytogenes* ribotypes** (n >10) **isolated from human listeriosis cases reported in Norway from 1992 to 2005**

DUP-ID	1030A	1038B	1039C	1042B	1042C	Total
Lineage	II	I	II	I	I	
Patients 1992	1	3	1		6	11
Patients 1993	1	1			1	3
Patients 1994	1	2				3
Patients 1995	3	2		5		10
Patients 1996		3	1	2		6
Patients 1997	1	3				4
Patients 1998		1	1			2
Patients 1999		2	1	1	1	5
Patients 2000	2	1			1	4
Patients 2001	1	1	2	1		5
Patients 2002	1	2	6	1	1	11
Patients 2003	4	1				5
Patients 2004	12		1	1	1	15
Patients 2005	4			1	1	6
Total	31	22	14	12	12	90

Table 3. **The association between ribotypes and correlation** (Sherman's r(p) **from patients and food sources; lineage I - below diagonal, lineage II - above diagonal n- number of isolates**

		Patients	FishR	FishPE	FishRTE	ChicR	ChicRTE	MeatR	MeatPE	MeatRTE
Patients	r(p)	1	0,418	0,328	0,301	0,368	0,341	0,364	0,294	0,323
	n		22	22	22	22	22	22	22	22
FishR	r(p)	0,656	1	0,455*	0,328	-0,006	0,446*	0,229	0,120	0,182
	n	9		22	22	22	22	22	22	22
FishPE	r(p)	0,600	0,543	1	0,724**	0,362	0,364	0,325	0,413	0,595**
	n	9	9		22	22	22	22	22	22
FishRTE	r(p)	0,273	0,085	0,652	1	0,367	0,308	0,438*	0,546**	0,603**
	n	9	9	9		22	22	22	22	22
ChicR	r(p)	0,783*	0,696*	0,821**	0,350	1	0,462*	0,531**	0,404	0,445*
	n	9	9	9	9		22	22	22	22
ChicRTE	r(p)						1	0,492*	0,332	0,462*
	n	-	-	-	-	-		22	22	22
MeatR	r(p)							1	0,490*	0,718**
	n	-	-	-	-	-			22	22
MeatPE	r(p)	0,829**	0,655	0,887**	0,556	0,946**			1	0,599**
	n	9	9	9	9	9	-	-		22
MeatRTE	r(p)	0,839**	0,705*	0,882**	0,564	0,923**			0,994	1
	n	9	9	9	9	9	-	-	9	22

** - Correlation is significant at the 0.01 level (2-tailed)
* - Correlation is significant at the 0.05 level (2-tailed)

A previous study done in the Nordic countries concluded that this ribotype was the most common ribotype found in the food industry, being present in 10 of the 12 food processing plants investigated (Suihko et al. 2002). In a survey done in America, DUP-1039C was isolated from 48.9% of the samples (Norton et al. 2001a). This shows that some *L. monocytogenes* ribotypes are globally distributed with a high prevalence. On the other hand, Sauders et al. (2004) found DUP-1042C to be exclusively isolated from foods, while in the present study this ribotype was found in 12 out of the human isolates (n=137) and 12 out of 188 food and environmental isolates (Table 1). In a survey done by Gray et al. (2004), DUP-1062A was the most common ribotype found among the food isolates. This ribotype was not isolated at all from the 188 food and environmental samples in the present study, nor among 226 smoked salmon isolates ribotyped in another survey done earlier in Norway (Klæboe et al. 2010). In another survey done in Norway, a rare ribotype (DUP-1023C) was dominating in two salmon processing plants (Klæboe et al. 2006) with 50 of 110 isolates. This is an uncommon ribotype found only sporadically elsewhere; supporting certain subtypes may be largely plant and/or location specific

The results of ribotyping were used to assign the isolates into lineages (Table 1). The majority of human listeriosis outbreaks worldwide have been linked to lineage I. (Orsi et al. 2011). Comparisons of the relative frequencies of *L. monocytogenes* lineages I and II from human listeriosis cases have consistently found that lineage I is overrepresented among human clinical isolates (Jeffers et al. 2001, Gray et al. 2004, Ward et al. 2004). Sauders et al. (2006) found a significant association between lineage I and human isolates, and the common consensus has been that human clinical isolates of *L. monocytogenes* are associated with lineage I. In the present study, 55.6% of the human clinical isolates surprisingly were classified as lineage II, while 41.6% of the human isolates were assigned to lineage I. The association between lineages and human listeriosis cases, however, may vary by regions. In particular lineage II (serotype 1/2a) strains appear to be more common in Northern Europe (Lukinmaa et al. 2003, Parihar et al.. 2008) and are in accordance with the results of the present study. The genetic diversity is showed in Fig 1. Cluster 1 and 3 consisted of lineage I isolates, while cluster 2 was lineage II isolates. One exception, DUP-1062D which is a lineage II strain was assigned in the lineage I-cluster 1. This strain (n=14) was isolated from ready to eat food, raw food and production environment but were not found among the human isolates.

The ribotyping, however, has limited discriminatory power (Fugett et al. 2007) but the automated riboprinting is rapid, reliable and reproducible, and the data are stored and automatically compared to other ribotypes. In conclusion, the population of *L. monocytogenes* from Norwegian patients and foods is very diverse. Epidemiological data is lacking, but we suggest that contaminated products might be source for listeriosis cases in Norway. Control measures that prevent the occurrences of high levels of all subtypes of *L. monocytogenes* contamination at consumption still has to be the most important strategy to reduce the rates of human listeriosis cases (FAO/WHO, 2004).

Acknowledgements

The bacterial strains from the human clinical cases were kindly made available by The Norwegian Institute of Public Health. We thank Animalia, Oslo, and Norwegian Veterinary College, Oslo for providing the isolates from meat, food and the environmental sources. For helpful comments and improvements of the manuscript and help to interpret the ribotypes we thank Professor Martin Wiedmann, Department of Food Science, Cornell University, Ithaca.

References

1. Autio T., Hielm S., Miettinen M., Sjoberg A. M., Aarnisalo K., Bjorkroth J., Mattila-Sandholm T., Korkeala H. Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. Appl Environ Microbiol, 1999. Vol. 65. P. 150–155.

2. Brosch R., Chen J. and Luchansky J. B. Pulsed-field fingerprinting of listeriae: identification of genomic divisions for *Listeria monocytogenes* and their correlation with serovar. Appl Environ Microbiol, 1994. Vol. 60. P. 2584–2592.

3. CDC. Outbreak of listeriosis -Northeastern United States, *JAMA*, 2002. 2260.

4. Chen Y. H., Ross H., Scott V. N. Gombas D. E. *Listeria monocytogenes*: low levels equal low risk. J Food Prot., 2003. Vol. 66. P. 570–577.

5. Cossart P. Interactions of the bacterial pathogen *Listeria monocytogenes* with mammalian cells: bacterial factors, cellular ligands, and signaling. Folia Microbiol. (Praha), 1998. Vol. 43. P. 291–303.

6. den Bakker H. C., Bowen B. M., Rodriguez-Rivera L. D., Wiedmann M. FSL J1-208: a virulent uncommon phylogenetic lineage IV *Listeria monocytogenes* strain with a small chromosome size and putative virulence plasmid carrying internalin-like genes. Appl Environ Microbiol., 2012. Vol. 78(6). P. 1876–1889.

7. Doyle M. E., Mazzotta A. S., Wang T., Wiseman D. W., Scott V. N. Heat resistance of *Listeria monocytogenes*. J Food Prot., 2001. Vol.4. P. 410–429.

8. FAO/WHO. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: Interpretative summary. Microbiological Risk Assessment. 2004. Series 4.

9. Farber J. M., Peterkin P. I. *Listeria monocytogenes* a food-borne pathogen. Microbiol. Rev., 1991. Vol. 55. P. 476–511.

10. Fugett E. B., Schoonmaker-Bopp D., Dumas N. B., Corby J., Wiedman M. Pulsed field gel electrophoresis (PFGE) analysis of temporally matched *Listeria monocytogenes* isolates from human clinical cases, foods, ruminant farms, and urban and natural environments reveals source associated as well as widely distributed PFGE types. J Clin Microbiol., 2007. Vol. 45. P. 865–873.

11. Gellin B. G., Broome C. V. Listeriosis. Journal of the American Medical Association. 1989. Vol. 261. P. 1313–1320.

12. Gray M. J., Zadoks R. N., Fortes E. D., Dogan B., Cai S., Chen Y., Scott V. N., Gombas D. E., Boor K. J., Wiedmann M. *Listeria monocytogenes* isolates from foods and humans form distinct but overlapping populations. Appl Environ Microbiol., 2004.Vol. 70. P. 5833–5841.

13. Heir E., Lindstedt B. A., Røtterud O. J., Vardund T., Kapperud G.,Nesbakken T. Molecular epidemiology and disinfectant susceptibility of Listeria monocytogenes from meat processing plants and human infections. Int J Food Microbiol. 2004.Vol. 96. P. 85–96.

14. Hoffman A. D., Gall K. L., Norton D. M.,Wiedmann M. *Listeria monocytogenes* contamination patterns for the smoked fish processing environment and for raw fish. J Food Prot. 2003. Vol. 66. P. 52–60.

15. Hofshagen,M., Nygård K., Hauge, K. Zoonoserapporten 2005 (Zoonotic report 2005), The Norwegian Institute of Veterinary Science. 2005.

16. Jeffers G. T., Bruce J. L., McDonough P. L., Scarlett J., Boor K. J.,Wiedmann M. Comparative genetic characterization of *Listeria monocytogenes* isolates from human and animal listeriosis cases. Microbiology. 2001. Vol. 147. P. 1095–1104.

17. Jemmi T., Stephan R. *Listeria monocytogenes*: food-borne pathogen and hygiene indicator. Rev Sci. Tech. 2006. Vol. 25. P. 571–580.

18. Klæboe H., Rosef O., Sæbø M. Longitudinal studies on *Listeria monocytogenes* and other Listeria species in two salmon processing plants. Int J Environ. Health. Res. 2005. Vol. 15. P. 71–77.

19. Klæboe H., Rosef O., Fortes E., Wiedman M. Ribotype diversity of *Listeria monocytogenes* isolates from two processing plants in Norway. Int J Environ. Health. Res. 2006. Vol. 16. P. 375–383.

20. Klæboe H., Lunestad B. T., Borlaug K., Paulauskas A., Rosef O. Persistence and diversity of of *Listeria monocytogenes* isolates in Norwegian processing plants. Vet. Med. Zoot. 2010. Vol. 50 (72). P. 42–47.

21. Lassen L.,Caougant D. A. Listeria-utbrudd i Trøndelag. In MSIS-report, The Norwegian Institute of Public health. 1992. P. 43–44.

22. Lindemann R. A historical birth tragedy. Neonatal infections still of interest today as they were 300 years ago.,Tidsskr Nor Laegeforen. 1990. Vol. 110. P. 3860–3862.

23. Lukinmaa S., Miettinen M., Nakari U-M., Korkelala H., Siitonen A. *Listeria monocytogenes* isolates from invasive infections: Variation of sero- and genotypes during an 11-year period in Finland. J Clin Microbiol., 2003.Vol. 41. P. 1694–1700.

24. McLauchlin J., Mitchell R. T., Smerdon W. J., Jewell K. *Listeria monocytogenes* and listeriosis: a review of hazard characterisation for use in microbiological risk assessment of foods. Int J Food Microbiol., 2004.Vol. 92. P. 15–33.

25. Moorhead S. M., Dykes G. A., Cursons R. T. An SNP-based PCR assay to differentiate between *Listeria monocytogenes* lineages derived from phylogenetic analysis of the sigB gene. J Microbiol Methods, 2003.Vol. 55. P. 425–432.

26. Nesbakken T., Kapperud G., Caugant D. A. Pathways of Listeria monocytogenes contamination in the meat processing industry. Int J Food Microbiol., 1996.Vol. 31. P. 161–171.

27. Norton D. M., McCamey M. A., Gall K. L., Scarlett J. M., Boor K. J.,Wiedmann M. Molecular studies on the ecology of *Listeria monocytogenes* in the smoked fish processing industry.. Appl Environ Microbiol., 2001a Vol. 67. P. 198–205.

28. Norton D. M., Scarlett J. M., Horton K., Sue D., Thimothe J., Boor K. J.,Wiedmann M. Characterization and pathogenic potential of *Listeria monocytogenes* isolates from the smoked fish industry. Appl Environ Microbiol., 2001b. Vol. 67. P. 646–653.

29. Orsi R.H., Bakker H.C. ,Wiedman M. *Listeria monocytogenes* lineages: Genomics, evolution, ecology, and phenotypic characteristics. Int J Med Microbiol., 2011. Vol. 301.P. 79–96.

30. Parihar V. S., Lopez-Valladares G., Danielsson-Tham M. L., Peiris I., Helmersson S., Unemo M., Andersson B., Arneborn M., Bannerman E., Barbuddhe S., Bille J., Hahdu L., Johansson J. C., Lödahl M., Möllerberg G., Ringber H., Rocourt J., Tjernberg I., Ursing J., Henriques-Nordmark B.,Tham W. Characterization of human invasive isolates of *Listeria monocytogenes* in Sweden 1986-2007. Foodborne Pathog Dis., 2008. Vol. 5. P. 755–761.

31. Rasmussen O. F., Skouboe P., Dons L., Rossen L., Olsen J. E. *Listeria monocytogenes* exists in at least three evolutionary lines: evidence from flagellin, invasive associated protein and listeriolysin O genes. Microbiology, 1995. Vol. 141. P. 2053–2061.

32. Roberts A., Nightingale K., Jeffers G., Fortes E., Kongo J. M., Wiedmann M. Genetic and phenotypic characterization of *Listeria monocytogenes* lineage III. Microbiology, 2006. Vol. 152. P. 685–693.

33. Rocourt J., Hogue A., Toyofuku H., Jacquet C., Schlundt J. Listeria and listeriosis: risk assessment as a new tool to unravel a multifaceted problem. Am J Infect Control., 2001.Vol. 29. P. 225–227.

34. Rørvik L. M., Skjerve E., Knudsen B. R.,Yndestad M. Risk factors for contamination of smoked salmon with *Listeria monocytogenes* during processing. Int J Food Microbiol., 1997.Vol. 37. P. 215–219.

35. Rørvik L. M., Aase B., Alvestad T., Caugant D. A. Molecular epidemiological survey of *Listeria monocytogenes* in seafoods and seafood-processing plants. Appl Environ Microbiol., 2000. Vol. 66. P. 4779–84.

36. Rørvik L. M., Aase B., Alvestad T. and Caugant D. A. Molecular epidemiological survey of *Listeria monocytogenes* in broilers and poultry products. J Appl Microbiol., 2003. Vol. 94. P. 633–640.

37. Sauders B. D., Mangione K., Vincent C., Schermerhorn J., Farchione C. M., Dumas N. B., Bopp D., Kornstein L., Fortes E. D., Windham K.,Wiedman M. Distribution of *Listeria monocytogenes* molecular subtypes among human and food isolates from New York State shows persistence of Human Disease-Associated Listeria monocytogenes strains in retail environments. J Food Prot., 2004. Vol. 67. P. 1417–1428.

38. Sauders B. D., Schukken Y., Kornstein L., Reddy V., Bannerman T., Salehi E., Dumas N., Anderson B. J., Massey J. P.,Wiedmann M. Molecular epidemiol-

ogy and cluster analysis of human listeriosis cases in three U.S. states. J Food Prot., 2006. Vol. 69. P. 1680–1689.

39. Senczek D., Stephan R.,Untermann F. Pulsed-field gel electrophoresis (PFGE) typing of Listeria strains isolated from a meat processing plant over a 2-year period. Int J Food Microbiol., 2000. Vol. 62. P. 155–159.

40. Suihko M. L., Salo S., Niclasen O., Gudbjornsdottir B., Torkelsson G., Bredholt S., Sjoberg A. M.,Gustavsson P. Characterization of *Listeria monocytogenes* isolates from the meat, poultry and seafood industries by automated ribotyping. Int J Food Microbiol., 2002.vol. 72. P. 137–146.

41. Tsai Y. H., Maron S. B., McGann P., Nightingale K. K., Wiedmann M., Orsi R. H. Recombination and positive selection contributed to the evulotion of *Listeria monocytogenes* lineage III and IV, two distinct and well supported uncommon *L. monocytogenes* lineages. 2011. Infect Genet Evol., 2011. Vol. 11. P. 1881–1890.

42. Ward T., Gorski L., Borucki M., Mandrell R., Hutchins J., Pupedis K. Intraspecific phylogeny and lineage group identification based on the prfA virulence gene cluster of *Listeria monocytogenes*. J Bacteriology. 2004. Vol. 186. P. 4994–5002.

43. Wiedmann M., Bruce J. L., Keating C., Johnson A. E., McDonough P. L., Batt C. A. Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. Infect Immun., 1997. Vol. 65. P. 2707–2716.

44. Zhou X., Jiao X. Molecular grouping and pathogenic analysis of *Listeria monocytogenes* of clinical and food origin. J Food Control, 2005. Vol. 16. P. 867–872.

Received 20 February 2012
Accepted 7 June 2012