Occurrence and Characteristics of *Listeria monocytogenes* in Ready-to-eat Food from Retail Market in the Czech Republic

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Abstract: The study objectives were to test ready-to-eat food from the retail market in the Czech Republic for the presence of *L. monocytogenes* and, based on typing methods, to investigate probable causes of contamination. A total 2180 samples of ready-to-eat food (meat, dairy, fish, delicatessen and confectionery products and fresh fruit and vegetables) were analysed qualitatively and quantitatively. *L. monocytogenes* isolates were characterised by serotyping and macrorestriction analysis after digestion with the restriction enzyme *Asc*I. In 2004–2008 *L. monocytogenes* was most often detected in delicatessen (5.2%), meat (3.4%) and dairy products (1.8%). In the analysed samples, *L. monocytogenes* was mostly present at counts lower than 10^2 CFU/g. Only in 2004, higher counts of *L. monocytogenes* were found in two heat-processed meat products (10^3 CFU/g). The obtained macrorestriction patterns helped in tracing the source of contamination and routes of the spread of *L. monocytogenes* in the manufacturing plant and retail market.

Keywords: *L. monocytogenes*; foodstuff; typing methods; hygiene

L. monocytogenes is a Gram-positive, facultative anaerobic opportunistic intracellular bacterial pathogen whose primary route of transmission to humans is the consumption of contaminated food (Vázquez-Boland *et al.* 2001). The invasive form of listeriosis is observed primarily in high-risk population groups such as the elderly, individuals with lowered immunity, pregnant women and newborns. Listeriosis is a low prevalence disease and in 2007, the reported incidence rates were 0.3 cases per 100 000 population in EU and 0.5 cases per 100 000 population in the Czech Republic (EFSA Zoonosis Report 2009). Nevertheless, the seriousness of this food-borne zoonosis lies in high case fatality rates reaching up to 30% (Denny & Mc Lauchlin 2008).

The bacterium *L. monocytogenes* can be isolated from various sources such as soil, water, plants, feeds and silage, as well as from the environment in food industry plants and from foods (Farber & Peterkin 1991; Fugett *et al.* 2007). Important characteristics of *L. monocytogenes* are psychrotrophy and tolerance to high concentrations of salt (NaCl) and to low pH (Vázquez-Boland *et al.* 2001). The presence of listeria in foods is associated, among others, with the ability to persist in the environment of food plants (Miettinen *et al.* 1999; Thévenot *et al.* 2006; López *et al.* 2008) and to form biofilm on the surfaces of the food processing equipment (Borucki *et al.* 2003).

Ready-to-eat food are the most important source of both sporadic cases and outbreaks of listeriosis in humans (Westrell *et al.* 2009). The disease can develop after consumption of a wide range of foods such as meat products (De Cesare *et al.* 2007), dairy products (especially in ripened cheeses) (Rudolf & Scherer 2001), delicatessen products (Chao *et al.* 2006), fish and seafood products

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Projects No. MSM 6215712402 and No. 2B08050, and by the EU Integrated Project BIOTRACER, Contract 036272 in the 6th RTD Framework.

(Inoue *et al.* 2000) and vegetables (Cordano & Jacquet 2009). Some countries such as the USA have zero tolerance for the presence of *L. monocytogenes* in these foods in their legislation. In the EU countries, a limit of less than 10^2 CFU/g has been set for ready-to eat food in the retail market by Commission Regulation (EC) No 2073/2005. This limit is considered safe for consumers.

Molecular biology-based typing methods are increasingly used for the detection of sources and routes of food chain contamination with *L. monocytogenes*. Molecular typing of food isolates is a valuable tool for the confirmation of suspected vehicles in both sporadic cases and outbreaks of listeriosis (Autio *et al.* 2002; Fugett *et al.* 2007). A frequently used method with high discriminatory potential and reproducibility is the macrorestriction analysis of bacterial genome followed by pulsed-field gel electrophoresis (PFGE) (Autio *et al.* 2002; Fugett *et al.* 2007; Filiousis *et al.* 2009).

MATERIAL AND METHODS

Range of analysed foods. In 2004 through 2008, 2180 samples of ready-to-eat food from the retail in the Czech Republic were analysed. The samples were collected within the Project of the Ministry of Health of the Czech Republic, the System of Monitoring the Environmental Impact on Population Health of the Czech Republic, Subsystem IV (http://www.chpr.szu.cz/monitoring.htm). The selection of the test commodities was based on the food consumption basket and on the role which they played in previous food-borne outbreaks in the Czech Republic and other countries. We analysed meat products (ham, heat-processed sausages, long-life heat-processed and long-life fermented meat products), dairy products (pasteurised cow's milk, semi-hard cheeses, ripened cheeses, ice creams and butter), fish products (marinated fish, smoked mackerel), delicatessen products (salads with vegetables, sausages and mayonnaise), confectionery products, fresh vegetables and fruit. The range and numbers of sampled foods are summarised in Table 1.

L. monocytogenes ***detection and quantification.*** The detection of *L. monocytogenes* in food samples and its enumeration in positive samples were performed according to ČSN EN/ISO 11290 – 1, 2, using culture media Fraser and ALOA (BIO-RAD, France).

Serotyping. Serotyping was performed by the slide agglutination method using commercially available antisera (DENKA SEIKEN, Japan). Serotyping results were obtained by combining slide agglutination and a multiplex PCR (Borucki & Call 2003; Doumith *et al.* 2004) using PPP polymerase (Top-Bio, Czech Republic) and GENERI BIOTECH primers (Czech Republic).

Pulsed-field gel electrophoresis (PFGE). Macrorestriction analysis after digestion with the restriction endonuclease *Asc*I (BioLabs, The United Kingdom) was carried out according to the PulseNet Europe Protocol (2002) and the results were interpreted based on the criteria of Tenover *et al.* (1995).

RESULTS AND DISCUSSION

In 2004–2008, *L. monocytogenes* was detected in 55 (2.5%) of 2180 analysed food samples. *L. monocytogenes* was found in all analysed types of foods but fresh fruit and was most frequently detected

Table 1. Analysed food samples and positive findings of *L. monocytogenes*

Commodity	No of analysed samples	No (%) of positive samples
Meat products	1044	36 (3.4)
Dairy products	549	10 (1.8)
Fish products	120	2 (1.7)
Delicatessen products	96	5 (5.2)
Confectionery products	108	1 (0.9)
Fresh vegetables	180	1 (0.5)
Fresh fruit	83	0

in delicatessen products (5.2%), meat products (3.4%) and dairy products (1.8%) (Table 1). In the EU, in 2007, the highest *L. monocytogenes* positivity rates were reported in fish products (18.3%), particularly in smoked fish, where the limit of 10^2 CFU/g was most frequently exceeded (EFSA Zoonosis Report 2009). In our study *L. monocytogenes* was detected in 1.7% of fish products and the limit count of 10^2 CFU/g was not exceeded in any of the analysed samples. The discrepancy can be explained by a lower number of the analysed products or their types. Our study was aimed at two types of products only: marinated fish with an acid pH and smoked mackerel.

In 96 analysed delicatessen products, *L. monocytogenes* was only detected in 2004 (4 isolates) and 2006 (1 isolate). All isolates originated from the same type of vegetable salad with mayonnaise. The bacterial count did not exceed 10^2 CFU/g in any of the analysed samples. In the EU in 2007, the *L. monocytogenes* positivity rate in this type of products was about 4.6% (EFSA Zoonosis Report 2009). In some countries, *L. monocytogenes* was detected even in 13% (32/245) of delicatessen products from the retail market (Chao *et al*. 2006).

The EFSA annual report (EFSA Zoonosis Report 2009) indicates the highest detection rates of *L. monocytogenes* in the EU member states for meat and fish products and cheeses. In this study *L. monocytogenes* was most often isolated from meat and dairy products, hence we focused our attention on the characterisation of isolates from these types of commodities. In the study period, *L. monocytogenes* was detected in 36 (3.4%) meat product samples. Nevertheless, with the exception of two meat products from 2004, bacterial counts higher than 10^2 CFU/g were not found in any of the analysed samples (Table 2). Fermented products (3.3% positive findings of *L. monocytogenes*) in comparison with results determined by De Cesare *et al.* (2007) were contaminated less often. The most frequent *L. monocytogenes* serotype was 1/2a (44.4%), followed by 1/2c (19.4%) and 1/2b (16.7%). The predominance of serotype 1/2a in meat products has been confirmed by Bĕrziņs *et al*. (2009). Detailed data on the detection and serotyping of *L. monocytogenes* are given in Table 2.

Thirty-six isolates of *L. monocytogenes* were obtained from meat products, with 83% of these isolates originating from sliced products. Undesirable bacterial contamination of meat products can occur either directly in the manufacturing process or as a result of subsequent handling, storage or distribution. The source and route of contamination can be traced e.g. by macrorestriction analysis (Thévenot *et al.* 2006; López *et al.* 2008). One of the possible causes of product contamination in the retail market is inadequate cleaning of slicing machines and consequent transmission of *L. monocytogenes* between the product and slicer surface (Sheen 2008). In our study, this hypothesis is supported by the detection of an identical clone of *L. monocytogenes* in products from different producers supplying the same shop (Table 3).

L. monocytogenes was detected in ten (1.8%) of 549 analysed dairy product samples. Among the analysed products (pasteurised cow's milk, semi-hard cheeses, ripened cheeses, ice creams and butter), the most frequent source of *L. monocytogenes* were mainly blue-veined cheeses (9/60). One positive isolate was detected also in 120 analysed ice creams. The highest *L. monocytogenes* detection rates were observed in 2006 (4.6%) and 2007 (2.7%). This increased occurrence was attributable to the

Table 2. Positive findings and serotypes of *L. monocytogenes* from meat products

Commodity	No (%) of isolates	Count (CFU/g)	Serotype					
			1/2a	1/2b	1/2c	4b	4ab	4d
Ham	8 (6.7)	$< 1 \times 10^2$	4	3	1	0	0	0
	1 (0.8)	5.8×10^3	0	0	1	0	0	0
Heat-processed meat products	16 (2.3)	$< 1 \times 10^2$	9	3	2	1	1	0
	1 (0.1)*	1×10^3	1	0	0	0	0	0
Long-life heat-processed meat products	6 (5.0)	$< 1 \times 10^2$	4	1	1	0	0	0
Long-life fermented meat products	4 (3.3)	$< 1 \times 10^2$	1	0	2	0	0	1

*bacon

Table 3. Typing results for *L. monocytogenes* strains isolated from meat products obtained in one supermarket in 2008

City	Commodity	Producer	Serotype	Pulsotype
Jablonec nad Nisou	Salami Vysočina	A	1/2a	713
	Salami Herkules	A	1/2a	713
	Salami Turist	B	1/2a	713
	Salami Gothaj	C	1/2b	505
	Pork ham	D	1/2b	505

Table 4. Positive findings, serotypes and pulsotypes of *L. monocytogenes* from dairy products

Commodity	Year of isolation	Producer	No of isolates	Count (CFU/g)	Serotype		Pulsotype
					1/2a	1/2b	
Blue-veined cheese	2004	E	1	$< 1 \times 10^2$	1	0	719
	2006	E	5	$< 1 \times 10^2$	5	0	719
	2007	E	3	$< 1 \times 10^2$	3	0	719
Ice cream	2005	F	1	$< 1 \times 10^2$	0	1	525

contamination during the manufacturing process in a plant of the leading producer of this type of cheese in the Czech Republic (Table 4).

The typing of *L. monocytogenes* isolated from final products of different producers is a valuable tool for tracing clones specific to particular products or producers (Autio *et al.* 2002). All analysed strains from blue-veined cheeses in our study were classified into serotype 1/2a and were clonally identical (pulsotype 719). These results indicate repeated contamination of ripened cheeses at the producer level over several years and possible presence of persistent strains of *L. monocytogenes* in the manufacturing plant. In 2008, all blue-veined cheese samples from producer E were negative, probably as a result of the remedial measures taken in the dairy plant. Similarly as in our study, Brito *et al.* (2008) used macrorestriction analysis to trace the routes of spread of listeria in a food-processing plant and the retail market. They revealed the link between the contamination of cheeses with clonally identical strains of *L. monocytogenes* of serotype 1/2a in the retail market and the manufacturing plant.

CONCLUSION

The results of typing methods provide important information about the characteristics of *L. monocytogenes* strains isolated from foods and enable the identification of sources and routes contamination of food chain, including retail market.

References

Autio T., Lundén J., Fredriksson-Ahomaa M., Björkroth J., Sjöberg A.M., Korkeala H. (2002): Similar *Listeria monocytogenes* pulsotypes detected in several foods originating from different sources. International Journal of Food Microbiology, **77**: 83–90.

Běrziņs A., Terentjeva M., Korkeala H. (2009): Prevalence and genetic diversity of *Listeria monocytogenes* in vacuum-packaged ready-to-eat meat products at retail markets in Latvia. Journal of Food Protection, **72**: 1283–1287.

Borucki M.K., Call D.R. (2003): *Listeria monocytogenes* serotype identification by PCR. Journal of Clinical Microbiology, **41**: 5537–5540.

Borucki M.K., Peppin J.D., White D., Loge F., Call D.R. (2003): Variation in biofilm formation among strains of *Listeria monocytogenes*. Applied and Environmental Microbiology, **69**: 7336–7342.

Brito J.R.F., Santos E.M.P., Arcuri E.F., Lange C.C., Brito M.A.V.P., Souza G.N., Cerqueira M.M.P.O., Beltran J.M.S., Call J.E., Liu Y., Porto-Fett A.C.S., Luchansky J.B. (2008): Retail survey of Brazilian milk and minas frescal cheese and a contaminated dairy

plant to establish prevalence, relatedness, and sources of *Listeria monocytogenes* isolates. Applied and Environmental Microbiology, **74**: 4954–4961.

Chao G., Deng Y., Zhou X., Xu Q., Qian X., Zhou L., Zhu B. (2006): Prevalence of *Listeria monocytogenes* in delicatessen food products in China. Food Control, **17**: 971–974.

Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs as last amended by Commission Regulation (EC) No 1441/2007.

Cordano A.M., Jacquet CH. (2009): *Listeria monocytogenes* isolated from vegetable salads sold at supermarkets in Santiago, Chile: Prevalence and strain characterisation. International Journal of Food Microbiology, **132**: 176–179.

De Cesare A., Mioni R., Manfreda G. (2007): Prevalence of *Listeria monocytogenes* in fresh and fermented Italian sausages and ribotyping of contaminating strains. International Journal of Food Microbiology, **120**: 124–130.

Denny J., Mc Lauchlin J. (2008): Human *Listeria monocytogenes* infections in Europe – an opportunity for improved European surveillance. Eurosurveillance, **13**: 32–36.

Doumith M., Buchrieser C., Glaser P., Jacquet C., Martin P. (2004): Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. Journal of Clinical Microbiology, **42**: 3819–3822.

EFSA (2009): The community summary report on trends and sources of zoonoses and zoonotic agents in the European Union in 2007. The EFSA Journal: 223.

Farber J.M., Peterkin P.I. (1991): *Listeria monocytogenes*, a food-borne pathogen. Microbiological Reviews, **55**: 476–511.

Filiousis G., Johansson A., Frey J., Perreten V. (2009): Prevalence, genetic diversity and antimicrobial susceptibility of *Listeria monocytogenes* isolated from open-air food markets in Greece. Food Control, **20**: 314–317.

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

Inoue S., Nakama A., Arai Y., Kokubo Y., Maruyama T., Saito A., Yoshida T., Terao M., Yamamoto S., Kumagai S. (2000): Prevalence and contamination levels of *Listeria monocytogenes* in retail foods in Japan. International Journal of Food Microbiology, **59**: 73–77.

López V., Villatoro D., Ortiz S., López P., Navas J., Dávila J.C., Martinéz-Suárez J.V. (2008): Molecular tracking of *Listeria monocytogenes* in an Iberian pig abattoir and processing plant. Meat Science, **78**: 130–134.

Miettinen M.K., Björkroth K.J., Korkeala H.J. (1999): Characterization of *Listeria monocytogenes* from an ice cream plant by serotyping and pulsed-field gel electrophoresis. International Journal of Food Microbiology, **46**: 187–192.

PulseNet Europe Protocol (2002): Standardized protocol for molecular subtyping of *Listeria monocytogenes* by Pulsed-Field Gel Electrophoresis (PFGE). Available at http://www.pulsenet-europe.org

Rudolf M., Scherer S. (2001): High incidence of *Listeria monocytogenes* in European red smear cheese. International Journal of Food Microbiology, **63**: 91–98.

Sheen S. (2008): Modeling surface transfer of *Listeria monocytogenes* on salami during slicing. Journal of Food Science, **73**: 304–311.

Tenover F.C., Arbeit R.D., Goering R.V., Mickelsen P.A., Murray B.E., Persing D.H., Swaminathan B. (1995): Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. Journal of Clinical Microbiology, **33**: 2233–2239.

Thévenot D., Delignette-Muller M.L., Christieans S., Leroy S., Kodjo A., Vernozy-Rozand C. (2006): Serological and molecular ecology of *Listeria monocytogenes* isolates collected from 13 French pork meat salting-curing plants and their products. International Journal of Food Microbiology, **112**: 153–161.

Vázquez-Boland J.A., Kuhn M., Berche P., Chakraborty T., Domínguez-Bernal G., Goebel W., González-Zorn B., Wehland J., Kreft J. (2001): *Listeria* pathogenesis and molecular virulence determinants. Clinical Microbiological Reviews, **4**: 584–640.

Westrell T., Ciampa N., Boelaert F., Helwigh B., Korsgaard H., Chríel M., Ammon A., Mäkela P. (2009): Zoonotic infections in Europe in 2007: a summary of the EFSA-ECDC annual report. Eurosurveillance, **14**: 1–2.

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