Outdoor Environment as a Source of *Listeria monocytogenes* in Food Chain

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Abstract

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We monitored the presence of *Listeria monocytogenes* in environmental sources and to evaluate the phenotypic and molecular characteristics of the isolates recovered. *L. monocytogenes* was isolated in 12 (11.2%) of the 107 samples from the wild, farm environment, and vegetation. Most isolates (83.3%) were of serotype 1/2a and the remainder (2) were of serotype 4b. All 12 isolates were susceptible to the whole range of antimicrobials tested. These12 strains were carriers of the virulence genes *prfA*, *hlyA*, *actA*, *plcA*, *plcB*, *inlA*, *inlB*, *inlC*, and *inlJ*. The detection of the *inlA* gene in 4 (33.3%) of 12 strains using the PCR-RFLP suggests the potential of some of these strains to penetrate into epithelial cells of the intestinal barrier. Macrorestriction analysis also confirmed clonal identity of some environmental isolates with food and human isolates. These results indicate that the external environment is a source of potentially pathogenic strains of *L. monocytogenes*.

Keywords: virulence; listeria; antimicrobial resistance; restriction fragment length polymorphism; pulsed-field gel electrophoresis (PFGE)

Listeria monocytogenes is a ubiquitous saprophytic bacterium, adapted to life in soil and decaying vegetation, but also in the cytosol of eukaryotic host cells (Freitag *et al.* 2009). *L. monocytogenes* can also be isolated from the surface and underground waters, improperly fermented silage, sewage sludge, slaughter wastes, animal and human faeces, foodstuffs, and food industry plants (Farber & Peterkin 1991; Ivanek *et al.* 2006). In both animals and susceptible humans, *L. monocytogenes* causes serious invasive disease. The main source of listeria to animals is contaminated feed, and that to humans is food for direct consumption (Vázquez-Boland *et al.* 2001).

The presence of *L. monocytogenes* in farm animals is mainly associated with cattle and sheep farming, and less often with pig and poultry farming. In ruminants, the disease manifests itself primarily by neurological symptoms or abortion, and very uncommonly, by mastitis. However, as a rule, *L. monocytogenes* passes asymptomatically through the gastrointestinal tract to be excreted in faeces (Ivanek *et al.* 2006; Esteban *et al.* 2009). Fecal contamination of soil, vegetation, and surface water is the major source of *L. monocytogenes* in primary food production (Ivanek *et al.* 2006).

Pathogenicity of *L. monocytogenes* is due to its capacity of penetrating into and proliferating in

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various types of host cells, including non-phagocytic cells. The intracellular cycle of *L. monocytogenes* starts with the invasion of host cells mediated by the surface proteins internalins A (encoded by *inlA* gene) and B (*inlB*) (Bierne *et al.* 2007). An essential prerequisite for *L. monocytogenes* proliferation and replication in the cytosol is the bacterial escape from phagolysosomes, mediated by listeriolysin O (*hlyA*) in combination with phospholipases, phosphatidylinositol (*plcA*), and phosphatidylcholine (*plcB*) phospholipases C. The intracellular motility of *L. monocytogenes* and spread from cell to cell are due to an actin polymerisation protein (*actA*). The PrfA (positive regulatory factor A) protein is crucial to the transcriptional regulation of the above-mentioned genes located in LIPI-1 (*Listeria* pathogenicity island 1) and in part also of *inlA* and *inlB* genes (Gray *et al.* 2006; Freitag *et al.* 2009).

Variation in the pathogenic potential of *L. monocytogenes* isolated from foodstuffs and the environment has been reported. This may be due to the deletion of one or more genes encoding the key virulence factors (Doumith *et al.* 2004a). However, a number of studies have found such genes to be stable parts of the genome of *L. monocytogenes* (Jaradat *et al.* 2002; Doumith *et al.* 2004a). The reduced virulence of *L. monocytogenes* may also be a result of point mutations of the above-mentioned genes. Sequencing analysis of the *inlA* gene has identified point mutations leading to the production of a truncated InlA. *L. monocytogenes* strains producing a truncated form of InlA protein have a reduced capacity of invading tissue culture epithelial cells. For a rapid screening of these potentially non-invasive *L. monocytogenes* strains, PCR-RFLP (restriction fragment length polymorphism) can be used to identify the *inlA* gene polymorphism (Rousseaux *et al.* 2004).

MATERIAL AND METHODS

From February to July 2010, 107 environmental samples collected in the Czech Republic, particularly in Brno and its surroundings, were analysed (Table 1). The samples were obtained from the outdoor environment (soil, mud, surface water, silt, compost, sand), urban environment (soil, sandpits), animal farm environment (soil, bedding, feed, sand, faeces), vegetation (aquatic algae, leaves, grass, moss), and wild animals (wireworm, earthworms, leeches, grubs, dead fish).

Table 1. Origin and number of analysed and positive samples for *L. monocytogenes*

Origin of samples		Number of analysed samples	Number (%) of positive samples
Environment	wild	56	6 (10.7)
	city	20	0
	farm	14	3 (21.4)
Vegetation		11	3 (27.3)
Animal		6	0
Total		107	12 (11.2)

Detection of L. monocytogenes. The samples were analysed in compliance with EN/ISO 11290-1:2004, using a modified protocol with the following culture media: Buffered Peptone Water (BPW), Fraser broth, and ALOA (Bio-Rad, Paris, France). The modifications made to the protocol were as follows: (a) homogenisation of 5 g to 25 g of the test sample in BPW (at a ratio of 1 to 9), incubation at 37°C for 24 h, (b) subsequent inoculation of 1 ml of culture into 10 ml of Fraser broth, incubation at 37°C for 24 h, and (c) plating on ALOA (incubation at 37°C for 24 h). When water was analysed, 1 ml of sample was inoculated into 9 ml of BPW and cultured at 37°C for 24 hours. Other steps were identical to those specified in the standard protocol.

Serotyping. Serotyping was performed by the slide agglutination method using commercially available antisera (Denka Seiken, Tokyo, Japan) and subsequently confirmed by multiplex PCR (Doumith *et al.* 2004b) using PPP polymerase (Top-Bio, Prague, Czech Republic) and primers synthesised by Generi Biotech (Hradec Králové, Czech Republic).

Resistance to antimicrobials. Phenotypic resistance testing was performed by the disk diffusion method on Mueller-Hinton agar (Bio-Rad, Paris, France). The antimicrobials tested and interpretation criteria used are shown in Table 2.

Detection of virulence genes. Four PCR assays were performed to detect the following virulence genes: (1) *prfA* (D'Agostino *et al.* 2004) and *plcA* (Jaradat *et al.* 2002), (2) *hlyA* (Aurora *et al.* 2008) and *actA* (Jaradat *et al.* 2002), (3) *plcB* (Jaradat *et al.* 2002), and (4) *inlA, inlC, inlJ* (Liu *et al.* 2007) and *inlB* (Jaradat *et al.* 2002). All assays used primers synthesised by Generi Biotech (Hradec Králové, Czech Republic), PPP polymerase (Top-Bio, Prague, Czech Republic) or a Qiagen Multiplex PCR Kit (Bio-Consult, Prague, Czech Republic).

Table 2. Tested antimicrobials with their concentrations and interpretation criteria used

Antimicrobials	Concentration of disk (μg)(Oxoid, UK)	Interpretation criteria
Trimethoprim	5	CLSI (2006) (criteria for *Staphylococcus* spp.)
Gentamicin	10	
Ampicillin	10	CLSI (2006) (criteria for *Enterococcus* spp.)
Penicillin	10	
Tetracyclin	30	
Erythromycin	15	
Chloramfenicol	30	
Streptomycin	10	Comité de l'antibiogramme de la Société Française de Microbiologie (2005)
Vancomycin	30	

PCR-RFLP analysis of inlA polymorphism. Potentially non-invasive *L. monocytogenes* strains were screened by PCR-RFLP based on *inlA* polymorphism (Rousseaux *et al.* 2004). The 733 bp *inlA* fragment was amplified using primers seq01 and seq02 (Rousseaux *et al.* 2004) (Generi Biotech, Hradec Králové, Czech Republic). The amplified product was cleaved with restriction endonuclease *Alu*I (BioLabs, Hitchin, UK) and PCR-RFLP fragments were separated by electrophoresis on 3.5% gel (SERVA Electrophoresis GmbH, Heidelberg, Germany).

Pulsed-field gel electrophoresis (PFGE). Macrorestriction analysis using endonuclease *Asc*I (BioLabs, Hitchin, UK) was performed according to the protocol PulseNet Europe (2002).

RESULTS AND DISCUSSION

L. monocytogenes was detected in 12 (11.2%) of 107 environmental samples analysed in this study. All positive samples were collected in areas characterised by high humidity or the presence of either farm or wild warm-blooded animals (Table 3), which is in line with the reports from other countries (Welshimer & Donket-Voet 1971; Weis & Seeliger 1975; Colburn *et al.* 1990; Lyautey *et al.* 2007). The highest proportion of *L. monocytogenes* isolates (66.7%) was found in the mud samples from the vicinity of water courses and in the soil samples from the areas where animals live.

Most *L. monocytogenes* isolates (10/12) were of serotype 1/2a. Only two isolates from different sites of the same area were classified into serotype 4b. Both strains of this serotype were of the same clonal type (pulsotype 204). Thus, it appears that 1/2a is the most widespread serotype in the Czech Republic, found not only in foodstuffs and humans (Karpíšková & Gelbíčová 2008) but also in the outdoor environment.

None of the 12 isolates recovered showed resistance to antimicrobials (ampicillin, penicillin, gentamicin, and trimetoprim) used in the treatment of human listeriosis. The environmental isolates were also susceptible to other antimicrobials tested. Although *L. monocytogenes* is commonly susceptible to a wide range of antimicrobials, some authors have reported tetracycline resistance in environmental strains of *L. monocytogenes* (MacGowan *et al.* 1994) or multidrug resistance to ampicillin, erythromycin, tetracycline, dicloxacillin, and co-trimoxazole (Rodas-Suárez *et al.* 2006). The monitoring of *L. monocytogenes* resistance to antimicrobials is currently needed as well as the development of international criteria for the evaluation of resistant strains.

All genes involved in the pathogenicity of *L. monocytogenes* (*prfA*, *hlyA*, *plcA*, *plcB*, *actA*, *inlA*, and *inlB*) were detected in the environmental isolates. However, some studies have also reported avirulent strains of *L. monocytogenes* recovered from foodstuffs (Chen *et al.* 2009a; Kaur *et al.* 2010) or from faeces of captive wild animals (Kalorey *et al.* 2006) that typically lack some of these virulence genes. Whether the strains lacking *prfA* or *hlyA* (Kalorey *et al.* 2006; Kaur *et al.* 2010) that are commonly used for the detection of *L. monocytogenes* were correctly identified to the species level by phenotypic methods remains a question. Based on the results of this study and other studies (Chen *et al.* 2009a; Mammina *et al.* 2009), the *inlC* and *inlJ* genes are also stable

parts of the genome of *L. monocytogenes*, but their roles in the virulence remain unclear (Bierne *et al.* 2007). Nevertheless, other authors (Chen *et al.* 2009a,b) have reported the absence of the *inlC* and *inlJ* genes in *L. monocytogenes* strains of serotype 4a exhibiting a lower virulence. This is explained by a higher genetic relatedness between serotype 4a and the non-pathogenic species *L. innocua* that is not a producer of InlC and InlJ proteins either (Chen *et al.* 2009b).

An important role in the pathogenicity of *L. monocytogenes* is played by internalin A that mediates the interaction with E-cadherin of epithelial cells, thus enabling the passage through the intestinal barrier (Bierne *et al.* 2007). By the PCR-RFLP method for the detection of the *inlA* gene polymorphism, the strains tested were classified into four of the five profiles described previously (Rousseaux *et al.* 2004). Most strains tested were of profile 1 (6/12). The strains of profiles 1 and 4 have been found to produce the truncated form InlA and to have a lower capability of invading tissue culture epithelial cells (Caco-2) (Rousseaux *et al.* 2004; Lyautey *et al.* 2007). However, in our study, these profiles were also detected in the strains that are clonally identical to those recovered from human listeriosis cases, i.e. in a strain of pulsotype 713 and PCR-RFLP profile 4, and in strains of pulsotypes 716 and 735 and of PCR-RFLP profile 1. Therefore, it can be stated that some strains of profiles 1 and 4 may also be producers of the functional form of InlA (Rousseaux *et al.* 2004). Nevertheless, from our results, it cannot be concluded unambiguously whether these strains actually produce an 80 kDa InlA or whether other factors also play a role in their invasiveness. On the other hand, 4 (33.3%) of the 12 strains classified into profiles 2 and 3 are potentially invasive strains in which functional internalin A was reported by others (Rousseaux *et al.* 2004).

Macrorestriction analysis revealed 11 different pulsotypes among the isolates recovered. Some of them were detected for the first time (774, 777), while others have already been found not only in food chain isolates but also in human isolates (as documented by the database of the National Institute of Public Health, National Reference Laboratory for Listeria, Prague, Czech Republic). *L. monocytogenes* clones of serotype 1/2a and of pulsotypes 713, 716, and 738 as well as the clone of serotype 4b and pulsotype 204 were recovered from sporadic human listeriosis cases from 2007 through 2010. The *L. monocytogenes* strain of serotype 1/2a and pulsotype 735 detected in waste from a drain in the farm with livestock (sewer of livestock farm) belongs to the same clone as that implicated in the listeriosis outbreak related to vacuum packed ham in the South Bohemian Region at the turn of

Table 3. Characteristics of *L. monocytogenes* strains of environmental origin

Origin of strains	Serotype	Pulsotype	LIPI-1[a] *prfA*, *hlyA*, *plcA*, *plcB*, *actA*	Internalins[a] *inlA*, *inlB*, *inlC*, *inlJ*	PCR-RFLP profile
Mud from the bank	1/2a	769	+	+	1
Mud from the stream	1/2a	701	+	+	1
Mud from the stream in the place where animals goes to drink	1/2a	709	+	+	4
Mud from the wood small lake	4b	204	+	+	2
Soil from the game-preserve of boars	1/2a	777	+	+	1
Soil by the rack in the wood	4b	204	+	+	2
Decaying foliage from the bank	1/2a	713	+	+	4
Algae from the water reservoir	1/2a	762	+	+	3
Decaying foliage from the bank	1/2a	716	+	+	1
Soil in the farm with horses	1/2a	738	+	+	3
Soil in the farm with swines	1/2a	774	+	+	1
Waste from the drain in the farm with livestock	1/2a	735	+	+	1

[a]+ or – indicate the presence or the absence of mentioned *L. monocytogenes*

2008/2009 (Karpíšková & Gelbíčová 2009). Interestingly, the strain of serotype 1/2a and pulsotype 713 is classified into the persistent clone which has long been detected in a Czech plant making soft-ripened cheeses (Gelbíčová *et al.* 2008). These results are in accordance with those reported in other countries (Fugett *et al.* 2007), showing that some *L. monocytogenes* clones are associated with specific sources, while others are widespread and can be found among the isolates from the farm environment, foodstuffs, environmental sources, or humans. Therefore, to facilitate epidemiological investigations and identification of *L. monocytogenes* sources, it is necessary to maintain an extensive database with phenotypic and genotypic characteristics of strains of different origin.

CONCLUSION

The bacterium *L. monocytogenes* can be isolated from the external environment, in particular from wet areas or from those where animals are present. The predominance of serotype 1/2a and susceptibility of the isolates to antimicrobials are in line with the available data on food and human isolates from all over the Czech Republic. The results obtained have confirmed that the environment is a source of potentially pathogenic *L. monocytogenes* strains that carry a basic set of virulence genes and have the potential for invading the host epithelial cells.

References

Aurora R., Prakash A., Prakash S., Rawool D.B., Barbuddhe S.B. (2008): Comparison of PI-PLC based assays and PCR along with *in viv*o pathogenicity tests for rapid detection of pathogenic *Listeria monocytogenes*. Food Control, **19**: 641–647.

Bierne H., Sabet C., Personnic N., Cossart P. (2007): Internalins: a complex family of leucine-rich repeat-containing proteins in *Listeria monocytogenes*. Microbes and Infection, **9**: 1156–1166.

Chen J., Luo X., Jiang L., Jin P., Wei W., Liu D., Fang W. (2009a): Molecular characteristics and virulence potential of *Listeria monocytogenes* isolates from Chinese food systems. Food Microbiology, **26**: 103–111.

Chen J., Jiang L., Chen X., Lou X., Chen Y., Yu Y., Tian G., Liu D., Fang W. (2009b): *Listeria monocytogenes* serovar 4a is a possible evolutionary intermediate between *L. monocytogenes* serovars 1/2a and 4b and *L. innocua*. Journal of Microbiology and Biotechnology, **19**: 238–249.

Colburn K.G., Kaysner CH.A., Carlos Abeyta J.R., Wekell M.M. (1990): *Listeria* species in a California coast estuarine environment. Applied and Environmental Microbiology, **56**: 2007–2011.

CLSI (2006); Performance standards for antimicrobial susceptibilty testing; sixteenth informational supplement. NCCLS document M100-S16. Clinical and Laboratory Standards Institute, Wayne: 44–55.

Comité de l'antibiogramme de la Société Française de Microbiologie (2005): Concentrations et diamètres critiques pour les diverses classes d'antibiotiques. Société Française de Microbiologie:11–12.

D'Agostino M., Wagner M., Vazquez-Boland J.A., Kuchta T., Karpiskova R., Hoorfar J., Novella S., Scortti M., Ellison J., Murray A., Fernandes I., Kuhn M., Pazlarova J., Heuvelink A., Cook N.A. (2004): A validated PCR-based method to detect *Listeria monocytogenes* using raw milk as a food model-towards an international standard. Journal of Food Protection, **67**: 1646–1655.

Doumith M., Cazalet Ch., Simoes N., Frangeul L., Jacquet Ch., Kunst F., Martin P., Cossart C., Glaser P., Buchrieser C. (2004a): New aspects regarding evolution and virulence of *Listeria monocytogenes* revealed by comparative genomics and DNA arrays. Infection and Immunity, **72**: 1072–1083.

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

Esteban J.I., Oporto B., Aduriz G., Juste R.A., Hurtado A. (2009): Faecal shedding and strain diversity of *Listeria monocytogenes* in healthy ruminants and swine in Northern Spain. BMC Veterinary Research, **5**: 2.

EN ISO 11290-1:1996/Amd.1:2004: Microbiology of food and animal feeding stuffs-Horizonatl method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection Method.

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

Freitag N.E., Port G.C., Miner M.D. (2009): *Listeria monocytogenes* – from saprophyte to intracellular pathogen. Nature Reviews Microbiology, **7**: 623–628.

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.

Gelbíčová T., Šťástková Z., Pospíšilová M., Karpíšková R. (2008): Charakteristika izolátů *Listeria monocytogenes* z mléčných výrobků. Veterinářství, **58**: 324–326.

Gray M.J., Freitag N.E., Boor K.J. (2006): How the bacterial pathogen *Listeria monocytogenes* mediates the switch from environmental Dr. Jekyll to pathogenic Mr. Hyde. Infection and Immunity, **74**: 2505–2512.

Ivanek R., Gröhn Y.T., Wiedmann M. (2006): *Listeria monocytogenes* in multiple habitats and host populations: review of available data for mathematical modeling. Foodborne Pathogens and Disease, **3**: 319–336.

Jaradat Z.W., Schutze G.E., Bhunia A.K. (2002): Genetic homogeneity among *Listeria monocytogenes* strains from infected patients and meat products from two geographic locations determined by phenotyping, ribotyping and PCR analysis of virulence genes. International Journal of Food Microbiology, **76**: 1–10.

Kalorey D.R., Kurkure N.V., Warke S.R., Rawool D.B., Malík S.V.S., Barbuddhe S.B. (2006): Isolation of pathogenic *Listeria monocytogenes* in faeces of wild animals in captivity. Comparative Immunology, Microbiology and Infectious Diseases, **29**: 295–300.

Karpíšková R., Gelbíčová T. (2008): Charakteristika a prevalence klonů *Listeria monocytogenes* izolovaných od pacientů v letech 2001–2008 v České republice. Epidemiologie, mikrobiologie, imunologie, **57**: 137–140.

Karpíšková R., Gelbíčová T. (2009): The role of typing methods in epidemiological investigations of listeriosis. In: 3rd Congress of European Microbiologists FEMS. Sweden: 232.

Kaur S., Malík S.V.S., Bhilegaonkar K.N., Vaidya V.M., Barbuddhe S.B. (2010): Use of phospholipase-C assay, *in vivo* pathogenicity assays and PCR in assessing the virulence of *Listeria* spp. Veterinary Journal, **184**: 366–370.

Liu D., Lawrence M.L., Austin F.W., Ainsworth A.J. (2007): A multiplex PCR for species – and virulence – specific determination of *Listeria monocytogenes*. Journal of Microbiological Methods, **71**: 133–140.

Lyautey E., Lapen D.R., Wilkes G., McCleary K., Pagotto F., Tyler K., Hartmann A., Piveteau P., Rieu A., Robertson W.J., Medeiros D.T., Edge T.A., Gannon V., Topp E. (2007): Distribution and characteristics of *Listeria monocytogenes* isolates from surface waters of the south nation river watershed, Ontario, Canada. Applied and Environmental Microbiology, **73**: 5401–5410.

MacGowan A.P., Bowker K., McLauchlin J., Bennett P.M., Reeves D.S. (1994): The occurrence and seasonal changes in the isolation of *Listeria* spp. in shop bought food stuffs, human faeces, sewage and soil from urban sources. International Journal of Food Microbiology, **21**: 325–334.

Mammina C., Aleo A., Romani C., Pellissier N., Nicoletti P., Pecile P., Nastasi A., Pontello M.M. (2009): Characterization of *Listeria monocytogenes* isolates from human listeriosis cases in Italy. Journal of Clinical Microbiology, **47**: 2925–2930.

Pulse-net Europe (2002): Standardized protocol for molecular subtyping of *Listeria monocytogenes* by pulsed-field gel electrophoresis (PFGE): 10. Available at http://www.pulsenet-europe.org

Rodas-Suárez O.R., Flores-Pedroche J.F., Betancourt-Rule J.M., Quiñones-Ramírez E.I., Vázquez-Salinas C. (2006): Occurrence and antibiotic sensitivity of *Listeria monocytogenes* strains isolated from oysters, fish, and estuarine water. Applied and Environmental Microbiology, **72**: 7410–7412.

Rousseaux S., Olier M., Lemaître J.P., Piveteau P., Guzzo J. (2004): Use of PCR-restriction fragment length polymorphism of *inlA* for rapid screening of *Listeria monocytogenes* strains deficient in the ability to invade Caco-2 cells. Applied and Environmental Microbiology, **70**: 2180–2185.

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, **14**: 584–640.

Weis J., Seeliger H.P.R. (1975): Incidence of *Listeria monocytogenes* in nature. Applied Microbiology, **30**: 29–32.

Welshimer H.J., Donket-Voet J. (1971): *Listeria monocytogenes* in nature. Applied Microbiology, **21**: 516–519.

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