Original article

Invasiveness of *Listeria monocytogenes* strains isolated from animals in Poland

E. Wałecka-Zacharska[1], K. Kosek-Paszkowska[1], J. Bania[1], Z. Staroniewicz[2], M. Bednarski[3], A. Wieliczko

[1] Department of Food Hygiene and Consumer Health Protection,
Wrocław University of Environmental and Life Sciences, Norwida 31, 50-375, Wrocław, Poland
[2] Department of Pathology, Wrocław University of Environmental and Life Sciences,
Norwida 31, 50-375, Wrocław, Poland
[3] Department of Epizootiology with Clinic for Birds and Exotic Animals,
pl. Grunwaldzki 45, 50-366, Wrocław, Poland

Abstract

Animals are important reservoir of *Listeria monocytogenes*, a pathogen causing serious infections in both humans and livestock. However, data on invasiveness of *L. monocytogenes* strains of animal origin is very scarce. Ability of 18 *L. monocytogenes* strains of animal origin to invade HT-29 cells was investigated. Plaque forming assay was used to assess invasiveness and ability of the pathogen to spread in the cell line. Almost 40% of *L. monocytogenes* strains were weakly invasive. It was shown that strains from serogroup 4b exhibited the highest invasiveness, whereas serogroup 1/2b consisted of strains of invasiveness below 0.0001%. Analysis of translated *inlA* and *inlB* gene sequences revealed no premature stop codons. Lineage-specific mutations in low invasive strains were identified within *inlA* and *inlB* sequences. Our results demonstrate high incidence of low invasive animal *L. monocytogenes* strains, which may be at least partly explained by unique point mutations in the InlA and InlB.

Key words: *Listeria monocytogenes*, animals, invasiveness

Introduction

Listeria monocytogenes is a non-spore forming, facultative anaerobic Gram-positive bacterium, widespread in the environment (Farber and Peterkin 1991). The bacterium is a serious pathogen responsible annually for 1 662 invasive infections and 266 related deaths in the United States (Cartwright et al. 2013). *L. monocytogenes* was identified for the first time in 1926 as a cause of monocytosis in rodents.

Then listeric infections were reported among several mammalian species, including feral and domestic ruminants, and monogastric animals (Gray and Killinger 1966). The main factor in disease transmission among animals is poor quality silage (Wesley et al. 2002). The species comprises 13 serotypes, which are classified into four genetically distant lineages (Ragon et al. 2008). Serotypes 1/2b and 4b (lineage I) have been mainly isolated from human food-borne epidemics, as well as sporadic cases in humans and animals.

Correspondence to: E. Wałecka-Zacharska, e-mail: ewa.walecka@up.wroc.pl, tel.: +48 71 320 5 262

Table 1. *L. monocytogenes* strains used in the study.

Strain	Source	Lineage	Genotype actA	Genotype RAPD	Serotype	Invasiveness [%]	Plaque size [mm]
8z	pig (brain)	I	A1	R1	1/2b (3b)	2.75E-05 ± 7.78E-06	0.8 ± 0.09
9z	sheep (liver)	I	A1	R2	1/2b (3b)	7.46E-07 ± 1.26	0.37 ± 0.06
33z	goat (brain)	I	A1	R2	1/2b (3b)	7.2E-04 ± 3.2E-04	1.01 ± 0.12
34z	goat (brain)	I	A1	R2	1/2b (3b)	3.9E-06 ± 1.98E-06	1.05 ± 0.19
41z	sheep (liver)	I	A1	R3	1/2b (3b)	5.53E-07 ± 2.92E-07	1.03 ± 0.25
35z	sheep (liver)	I	A2	R4	4b (d,e)	20 ± 1.1	0.91 ± 0.15
36z	sheep (liver)	I	A2	R4	4b (d,e)	23.3 ± 9.3	1.16 ± 0.08
39z	sheep (liver)	I	A3	R5	4b (d,e)	12.6 ± 6.09	0.86 ± 0.12
40z	sheep (organs)	I	A3	R6	4b (d,e)	7.85 ± 3.6	0.8 ± 0.2
12z	pig (liver)	II	A6	R7	1/2a (3a)	5.2 ± 2.6	0.71 ± 0.17
13z	dog (spleen)	II	A6	R7	1/2a (3a)	2.6 ± 1.2	0.92 ± 0.16
42z	sheep (organs)	II	A6	R8	1/2a (3a)	2.75 ± 0.92	0.52 ± 0.12
43z	goat (brain)	II	A6	R8	1/2a (3a)	2.87 ± 1.41	0.65 ± 0.16
18z	sheep (brain)	II	A6	R9	1/2a (3a)	0.17 ± 0.08	0.84 ± 0.11
26z	sheep (spleen)	II	A5	R10	1/2a (3a)	0.43 ± 0.18	0.96 ±0.14
30z	sheep (brain)	II	A5	R11	1/2a (3a)	0.53 ± 0.31	0.7 ± 0.13
37z	sheep (foetus)	II	A4	R12	1/2a (3a)	0.0057 ± 0.0027	1.02 ± 0.17
38z	sheep (brain)	II	A4	R13	1/2a (3a)	0.073 ± 0.0038	1.09 ± 0.21

Serotypes 1/2a, 1/2c and 3a (lineage II) have been associated with sporadic cases of listeriosis, whereas serotypes 4a and 4c are generally isolated from animals (Vhzquez-Boland et al. 2001). It has been shown that serotypes 1/2a, 1/2b, 1/2c and 4b are most frequently isolated from the liver and spleen of experimentally infected mouse (Barbour et al. 2001).

Serotype 4b strains of human and food origin have been shown to present the highest invasion efficiency among *L. monocytogenes* isolates (Wałecka--Zacharska et al. 2013). Nonetheless, little is known about invasiveness of *L. monocytogenes* strains of animal origin.

Food contamination usually occurs by cross-contamination of the final product by *L. monocytogenes* present in food processing plant environments. Despite infected animals are infrequently linked to human infections, animal-derived food products not processed before consumption, as well as raw food of plant origin contaminated by manure from infected or shedding animals can directly cause human listeriosis (Nightingale et al. 2004).

Our aim was to determine the ability to invade and spread within HT-29 cells of 18 *L. monocytogenes* strains isolated from animals.

Materials and Methods

Growth of L. monocytogenes

The study was conducted on 18 *L. monocytogenes* strains isolated from independent listeriosis cases in animals (Table 1). All strains were classified into serogroups using multiplex PCR (Doumith et al. 2004). Assignment to lineages was based on *actA* gene partial sequence analysis (Zhou et al. 2005). RAPD-PCR was used to further differentiate the strains (Bania et al. 2009). Single colonies of *L. monocytogenes* were seeded into 5 ml of BHI and grown at 37°C with shaking at 180 rev min^{-1} for 6 hours. Then 50-μl aliquots from these cultures were used to inoculate 5 ml of fresh BHI and the bacteria were grown at 37° with shaking at 230 rev min^{-1} for 18 hours. Two to eight log CFU of bacteria were taken for infection of HT-29 and plated in duplicate onto BHI agar.

Cell line and culture conditions

The human adenocarcinoma cell line HT-29 (Institute of Immunology and Experimental Therapy, Wrocław, Poland) was cultured in DMEM (Dulbecco's modified Eagle's medium; Sigma-Aldrich, Poznań, Poland) supplemented with 10% heat-inactivated fetal calf serum (Invitrogen, Warsaw, Poland), 2 mmol l^{-1} glutamine, 100 IU ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin (Sigma-Aldrich) at 37°C in 5% CO_2.

Plaque-Forming Assay (PFA)

HT-29 cells were seeded onto 6-well plates and grown to obtain almost confluent monolayers. Twenty-four hours before infection, the standard

culture medium was replaced by DMEM without antibiotics. HT-29 cells were infected with 2 to 8 log CFU of bacteria for 2 h, then the medium was replaced by DMEM containing 100 μg/mL gentamicin (Sigma-Aldrich) and incubated for 1·5 h. Each well was then overlaid with DMEM containing 10 μg ml^{-1} gentamicin and 1·0 % low-melting-point agarose (Prona, Gdańsk, Poland). After 3 days of culture the number of plaques and plaque size were determined. Diameters of a minimum 10 randomly selected plaques per well were measured on digital images. Each assay was performed in duplicate and repeated at least three times. Invasiveness was expressed as the number of plaques per number of log CFU deposited.

Sequencing of inlA and inlB genes

Conventional PCR was performed. Briefly, 1 μl of appropriate DNA, obtained using DNA extraction kit (Eurx, Gdańsk, Poland), was added to a mix containing 450 nmol l^{-1} of primers 200 mmol l^{-1} dNTPs, 10 mol l^{-1} buffer Tris-HCl of pH 8·3, 50 mnol l^{-1} KCl, 1·5 mmol l^{-1} MgCl$_2$, 0·1% Triton X-100, and 2U of RED-Taq genomic polymerase (Sigma). Primers for *inlA* were from Kovačević et al. (2013).

Primers for *inlB*-for: GTTTTCGGACTATATC-TAGC and for *inlB*-rev: TTAT-TTCTGTGCCCTTAAATTAGCTGC were designed based on known *inlB* gene sequences using Molecular Beacon software (BioRad). The protocol was as follows: initial denaturation at 95°C for 3 min., followed by 32 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 2 min. Then PCR product was sequenced using the BigDyeTM Terminator Ready Reaction Cycle Sequencing kit (Genomed,Warsaw, Poland). Sequences were translated into proteins using BioEdit software (http:// http://www.mbio.ncsu.edu/bioedit/bioedit.html) and the presence of mutations was determined by comparing InlA and InlB sequences to *L. monocytogenes* EGDe strain (Glaser et al. 2001).

Statistical analysis

Each experiment was performed at least in triplicate. The mean values were used for statistical analysis (StatSoft, Krakow, Poland). Kruskal-Wallis test was used to assess differences in invasiveness and plaque size between lineages and serogroups. Significance was set at $p < 0.05$.

Results

Invasiveness and ability to spread in HT-29 cells of L. monocytogenes strains

The 18 *L. monocytogenes* strains were classified into three serogroups: 1/2a (3a) (9 strains), 1/2b (3b) (5 strains) and 4b (4d,e) (4 strains). Determination of partial *actA* gene sequence revealed 6 different *actA* genotypes, i.e. *actA* I-VI, and allowed strain assignment to lineage I (9 strains) and lineage II (9 strains). Thirteen different RAPD genotypes (R1-R13) were determined. No significant difference in the ability to spread in HT-29 cell monolayer was found between three serogroups and two lineages. Mean plaque size diameters were 0.85 ± 0.29 mm, 0.93 ± 0.15 mm and 0.82 ± 0.19 mm in serogroup 1/2b(3b), 4b(d,e) and 1/2a(3a), respectively. Analysis of *L. monocytogenes* ability to invade HT-29 cells revealed no significant differences of invasiveness between lineage I and II (invasiveness of lineage I was 7.08 ± 9.43%, invasiveness of lineage II was 1.62 ± 1.81%). Difference of invasiveness was noted between the serogroups. The most invasive were serogroup 4b(d,e) strains (15.4 ± 7.01%). Strains of invasiveness ranging from 5.2 ± 2.6% – 0.0057 ± 0.0027% were found in the serogroup 1/2a(3a). The serogroup 1/2b(3b) consisted of strains of invasiveness below 0.0001% (*actA* I type, RAPD genotypes R1, R2, R3).

Analysis of InlA and InlB sequences

Analysis of complete *inlA* gene sequences reveled that all *L. monocytognes* strains should produce full-length InlA protein, since no premature stop codons were detected in translated nucleotide *inlA* sequences. Twenty-nine amino-acid point mutations, from which seven were lineage I-specific, were found in the translated InlA protein sequence, as compared to InlA of EGDe strain (Table 2). Low invasive strains from lineage I (8z, 9z, 33z, 34z, 41z) possessed unique mutation Asn32Ser in the InlA sequence. In turn, the hallmark of lineage II low invasive strains were four amino acid substitutions in InlA, namely Ile157Leu, Asn546Asp, Val644Ile, Ala652Thr.

Forty-four point mutations were found in translated InlB protein sequence, including 12 lineage I-specific (Table 3). Low invasive strains from lineage I (8z, 9z, 33z, 34z, 41z) were distinguished by three amino acid substitutions, namely Asn76Asp, Asn371Asp, Ala390Val.

Table 2. Amino-acid residues that differ among the *L. monocytogenes* strains, based on the analysis of *inlA* gene.

Strain	Amino acid residue																
	32	51	94	118	142	157	187	416	420	426	454	461	474	476	494	495	500
EGDe, 30z	N	T	V	N	T	I	S	A	A	A	T	N	S	P	L	A	V
8z,9z,33z,34z,41z	S	·	L	D	·	·	·	E	P	V	A	·	N	S	·	·	A
36z	·	·	L	D	S	·	·	E	P	V	A	·	N	S	N	V	A
35z,39z,40z	·	·	L	D	S	·	·	E	P	V	A	·	N	S	·	·	A
12z,13z,18z,26z,42z	·	A	L	D	·	·	N	·	·	·	A	·	·	·	·	·	A
37z,38z	·	A	·	·	·	L	·	·	·	·	·	·	·	·	·	·	·
43z	·	A	L	D	·	·	N	·	·	·	A	Y	·	·	·	·	A

Strain	Amino acid residue											
	530	546	558	573	593	645	648	652	664	738	781	790
EGDe, 30z	H	N	N	D	A	V	S	A	A	D	L	M
8z,9z,33z,34z,41z	Y	·	·	·	·	·	T	·	T	N	I	V
36z	Y	·	D	·	·	·	T	·	T	N	I	V
35z,39z,40z	Y	·	D	·	·	·	T	·	T	N	I	V
12z,13z,18z,26z,42z	·	·	·	E	P	·	·	·	·	·	·	·
37z,38z	·	D	D	E	P	I	T	P	·	·	·	·
43z	·	·	·	E	P	·	·	·	·	·	·	·

Table 3. Amino-acid residues that differ among the *L. monocytogenes* strains, based on the analysis of *inlB* gene.

Strain	Amino acid residue																
	41	49	69	73	76	91	117	132	138	164	176	181	197	205	246	251	262
EGDe, 30z	P	S	A	N	N	V	A	V	L	P	I	V	Q	A	P	S	T
8z,9z,33z,34z,41z	·	P	L	S	D	I	·	I	·	L	L	I	E	S	S	T	I
35z, 36z	·	P	L	S	·	I	·	I	·	L	L	I	E	S	S	T	I
39z,40z	S	P	L	S	·	·	·	I	I	·	L	I	E	S	S	T	I
12z,13z,18z,26z,42z,43z	S	P	·	·	·	·	T	I	·	·	·	·	·	·	·	·	·
37z,38z	·	·	·	·	·	·	T	I	·	·	·	·	·	·	·	·	·

Strain	Amino acid residue																
	291	304	371	373	387	390	396	445	446	470	482	486	489	501	502	533	555
EGDe, 30z	T	I	N	N	V	A	A	G	K	M	R	S	S	K	H	G	K
8z,9z,33z,34z,41z	I	V	D	S	M	V	·	·	·	I	K	L	A	·	·	·	·
35z, 36z	I	V	·	S	M	·	·	·	E	I	K	L	A	T	L	D	I
39z,40z	I	V	·	S	M	·	·	S	E	I	K	L	A	T	L	D	I
12z,13z,18z,26z,42z,43z	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·
37z,38z	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

Strain	Amino acid residue								
	557	558	578	580	582	584	594	599	600
EGDe, 30z	T	R	A	N	V	R	K	C	Q
8z,9z,33z,34z,41z	·	·	·	·	·	·	·	·	·
35z, 36z	I	Q	V	S	I	W	T	R	T
39z,40z	I	Q	V	S	I	W	T	R	T
12z,13z,18z,26z,42z,43z	·	·	·	·	·	·	·	·	·
37z,38z	·	·	·	·	·	·	·	·	·

Discussion

Virulence of *L. monocytognes* population has been shown to be heterogenic. Eight to 21% of strains are estimated to be avirulent or weakly virulent (Roche et al. 2005). There is little information on pathogenicity of *L. monocytogenes* strains of animal origin. Therefore, in our study we used plaque-forming assay to describe ability of 18 *L. monocytogenes* strains isolated from animals to invade and spread in the human adenocarcinoma cell line.

Almost 40% of strains studied here were weakly invasive. In contrast to previous studies no differences were found between invasiveness values of isolates assigned to *L. monocytogenes* lineages I and II (Wiedmann et al. 1997, Zhou et al. 2005). Both lineage I and lineage II comprised invasive and weakly invasive strains, which varied in the ability to invade HT-29 cells up to 7.6 and almost 3 orders of magnitude, respectively. The most invasive were serogroup 4b(d,e) strains, which agrees with the results of our previous study (Wałecka-Zacharska et al. 2013).

Little is known about the molecular basis of virulence heterogeneity in *L. monocytogenes* population. Internalin InlA and InlB are the key surface proteins containing N-terminal leucine-rich repeats (LRRs) domain that interact with their respective host-cell receptors, leading to bacterial internalization (Bierne et al. 2007). It has been shown that loss of *inlA* and *inlB* genes seriously hamper *L. monocytogenes* ability to invade epithelial cells (Kim et al. 2005). A number of mutations in *inlA* gene, leading to premature stop codons (PMSCs), have been observed in *L. monocytogenes*. Such mutations result in production of truncated InlA, contributing to attenuated invasion efficiency of the bacteria (Nightingale et al. 2005, Nightingale et al. 2008). However, recent study by Roche et al. (2012) reports on low virulent *L. monocytogenes* strains expressing full length InlA. In our study, 7 out of 18 strains exhibited invasiveness under 0.1%, from which five had invasiveness below 0.0001%. Analysis of the translated InlA sequence revealed no PMSCs in all strains. Instead we have found a number of point mutations in the InlA (Table 2). Some of them were earlier reported to be lineage-specific (Ragon et al. 2008). Low invasive strains from lineage I possessed unique mutation (Asn32Ser) in the signal peptide of the *inlA* gene, whereas lineage II-strains were characterized by one amino acid substitution in LRR region (Ile157Leu) and three mutations in the domain B of InlA (Asn546Asp, Val644Ile, Ala652Thr).

Two mutations in LRR domain of InlB, namely Ala117Thr and Val132Ile, have been described. Since this region is crucial for interaction with the InlB receptor (Met) and Ala117Thr substitution leads to change of hydrophobicity of amino acid, the role of this mutation in the protein inactivation has been postulated (Temoin et al. 2008, Roche et al. 2012). We have identified 44 mutations in the InlB sequence (Table 3). We have found one mutation in the LRR domain (Asn76Asp) and two in the C-terminal region (Asn371Asp; Ala390Val) in low invasive lineage I strains.

Remaining mutations, including Ala117Thr, however, appeared in both invasive and weakly invasive strains. Whether mutations reported here play role in *L. monocytogenes* ability to invade HT-29 cells merits further confirmation.

Comparison of results presented here with our previous data on *L. monocytogenes* strains from human clinical cases (n=14) and food (n= 27) demonstrates that low invasive strains are more prevalent among animal population than human and food isolates (Wałecka-Zacharska et al. 2013).

In summary, results presented here demonstrate high incidence of low invasive *L. monocytogenes* strains of animal origin which are characterized by unique point mutations in InlA and InlB. Importance of these mutations requires further investigation.

Acknowledgements

References

Bania J, Żarczyńska A, Molenda J, Dabrowska A, Kosek-Paszkowska K, Wieckowska-Szakiel M, Rózalska B (**2009**) Subtyping of *Listeria monocytogenes* isolates by *actA* gene sequencing, PCR-fingerprinting, and cell-invasion assay. Folia Microbiol 54: 17-24.

Barbour AH, Rampling A, Hormaeche CE (**2001**) Variation in the infectivity of *Listeria monocytogenes* isolates following intragastric inoculation of mice. Infect Immun 69: 4657-4660.

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

Cartwright EJ, Jackson KA, Johnson SD, Graves LM, Silk BJ, Mahon BE (**2013**) Listeriosis outbreaks and associated food vehicles, United States, 1998-2008. Emerg Infect Dis 19: 1-9.

Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P (**2004**) Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J Clin Microbiol 42: 3819-3822.

Farber JM, Peterkin PI (**1991**) *Listeria monocytogenes*, a food-borne pathogen. Microbiol Rev 55: 476-511.

Glaser P, Frangeul L, Buchrieser C , Rusniok C, Amend A, Baquero F, Berche P, Bloecker H, Brandt P, Chakraborty T, Charbit A, Chetouan F, Couvé E, de Daruvar A, Dehoux P, Domann E, Domtnguez-Bernal G, Duchaud E, Durant L, Dussurget O, Entian KD, Fsihi H, Garcta-del Portillo F, Garrido P, Gautier L, Goebel W, Gómez-López N, Hain T, Hauf J, Jackson D, Jones LM, Kaerst U, Kreft J, Kuhn M, Kunst F, Kurapkat G, Madueno E, Maitournam A, Vicente JM, Ng E, Nedjari H, Nordsiek G, Novella S, de Pablos B, Pérez-Diaz JC, Purcell R, Remmel B, Rose M, Schlueter T, Simoes N,

Tierrez A, Vázquez-Boland JA, Voss H, Wehland J, Cossart P (**2001**) Comparative genomics of *Listeria* species. Science 294: 849-852.

Gray ML, Killinger AH (**1966**) *Listeria monocytogenes* and listeric infections. Bacteriol Rev 30: 309-382.

Kim H, Marquis H, Boor KJ (**2005**) Sigma B contributes to *Listeria monocytogenes* invasion by controlling expression of *inlA* and *inlB*. Microbiology 151: 3215-3222.

Kovačević J, Arguedas-Villa C, Wozniak A Tasara T, Allen KJ (**2013**) Examination of food chain-derived *Listeria monocytogenes* strains of different serotypes reveals considerable diversity in *inlA* genotypes, mutability, and adaptation to cold temperatures. Appl Environ Microbiol 79: 1915-1922.

Nightingale KK, Windham K, Martin KE, Yeung M, Wiedmann M (**2005**) Select *Listeria monocytogenes* subtypes commonly found in foods carry distinct nonsense mutations in *inlA*, leading to expression of truncated and secreted internalin A, and are associated with a reduced invasion phenotype for human intestinal epithelial cells. Appl Environ Microbiol 71: 8764-8772.

Nightingale KK, Ivy RA, Ho AJ, Fortes ED, Njaa BL, Peters RM, Wiedmann M (**2008**) *inlA* premature stop codons are common among *Listeria monocytogenes* isolates from foods and yield virulence-attenuated strains that confer protection against fully virulent strains. Appl Environ Microbiol 74: 6570-6583.

Nightingale KK, Schukken YH, Nightingale CR, Fortes ED, Ho AJ, Her Z, Grohn YT, McDonough PL, Wiedmann M (**2004**) Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. Appl Environ Microbiol 70: 4458-4467.

Ragon M, Wirth T, Hollandt F, Lavenir R, Lecuit M, Le Monnier A, Brisse S (**2008**) A new perspective on *Listeria monocytogenes* evolution. PLoS Pathog 4: e1000146.

Roche SM, Grepinet O, Kerouanton A, Ragon M, Leclercq A, Temoin S, Schaeffer B, Skorski G, Mereghetti L, Le Monnier A, Velge P (**2012**) Polyphasic characterization and genetic relatedness of low-virulence and virulent *Listeria monocytogenes* isolates. BMC Microbiol 12: 304.

Roche SM, Gracieux P, Milohanic E, Albert I, Virlogeux-Payant I, Témoin S, Grépinet O, Kerouanton A, Jacquet C, Cossart P, Velge P (**2005**) Investigation of specific substitutions in virulence genes characterizing phenotypic groups of low-virulence field strains of *Listeria monocytogenes*. Appl Environ Microbiol 71: 6039-6048.

Témoin S, Roche SM, Grépinet O, Fardini Y, Velge P (**2008**) Multiple point mutations in virulence genes explain the low virulence of *Listeria monocytogenes* field strains. Microbiology 154: 939-948.

Vhzquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Domtnguez-Bernal G, Goebel W, Gonzhlez-Zorn B, Wehland J, Kreft J (**2001**) *Listeria* pathogenesis and molecular virulence determinants. Clin Microbiol Rev 14: 584-640.

Wałecka-Zacharska E, Kosek-Paszkowska K, Bania J, Karptšková R, Stefaniak T (**2013**) Salt stress-induced invasiveness of major *Listeria monocytogenes* serotypes. Lett Appl Microbiol 56: 216-221.

Wesley IV, Larson DJ, Harmon KM, Luchansky JB, Schwartz AR (**2002**) A case report of sporadic ovine listerial menigoencephalitis in Iowa with an overview of livestock and human cases. J Vet Diagn Invest 14: 314--321.

Wiedmann M, Bruce JL, Keating C, Johnson AE, McDonough PL, Batt CA (**1997**) Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. Infect Immun 65: 2707-2716.

Zhou X, Jiao X, Wiedmann M (**2005**) *Listeria monocytogenes* in the Chinese food system: strain characterization through partial *actA* sequencing and tissue-culture pathogenicity assays. J Med Microbiol 54: 217-224.