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Listeria monocytogenes clones circulating in the natural environment of the Czech Republic and Slovakia

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Abstract: *Listeria monocytogenes* is not only a pathogen causing a serious food-borne disease in humans but can also occur as a saprophyte in the natural environment. This study aimed to evaluate the occurrence of *L. monocytogenes* obtained from the natural environment of the Czech Republic (hereinafter Czechia) and Slovakia in 2016–2018 and to compare the clonal relationship of strains circulating in the environment with the strains originating from the food chain and humans. Altogether, 217 samples of mud, surface water, vegetation and soil were collected in 61 locations. Samples were processed according to the modified EN ISO 11290-1 standard. The obtained *L. monocytogenes* isolates were characterised using serotyping, macrorestriction analysis, followed by pulsed-field gel electrophoresis, whole genome sequencing (WGS), and antimicrobial susceptibility testing. *L. monocytogenes* were detected in 8.8% of the examined samples and were isolated in 15 locations, mainly from the mud from the banks of the surface water sources. Altogether, 25 *L. monocytogenes* strains were obtained from 19 positive samples. Serotypes 1/2a, 4b, and 1/2b were detected among the strains. Twenty combined *AscI/Apa*I pulsotypes were obtained by macrorestriction analysis. Altogether, 12 sequence types (STs) were detected using Multi-locus sequence typing (MLST) by WGS, with ST451 being the most frequent. The core genome MLST analysis revealed a heterogeneous population of environmental strains. No phenotype resistance was detected by antimicrobial susceptibility testing. Screening of antimicrobial-resistance genes using the platform ResFinder revealed the genes *fosX* in 24 isolates and *bla*TEM-116 in one isolate. The occurrence of *L. monocytogenes* in various samples from natural environments within wide altitude range during different seasons of the year may highlight this bacterium's remarkable adaptability and exceptional tolerance to external factors. Serotype distribution of the strains circulating in the natural environment of Czechia and Slovakia seems to reflect distribution in the human population more than in the food chain.

Keywords: nature; serotyping; macrorestriction analysis; antimicrobial susceptibility; whole genome sequencing

In humans, *Listeria monocytogenes* can cause severe illness affecting mainly risk groups of population, e.g. pregnant women, new-borns, elderly and immunocompromised people (Vazquez-Boland et al. 2001; Gray et al. 2006). In nature, *L. monocytogenes* can usually be found in soil, surface water and decaying veg-

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etation. Potential reservoirs of this bacterium are wild and domesticated animals, especially cattle and small ruminants, which represent a significant source of faecal contamination of the farm environment (Nightingale et al. 2004; Khan et al. 2016). Proximity to farms appeared to be associated with higher detection rates of *L. monocytogenes* in nature (Sauders et al. 2006; Strawn et al. 2013). The presence of *L. monocytogenes* in the natural environment represents a permanent source of contamination for the human food chain at the level of primary food production (Khan et al. 2016). Development of agriculture and the associated creation of artificial agroecosystems can contribute to the transmission of *L. monocytogenes* into the human food chain through contaminated crops and livestock products (Vivant et al. 2013). The ability of *L. monocytogenes* to survive and multiply under adverse stress conditions in the complex ecosystem of nature is affected by external factors such as water content, temperature, pH, soil matrix composition or by the presence of a competitive microbiota (McLaughlin et al. 2011). Internal factors facilitating adaptation to external environments possibly include the expression of specific genes, e. g. genes encoding cell transport mechanisms, carbohydrate-catalysing enzymes or genes allowing utilisation of carbon or nitrogen compounds in soil (Vivant et al. 2013; Lourenco et al. 2022).

Prevalence and diversity of *L. monocytogenes* have been monitored in various natural environment-associated sources, e.g. in flowing and standing water (Raschle et al. 2021; Gorski et al. 2022), in wastewater (Paillard et al. 2005; Serhat et al. 2021), in soil (Claxton et al. 2021), in vegetation (Linke et al. 2014) and in faeces of wild animals (Wang et al. 2017; Cao et al. 2018). In Czechia, a study assessing the occurrence of *L. monocytogenes* in the wild, farm environment and in vegetation was performed in 2010 (Gelbíčová and Karpíšková 2012).

The present study aimed to evaluate the occurrence of *L. monocytogenes* in the natural environment of Czechia and Slovakia, to characterise the obtained isolates, and, based on their genetic characteristics, to specify the *L. monocytogenes* circulating in the natural environment of Czechia and Slovakia and to compare the clonal relationship of strains circulating in the environment with the strains originating from the food chain and humans.

MATERIAL AND METHODS

Environmental sampling. A total of 217 environmental samples (mud from the banks of surface water sources, surface water, vegetation, and soil) were investigated (see Electronic Supplementary Material, ESM, Supplementary Table S1). For each sample, the following information was recorded: sampling location, the sampling point's altitude, and the sampling date. Samples were collected in 54 locations across Czechia (208 samples) and seven locations across Slovakia (9 samples). One to twenty samples were taken from each site. Based on the nature and type of land used by humans in the sampling point, all collected samples were further differentiated into samples originating from countryside/forest areas (153 samples, surroundings of villages, forests and wildlife with variable occurrence and movement of people), samples from agricultural areas (13 samples, cultivated fields and meadows, compost, silage), samples from urban areas (13 samples, towns, centres of populated areas with high occurrence and movement of people) and samples from recreation areas (38 samples, natural areas intended for recreation with high occurrence and movement of people).

Sampling took place from July 2016 to September 2018 during spring (March–May), summer (June–August) and autumn (September–November) seasons. No sample was collected in winter. The highest proportion of samples was collected in summer (103/217, 47.5%), followed by autumn (60/217, 27.7%) and spring season (54/217, 24.9%).

Sampling was carried out in lowlands (altitude 0–300 m), hills (altitude 300–600 m), highlands (altitude 600–900 m) and mountains (altitude above 900 m). Most samples were collected in hills (125 samples, 57.6%) and lowlands (77 samples, 35.5%), followed by highlands (10 samples, 4.6%) and mountains (5 samples, 2.3%). Samples were collected into sterile plastic 50 mL tubes (mud, water) or 100 mL plastic containers (vegetation, soil) (Dispolab, Brno, Czechia) and were transported to the laboratory as soon as possible.

Isolation of *L. monocytogenes*. Samples were processed immediately after delivery to the laboratory. First, non-selective enrichment of 25 g of the sample in buffered-peptone water (Oxoid, Ltd., Basingstoke, United Kingdom) followed by selective enrichment in Fraser Broth (Oxoid, Ltd., Basingstoke, United Kingdom) were performed. Then, were proceeded according to EN ISO 11290-1 standard (Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method. International Organization for Standardization, Geneva) with subsequent inoculation on the chromogenic agar media

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ALOA (Oxoid, Basingstoke, United Kingdom) and Rapid'*L.mono* (Biorad, Hercules, California, USA). Suspected *L. monocytogenes* colonies were selected based on their morphology. From each *L. monocytogenes* positive sample, a total of three suspected colonies per plate were further characterised. Individual *L. monocytogenes* isolates were distinguished by serotyping and macrorestriction analysis and were stored in brain heart infusion (BHI) containing 20% glycerol at –75 °C for further analysis.

Serotyping. Slide agglutination using commercially available antisera (Denka Seiken, Tokyo, Japan) in combination with the PCR assay (Doumith et al. 2004) were used.

Macrorestriction analysis. Macrorestriction analysis followed by pulsed-field gel electrophoresis (PFGE) was performed in accordance with the EU Reference Laboratory protocol (Roussel et al. 2014) using restriction enzymes *Asc*I and *Apa*I (New England BioLabs, Ipswich, Massachusetts, USA). The results were analysed by BioNumerics software (version 5.2, AppliedMaths, Sint-Martens-Latem, Belgium). The parameters of the analysis were as follows: coefficient Dice, dendrogram type UPGMA, optimisation 1.1% and band position tolerance 1.0%. *Salmonella* Braenderup H9812, restricted with *Xba*I enzyme (Takara, Kyoto, Japan), was used as a molecular weight marker. Strains originating from one sample, differing based on the results of macrorestriction analysis, were selected for whole genome sequencing and antimicrobial susceptibility testing.

Whole genome sequencing (WGS). The extraction of genomic DNA from the strains was carried out using the DNeasy Blood & Tissue kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). The preparation of sequencing libraries and the sequencing process were carried out by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES – Plouflagran laboratories, 19 strains) and the ICM Brain & Spine Institute in Paris, France (6 strains). Genome assembling was performed using the Ridom SeqSphere+ software (Ridom GmbH, Munich, Germany) and its integrated part Velvet (version 1.1.04).

Multi-locus sequence typing (MLST) based on the sequencing of seven housekeeping genes (*abcZ, bglA, cat, dapE, dat, ldh, lhkA*) according to Ragon et al. (2008) was performed. Core genome multi-locus sequence typing (cgMLST) was performed at the level of 1701 genes, according to Ruppitsch et al. (2015). The mentioned analyses were performed using Ridom SeqSphere+ software. The WGS data were released to the European Nucleotide Archive (ENA), and the Sample Accession is available in the ESM (Table S1).

Antimicrobial susceptibility testing and detection of antimicrobial-resistance genes. Phenotypic resistance to seven selected antimicrobials was tested by disk diffusion method using commercially available antibiotic disks (Oxoid, Ltd., Basingstoke, England) on Mueller-Hinton agar (MH, Oxoid, Ltd., Basingstoke, England). The following antimicrobials and their concentrations were used: penicillin (1 U), erythromycin (15 µg), gentamicin (10 µg), meropenem (10 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg) and ampicillin (2 µg). The results were evaluated according to EUCAST criteria (EUCAST 2021) and for gentamicin and tetracycline according to Vela et al. (2001).

Detection of genotypic resistance, i.e. screening of antimicrobial-resistance genes, was performed on the WGS data obtained from the isolates using the online platform ResFinder 4.1 (Florensa et al. 2022).

Statistical analysis. Prevalence of *L. monocytogenes* in relationship to *i*) source of isolation, *ii*) geomorphological area, and *iii*) season of the year was statistically evaluated using the Fisher's exact test (package stats) within the project R: A language and environment for statistical computing (R Core Team 2018).

RESULTS AND DISCUSSION

Prevalence of *L. monocytogenes* in the natural environment. The study focused on assessing the occurrence and characteristics of *L. monocytogenes* obtained from the natural environment during three years in the Czech and Slovak regions. 15 out of 61 investigated sampling locations (24.6%) were positive for the presence of *L. monocytogenes*. The bacterium was detected in 19 out of 217 analysed samples with an overall prevalence of 8.8%, which was comparable to the results obtained in other studies, e.g. 6% in Austria (Linke et al. 2014), 4.4% in the USA (Sauders et al. 2006), 13.3% in Iran (Nassirabady et al. 2015) and 11.2% in Czechia (Gelbíčová and Karpíšková 2012). The results of these studies may differ depending on their type, sampling methodology and the detection method used. *L. monocytogenes* were primarily obtained from mud from the banks of the surface water sources (12 out of 70 samples, 17.1%), followed by vegetation (4 out of 52 samples, 7.7%), surface water (2 out of 55 samples, 3.6%) and soil (1 out of 40 samples, 2.5%). In total, 25 *L. monocytogenes* strains were obtained (ESM, Table S1).

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Assessment of the impact of sample origin. *L. monocytogenes* was found in all categories of the investigated samples, with the significantly highest prevalence observed in mud from the banks of the surface water sources (8.3%) in comparison to other sources ($P < 0.05$). Most frequently, *L. monocytogenes* occurred in mud from the banks of ponds (7 positive samples). *L. monocytogenes* was also found in various types of vegetation: moss, plants, and decaying leaves (4 positive samples), less frequently in surface water of ponds and rivers (2 positive samples), and rarely in uncultivated (forest) soil (1 positive sample). The significantly higher prevalence of *L. monocytogenes* found in mud (17.1%) compared to that found in soil (2.5%) could be linked to different humidity levels. However, it is necessary to consider other contributing factors, such as the type of sampling area, climate conditions and proximity to wild/domesticated animals.

Positive samples mostly (but not exclusively) represented typical isolation sources of *L. monocytogenes* from nature with high humidity, rich in organic nutrients, proximity to animal farms or wild animals or proximity to human activities. Most of the positive samples (13/19) were collected in countryside/forest areas, followed by recreation (5/19) and the urban regions (1/19).

In studies conducted in the USA and Ireland, the prevalence of *L. monocytogenes* in various cultivated and uncultivated soils was higher compared to our research, up to 50% (Nightingale et al. 2004; Fox et al. 2009; Sauders et al. 2012; Claxton et al. 2021). Higher prevalence in flowing and standing water sources compared to our study was also observed by authors from Switzerland, Iran, Nigeria, and the USA, 13, 13.3, 33.3, and 41.9%, respectively (Raschle et al. 2021; Nassirabady et al. 2015; Mawak et al. 2009; Gorski et al. 2022).

Assessment of the geomorphological aspects. Based on the geomorphological aspects, the detected prevalence was as follows: mountains (40%), highlands (10%), lowlands (9%), and hills (7.2%), with the highest prevalence, observed in the mountains (40%, statistically insignificant, $P > 0.05$) compared to the other areas.

The largest numbers of different strains were obtained in hills (13 strains out of 9 positive samples) and lowlands (8 strains out of 7 positive samples), which could be associated with increased heterogeneity of *L. monocytogenes* in these areas, possibly due to dispersion of various strains via water courses (Linke et al. 2014), or their transfer and spread via potential animal vectors.

The samples were collected at a wide altitude range of 170 to 1 837 m, and *L. monocytogenes* was detected at a range of 173 to 1 380 m. This wide range of detection may be linked to the tolerance of *L. monocytogenes* to variable temperatures and its adaptability to various external conditions.

The psychotropic nature of *L. monocytogenes* led the authors Linke et al. (2014) to suggest its abundance in cold-climate areas at higher altitudes, which was not confirmed in their study; our results, however, are in accordance with this presumption. The high level of adaptability and exceptional resistance of *L. monocytogenes* to inhospitable environmental conditions was shown by two positive findings in the mountains at the altitudes of 1 099 m (location 54) and 1 380 m (location 60). In both cases, the place of origin was mud from the water spring serving as drinking water source for tourists and wild animals. The presence of *L. monocytogenes* near these water sources may be linked to an increase in the movement of people and wildlife around the springs and could also be associated with the reduction of competitive microbiota due to specific year-round conditions (low average annual temperature, high average annual rainfall, long-lasting snow cover). From this point of view, particularly interesting is the isolation of two different strains (LV1200, LV1201) from mud near the river spring in location 60, with an average annual temperature of 1.7 °C, an average yearly rainfall of 1 064 mm and a snow cover lasting approximately from October/November to April/May [Czech Hydrometeorological Institute (Český hydrometeorologický ústav)].

Assessment of the seasonal aspects. The alternation of seasons in the temperate zone could be another important factor influencing the circulation of *L. monocytogenes* in the natural environment. During the annual cycle, the population of *L. monocytogenes* is affected by multiple factors, including temperatures and their fluctuation, water availability, presence and activity of potential vectors and sufficient supply of organic nutrients.

Regarding the seasonal aspects, the prevalence revealed in our study was as follows: autumn (15%), summer (8.7%) and spring (1.9%). Welshimer and Donker-Voet (1971) assumed that higher humidity and an increased proportion of decomposing organic matter in the spring season could lead to a higher detection rate of *L. monocytogenes* in nature. In our study, however, spring was the season with the significantly lowest prevalence ($P < 0.05$), whereas the significantly highest prevalence was observed in autumn

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($P < 0.05$). Authors from the United States obtained different results. Sauders et al. (2006) observed an almost steady prevalence in the spring, summer, and autumn periods (1.3, 1.8, and 1.2%, respectively), while Gorski et al. (2022) observed the highest prevalence during winter and spring seasons.

Spring and autumn in the temperate zone have relatively similar characteristics (e.g. low temperatures, high humidity), and due to the psychotropic nature of *L. monocytogenes*, its higher abundance in the natural environment during these seasons could be expected. However, specific conditions during these seasons in a particular year could be very variable, and thus the results of individual studies may differ. The highest prevalence of *L. monocytogenes* during the autumn season in our study could be associated with sufficient amounts and increased availability of organic nutrients or increased movement and activity of potential vectors (especially wild animals) compared to the spring season.

Characterisation of the environmental strains and comparison of their serotypes and sequence types (STs) with *L. monocytogenes* in the food chain and humans. Subtypes of *L. monocytogenes* in the natural environment of Czechia and Slovakia were revealed based on the pheno- and genotyping methods used (see ESM). Serotyping revealed three serotypes, with the most frequent serotype 1/2a (19/25, 76%), followed by 4b (5/25, 20%) and 1/2b (1/25, 4%). Lineage II strains (19/25, 76%) were distinctly more prevalent than lineage I strains (6/25, 24%). Serotype 1/2a also prevailed in nature in a previous Czech study (Gelbíčová and Karpíšková 2012), as well as in other countries: Austria, France, Russia and Switzerland (Locatelli et al. 2013; Linke et al. 2014; Voronina et al. 2015; Raschle et al. 2021). On the contrary, serotype 4b was reported to be the most frequent in the USA (Gorski et al. 2022) and in France (Paillard et al. 2005). Similarly to our results, rare detection of serotype 1/2b in nature was reported in France and Russia (Paillard et al. 2005; Voronina et al. 2015).

Serotype 1/2a prevailed in Czechia and Slovakia, also in strains isolated from human listeriosis cases and from foods (Tomáštíková et al. 2019; Kubicová et al. 2021). In both countries, the proportion of individual serotypes in the environmental strains was similar to their distribution in strains from the human population. However, the distribution of serotypes in foods seems slightly different based on the type of food investigated (Table 1).

No strain of serotype 1/2c was detected in the natural environment, although this serotype has been observed to a small extent in food sources in both countries but not in human population.

Altogether, 20 combined *Asc*I/*Apa*I pulsotypes were obtained using macrorestriction analysis (Figure 1). The most frequent pulsotypes were 735/2 ($n = 4$, serotype 1/2a), 211/37 ($n = 2$, serotype 4b), and 846/48 ($n = 2$, serotype 1/2a). It was possible to observe a specific pulsotype detected in strains originating from different lo-

Table 1. Distribution of *Listeria monocytogenes* serotypes isolated from the natural environment in this study compared to serotypes detected in human and food sources in other studies from Czechia (CZ) and Slovakia (SK)

Serotype (%)	Environmental sources	Human sources		Food sources	
	CZ + SK (this study)	CZ (Tomáštíková et al. 2019)	SK (Kubicová et al. 2021)	CZ (Tomáštíková et al. 2019)	SK (Kubicová et al. 2021)
1/2a	76	57.3	41.5	54.7	72* 38.6** 64.4***
4b	20	27.4	31.7	18	7.9* 22.1** 4.4***
1/2b	4	15.4	24.4	23.4	14.3* 35.7** 12.6***
1/2c	ND	ND	ND	3.9	3.1* 2.2** 16.3***

* milk/milk products; ** delicatessen products; *** meat/meat products; ND – not detected

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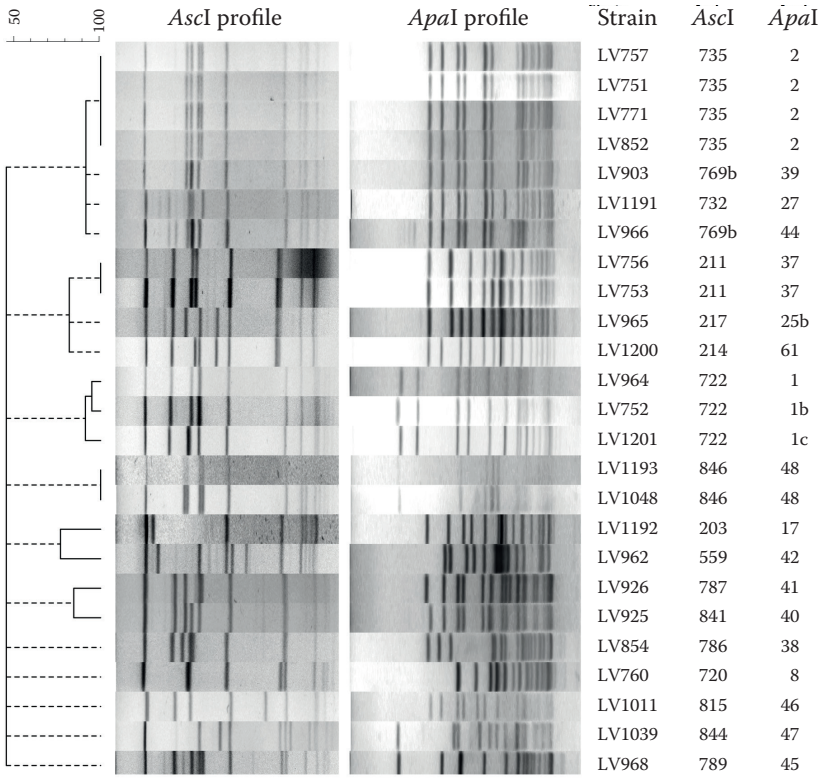

Figure 1. Dendrogram based on the macrorestriction analysis of 25 *Listeria monocytogenes* strains isolated from the natural environment

cations. Pulsotype 735/2 (serotype 1/2a) was detected in strains obtained from four geographically distinct locations, and pulsotypes 211/37 (serotype 4b) and 846/48 (serotype 1/2a) were each detected in strains from two different locations. The higher detection rate of these pulsotypes and their occurrence in multiple locations could be connected with their abundance in the natural environment of Czechia and Slovakia. Pulsotype 735/2 was also detected in the natural environment (livestock farm) in a previous Czech study (Gelbíčová and Karpíšková 2012) and circulated as well in the food chain and humans in Czechia (Gelbíčová et al. 2019).

In total, 12 STs assigned to 12 clonal complexes (CCs) were detected using MLST by WGS. The most prevalent ST in our study, ST451 (n = 7, serotype 1/2a), has been observed in farm animals and environment in the USA and Switzerland (Dreyer et al. 2016; Steckler et al. 2018) while its occurrence in other sources (humans, foods) seems to be rare (Pasteur MLST database). ST1, the second most common ST found in our study (n = 4, serotype 4b), has been associated mostly with human infections, especially in Europe (Althaus et al. 2014; Jensen et al. 2016; Maury et al. 2016) and in Australia (Jennison et al. 2017), although its occurrence in the natural environment has also been abundant (Pasteur MLST database; Papić et al. 2019; Gorski et al. 2022). A strong affinity of ST1 to cause clinical infections was observed in farm animals (Dreyer et al. 2016). The third most frequent sequence type in our study, ST37 (n = 3, serotype 1/2a), seems to be primarily a natural environment-associated one, frequently observed in farm-related environments (Pasteur MLST database; Dreyer et al. 2016). ST37 was a predominant sequence type found in Austrian soil samples (Linke et al. 2014).

ST451, ST1, and ST37, together with other STs detected in this study (ST6, ST121, ST21, ST87), have also been observed in the strains originating from humans and foods in Czechia (Tomáštíková et al. 2019), although the proportion of individual STs in the natural environment, humans and foods seems to be different. ST121, the most frequently detected ST from foods, was detected only in one environmental strain and ST8, the most prevalent ST in human strains, was not detected in the environmental strains at all. In Slovakia, ST451 was the most prevalent ST in the food (milk/milk products) strains; this study, however, does not include strains from the natural environment (Kubicová et al. 2021).

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Comparison of environmental strains at the level of 1 701 core genes using cgMLST showed high levels of heterogeneity with variable levels of genetic relatedness from 2 to 1 637 differing alleles among the strains (Figure 2).

Strains clustered based on their sequence types; the clustering did not show any dependence on sampling location, geomorphological area, sample origin and season of collection. A very close genetic relationship (≤ 10 differing alleles) was observed only in two strains of ST20 (*Asc*I/*Apa*I pulsotypes 841/40 and 787/41) isolated at the same time from the same sample and location. A relatively close genetic relationship (15–31 differing alleles) was observed among the strains of ST451, although these strains belonged to four different *Asc*I/*Apa*I pulsotypes (735/2, 769b/39, 769b/44, and 732/27) and each was obtained from a geographically distinct location. Also, strains belonging to the frequent pulsotypes 211/37 (ST1) and 846/48 (ST18) showed a relatively close genetic relationship (maximally 40 differing alleles between the strains of each pulsotype).

Antimicrobial susceptibility. No phenotypic resistance was found by the disk diffusion method in *L. monocytogenes* strains in this study, which is in accordance with a previous Czech study conducted in 2010 (Gelbíčová and Karpíšková 2012). Phenotypic resistance in *L. monocytogenes* from the natural environment was detected in some countries. Erythromycin-resistant strains were found in sludge in France (Granier et al. 2011). Soil-originating strains resistant to erythromycin, ciprofloxacin and linezolid were detected in Austria (Linke et al. 2014). Clindamycin, oxacillin, and erythromycin-resistant strains were obtained from the faeces of wild animals (Wang et al. 2017; Cao et al. 2018). Differences between our and foreign studies may be associated with different criteria for evaluating results obtained. In all mentioned studies, except the Austrian study (Linke et al. 2014), criteria according to the Clinical & Laboratory Standards Institute (CLSI)

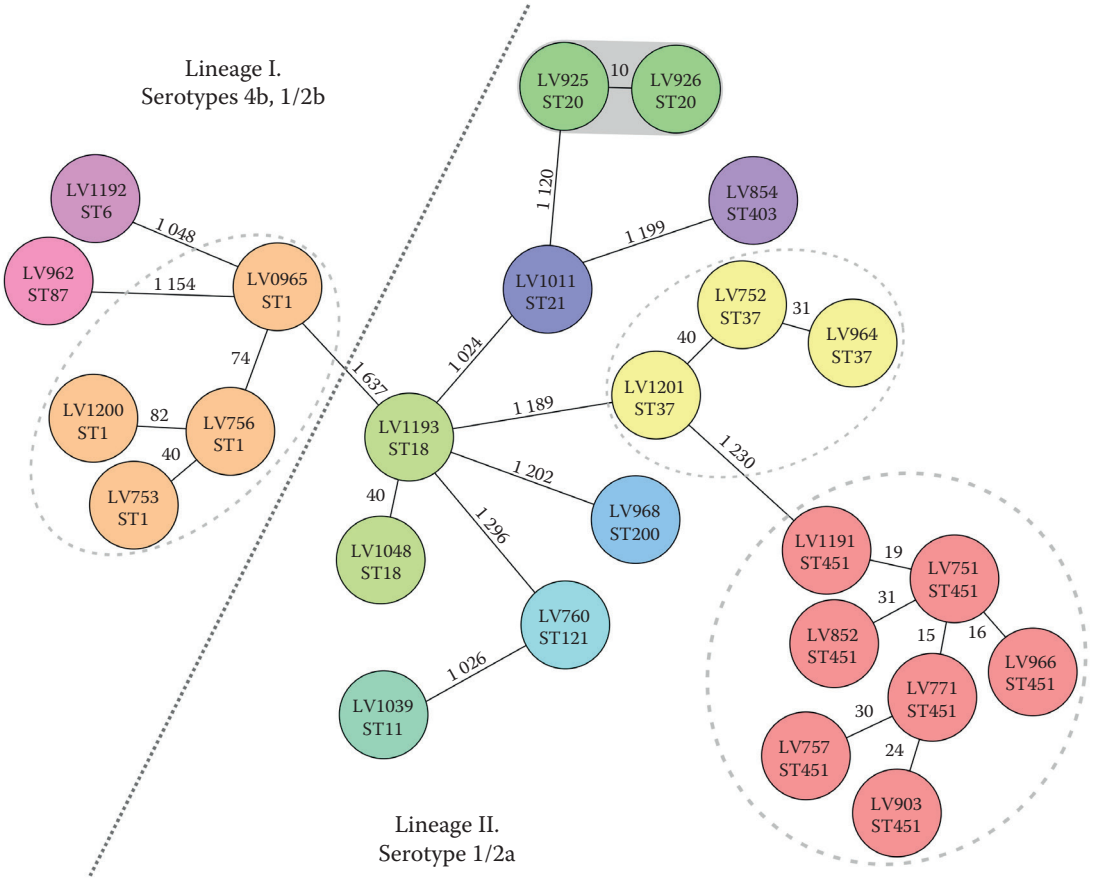

Figure 2. Ridom SeqSphere+ minimum spanning tree based on Core genome multi-locus sequence typing (cgMLST) of 25 *Listeria monocytogenes* isolates from the natural environment

Strains were compared based on the 1 701 loci according to Ruppitsch et al. (2015). The numbers on the connecting lines represent the numbers of target genes with different alleles.

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were used, compared to our study, which was assessed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendation.

Screening of antimicrobial-resistance genes using the ResFinder tool revealed the *fosX* gene in 24 isolates and the *bla*TEM-116 gene in one isolate (ESM, Table S1). *fosX* gene encodes intrinsic resistance to fosfomycin in the genus *Listeria* and is considered part of the core genome of *L. monocytogenes* (Scortti et al. 2018). Thus its presence in almost all isolates could be expected. *bla*TEM-116 gene encodes resistance against a broad spectrum of cephalosporines. This gene is closely related to *bla*TEM-1, the antimicrobial-resistance gene frequently detected in Gram-negative microorganisms (Balsalobre et al. 2010). In *L. monocytogenes,* a high occurrence of the *bla*TEM gene was described, e.g. in isolates recovered from irrigation water and agricultural soil in South Africa (Iwu and Okoh 2020).

CONCLUSION

This study offers a detailed insight into the occurrence and characteristics of *L. monocytogenes* strains originating from the natural environments of two Central European countries. *L. monocytogenes* was detected mostly in mud from the banks of surface water sources, with the highest prevalence observed in mountains and highlands, especially in autumn. The occurrence of *L. monocytogenes* in various samples from natural environments within a wide altitude range across different year seasons may highlight a remarkable adaptability and exceptional tolerance of this bacterium to external factors. Based on the typing methods, high heterogeneity of the environmental strains was detected. Serotype distribution of the strains circulating in the natural environment of Czechia and Slovakia seems to reflect distribution in the human population more than in the food chain, the proportion of STs in the strains from the natural environment, humans and foods seems to be different.

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REFERENCES

Althaus D., Lehner A., Brisse S., Maury M., Tasara T., Stephan R. (2019): Characterization of *Listeria monocytogenes* strains isolated during 2011–2013 from human infections in Switzerland. Foodborne Pathogens and Disease, 11: 753–758.

Balsalobre L.C., Dropa M., de Oliveira D.E., Lincopan N., Mamizuka E.M., Matté G.R., Matté M.H. (2010): Presence of *bla*TEM-116 gene in environmental isolates of *Aeromonas hydrophila* and *Aeromonas Jandaei* from Brazil. Brazilian Journal of Microbiology, 41: 178–9.

Cao X., Wang Y., Wang Y., Changyun Y. (2018): Isolation and characterization of *Listeria monocytogenes* from the black-headed gull feces in Kunming, China. Journal of Infection and Public Health, 11: 59–63.

Claxton M.L., Hudson L.K., Bryan D.W., Denes T. (2021): *Listeria* spp. Isolated from soil samples collected in the Great Smoky Mountains. Available at www.biorxiv.org (accessed Dec 09, 2022).

Český hydrometeorologický ústav (2021): ČHMÚ nowcasting webportal. Available at https://www.chmi.cz (accessed Oct 19, 2021).

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.

Dreyer M., Aguilar-Bultet L., Rupp S., Guldimann C., Stephan R., Schock A., Otter A., Schüpbach G., Brisse S., Lecuit M., Frey J., Oevermann A. (2016): *Listeria monocytogenes* sequence type 1 is predominant in ruminant rhombencephalitis. Scientific Reports, 6: 36419.

EUCAST (The European Committee on Antimicrobial Susceptibility Testing) (2021): Breakpoint tables for interpretation of MICs and zone diameters, Version 11.0. Available at www.eucast.org (accessed Nov 17, 2021).

Florensa A.F., Kaas R.S., Clausen P.T.L.C., Aytan-Aktug D., Aarestrup F.M. (2022): ResFinder – an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. Microbial Genomics, 8: 748.

Fox E., O'Mahony T., Clancy M., Dempsey R., O'Brien M., Jordan K. (2009): *Listeria monocytogenes* in the Irish dairy farm environment. Journal of Food Protection, 72: 1450–1456.

Gelbíčová T., Karpíšková R. (2012): Outdoor environment as a source of *Listeria monocytogenes* in food chain. Czech Journal of Food Sciences, 30: 83–88.

Gelbíčová T., Florianová M., Tomáštíková Z., Pospíšilová L., Koláčková I., Karpíšková R. (2019): Prediction of persistence of *Listeria monocytogenes* ST451 in a rabbit meat

https://doi.org/10.17221/234/2022-CJFS

processing plant in the Czech Republic. Journal of Food Protection, 82: 1350–1356.

Gorski L., Cooley M.B., Oryang D., Carychao D., Nguyen K., Luo Y., Weinstein L., Brown E., Allard M., Mandrell R.E., Chen Y. (2022): Prevalence and clonal diversity of over 1,200 *Listeria monocytogenes* isolates collected from public access waters near produce production areas on the Central California Coast during 2011 to 2016. Applied and Environmental Microbiology, 88: 35722.

Granier S.A., Moubareck C., Colaneri C., Lemire A., Roussel S., Dao T.T., Courvalin P., Brisabois A. (2011): Antimicrobial resistance of *Listeria monocytogenes* isolates from food and the environment in France over a 10-year period. Applied and Environmental Microbiology, 77: 2788–2790.

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

Iwu C.D., Akoh A.I. (2020): Characterization of antibiogram fingerprints in *Listeria monocytogenes* recovered from irrigation water and agricultural soil samples. PLoS One, 15: 228956.

Jennison A.V., Masson J.J., Fang N.X., Graham R.M., Bradbury M.I., Fegan N., Gobius K.S., Graham T.M., Guglielmino C.J., Brown J.L., Fox E.M. (2017): Analysis of the *Listeria monocytogenes* population structure among isolates from 1931 to 2015 in Australia. Frontiers in Microbiology, 8: 603.

Jensen A.K., Björkman J.T., Ethelberg S., Kiil K., Kemp M., Møller Nielsen E. (2002): Molecular typing and epidemiology of human listeriosis cases, Denmark, 2002–2012. Emerging Infectious Diseases, 22: 625–633.

Khan I., Khan J., Miskeen S., Tango C.N., Park Y.S., Oh D.H. (2016): Prevalence and control of *Listeria monocytogenes* in the food industry – a review. Czech Journal of Food Sciences, 34: 469–487.

Kubicová Z., Roussel S., Benjamin F., Cabanová L. (2021): Genomic diversity of *Listeria monocytogenes* isolates from Slovakia (2010 to 2020). Frontiers in Microbiology, 12: 729050.

Linke K., Rückerl I., Brugger K., Karpiskova R., Walland J., Muri-Klinger S., Tichy A., Wagner M., Stessl B. (2014): Reservoirs of *Listeria* species in three environmental ecosystems. Applied and Environmental Microbiology, 80: 5583–5592.

Listeria Sequence Typing Home Page. Institut Pasteur MLST databases and software [dataset]. Available at http://bigsdb.pasteur.fr/listeria/ (accessed Jan 2, 2019).

Locatelli A., Depret G., Jolivet C., Henry S., Dequiedt S., Piveteau P., Hartmann A. (2013): Nation-wide study of the occurrence of *Listeria monocytogenes* in the French soils using culture-based and molecular detection methods. Journal of Microbiological Methods, 93: 242–250.

Lourenco A., Linke K., Wagner M., Stessl B. (2022): The saprophytic lifestyle of *Listeria monocytogenes* and entry into the food-processing environment. Frontiers in Microbiology, 13: 789801.

Maury M.M., Tsai Y.H., Charlier C., Touchon M., Chenal-Francisque V., Leclercq A., Criscuolo A., Gaultier C., Roussel S., Brisabois A., Disson O., Rocha E.P.C., Brisse S., Lecuit M. (2016): Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. Nature Genetics, 48: 308–313.

Mawak J.D., Dashen M.M., Idolor A.J., Chukwu O.O.C. (2009): Occurrence of *Listeria monocytogenes* in irrigation water and vegetables at Jos, Plateau state, Nigeria. International Journal of Tropical Agriculture and Food Systems, 3: 279–282.

McLaughlin H.P., Casey P.G., Cotter J., Gahan C.G.M., Hill C. (2011): Factors affecting survival of *Listeria monocytogenes* in soil samples. Archives of Microbiology, 193: 775–785.

Nassirabady N., Meghdadi H., Alami A. (2015): Isolation of *Listeria monocytogenes* in Karunriver (environmental sources rural and urban) by culture and PCR assay. International Journal of Enteric Pathogens, 3: e21829.

Nightingale K.K., Schukken Y.H., Nightingale C.R., Fortes E.D., Ho A.J., Her Z., Grohn Y.T., McDonough P.L. (2004): Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. Applied and Environmental Microbiology, 70: 4458–4467.

Paillard D., Dubois V., Thiebaut R., Nathier F., Hoogland E., Caumette P., Quentin C. (2005): Occurrence of *Listeria* spp. In effluents of French urban waste water treatment plants. Applied and Environmental Microbiology, 71: 7562–7566.

Papić B., Pate M., Félix B., Kušar D. (2019): Genetic diversity of *Listeria monocytogenes* strains in ruminant abortion and rhombencephalitis cases in comparison with the natural environment. BMC Microbiology, 19: 299.

R Core Team (2018). The R Project for Statistical Computing. R Foundation for Statistical Computing. Available at https://www.r-project.org (accessed Apr 15, 2019).

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

Raschle S., Stephan R., Stevens M.J.A., Cernela N., Zurfluh K., Muchaamba F., Nüesch-Inderbinen M. (2021): Environmental dissemination of pathogenic *Listeria monocytogenes* in flowing surface waters in Switzerland. Scientific Reports, 11: 9066.

Roussel S., Michelon D., Lombard B., Lailler R. (2014): Molecular typing of *Listeria monocytogenes* strains isolated from food, feed and animals: state of play and standard operation procedures for pulsed field gel electrophoresis (PFGE) typing, profile interpretation and curation. EFSA Supporting Publications, 11: 81.

https://doi.org/10.17221/234/2022-CJFS

Ruppitsch W., Pietzka A., Prior K., Bletz S., Fernandez H.L., Allerberg F., Harmsen D., Mellmann A. (2015): Defining and evaluating a core genome multilocus sequence typing scheme for whole-genome sequence-based typing of *Listeria monocytogenes*. Journal of Clinical Microbiology, 53: 2869–2876.

Sauders B.D., Overdevest J., Fortes E., Windham K., Schukken Y., Lembo A., Wiedmann M. (2012): Diversity of *Listeria* species in urban and natural environment. Applied and Environmental Microbiology, 78: 4420–4433.

Sauders B.D., Zeki Durak M., Fortes E., Windham K., Schukken Y., Lembo Jr. A.J., Akey B., Nightingale K.K., Wiedmann M. (2006): Molecular characterization of *Listeria monocytogenes* from natural and urban environments. Journal of Food Protection, 69: 93–105.

Scortti M., Han L., Alvarez S., Leclercq A., Moura A., Lecuit M., Vazquez-Boland J. (2018): Epistatic control of intrinsic resistance by virulence genes in *Listeria*. PLoS Genetics, 4: e1007727.

Serhat A., Hüseyin B.D., Harun H., Nurhan E.O., Yeliz Y., Zafer S.G. (2021): Prevalence and molecular characterization of *Listeria monocytogenes* isolated from wastewater of cattle slaughterhouses in Turkey. Journal of Applied Microbiology, 132: 1518–1525.

Steckler A.J., Cardenas-Alvarez M.X., Townsend Ramsett M.K., Dyer N., Bergholz T.M. (2018): Genetic characterization of *Listeria monocytogenes* from ruminant listeriosis from different geographical regions in the U.S. Veterinary Microbiology, 215: 93–97.

Strawn L.K., Fortes E.D., Bihn E.A., Nightingale K.K., Gröhn Y.T., Worobo R.W., Wiedmann M., Bergholz P.W. (2013): Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. Applied and Environmental Microbiology, 79: 588–600.

Tomáštíková Z., Gelbíčová T., Karpíšková R. (2019): Population structure of *Listeria monocytogenes* isolated from human listeriosis cases and from ready-to eat foods in the Czech Republic. Journal of Food and Nutrition Research, 58: 99–106.

Vazquez-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 Microbiology Reviews, 14: 584–640.

Vela A.I., Fernández-Garayzábal J.F., Latre M.V., Rodríguez A.A., Domínguez L., Moreno M.A. (2001): Antimicrobial susceptibility of *Listeria monocytogenes* isolated from meningoencephalitis in sheep. International Journal of Antimicrobial Agents, 17: 215–220.

Vivant A.L., Garmyn D., Piveteau P. (2013): *Listeria monocytogenes*, a down-to-earth patogen. Frontiers in Cellular and Infection Microbiology, 3: 87.

Voronina O.L., Ryzhova N.N., Kunda M.M., Kurnaeva M.A., Semenov A.N., Aksenova E.I., Egorova I.Y., Kolbasov D.V., Ermolaeva S.A., Gintsburg A.L. (2015): Diversity and pathogenic potential of *Listeria monocytogenes* isolated from environmental sources in the Russian Federation. International Journal of Modern Engineering Research, 5: 5–15.

Wang Y., Lu L., Lan R., Salazar J.K., Liu J., Xu J., Ye C. (2017): Isolation and characterization of *Listeria* species from rodents in natural environments in China. Emerging Microbes & Infections, 6: e44.

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

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