

## Population structure of *Listeria monocytogenes* isolated from human listeriosis cases and from ready-to-eat foods in the Czech Republic

ZUZANA TOMÁŠTIKOVÁ – TEREZA GELBÍČOVÁ – RENATA KARPÍŠKOVÁ

### Summary

The study investigated population structure of *Listeria monocytogenes* strains originating from human listeriosis cases and from ready-to-eat (RTE) foods in the Czech Republic in 2013–2016. A total of 117 isolates, both from sporadic and epidemic cases, and 128 isolates mainly from meat and dairy products were characterized using serotyping and multi-locus sequence typing (MLST). Serotypes 1/2a, 1/2b, 1/2c and 4b were detected with serotype 1/2a prevailing both in the human population (57.3 %) and in foods (54.7 %). MLST detected 32 sequence types (STs) belonging to 28 clonal complexes (CCs) with STs occurring exclusively in humans, in foods and simultaneously in foods and humans. Phylogenetic analysis revealed strains belonging to the evolutionary lineages I and II. The population structure of *L. monocytogenes* showed a high level of heterogeneity. Strains of lineage I and II showed the same potential to cause listeriosis in human population. Strains of ST8 (serotype 1/2a) and ST6 (serotype 4b) were dominant among human isolates. Strains of ST121 (1/2a), ST8 (1/2a), ST2 (4b) and ST3 (1/2b) prevailed in food isolates. Within the study, 16 STs were detected, the presence of which in RTE foods can pose an increased risk of illness to consumers in the Czech Republic.

### Keywords

*Listeria monocytogenes*; serotyping; multi-locus sequence typing; sequence type; clonal complex

*Listeria monocytogenes* is not only a causative agent of human disease, but this bacterium is also found in foods and natural environment [1]. Despite the fact that the number of human cases of listeriosis is small, in comparison with other bacterial pathogens causing foodborne diseases, the high rate of mortality associated with this infection makes it a significant health concern [2].

In 2016, member states of the European Union reported 2536 cases of human listeriosis with an incidence equivalent to 0.47 cases per 100 000 of population. Listeriosis has been showing a rising trend in the European Union since 2008. In the Czech Republic, 30 to 40 listeriosis cases are reported annually for an extended period [3], with an increase reported in 2016 (47 cases) due to a local outbreak [4].

The ubiquitous distribution, together with the ability to propagate and grow under variable con-

ditions, facilitates the entry of *L. monocytogenes* into the human food chain. Contamination of the raw ingredients can then lead to colonization of the food processing environment [5]. The consumption of foods contaminated with *L. monocytogenes* results in the development of disease, primarily amongst risk groups of the population, namely pregnant women, newborns, the elderly and immunocompromised individuals [6]. Eating ready-to-eat (RTE) foods poses the highest risk of contracting listeriosis to the consumers [7].

Typing of *L. monocytogenes* at a sub-species level is not only an important tool for epidemiological investigative purposes, but it also allows scientific monitoring of the sources and pathways for this pathogen to spread along the entire food chain. The first-line typing method is serotyping [8]. Among the methods allowing a high level of discrimination, sequencing methods find

**Zuzana Tomáštková**, Department of Bacteriology, Veterinary Research Institute, Hudcova 296/70, 621 00 Brno, Czech Republic; Department of Milk Hygiene and Technology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1946/1-3, 61242 Brno, Czech Republic.

**Tereza Gelbíčová, Renata Karpíšková**, Department of Bacteriology, Veterinary Research Institute, Hudcova 296/70, 62100 Brno, Czech Republic.

Correspondence author:

Zuzana Tomáštková, e-mail: tomastikova@vri.cz, tel.: +420 605 009 226

their most valuable application in epidemiological studies. In particular, it is the multi-locus sequence typing (MLST), which enables us to classify *L. monocytogenes* into sequence types (STs) and clonal complexes (CCs) on the basis of sequencing of seven housekeeping genes (*abcZ*, *bglA*, *cat*, *dapE*, *dat*, *ldh*, *lhcA*) [9]. To date, four evolutionary lineages [10], four serogroups, 13 serotypes [8] and more than one thousand CCs and STs of *L. monocytogenes* have been described [11]. The results of current research suggest that strains of certain CCs are largely found in the human population, and alternatively in foods. They also have a variable potential to cause a certain type of infection [12]. Strains of a particular ST or belonging to a certain CC are more often associated with the ability to persist in food processing environment, e.g. ST121 [13, 14], ST8 [15, 16] and ST9 [17].

The aim of the study was to provide an overview of the population structure of *L. monocytogenes* strains isolated from human listeriosis cases and RTE foods from the retail market in the Czech Republic in 2013–2016. This study is based on the hypothesis that the population of *L. monocytogenes* is heterogeneous in the Czech Republic. Some clones have the potential to cause disease in humans, whereas other clones are primarily found in foods or adapt themselves to the food processing environment and their occurrence in humans is uncommon or zero. A detailed study of *L. monocytogenes* isolates obtained from humans and a comparison of their properties with isolates from foods can help predict latent outbreaks of listeriosis and can gain a better understanding of health risks associated with the consumption of foods contaminated with *L. monocytogenes*.

## MATERIALS AND METHODS

### Bacterial isolates

A total of 245 isolates of *L. monocytogenes* obtained from RTE foods ( $n = 128$ ) and from human cases of listeriosis ( $n = 117$ ) in the Czech Republic were analysed between 2013 and 2016 in this study. Isolates from humans were referred from clinical laboratories during routine epidemiological surveillance from the whole Czech Republic and were isolated largely from blood cultures ( $n = 81$ ) and cerebrospinal fluid ( $n = 17$ ), or from skin swabs, body cavities and cerebral tissue ( $n = 14$ ). The isolation site was not specified for five isolates. Isolates from RTE foods were obtained from meat products ( $n = 92$ ), dairy products ( $n = 21$ ), delicatessen ( $n = 10$ ) and vegetables and fruits ( $n = 5$ ). Isolates from foods were obtained within

the state inspections of foods of animal and plant origin ( $n = 105$ ) and as part of research activity of the Veterinary Research Institute in Brno, Czech Republic ( $n = 23$ ). *L. monocytogenes* isolates were stored at  $-75\text{ }^{\circ}\text{C}$  in brain heart infusion (BHI) medium (Oxoid, Basingstoke, United Kingdom) containing 20% (v/v) glycerol. Prior to typing, the isolates were inoculated on blood agar (LabMediaServis, Jaroměř, Czech Republic) and incubated under aerobic conditions for 24 h at  $37\text{ }^{\circ}\text{C}$ .

### Serotyping

The isolates were serotyped by multiplex polymerase chain reaction (PCR) [8] using primers synthesized by Generi Biotech (Hradec Králové, Czech Republic) and PPP polymerase (Top-Bio, Vestec, Czech Republic) in combination with rapid slide agglutination using commercially available antisera (Denka Seiken, Tokyo, Japan).

### Multi-locus sequence typing (MLST)

DNA extraction for MLST and amplification of PCR products were performed according to RAGON et al. [9] with the use of primers described in the study of HAASE et al. [18]. Sequencing of purified amplicons using universal sequencing primers was performed at Eurofins Genomics (Ebersberg, Germany). The isolates were classified into STs and CCs using Ridom SeqSphere+ software (version 3.0; Ridom, Münster, Germany).

## RESULTS AND DISCUSSION

The study provided an overview of the population structure of *L. monocytogenes* strains of human and food origin monitored during a four-year period in the Czech Republic. Serotypes 1/2a, 4b, 1/2b and 1/2c (Fig. 1) were detected by serotyping. Serotype 1/2a prevailed among human (67/117) and food strains (70/128). Among human strains, the second most common serotype was 4b ( $n = 32$ ) followed by 1/2b ( $n = 18$ ). In the food strains, serotype 1/2b ( $n = 30$ ) was the second most frequent, followed by 4b ( $n = 23$ ) and 1/2c ( $n = 5$ ). Serotype 1/2a strains prevailed among human and food isolates not only during the whole observed period, but also in individual years.

The predominant occurrence of serotype 1/2a strains among those isolated from human cases of listeriosis was also reported in Switzerland, Denmark and Italy [19–21]. Among strains isolated from RTE foods, serotype 1/2a strains predominated in Finland, Estonia and Japan [22–24]. Serotype 1/2c strains were only detected among strains

from foods. In the Czech Republic, strain of 1/2c serotype was involved in one case of congenital listeriosis to date [25, 26] and several sporadic cases of human listeriosis caused by 1/2c serotype strains were occasionally reported abroad [19, 20, 27, 28].

The distribution patterns of strains of different serotypes in the human population vary according to geographical locations and may be affected by the range and types of foods consumed in different countries. There may be a link between serotypes and specific foods, e.g. strains of serotype 1/2c are often isolated from meat and meat products, and only occasionally from other foods [13, 29]. The ability of strains of various serotypes to resist the effects of external environment can influence their survival and multiplication in food matrices. For example, authors from the United Kingdom and Canada observed that serotype 1/2a strains were more resistant to low temperatures compared with serotype 4b strains [30, 31].

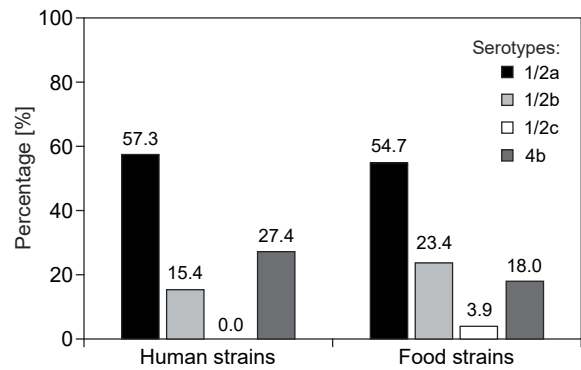
By MLST analysis, 32 STs assigned to 28 CCs were detected (Fig. 2). The group of strains was divided into two evolutionary lineages: lineage II strains were more frequent ( $n = 142$ ) than lineage I strains ( $n = 103$ ). In the present study, the following STs were detected: STs which occur exclusively in humans ( $n = 11$ ), STs which are unique to foods ( $n = 5$ ) and STs occurring in both foods and humans ( $n = 16$ ).

ST1348 (CC87, serotype 1/2b), ST1350 (CC26, serotype 1/2a) and ST1354 (CC2, serotype 4b) were newly detected and included in the Pasteur Institute MLST database [11].

According to the distribution of STs within the evolutionary lineages, it can be concluded from this study that lineage I and II strains show a comparable potential to cause human disease in the Czech Republic. In lineage II, STs which are exclusive to strains derived from foods occurred more frequently than in lineage I. Also, STs which are common to food and human strains were found more frequently in lineage II (Fig. 2). This may be related to the long-term higher occurrence of lineage II strains in the Czech Republic, notably with the predominance of serotype 1/2a strains in both the human population and in foods [32].

Most of the sporadic cases of listeriosis were caused by strains of STs found exclusively in the human population, except for 5 cases associated with strains of ST1350 (CC26). The absence of ST1350 strains in RTE foods, together with the geographical and time distribution of cases (5 regions of the Czech Republic, in which this ST was detected during the whole year 2014), did not allow tracing back this potential outbreak.

The absence of human-specific STs in strains



**Fig. 1.** *Listeria monocytogenes* serotypes obtained from human listeriosis cases and from ready-to-eat foods in 2013–2016 in the Czech Republic.

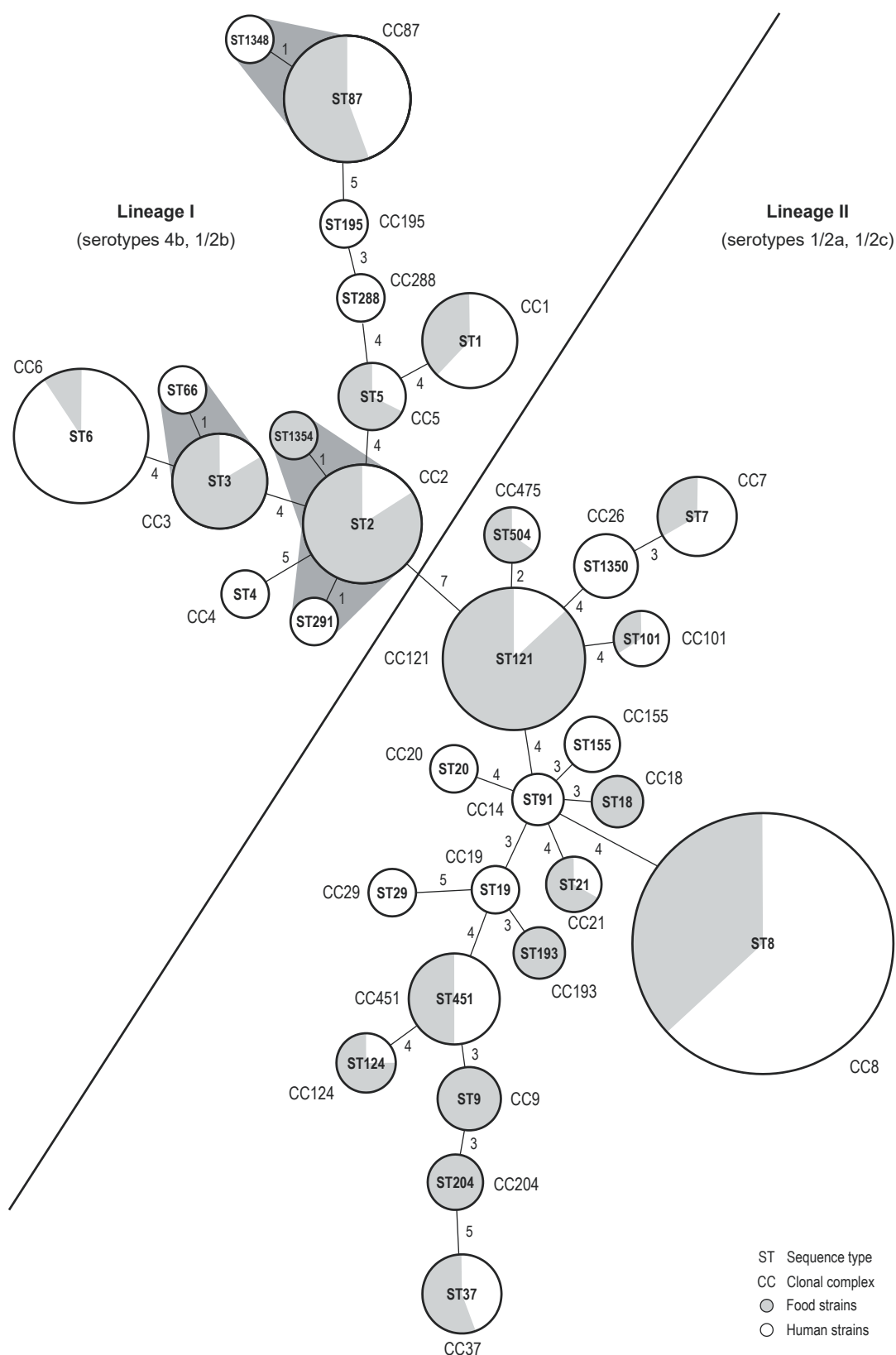
Number of human listeriosis cases  $n = 117$ , number of ready-to-eat foods  $n = 128$ .

from RTE foods suggests that the food vehicle, through which the infection reached the patients, could be foods which do not come under the control of state inspections, such as non-industrial products or imported foods. Strains of some of these STs were implicated in a number of human listeriosis cases in other countries, e.g. ST4 strains were frequently detected in humans in France [12] and ST29 strains are commonly described in outbreaks of listeriosis in US and Europe [33]. The rare occurrence of ST4 and ST29 in human strains in our study may be connected with a low exposure of the Czech Republic's human population to foods with the presence of strains of these STs due to their eating habits and distinct types of foods consumed in different countries.

Out of the STs only found in RTE food-derived strains, ST9 ( $n = 5$ , serotype 1/2c) and ST204 ( $n = 3$ , serotype 1/2a) were the most common. ST9 strains were isolated exclusively from meat products. This is in accordance with the studies carried out in Spain, Italy and Switzerland where the occurrence of ST9 strains was associated with the meat-processing industry [13, 29, 34].

The ST204 strains were isolated from ripening cheeses from two different producers in whose processing plants strains of this ST persisted for a long period [35]. ST204 strains were also detected in the environment of milk processing plants in Austria [36].

Two other studies found persistent ST204 and ST9 strains in food processing facilities [13, 37]. Considering the ability of *L. monocytogenes* to colonize manufacturing environments, this feature can be genetically determined. In ST9 and ST204 strains, stress survival islet SSI-1 gene complex, involved in increased resistance to adverse environ-



**Fig. 2.** Ridom SeqSphere+ Minimum spanning tree based on multi-locus sequence typing of 245 *Listeria monocytogenes* strains of human and ready-to-eat food origin in 2013–2016 in the Czech Republic.

Each circle represents sequence type and circle size is proportional to the number of strains. Clonal complexes are indicated by dark grey shading and listed next to each circle. Connecting lines represent phylogenetic relatedness according to the number of allelic differences.

mental conditions, was detected in several studies [12, 31, 38].

The largest group of STs in our study were those detected simultaneously in varying proportions in strains derived from the human population and RTE foods (Tab. 1, Fig. 2). Due to the ability of these STs to be involved in human listeriosis, their occurrence in RTE foods may put consumers in the Czech Republic at an increased risk of disease.

ST8 (serotype 1/2a) strains were predominant during the period of our study (Tab. 1, Fig. 2). Almost 21 % (51/245) of all tested strains were classified into this ST. During the monitoring period of our study, there was an outbreak of listeriosis in the Czech Republic associated with the predominant occurrence of ST8 strains in the human population [4]. ST8 was also the second most common ST in strains from RTE foods. A high number of human listeriosis cases with the involvement of ST8 strains was also reported by authors from Switzerland, Denmark and Netherlands [19, 20, 39]. ST8 strains occurrence in epidemic cases was also reported by authors from Denmark [40].

Whereas sporadic listeriosis cases were primarily associated with ST6 (serotype 4b), strains of this ST were rarely detected in RTE foods (Tab. 1, Fig. 2). Frequent occurrence in humans can be associated with a potentially higher virulence of CC6 strains [12], which can be connected with the

frequent occurrence of CNS-associated infections [20, 39]. In our study, ST6 was the sequence type with the highest involvement in CNS cases (6/19, 31.6 %). At present, European Union has been monitoring the outbreak occurrence of ST6 infections ongoing in five member states since 2015 [41].

Out of the strains isolated from RTE foods, ST121 (serotype 1/2a, Tab. 1, Fig. 2) was found most frequently. The low incidence of ST121 strains in humans might be linked to its potentially attenuated virulence and frequent occurrence of premature stop codon (PMSC) mutations in the *inlA* gene encoding internalin A, one of the key virulence factors of *L. monocytogenes* [12, 42, 43]. Our finding of the frequent occurrence of ST121 strains from foods is in accordance with studies in other European countries [13, 14, 29, 34] and is possibly associated with the higher ability of ST121 strains to persist in the food production environments [14].

In the strains from RTE foods, the frequency of ST2 (serotype 4b) and ST3 (serotype 1/2b) was significant (Tab. 1, Fig. 2). The finding of the low frequency of ST2-associated listeriosis cases in the Czech Republic differs from the studies performed in Denmark and France where ST2 strains significantly contributed to the disease in humans [12, 20]. However, the isolation of ST2 strains from foods is not unusual abroad [11]. ST3 strains are

**Tab. 1.** Representation and numbers of *Listeria monocytogenes* sequence types occurring both in humans and ready-to eat foods in 2013–2016 in the Czech Republic.

Serotype	Sequence type	Clonal complex	Number of strains		Type of ready-to eat food
			Human origin	Food origin	
1/2a	ST7	CC7	6	3	Meat product, dairy product
	ST8	CC8	32	19	Meat product, dairy product, delicatessen
	ST21	CC21	1	2	Dairy product
	ST37	CC37	4	5	Meat product, dairy product, delicatessen, vegetable or fruit
	ST101	CC101	2	1	Dairy product
	ST121	CC121	3	20	Meat product, dairy product, vegetable or fruit
	ST124	CC124	1	3	Meat product
	ST155	CC155	1	2	Meat product
	ST451	CC451	6	6	Meat product, dairy product, delicatessen
	ST504	CC475	1	2	Meat product
1/2b	ST3	CC3	3	15	Meat product, dairy product, delicatessen
	ST87	CC87	9	11	Meat product, delicatessen
	ST5	CC5	2	4	Meat product, dairy product, vegetable or fruit
4b	ST1	CC1	8	5	Meat product, dairy product, delicatessen, vegetable or fruit
	ST2	CC2	3	15	Meat product, delicatessen
	ST6	CC6	19	2	Meat product, dairy product

described abroad as representing a predominant clone with frequent occurrence in both the human population and in foods [12, 27, 28, 31]. In Brazil, the occurrence of persistent ST2 and ST3 strains was noted in milk processing environments [44].

## CONCLUSIONS

The population structure of *L. monocytogenes* in the Czech Republic showed high heterogeneity in 2013–2016. The potential of the strains of evolutionary lineages I and II to cause human disease was comparable. Strains of *L. monocytogenes* ST8 (serotype 1/2a) and ST6 (serotype 4b) predominated in the human population. In RTE foods, ST121 (1/2a), ST8 (1/2a), ST2 (4b) and ST3 (1/2b) strains were often identified. Within the study, 16 sequence types were detected, the presence of which in RTE foods can pose an increased risk of illness to consumers in the Czech Republic.

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