A survey on prevalence and sources of *Listeria monocytogenes* in ripened and steamed cheeses from the retail market in the Czech Republic

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Summary

The aim of the study was to determine the prevalence and characteristics of *Listeria monocytogenes* in ripened and steamed cheeses from the retail market in the Czech Republic examined between 2007 and 2016. The examination to detect and enumerate *L. monocytogenes* in samples was carried out at the time of purchase and at the end of the shelf-life. The isolates recovered from the samples were characterized by serotyping, clonogrouping by polymerase chain reaction (PCR) and by macrorestriction analysis. *L. monocytogenes* was detected in 20 of 387 (5.2%) originally packaged cheeses from different producers. The counts of *L. monocytogenes* did not exceed the limit of 100 CFU·g^{-1} in any of the samples. *L. monocytogenes* was detected most frequently in blue-veined cheeses from one producer (28.9%). Probably persistent *L. monocytogenes* strains were found in blue-veined cheeses and smear-ripened cheeses from two producers. With respect to the fact that *L. monocytogenes* is able to persist in food-processing facilities, it is necessary to carry out regular monitoring of *Listeria* occurrence in the food-processing environment. As *L. monocytogenes* can multiply at refrigeration temperatures, it is necessary to set the appropriate use-by date and provide information to consumers.

Keywords

Listeria monocytogenes; cheese producers; persistence; typing

The ability of *Listeria monocytogenes* to persist for long periods in the environment of food production systems and food-processing facilities is the main factor contributing to food contamination leading, in some cases, to human listeriosis. Even though *L. monocytogenes* is a facultative intracellular pathogen, it is able to survive and proliferate in environments of a wide pH range (4.7 to 9.2), high salinity (10%) and primarily at refrigeration temperatures (from –0.5 °C to 9.3 °C) [1]. Bacteria *L. monocytogenes* were isolated from raw milk [2], meat [3], but also from ready-to-eat food products, such as meat, fish and delicatessen products, ripened cheese and vegetables [4–6]. Foods that do not exceed the limit of 100 CFU·g^{-1} for *L. monocytogenes*, even if consumed in large quantities, pose only a negligible risk of developing listeriosis to a population of healthy humans. The Commission Regulation No. 2073/2005 on microbiological criteria for foodstuffs is also based on this limit [7]. In 2014, most non-compliant products (with counts of *L. monocytogenes* > 100 CFU·g^{-1}) in the retail market of the European Union countries were found in the category of smoked fish [8], similar to previous years.

Cheeses are usually considered as safe and nutritious food, but some types of cheeses may represent a potential source of undesirable bacteria, such as *L. monocytogenes*, *Staphylococcus aureus* or *Escherichia coli*. Soft ripened cheeses with a high moisture content or raw milk products are in the higher risk category of *L. monocytogenes*

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occurrence [9]. Human listeriosis outbreaks are often associated with the consumption of ripened cheeses. Smear-ripened cheeses were implicated in an extensive outbreak in the Czech Republic in late 2006 and early 2007 [10]. In 2009–2010, an international listeriosis outbreak was associated with the consumption of ripened sour milk curd cheese "Quargel" in Austria (25 cases), Germany (8 cases) and the Czech Republic (1 case) [11]. Furthermore, outbreaks attributed to ripened cheeses were reported for example in Italy [12], Portugal [13] and USA [14].

L. monocytogenes is capable of growing on the surface of cheeses during the ripening process, especially in cheeses with mould on the surface or inside the matter, and in smear-ripened cheeses. Microorganisms present on the cheese surface usually do not affect the counts of *L. monocytogenes* as a result of competitive inhibition. On the other hand, increased pH supports the growth of *Listeria* [15]. The colonization of cheeses with microorganisms is influenced by the milk feedstock, but especially by the food-processing environment and technological equipment. Bacterial contamination via technological equipment and food-processing environment has been implicated as the main cause of *L. monocytogenes* transmission. Due to cheese handling in the retail market (cutting, weighing, packaging), retail environment may also present a risk of contamination [16]. Despite the application of the Hazard Analysis and Critical Control Point (HACCP) system and compliance of sanitation programmes, persisting strains of *L. monocytogenes* are often found in the food-processing environment, as documented by studies conducted in 19 European cheese-processing facilities [17].

The aim of this study was to determine the prevalence of *L. monocytogenes* in ripened and steamed cheeses purchased from the retail market in the Czech Republic and to identify probable sources of their contamination, with the use of appropriate typing methods.

MATERIALS AND METHODS

Cheese sampling, detection and enumeration of *L. monocytogenes*

A total of 387 samples of ripened and steamed cheeses purchased from the retail market in the Czech Republic were examined in 2007–2016. The cheeses were made by various producers in the Czech Republic (238 samples), Germany (59), France (30), Poland (19), Slovakia (22), Denmark (11), Italy (4), Austria (3) and Lithuania (1). The numbers of examined cheese types and positive findings of *L. monocytogenes* are shown in Tab. 1. All samples were analysed at the time of the purchase. Since 2009, cheeses were also examined at the end of the shelf-life after being stored at 6 °C. Detection and enumeration of *L. monocytogenes* were carried out according to ISO 11290 [18]. The obtained isolates of *L. monocytogenes* were kept in brain heart infusion (BHI) medium with 20% glycerol at –75 °C. Before typing, the isolates were grown on blood agar (LabMediaServis, Jaroměř, Czech Republic) under aerobic conditions for 24 h at 37 °C.

Serotyping

Serotyping was performed by the slide agglutination method, using commercially available antisera (Denka Seiken, Tokyo, Japan) and subsequently confirmed by multiplex polymerase chain reaction (PCR) [19, 20] using PPP polymerase (Top-Bio, Brno, Czech Republic) and primers synthesized by Generi Biotech (Hradec Králové, Czech Republic).

Clonogrouping

Isolates within the serotype 1/2a were divided by clonogrouping [21] into specific clonal complexes using Qiagen Multiplex PCR Kit (Dynex, Buštěhrad, Czech Republic) and primers synthesized by Generi Biotech.

Pulsed field gel electrophoresis

Macrorestriction analysis using endonuclease *Asc*I (New England BioLabs, Ipswich, Massachusetts, USA) was performed according to the EU Reference Laboratory protocol (Anses, Paris, France) [22] and results were analysed by the software BioNumerics version 5.1 for analysis (AppliedMaths, Sint-Martens-Latem, Belgium).

Tab. 1. Detection and enumeration of *L. monocytogenes* in different types of cheeses examined in 2007–2016.

Cheese type	Number of samples		Positive samples [%]
	Examined	Positive	
With mould inside the matter	135	12	8.9
With mould on the surface	132	1	0.8
Double-mould	8	0	0
Smear-ripened	81	4	4.9
Ripened	17	1	5.9
Steamed	14	2	14.3

RESULTS AND DISCUSSION

Ripened cheese is risk commodity regarding *L. monocytogenes* contamination [23]. During the monitoring period, the prevalence of *L. monocytogenes* in ripened and steamed cheeses from the retail market in the Czech Republic was 5.2%. Apart from two positive findings in cheeses produced in Poland (isolation at the end of shelf-life in 2009, prevalence of *L. monocytogenes* 5.3%) and in Slovakia (isolation at the end of shelf-life in 2016, prevalence of *L. monocytogenes* 4.5%), *L. monocytogenes* was only detected in cheeses from Czech producers (7.6%). The predominant serotype in the analysed cheeses was 1/2a, similar to other studies [16, 17]. In the last decade, serotype 1/2a has become the most common serotype implicated in listeriosis outbreaks in Europe and North America [24]. The clonogrouping PCR assay was performed on strains of serotype 1/2a. The IIa/IIc PCR assay was designed for rapid identification of predominant serogroup IIa clones CC7, CC8, CC121, and CC155 [21]. Most of the major clones were involved in outbreaks of listeriosis [25, 26]. Only the strain of pulsotype 702 was classified as CC155. The rest of strains did not belong to target clones (Tab. 2). The reason may be the large heterogeneity of strains belonging to serotype 1/2a.

The overall prevalence of *L. monocytogenes* in cheeses in the EU member states was relatively low in 2014, similar as in previous years, as well as the numbers of unsatisfactory results. For soft and semi-soft cheeses at retail level, the legislative limit of 100 CFU·g^{-1} was exceeded only in 0.8% of samples [8]. In our study, counts of *L. monocytogenes* > 100 CFU·g^{-1} were not detected in any of the tested cheeses either at the time of purchase or at the end of the shelf-life. Lambertz et al. [5] detected *L. monocytogenes* in 0.4% (2/525) of ripened cheeses, with the limit of 100 CFU·g^{-1} being exceeded in one of the mould-ripened cheeses. Similarly, Véghová et al. [27] detected low-level positive findings of *L. monocytogenes* in ewes' milk products including also final products from cheese-processing facilities in Slovakia. In a study conducted in the United Kingdom between 2004 and 2005, *L. monocytogenes* was detected in 1% (17/1819) of cheeses (fresh, ripened and semi-hard) made from raw milk and in 0.2% (4/2618) of cheeses made from pasteurized milk. Regarding pasteurized milk cheeses, the number of samples tested positive (3.3%) in case the cheeses were cut before being sold at retail was higher in comparison with pre-packaged cheeses (1.1%) [16]. On the other hand, Rudolf and Scherer [23] detected *L. monocytogenes* in smear-ripened cheeses, at a higher rate in those made from pasteurized milk (8%, 13/163) than in cheeses made from raw milk (4.8%, 8/166). The highest numbers of positive samples in the mentioned study were found among cheeses produced in Italy (17.4%, 4/23), Austria (10%, 1/10) and Germany (9.2%, 11/120). This was likely due to the fact that the range of tested cheese types varied between the studies.

In our study, cheeses packaged by the producer were only tested. The highest incidence of *L. monocytogenes* was observed in blue-veined cheeses (3.1%). Except one isolate, all were recovered from cheeses produced by the same manufacturer. In one case, *L. monocytogenes* was found only at the end of the shelf-life. All isolated *L. monocytogenes* strains displayed identical serotype and pulsotype. Similarly, four strains of *L. monocytogenes* isolated from smear-ripened cheeses from one producer in the Czech Republic showed clonal compliance (Tab. 2).

L. monocytogenes was isolated from these two types of cheeses during the whole monitoring period from 2007 till the end of 2015. This fact confirmed that *L. monocytogenes* strains probably persisted in the environment of the given food-processing facilities over several years. Our conclusions also confirm the results obtained by

Tab. 2. Results of typing of isolates from ripened and steamed cheeses from producers with positive detection of *L. monocytogenes*.

Cheese type	Country of origin	Designation of producers	Number of samples		Serotype	Pulsotype
			Examined	Positive		
With mould inside the matter	Czech Republic	A	38	11	1/2a	719
	Poland	B	1	1	1/2a	759
With mould on the surface	Czech Republic	C	8	1	1/2a	702
Smear-ripened	Czech Republic	D	25	4	1/2a	713
Ripened	Slovakia	E	3	1	1/2a	722
Steamed	Czech Republic	F	5	2	1/2b	503

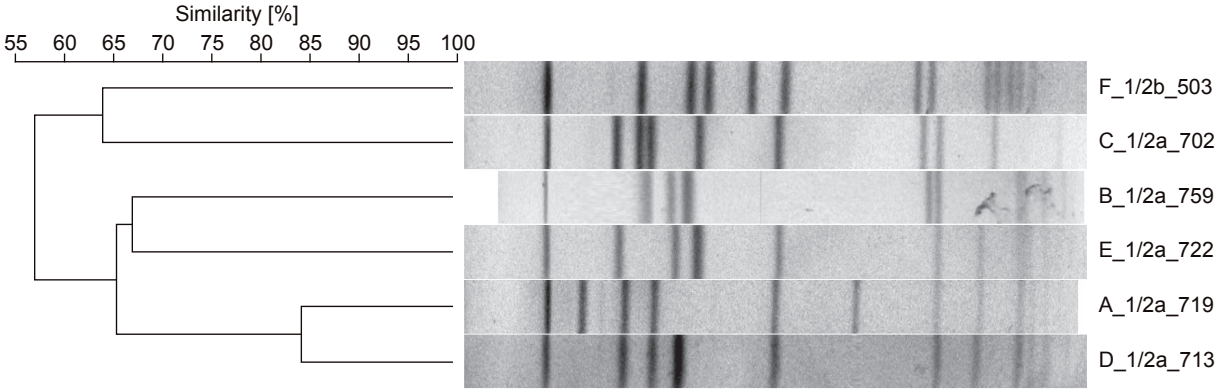

Fig. 1. Pulsed field gel electrophoresis of a macrorestriction product by*AscI* and the dendrogram for *L. monocytogenes* strains obtained from cheeses.

A–F – designation of producers; 1/2a and 1/2b – serotypes, 503; 702, 759, 722, 719, 713 – designation of pulsotypes.

Stessl et al. [17] investigating the persistence of *L. monocytogenes* in 11 cheese-processing facilities in Austria, six in Ireland and two in the Czech Republic. In six of them, including one Czech blue-veined cheese producer, *L. monocytogenes* strains persisting in food-processing facilities were demonstrated in the processing environment and in final products. Intensive production, together with high humidity in the food-processing facilities, residual water on the floors and technological equipment can increase a risk of spread of *L. monocytogenes* via aerosols, and reduce effectiveness of disinfection. In order to eradicate *L. monocytogenes* from a food-processing environment, it is also important to implement a systematic approach to monitoring of the environment, including compliance with the recommendations of drying of the surfaces after their cleaning [28].

In 2014, *L. monocytogenes* was isolated from two samples of steamed cheese produced by another Czech manufacturer at the end of their shelf-life (Tab. 2). However, in the case of two strains of serotype 1/2b and identical pulsotype, isolated in the same year from one manufacturer, it cannot be concluded whether or not it was a persistent strain. On the other hand, due to clonal identity of the isolates obtained from cheeses packed in the manufacturing plant, it is clear that contamination of final products occurred at the manufacturer's level. Persistent strains isolated from final products of producer A and D showed very similar pulsotypes in contrast with non-persistent strains (Fig. 1). It is possible that these persistent strains had some specific characteristics that facilitated their adaptation and survival. However, it is still unclear whether the persistence phenomenon in *L. monocytogenes* is genetically encoded or it is only a result of better adaptation of some strains to food and environmental factors [17].

Numerous studies documented the colonization of the cheese-processing environment with *L. monocytogenes* strains and consequent contamination of final products [28–30]. Even though storage of products at lower temperatures generally slows down the growth of *L. monocytogenes*, an increase up to *log* 7.7 CFU·g^{-1} occurred during storage at 4 °C in sour milk curd cheese quargel, which was in late 2009 and early 2010 involved in international outbreak of listeriosis. Psychrotrophic nature of *Listeria* in combination with storage time of cheeses, from several weeks up to several months, may thus lead to their undesirable multiplication even if the cold chain is maintained [31]. This finding was confirmed by our study of five cheeses in which the counts of *L. monocytogenes* increased during storage and the pathogen was only detected at the end of the shelf-life.

CONCLUSION

In terms of *L. monocytogenes* prevalence, the level of microbiological quality of ripened and steamed cheeses available on the market in the Czech Republic is good. The obtained results confirmed occurrence of *L. monocytogenes* in final products in the retail market. However, none of the products exceeded the limit of 100 CFU·g^{-1} set by the current legislation. Furthermore, the presence of persistent *L. monocytogenes,* which contaminated the cheeses during manufacture, was confirmed in products from two Czech producers. Both strains were of serotype 1/2a, but of distinct pulsotypes.

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