Genetic variety of food and veterinary isolates of *Listeria monocytogenes* in Latvia

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Introduction

Listeriosis is a food-borne/feed-borne infectious disease associated with a high mortality rate and can affect both humans and animals. *Listeria monocytogenes* is ubiquitous distributed in environment, but it may turn to an intracellular pathogen with a high stress tolerance. It is often detected in food producing plants and can be as a part of enteric flora of food animals, mostly ruminants. It can be divided in four genetic lineages and 13 serotypes from which serotypes 4b and 1/2b (both lineage I) and serotype 1/2a (lineage II) are associated with most of human cases of listeriosis. The aim of this study was to analyse and compare serogroups, sequence types (ST) and genetic features of *L. monocytogenes* isolates related to food and veterinary sources in Latvia.

Sample collection

Isolates (N=71) from food or food producing plants' environment were collected in 2017-2018 under national surveillance programme for *L. monocytogenes*.
Veterinary isolates (N=134) were obtained in 2013-2018 mainly from aborted foetus of cattle and small ruminants, several isolates were obtained from mastitis milk and one sample from ruminant brain tissue.

Methods

Detection of *L. monocytogenes* was performed in accordance with ISO 11290-1 and ISO 11290-2. Isolated *Listeria* spp. strains was confirmed by mass-spectroscopy. DNA from fresh culture was extracted with QIAamp DNA Mini Kit (Qiagen) and used for whole genome sequencing (WGS).

❑ Nextera XT Library construction (Illumina)

❑ Illumina MiSeq 2x300 bp paired reads

❑ Velvet genome assembly

❑ Ridom SeqSphere+ (Ridom) for ST and cgMLST allele calling, serotype prediction

❑ PROKKA for genome annotation.

Contingency tables and Fisher's exact test for significance of association was used and p < 0.05 was set.

Results

In total 205 whole genome sequences was analysed with following quality parameters for assembled genomes: average coverage 50x, average countig count – 70, average N50 = 246176.

Serogroup	Food isolates	Veterinary isolates	P value
IIa	40/71	124/133	**7.66e-10**
IIb	20/71	0/134	**3.43e-9**
IIc	4/71	5/134	0.38
IVb	4/71	4/134	0.28

Table 1. Distribution of food and veterinary *L.monocytogenes* isolates between various serogroups.
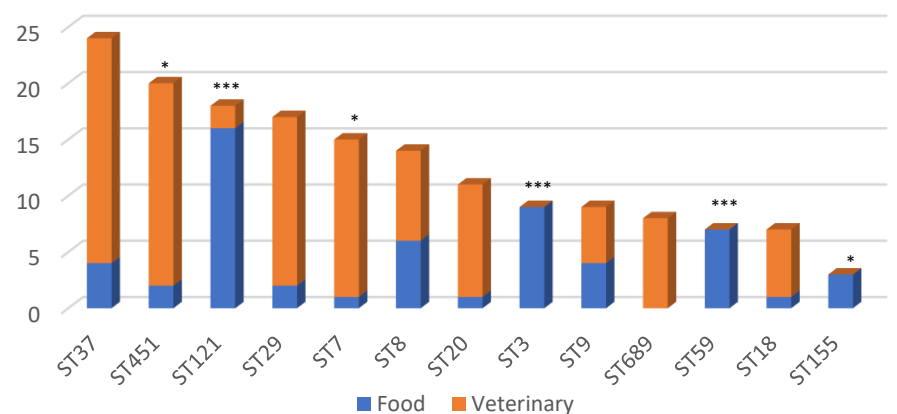
L. monocytogenes ST distribution by source

Figure 1. The most common sequence types in absolute numbers of *L. monocytogenes* isolated from food and veterinary sources and association of isolation source with individual sequence types. *p<0.05; ***p<0.0005

Analysis at individual gene level demonstrated average 2934 genes per one sequence of food isolate and 2869 genes per one sequence of veterinary isolate and **more than 300 genes** from various pathways including metabolism of toxic heavy metals (cadmium, arsenic, nickel, copper) **were significantly associated with *L.monocytogenes* living environment** (Fisher exact test, P-value < o.o5).

Conclusion

❑ Serogroup IIa is predominant in *L.monocytogenes* isolated from veterinary sources (ruminant abortion clinical material) and serogroup IIb is significantly associated with food source.

❑ We identified four *L.monocytogenes* STs – ST121, ST3, ST59 and ST155 – associated with isolates from food source and two STs – ST451 and ST7 – associated with isolates from clinical veterinary samples.

❑ Genes involved in metabolism of toxic heavy metals can be related to *L.monocytogenes* living environment.

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