Diversity and Pathogenic Potential of *Listeria monocytogenes* Isolated from Environmental Sources in the Russian Federation

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Highlights:

- *L. monocytogenes* environmental isolates from Russian European part were investigated by MLST and MvLST.
- Twelve STs (sequence types), including three novel ones were identified.
- Five STs belonged to the globally distributed epidemic clones (EC) VII, V, VI and III.
- European part and Far Eastern region of Russia are populated by different *L. monocytogenes* phylogenetic lineages.
- MLST and internalin genes are useful in *L. monocytogenes* epidemiological monitoring.

ABSTRACT: *The foodborne pathogen Listeria monocytogenes is also widely spread in nature. We report a survey of L.monocytogenes in Natural Parks of the densely populated Central Federal Region of Russia. Our study revealed the prevalence of phylogenetic lineage II, serovar 1/2a isolates, further classified into 11 sequence types (STs), three of which (756, 757, 758) we first described. Only one isolate from the dappled deer's faeces belonged to the phylogenetic lineage I, serovar 1/2b (ST5). All novel STs were single locus variants of known STs. Five STs belonged to the previously established epidemic clones VII, V, VI and III. Multi-virulent-locus-sequence-typing (MvLST) based on the internalins-genes-profile (IP) revealed six new IPs. Comparison of the concatenated MLST (multi-locus-sequence-typing) and IP sequences of the strains from two distinct areas of Russia, the European and the Far Eastern part, demonstrated considerable differences between them. In spite of the prevalence of global STs in both areas, phylogenetic lineage I strains predominated in the Far East region, while lineage II strains were mainly isolated in the European part. Our findings highlight the importance of epidemiological monitoring of the natural foci of L.monocytogenes infection and demonstrated usefulness of the combination of MLST and IP methods for this purpose.*
Keywords: Listeria monocytogenes, natural sources, MLST, internalin genes, epidemic clone, phylogenetic lineage.

I. Introduction

Listeriosis is the second most frequent cause of death from foodborne bacterial infection in Europe and the USA, after salmonella [[1]]. The incidence of listeriosis in Europe was 0.3-0.8 per 100,000 inhabitants in 2011 [[2]]. In Russia, listeriosis cases have been registered since 1992. According to the report of the Moscow Service for Supervision of Consumer Rights Protection and Human Welfare, 56 cases of this disease were registered in the Moscow region from 2010 to 2012, including 23 pregnant women and 8 fatalities [[3]]. These incidences of listeriosis in the densely populated Moscow region and in the whole of the Central Federal Region (CFR) demonstrate the need for constant surveillance for Listeria monocytogenes.

At the same time, environmental conditions of the CFR appear to be suitable for the *L. monocytogenes* persistence. The fauna includes more than 50 species of wild mammals; forests, occupying 44-55% of the land, are represented by parvifoliate and broad-leaved in Tver, Moscow and Kaluga regions and dominated by Pinus sylvestris in Vladimir region. The extensive river net of the Volga watershed, lakes and water reservoirs are

characterized CFR, as well as wide spectrum of soil types, including fertile dark carbonate soils and Alfisols that were found predominantly at Vladimir region; sod alluvial soils at bottomlands of Oka and Klyazma rivers; loamy soils at Vladimir, Kaluge and Moscow regions; sandy loam soils at Tver region; podzol-marsh soils at Kaluga and Tver regions.

Unfortunately, current *L. monocytogenes* surveillance programs place a higher emphasis on ruminant farms and retail establishments rather than natural environments. Only a few studies on *L. monocytogenes* persistence in natural environments have been conducted in the world. Rare examples of such studies, which include water-quality monitoring by Agriculture and Agri-Food Canada [[4], [5], [6]], and by the laboratory of geology associated with the Swiss Federal Institute of Technology in Lausanne (GEOLEP) [[7]], have demonstrated the importance of constant monitoring of environmental water resources for human health hazards. Thus, taking into account the importance of the pristine environment for recreational activities of the large CFR population, we chose the Natural Parks of this region to conduct the survey of *L. monocytogenes*.

II. Materials And Methods

2.1 Biological samples. A total of 2127 specimens were collected in the period 2006 to 2013 from three National Parks (Zavidovo National Park, Tarusa Natural Reserve, and Suzdal Hunting Ground) and from the Ivankovskoe water storage reservoir (Fig. 1).

Specimens were tested for the presence of *L. monocytogenes* according to Instructional Guidelines [[8]], which are similar to the FDA isolation protocol [[9]]. For each specimen, up to 10 esculin-positive colonies with the typical *L. monocytogenes* morphology were isolated, characterized bacteriologically, and verified with *L. monocytogenes*-specific PCR, as described [[10]]. One isolate from each sample was used in the further analysis.

Sixteen *L. monocytogenes* strains isolated from the specimens were included in molecular-genetic analysis. They are shown in Table 1: five from dappled deer faeces (*Cervus nippon*), four from wild board faeces (*Sus scrofa*), four from fishes, two from environmental specimens, and one from red deer faeces (*Cervus elaphus*). Collection strain "766", previously characterized serologically, was used as a reference.

2.2 Serotyping. The isolates were subtyped into serovars by the classical method [[11]] and by multiplex PCR [[12]].

2.3 Determination of *L. monocytogenes* virulence. Virulence of the isolates was estimated by the 50% lethal dose (LD50), determined by the probit method, using laboratory white outbred Swiss mice, weighing14-16 g. For each strain, five out of six mice were injected intraperitoneally with 0.5 ml dilutions of overnight agar-grown culture, washed with PBS. Dilutions were 1.0×10^9, 2.0×10^8, 4.0×10^7, 8.0×10^6, and 1.6×10^6 CFU per animal. All strains with LD50s from 1.0×10^4 to 1.6×10^7 CFU per mouse were considered virulent. A strain was considered non-virulent if it did not cause a death, in the injected group of mice, seven days after injection with 1.0×10^9 CFU [[13]].

2.4 PCR analyses. For DNA amplification, the following reagents were used: 5 u/μl Hot-Rescue DNA polymerase, 10x Taq PCR buffer (N.F. Gamaleya Institute for Epidemiology and Microbiology), 5 mM dNTP (Medigen) and primers (Syntol). Lysates of overnight *L. monocytogenes* cultures were prepared as described by Ermolaeva, et al., 2003 [[10]], and appropriately diluted relative to controls.

2.5 Multi locus sequence typing (MLST) analysis. The *L. monocytogenes* MLST scheme included internal fragments of the following seven housekeeping genes: *abcZ* (ABC transporter), *bglA* (beta glucosidase), *cat* (catalase), *dapE* (succinyl diaminopimelate desuccinylase), *dat* (D-amino acid aminotransferase), *ldh* (L-lactate dehydrogenase) and *lhkA* (histidine kinase). MLST was performed as described [[14], [15]], with the following modifications: primers for *abcZ*, *cat*, *dapE*, and *dat* were from [[14]] and for *bglA*, *ldh*, *lhkA* from [[15]].The following changes were made to the amplification conditions (94°C - 4 min; (94°C – 30 s, 52°C – 30 s, 72°C – 2 min) x 25; 72°C – 10 min): the number of cycles was increased to 35; melting temperatures (Tm) were 55°C for *abcZ*, *dapE*, *dat* and *cat*, 65°C for *bglA* and *lhkA*. For *lhkA* in three cases the Tm needed to be lowered to 58°C or 56°C. The recommended primers were used for sequencing six of the targets [[14], [15]]; however the ldh amplicons could only be sequenced with PCR primers from [[15]].

2.6 Multi-virulent-locus sequence typing (MvLST). Four internalin genes (*inlA*, *inlB*, *inlC*, *inlE*) that were previously used for MvLST [[16]], were used in the present study and formed internalin gene profile (IP). PCR amplification and sequencing of *inlA* and *inlC* internal fragments were performed with primers from [[16]]. For PCR amplification and sequencing of the internal *inlE* fragment, the following primers were used: inlEF 5'-

AAGCGGATGTAACAGACGAAG-3' and intER 5'-GATAAAATACGGGTTGGTTGG-3'. We identified a discrepancy between *inlB* sequences presented in GenBank and the *inlB* primers from [[16]] that prompted us to design new primers complementary to conserved *inlB* fragments: InlB-146F 5'-CAGATGATGCTTTTGCAGAA-3' and InlB-1229R 5'-GCATTCCCGCGAATATATTT-3'. For sequencing the 1083 bp amplicon, PCR primers and InlB-610R 5'-GTGGCACAATATCACTAATTT-3' were used.

PCR reactions for *inlC* and *inlE* were carried out under the following: 95°C - 3 min, (95°C - 20s ,55°C – 20s, 72°C – 30s) x 34, 72°C – 5min. For *inlA*, the Tm was increased to 58°C and for *inlB* the following parameters were changed: Tm = 54°C, denaturation time was 30s, and elongation time was 1 min.

2.7 PCR products sequencing. PCR products were sequenced according to the BigDye Terminator 3.1 Cycle Sequencing protocol for the Genetic Analyzer 3130 of Applied Biosystems/Hitachi. The electrophoretic DNA separation was performed in 50-cm capillaries, with POP7 polymer.

2.8 Data analysis. Allelic numbers and allelic profiles (genotypes, sequence types (STs)) were determined by use of the *L. monocytogenes* MLST database [[17]]. MLST gene sequences were aligned by use of ClustalW2 [[18]]. Sequencing data were analyzed in goeBURST [[19]], SplitsTree [[20]], and MEGA 6.0 [[21]]. DnaSP v.4 was used for nucleotide diversity index and recombination parameter calculations [[22]]. The Simpson's index of diversity (D) calculation was performed according to Hunter and Gaston, 1988 [[23]].

The phylogenetic analysis of *L. monocytogenes* genotypes (MLST and IP profiles) was based on concatenated sequences of MLST loci (*abcZ*, *bglA*, *cat*, *dapE*, *dat*, *ldh*, *lhkA*) and IP loci (*inlA*, *inlB*, *inlC*, *inlE*), with a total length of 5698 bp. Dendrograms were constructed with Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods.

Genetic distances between *L. monocytogenes* genotypes were evaluated with the Tamura-Nei method [[24]], which was chosen as an optimal evolution distance model derived from Modeltest, based on the Akaike information criterion [[25]]. The evolutionary history was inferred by using the ML method, based on the General Time Reversible model [[26]]. Initial tree(s) for the heuristic search were obtained automatically by applying NJ and BioNJ algorithms to a matrix of pairwise distances, estimated by use of the Maximum Composite Likelihood approach, and then selecting the topology with the superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (2 categories [+G, parameter = 0.1000]). Trees were constructed with MEGA 6.0 [[21]]. Bootstrap analyses were performed with 1,000 replicates.

2.9 Nucleotide sequences. New MLST alleles and STs were managed by curators of the Institute Pasteur MLST system (Paris, France). All strains were deposited in *L. monocytogenes* MLST database [[17]] with ids, as indicated in Table 1. Sequences of the internalin genes were deposited in GenBank under the accession numbers: inl A: KM670461 - KM670477; inl B: KM670512 - KM670528; inl C: KM670478 - KM670494, inl E: KM670495 - KM670511.

III.   Results

3.1 Classification of *L. monocytogenes* isolates. *L. monocytogenes* is widespread in the natural environment. The heavily frequented Natural parks of the Russian CFR, especially in hunting and fishing seasons, are closely monitored by the State Science Institution National Research Institute of Veterinary Virology and Microbiology of Russian Academy of Agricultural Sciences (SSINRRIVVandM). The results of their periodic environmental sampling and wildlife surveys have determined that there is a need for further investigation of *L. monocytogenes* distribution in this pristine environment. Sixteen *L. monocytogenes* strains isolated in this study (fourteen from wild animals and two from the environment) and one reference strain, were characterized (Table 1). Phenotypical and serological analyses showed that all but one of the strains belong to serovar 1/2a of phylogenetic lineage II. Isolate 699-09, obtained from dappled deer faeces in the Tver region, belongs to serovar 1/2b. MLST analysis of 699-09 confirmed its difference from other isolates by identifying it as ST5 of phylogenetic lineage I. Phylogenetic lineage II strains were subdivided into 11 STs (Table 1), three of which we described for the first time (ST 756, 757, 758).

3.2 Placement of *L. monocytogenes* STs within the common ST network. We performed a phylogenetic analysis of clonal relationships among *L. monocytogenes* strains in the MLST database. Using the goeBURST program, strains were placed within the common ST net according to ST (Fig. 2). Four of our ST7 isolates and one ST111, a single locus variant (SLV) of ST7, belonged to the clonal complex (CC) 7. ST18, ST756, and ST757 isolates were identified as CC18 members. Three ST14 isolates belonged to the extensive CC101, which also includes ST425, ST20, and ST11. ST20 and ST11 formed subclones inside this CC. ST758 was the member of CC8 and ST394 belonged to CC415. ST5 formed the separated subclone within CC1 (not shown).

3.3 Epidemiological importance of isolates. To evaluate the epidemiological importance of *L. monocytogenes* isolates, we compared them to known epidemic strains. Among isolates, we identified STs of CC7, CC8, ST11 and CC5 belonged to the epidemic clones (EC) VII, V, VI, III, respectively, according to Cantinelli, et al., 2013 [[27]]. Therefore, eight strains of five STs, isolated from the CFR natural parks, could potentially threaten human and animal health.

3.4 Virulence potential of isolates. MvLST was used to predict the virulence potential of isolates. This method places the internalin genes sequences of each strain into a category or IP, with a distinct virulence potential associated with it [[16]].

The four internalin genes included in the IPs, *inlA* [[28]], *inlB* [[29]], *inlC* [[30]], and *inlE* [[31]], encode a family of virulence-associated proteins that are common to all phylogenetic lineages [[32]]. *InlA* and *InlB* are important in the early stages of the cell invasion but interact with different receptors [[33]]. They cooperate with each other in the process of crossing intestinal, placental, and blood-cerebrospinal fluid barriers in humans [[33], [34]]. *InlC* functions in cell-to-cell spreading by promoting cell membrane protrusions [[35]]. The function of the *InlE* is unknown [[32]].

MvLST analysis subdivided strains into nine IPs (Table 1), six of which were novel (33-38). All CC7 strains had IP15. CC18 strains had the novel IP35. Two strains in ST14 group, isolated from different animals, had the same IP27, and the third strain (from a silver bream) had the new IP36, due to a substitution in *inlA*. It should be noted that three strains with different STs (20, 394, 758), belonging to different CCs (101, 415, 8), had IP13 or a SLV of IP13, which is IP34 and also an *inlA* substitution. Moreover, two of four IP37 internalin markers, found in the ST11 strain, were similar to IP27, found in the ST14 strains.

Experiments on mice suggested differences in the virulence of the IP36 and IP27 strains (Table 1). The IP27 strains demonstrated higher virulence, than the IP36 strain. The IP37 strain was highly virulent, too, and more virulent than the strain of ST5 from the first phylogenetic lineage. The CC7 strains with the same IP15 demonstrated similar virulence in mice.

Comparison of all 38 known IPs by the SplitTree program, subdivided them into two groups, consistent with their phylogenetic classification into lineages I or II (Fig. 3). The central node of IP 9, 13, 34, 35 and the line from IP16 to IP28 formed the border between two groups. This distinction allows us to infer not only CC-alleles specific for phylogenetic lineages [[27]], but also IP alleles.

3.5 Diversity of the IP and MLST genes. The estimation of the IP contribution into the method discriminatory power demonstrated the higher variability of STs, than IPs among analyzed strains: the D value for MLST was 0.934, while for IP it was 0.867. Addition of the IP genes to MLST scheme produced a D value of 0.949, which did not significantly improve the discriminatory power. These observations are in agreement with conclusions of Cantinelli, 2013, based on a different MvLST scheme (*prfA*, *inlB*, *inlC*, *clpP*, *dal*, *lisR*, *inlA* and *actA*) [[27]].

The detailed analysis of the nucleotide diversity in MLST and IP genes shown in Table 2, provides additional evidence supporting the hypothesis that the virulence-associated genes evolve faster than house-keeping genes [[36]]. In spite of a higher number of alleles and higher allelic diversity for the MLST genes, the number of polymorphic cites (5.8% for MLST vs 7.09% for IP genes) and especially non-synonymous replacements in nucleotide sequences, was higher in IP genes (13.0% for MLST vs 38.1% for IP concatenated sequences).

The number of distinct alleles per gene was the highest for *cat* (8), *dapE* (7), *ldh* (7) (MLST), and for *inlA* (6) (IP). The highest nucleotide variability was shown by the MLST *dat* gene (12.31%) and the IP *inlE* gene (17.02%). Last data suggested the validity of the *inlE* including in MvLST. Table 2 shows significant recombination potential for the following genes: *bglA* (R=7.4) and cat (R=0.3), from MLST analysis, and *inlA* (R=2.7), from IP analysis. These results demonstrated that in the given strains' group the recombination is infrequent as in MLST, so in chosen MvLST genes, but suggested earlier revealed recombination events in *inlA* gene [[15], [27]]

3.6 World distribution and various detected ST. World distribution and sources of various STs can be obtained from the Institute Pasteur *L. monocytogenes* database [[17]]. From the twelve STs detected for the given strains ST7 was the most representative (142 strains) in the mentioned database. The ST7 strains have been isolated from different sources in many countries, but most frequently from animals and the environment (77.5% of all sources). ST11, ST14, ST18, ST20 were less common with only 14-24 strains belonging to each ST. Nevertheless, these STs were widely distributed among different countries and continents. The sources of these strains were also primarily animals and the environment (47.6% for ST14 to 99.7% for ST20). ST111 (SLV of ST7) was isolated from a human individual in Germany and from three animals in France. ST394 was represented only by four strains, three of which were environmental. In contrast to the other STs, ST 5, which belonged to another phylogenetic lineage I, had 49 isolates in the database with most of them from human

sources. Meanwhile, the reporting even a few strains of a certain ST among human isolates places this ST in the number of potentially dangerous one.

3.7 Human and animals' contribution to *L. monocytogenes* spreading. Human activity can influence bacterial spreading among wildlife, especially if the ecological balance is managed by displacing big groups of animals. Several groups of Cervus nippon were relocated from the Far Eastern region (Pacific coast of Russia) to the CFR between 1950 and 1970. Also, in the 1990s, deer and wild boars were relocated between CFR Natural Parks. Consequently, we could expect to find common *L. monocytogenes* genotypes in samples from different National Parks of the CFR and between the European strains and strains from the Far East described previously [[37], [16]].

Indeed, there were common STs among isolates from different Natural Parks. CC7 strains were found in three regions (Kaluga, Tver, Vladimir) from the deer and the wild boars. ST14 was detected in two regions: in Tver (fish strains) and Vladimir (wild boar strain). These genotypes also showed similar IPs. These observations can be explained either by interchanges of *L. monocytogenes* strains between introduced and native animals, or by their prolonged circulation in these territories. Finding of ST14 in geographically remote locations might be due to animal relocation. For the widely distributed CC7 strains, prolonged circulation would be a better explanation because ST7 was detected not only among our CFR strains but also in strain '766', isolated from Siberia in 1955, and in the strain isolated from *Microtus arvalis*, in 1952, in the European part of the Russian Federation [[16]].

3.8 Comparison of the *L. monocytogenes* strains from distant regions of the Russian Federation. Comparison of strains from the CFR Natural Parks with strains from the Far East revealed that the STs were not the same; however, there were some notable similarities. These include two pairs of STs that were similar. ST313 (human aborted fetus, Far East) differed from ST20 (crucian carp, Natural Park) by a SLV in *ldh*, and ST19 (scallop Pectinidae sp., Far East) differed from ST11 (dappled deer, Natural Park) by one SLV in *cat* and one in *ldh*. Also, the ST314 strain isolated from the European *Myodes glareolus* [[16]], was a SVL of ST19 and a DLV of ST11. Comparison of IPs also revealed some similarities; the IPs of ST313, ST314, and ST19 strains were the same and differed in only one position from IP found in ST20 strains (*inlC* locus). In contrast, the IP of the ST11 strain differed in all loci (Table 1).

The relationship between all genotypes of the strains, isolated in the Russian Federation is presented by the phylogenetic tree, based on the concatenated sequences of the MLST and IP loci for each strain (Fig. 4). Thirty-eight allelic profiles (MLST and IP loci) were included in the analysis: seventeen were from the present study and twenty-one from the earlier works of Zaytseva et al., 2007 [[37]] and Adgamov et al., 2012 [[16]]. The data from earlier studies included profiles of fifteen Far Eastern (marked by the hash sign) and six European strains. All profiles formed the phylogenetic groups, which were generally corresponded to CCs. Within the phylogenetic lineage I clade (red points) representatives of CC2 (ST2, ST145) and CC315 (ST315) formed closer relationships with the sister subclade formed by representatives of CC1 (ST1, ST64). The basal branch was formed by the ST5 profile.

The phylogenetic lineage II clade (blue points) formed two separate subclades: one of them included representatives of CC7 (ST7 and ST111) and the other sister branch was formed by the CC8 representative ST758. The second clade was mixed and included representatives of CC101 (ST11, ST14, ST20, ST425), CC19 (ST19, ST314), CC155 (ST155) and CC415 (ST394). The basal and distantly related clade was formed by representatives of CC18 (ST18, ST756, ST757).

It should be noted that only three Far Eastern strain profiles belonged to the phylogenetic lineage II: one of ST313 and two of ST155. However, the ST155 strains had IP that differed in the *inlE* allele. At the same time, only two European strain profiles belonged to phylogenetic lineage I: ST64 (healthy human) and ST5 (dappled deer's faeces). These observations suggest that most Far Eastern strain profiles belonged to phylogenetic lineage I, which were of human, animal and environmental origin [[16], [37]] while most European strain profiles belonged to phylogenetic lineage II.

IV. Discussion

The analysis of *L. monocytogenes* isolates from the Natural Parks of the CFR in Russia revealed new allelic profiles for MLST and IP schemes and novel alleles. All new STs were the result of the SLV of wide spread STs. It is important to note the epidemical significance of strains isolated from wildlife. In particular, strains which belong to the EC VII, V, VI and III must be kept under an epidemiological surveillance, especially because some outbreaks had been caused by lineage II serotype 1/2a isolates (Orsi, et al., 2011).

Persistence of *L. monocytogenes* in the environment could be due to its presence in gastrointestinal tracts of humans and many mammals, which results in its incorporation into faecal flora [[38]]. One recent study

reported isolation of *L. monocytogenes* from stools of 1%–10% of carriers [38]. In another study, Nightingale et al., 2004 [39], verified that cattle contributed to the amplification of *L. monocytogenes* and its dispersal in the farm environment. Moreover, our surveillance of *L. monocytogenes* in Natural Parks, demonstrated that the faeces of the wild animals was the most common source of *L. monocytogenes*-positive samples. Managing wild animal populations may therefore be a strategy for controlling listeriosis in CFR and other Natural Parks.

The question of which phylogenetic lineages and serovars of *L. monocytogenes* may be regular inhabitants of pristine environments is one that is discussed in the literature. A review of this subject was presented by Orsi, et al. (2011) [32]. The results of our study support the existence of regional distinctions of the *L. monocytogenes* in the Russian Federation. Comparison of allelic profiles of the strains from two distinct areas of Russia, the European part and the Far Eastern part (the Pacific coastline), demonstrated considerable differences in occurrence of *L. monocytogenes* phylogenetic lineages. In spite of the occurrence of global STs in both areas, STs of phylogenetic lineage I (from a variety of sources) prevailed in the Far East [37], while phylogenetic lineage II prevailed in the European part of Russia.

The presence of particular local genotypes of bacteria in the Far East Region (FER) was demonstrated in an earlier investigation of *Burkholderia cepacia complex* [40]. *Burkholderia cepacia complex* STs 241 and 729, from this region, were not found in other areas of Russia, while other widespread STs were not found in the FER.

Regional distributions of animal genotypes were demonstrated by Abramson, et al., 2012 [[41]]. A wide-range phylogeographic study of the gray red-backed vole (*Craseomyc rufocanus*) revealed the prevalence of particular genotypes in certain regions of the FER. The study was carried on the basis of the mitochondrial cytochrome b gene and a fragment of the Y-chromosomal *SRY* gene. Type III *SRY* haplotype was detected only in Amursky, Khabarovsky, Primorsky Krai, and Kamchatka Peninsula region of the FER, while the distribution of type VI *SRY* haplotype appeared more limited, being detected only Primorsky Krai. These *SRY* haplotypes were typical for the gray red-backed vole from China. The type I *SRY* haplotype also appeared to have a limited distribution: it was detected in Sakhalin Island in the Russian Federation and Hokkaido Island in Japan. Cytochrome b gene analysis showed a similar distribution pattern. Subgroup C-2 cytochrome b haplotype was distributed in the FER and was partially sympatric with subgroup C-3 and group D haplotypes in Primorsky Krai [[41]].

Geographical distributions of *L. monocytogenes* strains associated with human listeriosis outside of Russia have been documented in other studies. Orsi, et al., 2011 [[32]], described differences in phylogenetic lineages of strains causing human listeriosis in Northern Europe and in the US. In Finland and Sweden, strains from phylogenetic lineage II (serovar 1/2a) were more frequently associated with human listeriosis [[42], [43]], but in the US listeriosis was more frequently associated with phylogenetic lineage I strains [[44]].

Therefore, further observations of *L. monocytogenes* occurrences among wildlife are necessary to expand our understanding of the interrelationships between *L. monocytogenes* and regional geography and ecology and how they influence the risks to human health.

Abbreviations
MLST - multi locus sequence typing;
ST - sequence type;
SLV - single locus variant;
DLV – double locus variant;
CC - clonal complex;
EC - epidemic clone;
MvLST - multi virulent locus sequence typing;
IP - interlanin genes profile;
D - Simpson's index of diversity;
CFR - Central Federal Region;
FER - Far East Region

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id MLST database	strain	source	year	place	abcZ	bglA	cat	dapE	dat	ldh	lhkA	ST	serovar	inlA	inlB	inlC	inlE	IP number	LD_{50min}-LD_{50max}, cfu x 10^6	virulence
2803	766	swine	1955	Novosibirsk region	5	8	5	7	6	2	1	7	1/2a	4	14	6	8	15	13.0-64.0	virulent
2804	134-08	ground from feeding area	2008	Kaluga region	5	8	5	7	6	53	1	111	1/2a	4	14	6	8	15	4.3-25.2	virulent
2805	164-09	dappled deer's faeces (Cervus nippon)	2009	Kaluga region	5	8	5	7	6	2	1	7	1/2a	4	14	6	8	15	no data	
2806	633-09	wild boar's faeces (Sus scrofa)	2009	Tver region	5	8	5	7	6	2	1	7	1/2a	4	14	6	8	15	4.4-30.0	virulent
2807	431-09	dappled deer's faeces (Cervus nippon)	2009	Vladimir region	5	8	5	7	6	2	1	7	1/2a	4	14	6	8	15	2.7-21.6	virulent
2799	69-06	bream (Abramis brama)	2006	Tver region	7	6	15	18	12	275	1	756	1/2a	12	13	6	6	35	4.6-23.2	virulent
2800	139-08	wild boar's faeces (Sus scrofa)	2008	Kaluga region	105	6	15	18	12	6	1	757	1/2a	12	13	6	6	35	4.4-21.8	virulent
2808	184-09	dappled deer's faeces (Cervus nippon)	2009	Kaluga region	7	6	15	18	12	6	1	18	1/2a	12	13	6	6	35	no data	
2809	319-0	red deer' faeces (Cervus elaphus)	2009	Tver region	7	6	15	8	6	2	14	425	1/2a	13	20	13	8	33	no data	
2810	71-06	silver bream (Blicca bjoerkna)	2006	Tver region	8	6	13	6	5	2	1		1/2a	12	14	11	11	36	5.5-28.2	virulent
2811	311-07	perch (Perca fluviatilis)	2007	Tver region	8	6	13	6	5	2	1		1/2a	11	14	11	11	27	1.2-6.1	highly virulent
2812	434-09	wild boar's faeces (Sus scrofa)	2009	Vladimir region	8	6	13	6	5	2	1	14	1/2a	11	14	11	11	27	0.2-1.2	highly virulent
2813	276v/o-07	crucian carp (Carassius)	2007	Tver region	17	13	3	6	5	7	1	20	1/2a	9	14	6	6	13	no data	
2814	243-09	dappled deer's faeces (Cervus nippon)	2009	Kaluga region	7	6	10	6	1	2	1	11	1/2a	11	19	13	11	37	0.5-2.7	highly virulent
2815	191-08	decaying plant residues	2008	Tver region	5	5	17	21	39	2	6	394	1/2a	9	14	6	6	13	no data	
2801	371-09	wild boar's faeces (Sus scrofa)	2009	Vladimir region	5	6	2	9	5	276	1	758	1/2a	12	14	6	6	34	no data	
2816	699-09	dappled deer's faeces (Cervus nippon)	2009	Tver region	2	1	11	3	3	1	7	5	1/2b	3	8	4	1	38	1.4-9.5	highly virulent

Table 1. Characterization of the *L. monocytogenes* strains, isolated in the Natural Parks of the CFR.
Taupe color – equal ST, ST of the same CC or identical IP; light grey color – new allele, SLV, new ST or ST with SLV

Table2. Polymorphism of MLST and IP genes among L. monocytogenes isolates.

Gene name	Template size, bp	No. of alleles/ allele diversity	polymorphic sites, %	No. of synonymous changes	Replacement changes	NonSyn /Syn changes	Pi (%) Nucleotide diversity	Recombination parameter, R
MLST								
abcZ	537	6 (0.779)	3.53	16	3	0.187	0.006	0.001
bglA	399	5 (0.662)	3.00	12	0	n.a	0.007	7.4
cat	486	8 (0.860)	6.37	29	4	0.137	0.020	0.3
dapE	462	7 (0.831)	8.00	33	6	0.181	0.025	0.001
dat	471	6 (0.794)	12.31	53	8	0.15	0.017	0.001
ldh	453	7 (0.662)	4.19	16	3	0.187	0.007	0.001
lhkA	480	4 (0.331)	3.12	14	2	0.142	0.004	0.001
c.s.	3288	12 (0.934)	5.80	173	26 (13.0%)	0.15	0.012	0.001
IP (MvLST)								
inlA	648	6 (0.824)	2.62	13	4	0.307	0.008	2.7
inlB	618	5 (0.574)	6.10	24	14	0.58	0.010	0.001
inlC	586	4 (0.566)	3.58	14	7	0.5	0.006	0.001
inlE	558	4 (0.735)	17.02	53	39	0.735	0.023	0.001
c.s.	2409	9 (0.890)	7.09	104	64 (38.1%)	0.615	0.012	0.001

c.s. - concatenated sequences, nonsyn – nonsynonymous, syn - synonymous
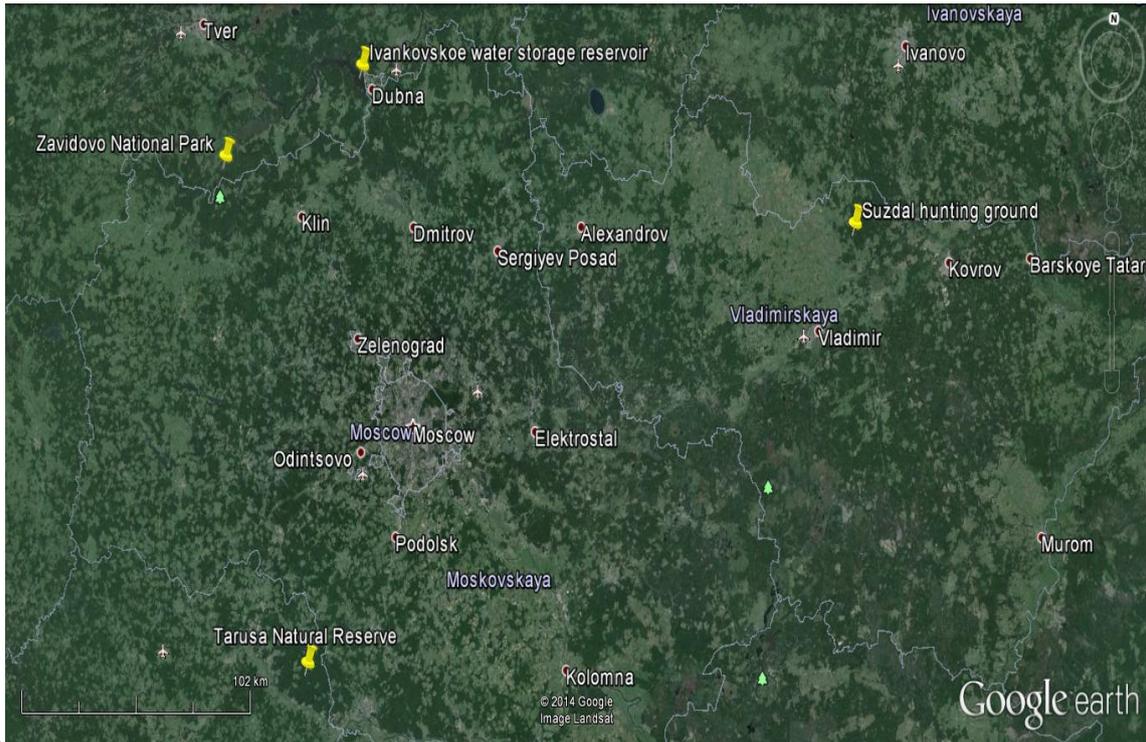
Figure 1. The map location of the Zavidovo National Park, Tarusa Natural Reserve and Suzdal Hunting Ground and Ivanovskoe water storage reservoir.
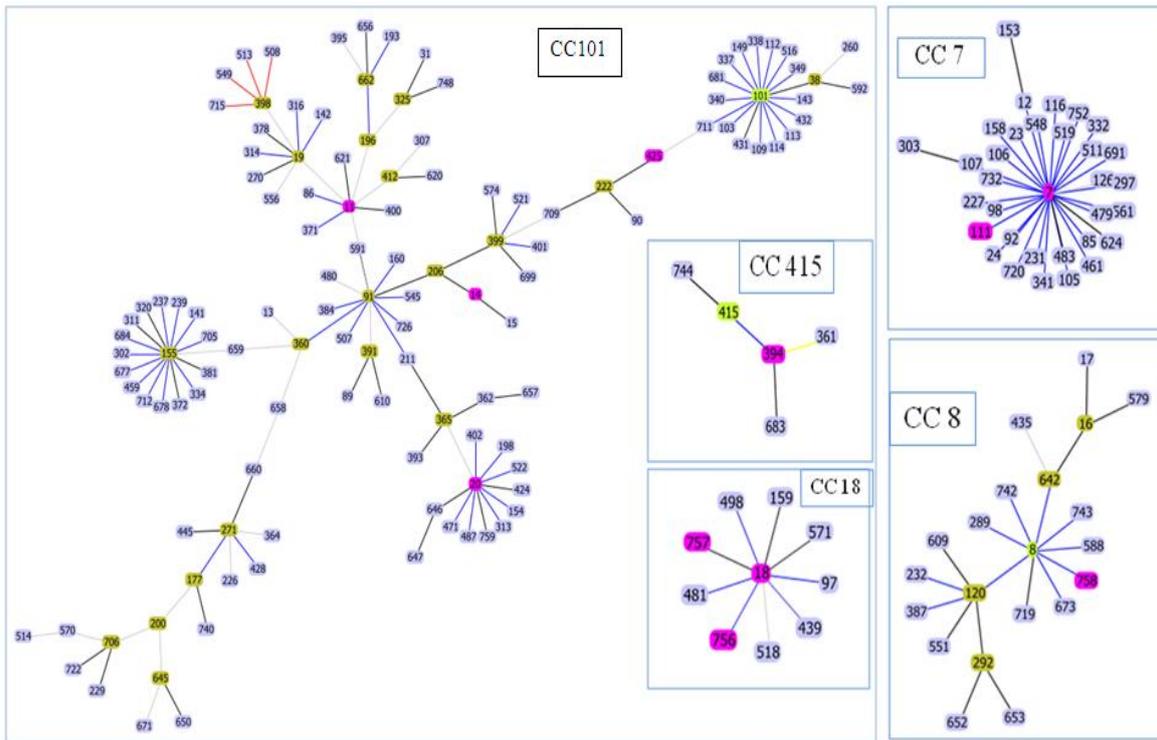
Figure 2. The fragments of the goeBURST analysis of the *L. monocytogenes* STs of the phylogenetic lineage I: CC, including STs from the present investigation (pink color). Light green - main ST in CC, yellow – main ST in subclone.
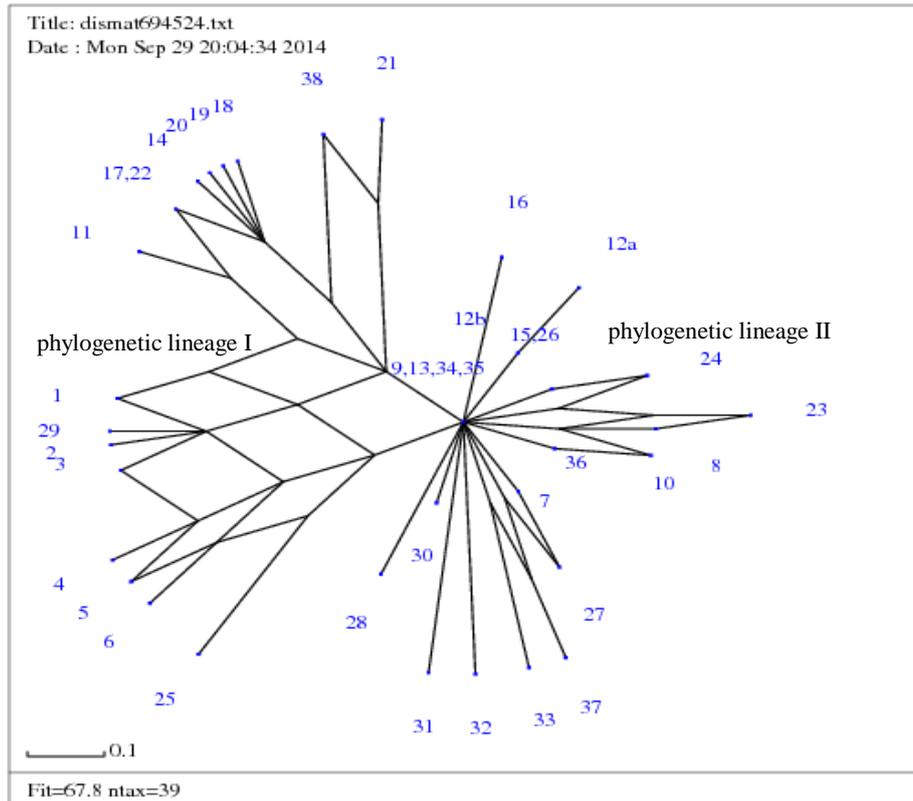
Figure 3. Phylogenetic tree on the base of the internalin genes (A, B, C, E) profiles. SplitsTree.
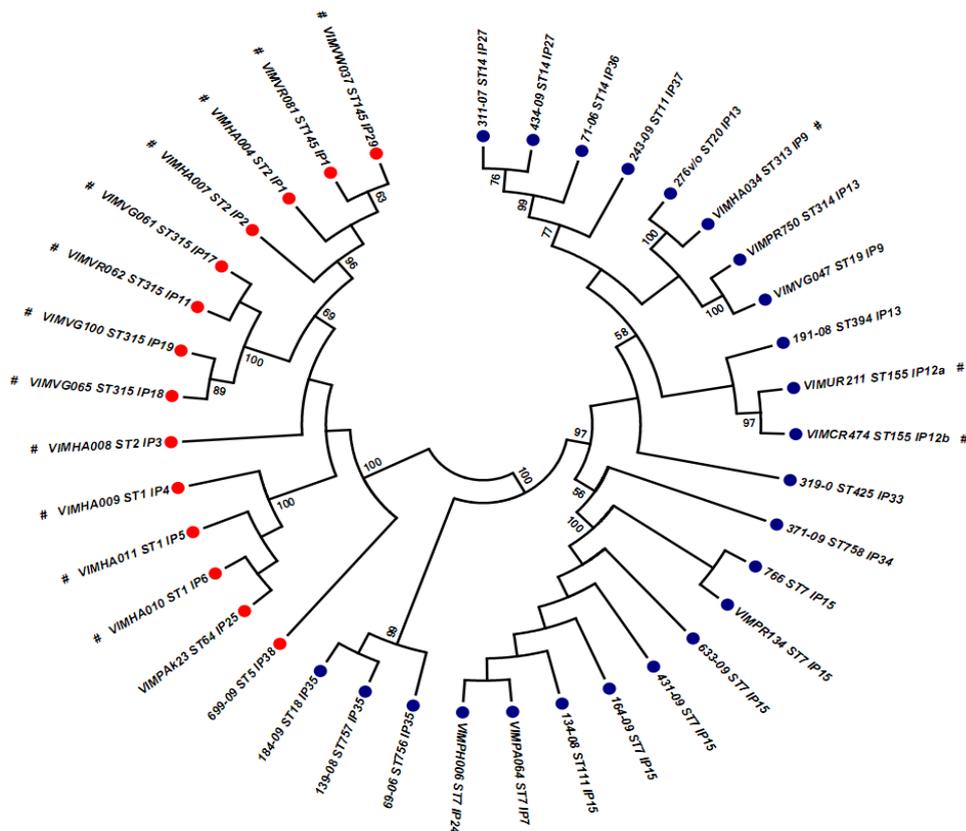
Figure 4 Phylogenetic tree, based on the concatenated sequences of the MLST and IP loci.
#- the Far Eastern strains, red points – phylogenetic lineage I strains, blue points – phylogenetic lineage II strains.