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LOW GENETIC DIVERSITY AND EPIDEMIOLOGICAL SIGNIFICANCE OF *LISTERIA MONOCYTOGENES* ISOLATED FROM WILD ANIMALS IN THE FAR EAST OF RUSSIA

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ABSTRACT

The causative agent of listeriosis, a serious disease of humans and animals, *Listeria monocytogenes* is a ubiquitous bacterium that inhabits both anthropogenic and pristine environments. We report *L. monocytogenes* isolation from wild animals, humans, food and the environment of a far eastern region of Russia. In total, 654 samples of internal organs of small rodents belonging to the *Muridae* and *Cricetidae* families, and 986 samples of the liver and muscles of mollusks and fish were examined to obtain seven and fourteen independent *L. monocytogenes* isolates, respectively. The wild animal isolates were compared with human (n=9), food (n=8) and environmental (n=3) isolates obtained in the same region. 20 of the 21 wild animal isolates belonged to the serovar 4b. The serovars 4b, 1/2a, 1/2b, and 4b, 1/2a, 1/2b, 1/2c were found among human and food isolates, respectively. All isolates were characterized into molecular subtypes by DNA sequencing of the 618 bp internal fragment of the house keeping gene *prs* and 621 bp internal fragment of the virulence gene *inlB*. Sequence analysis revealed 4 and 13 alleles for *prs* and *inlB* fragments, respectively. Distinct *prs* and *inlB* alleles clustered into two groups consistently with established phylogenetic lineages. Among isolates of every lineage, the nucleotide diversity of the *prs* fragment was low; the nucleotide diversity of the *inlB* fragment was low among wild animal isolates and higher among human isolates. All rodent isolates and 10 of 14 marine organism isolates carried the same allele of the *inlB* fragment, which was also found among environmental (2 of 3), food (2 of 8) and human (2 of 9) isolates.

Key words: pristine environment, *Listeria monocytogenes*, *inlB*, *prs*, wild animals, small rodents

The gram-positive bacterium *Listeria monocytogenes* is a facultative intracellular pathogen that causes an invasive infection named listeriosis both in humans and animals (for a review see Farber and Peterkin, 1991; McLauglin, 1997; Vazquez-Boland et al., 2001). Most cases of human listeriosis came from the consumption of *L. monocytogenes*-contaminated food (Meng and Doyle, 1997; Kathariou, 2002). *L. monocytogenes* is a common contaminant of anthropogenic environments such as ruminant and cattle farms or food-processing plants. Furthermore, *L. monocytogenes* is widely spread in pristine environments and is isolated from soil and water sources as well as from the feces of wild animals (Welshimer and Donker-Voet, 1971; Weiss and Seeliger, 1975; Somov and Litvin, 1988).

While all representatives of the *L. monocytogenes* species are considered pathogenic, some clones more frequently cause infection in humans. More than 90% of epidemic and sporadic cases of listeriosis were brought about by strains that belong to three (1/2a, 1/2b and 4b) of 13 described serovars (Seeliger and Hohne, 1979; Schuchat et al., 1991; Farber and Peterkin, 1991). The majority of epidemic cases of listeriosis were caused by a relatively narrow range of strains that belong to the serovar 4b (Piffaretti et al., 1989; Graves et al., 1994). This difference was suggested to be rather due to the host-specific tropism than to the higher incidence of these serotypes in food products. At least, serogroup 4b is less frequently isolated from food than other serogroups (Kathariou, 2002).

Substantially fewer studies were devoted to the analysis of the representation of various *L. monocytogenes* strains in animal disease. The prevalence of certain clones among strains responsible for infections in domestic animals was demonstrated by the multilocus enzyme electrophoresis, ribotyping and analysis of virulence gene alleles. Some clones were found both in human and animal cases, which suggested the role of domestic animals as a potential source of human infections (Boerlin and Piffaretti, 1991; Wiedmann et al., 1997; Vela et al., 2001; Jeffers et al., 2001).

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Many authors link the initial *L. monocytogenes* transmission on farms with the wild environment, which might harbor highly virulent clones. In the mid 1970th the occurrence of *L. monocytogenes* was demonstrated in the wildlife feeding grounds, plants and feces of wild animals and birds (Welshimer and Donker-Voet, 1971; Weiss and Seeliger, 1975; Somov and Litvin, 1988). It was suggested that wild animals may be exposed to *L. monocytogenes* via feeding. The successful multiplication of the pathogen in infected animals would help the selection of invasive clones and their further transmission.

The study of invasive clones is important to clear up a role of pristine environments as reservoirs of the pathogen. There are a number of published evidences on development of invasive diseases caused by *L. monocytogenes* in wild animals. Listeriosis in wild animals was recognized in Russia, Austria, Japan and other countries. Disease caused by *L. monocytogenes* was documented in wild turkeys, small rodents, wild boars and other wild mammals (Sixl et al., 1978; Hatkin et al., 1986; Brosch et al., 1988; Somov and Litvin, 1988; Hayashidani et al., 2002).

In this work, *L. monocytogenes* isolates were obtained from wild animals including small rodents and marine organisms that inhabit pristine environments in the Far East of Russia. With the aim to find invasive clones, we focused on the isolation of *L. monocytogenes* from internal organs including the liver, the spleen and the kidneys of small rodents, and the liver and muscles of mollusks and fish (Tables 1, 2). The wild animal isolates were compared with human, food and environmental isolates obtained in the same region. Combined data on samples tested are shown in the Table 1.

Samples were studied on the presence of *L. monocytogenes* according to the protocol that is similar to the FDA isolation protocol (Hitchins, 1995) and includes pre-enrichment and enrichment stages in the Fraser broth followed by plating on the selective Oxford and PALCAM agar (HiMedia, Inc., India). Up to 10 esculin-positive colonies with the typical morphology were characterized bacteriologically and tested with *L. monocytogenes*-specific PCR as previously

described (Ermolaeva et al., 2003). Only one isolate from each sample was used in the further analysis.

Small rodents were trapped in the forestry territory that surrounds the city of Vladivostok. The survey was part of a routine inspection of wild animals on the endemic infections and performed in compliance with the Russian Federation State protocols 3.1.099-96 and 3.1.088-96, and the guidelines of Vladivostok Institute of Microbiology. The distances between Vladivostok and the regions 1 and 2 (see Table 2) are 250 and 60 km, respectively. Both regions include mixed, mainly deciduous forests. The region 1 is about 200 km from the sea coast while the region 2 is in the close vicinity to the coast, hence the distance between the site of animal capture and the coastline does not exceed 1-2 km. Rodents were sacrificed by cervical translocation. The liver, spleen and kidneys were removed aseptically, chilled on ice until delivery to the laboratory, and kept frozen until a further assay. Seven independent *L. monocytogenes* isolates were obtained from the internal organs of 654 rodents, i.e. about 1% of studied animals were infected.

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Samples of marine organisms that inhabit internal bays of the Pacific Ocean were chilled on ice until delivery to the laboratory and kept frozen until further assay. The distances between Vladivostok and the bays T and R, and Vladivostok and the bay A are about 60 and 30 km, respectively. The distance between the bays T and R is about 3 km. Fourteen strains were isolated from 986 marine organisms tested in 1993 (Table 1). In 2005 the subsequent survey failed to isolate *L. monocytogenes* from 276 samples tested (data not shown). Annual variations in the incidence of *L. monocytogenes* may be responsible for the discrepancy between the results of the surveys.

Environmental samples were collected in March 1998 – November 1999 at the river Knevichi. The river does not flow through the urban territory. The site of sampling was about 40 km from the city of Vladivostok. 50 ml river water and 25-50 g soil samples were collected in sterile bags to deliver to the laboratory. Oceanic waters of internal bays of the Pacific Ocean were

studied in March 1998 – November 2001 in a similar way. Totally 299 environmental samples were studied. Three *L. monocytogenes* strains were isolated from 61 river water samples tested.

Five isolates of a human origin were obtained from aborted fetuses at the Plague-Control Station of the city of Khabarovsk in 2004 - 2005. Four strains were isolated from pregnant women at the last trimester of pregnancy without clinical manifestations of listeriosis that applied for the *L. monocytogenes* control to the Vladivostok Institute of Microbiology over the period from 1993 to 2005. The cervico-vaginal samples were obtained in the Institutional clinic and immediately investigated on *L. monocytogenes*. Food samples were obtained from local trading officers when they applied for microbiological control. Eight strains were isolated from 1395 samples examined over the period from 1999 to 2005. Totally, 41 independent isolates were obtained.

L. monocytogenes isolation from the internal organs of wild animals suggested that isolates were invasive. To prove it and to establish their virulence potential, the isolates obtained from small rodents were studied in experimental infection of laboratory mice. Groups of 5 non-inbred mice of 14-18 g were injected intraperitoneously with serial dilutions of the overnight *L. monocytogenes* culture in PBS. The virulence of the isolates was estimated using the 50 % lethal dose (LD_{50}) with the probit method (Table 2). All strains were virulent with LD_{50} varying in a range from $1x10^4$ to $1,6x10^7$ CFU per mouse (a strain was considered non-virulent if it did not cause a death among the group of 5 mice in 7 days after injection with 10^9 CFU (Takeuchi et al., 2003).

Serotyping of isolates was performed according to the standard method (Seeliger and Hohne, 1979). Serotyping revealed that all rodent and environmental isolates and all but one isolate from marine organisms belonged to the serovar 4b (Table 2). All three serogroups, which are usually revealed in clinical cases, namely 1/2a, 1/2b and 4b, were present among human isolates. Food isolates included 1/2a, 1/2b, 1/2c and 4b serovars. Results of serotyping were confirmed by revealing of serotype specific markers by using PCR as described by Doumith and colleagues (2004a). In conclusion, the distribution of *L. monocytogenes* serovars among human and food

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142 isolates obtained in the far eastern region of Russia was similar to the distribution of serovars
143 among isolates from Europe and Northern America (Farber and Peterkin, 1991; Kathariou, 2002).
144 In contrast, the noticeable prevalence of the 4b serovar was characteristic for invasive *L.*
145 *monocytogenes* isolated from wild animals that inhabit pristine environments of the studied region.
146 To further characterize isolates, strain-specific markers *inlG* and *inlH* were revealed. *inlG*
147 and *inlH* encode proteins of the internalin family, which includes membrane and secreted proteins
148 that are necessary for *L. monocytogenes* adhesion to and invasion into normally non-phagocytic
149 eukaryotic cells (Vazquez-Boland et al., 2001). These two genes are present or absent in certain
150 strains of particular *L. monocytogenes* serovars (Doumith et al., 2004b; Tsai et al., 2006). We
151 studied the occurrence of *inlG* and *inlH* with PCR. The primers used were G1: 5' -
152 TCACGGATCCAGCATTAGCG and G2: 5' -TGCACCTCCGATGAAAAGCG for *inlG*; H1: 5' -
153 GTTCGGGCAGAGAGCATCACG and H2: 5' - GTTGGATCATCGGGATTCGGG for *inlH*. The PCR was
154 run with 1 µl of lysate prepared as described previously (Ermolaeva et al., 2003) in the "Tertsik"
155 thermocycler (DNA Technology, Russia) for 35 rounds using the "fast" protocol as following: 20 s
156 at 94°C, 20 s at 55°C, 20 s at 72°C for the first 5 rounds followed by 30 cycles with timing reduced
157 up to 5 s.
158 Only isolates of serovars 1/2a and 1/2c carried *inlG* and *inlH* (Table 2), which is consistent
159 with previously published results (Doumith et al., 2004b; Tsai et al., 2006). Interestingly, *inlH* was
160 found in five isolates out of nine while this marker was reported to occur only rarely among isolates
161 from USA (Tsai et al., 2006). All *inlH*-carrying isolates originated from the studied region while the
162 absence of *inlH* was observed in isolates from imported food products (Table 2).
163 All isolates were characterized by partial sequencing of two genes, the virulence gene *inlB*
164 and the housekeeping gene *prs*. The sequences of the 621-bp internal fragment of the *inlB* gene,
165 which codes for a protein of the internalin family, and the 618-bp internal fragment of *prs*, which
166 codes for phosphoribosyl synthetase, were determined. Both genes were included in previously

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167 described MLST schemes, and their high variability was demonstrated (Cai et al., 2002; Zhang et
168 al., 2004; Nightingale et al., 2005; Tsai et al., 2006). Sequences were proofread and assembled in
169 Chromas version 1.45 (Copyright© 1996-1998 Conor McCarthy,
170 http://www.technelysium.com.au/chromas.html). Sequence data are available in the GenBank
171 database under the accession numbers EF056129-EF056210. DNA alignment was done with the
172 ClustalW1.83.XP (Thompson et al., 1994). Dendrograms were constructed using the neighbor-
173 joining algorithm included in the Mega 3.1 software package (Kumar et al., 2004). Descriptive
174 analysis of the sequence data was performed using DnaSP version 4.10 (Rozas et al., 2003).
175 Dendrograms revealed two major groups consistent for both genes (Fig. 1). One group
176 included isolates of serovars 4b and 1/2b, and another included serovars 1/2a and 1/2c. Therefore,
177 the groups were consistent with the major phylogenetic lineages 1 and 2, which were described
178 previously (Piffaretti et al., 1989; Wiedmann et al., 1997; Doumith et al., 2004a). DNA
179 polymorphism correlated with the lineage division. Calculation of the nucleotide diversity measure
180 π (Nei and Miller, 1990) demonstrated that the intra-lineage nucleotide diversity of the studied
181 DNA fragments was low in comparison with the nucleotide diversity between lineages (Table 3).
182 For *prs*, only one polymorphic site distinguished alleles among isolates of every lineage
183 (Table 3). All wild animal isolates carried the same *prs* allele, which is not surprising for strains
184 isolated from the same geographic region. In agreement with previously published results (Cai et
185 al., 2002; Nightingale et al., 2005), all substitutions in *prs* were synonymous.
186 For *inlB*, the intra-lineage DNA polymorphism was substantially higher. Twenty-four and
187 eight polymorphic sites were observed that gave rise to 9 and 4 alleles for lineage1 and lineage 2,
188 respectively (Table 3). Nevertheless, the analysis of lineage 1 isolates related to the source of
189 isolation demonstrated that the human isolates made the major contribution in the high nucleotide
190 diversity of *inlB* (Table 4). All rodent isolates grouped tightly in the *inlB* tree and carried the same
191 *inlB* allele (type 1) (Fig. 1, Table 2, 4). The type 1 *inlB* was found in ten of the fourteen marine

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192 isolates and two of the three environmental isolates. Thus, the same *inlB* allele was found in 17 of
193 the 21 wild animal isolates. Single nucleotide substitutions (from one to five) were found in three
194 lineage 1 isolates that carried *inlB* other than type 1. The isolate VIMVG047, which originated from
195 a marine organism and belonged to the lineage 2, was the only wild animal isolate that differed
196 significantly in the sequence of the *inlB* fragment. However, this difference seems to relate to the
197 well-established evolutionary fixed divergence between phylogenetic lineages (Piffaretti et al.,
198 1989; Wiedmann et al., 1997; Doumith et al., 2004b). In contrast, human lineage 1 isolates
199 belonged to different subgroups in the *inlB* tree. There were 16 fixed differences between the
200 subgroups that gave rise to 5 non-synonymous substitutions in the amino acid sequence. Two
201 human isolates carried the *inlB* allele (type 1) characteristic for wild animal isolates (Fig. 1, Table
202 2).

203 Previously published results demonstrated a substantial variability of the *inlB* gene among
204 lineage 1 strains, and in particular among 4b strains. Up to five non-synonymous substitutions were
205 observed among nine 4b strains partially sequenced by Zhang and colleagues (2004). All 4b strains
206 from human and animal clinical cases, which were analyzed by Tsai and colleagues (2006), carried
207 distinguishing *inlB* alleles. The diversity of *inlB* alleles among human isolates examined in this
208 work further supports the view on the *inlB* gene as variable. The low diversity of *inlB* among the
209 wild animal isolates is not consistent with these observations. We suggested that the low diversity
210 of *inlB* might be characteristic for the studied region and proposed a special role for small rodents in
211 the maintenance of the specific *inlB* allelic variant among the *L. monocytogenes* population. To
212 verify this hypothesis, an experimental assessment of *L. monocytogenes* invasiveness into murine
213 cells in dependence on the *inlB* allele is planned in our laboratory.

214 In conclusion, the analysis of invasive *L. monocytogenes* strains isolated from small rodents
215 and marine organisms that inhabit pristine environments in the Far East of Russia demonstrated (i)
216 the prevalence of the 4b serovar; (ii) the high incidence of the rare *inlH* gene among lineage 2

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isolates; (iii) the high frequency of certain alleles of *prs* and *inlB*. Taken together, our data suggests that invasive *L. monocytogenes* clones with close genetic markers are spread among wild animals that inhabit the pristine territories of the Far East of Russia. The isolation of strains with similar genetic markers from distant hosts suggested the transmission of *L. monocytogenes* in the natural ecosystems. While small rodents and marine organisms do not share feeding grounds, the transmission might be performed via more extended food chains. Our data are consistent with the suggestion that wild environments provide opportunities for the selection of invasive *L. monocytogenes* clones adapted for survival in mammals and thus might represent a reservoir for the transmission of this pathogen to humans.

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313 **FIGURE LEGEND.**

314 Fig. 1. Dendrograms were constructed by the Neighbor-Joining method for (A) *prs* partial
315 sequences; (B) *inlB* partial sequences. Brackets and source titles show isolation sources. The
316 frequency (in percent) for a given node to be observed in 1000 bootstrap replications is shown
317 for nodes observed in ≥65% replications.

318 Table 1. Combined data on *L. monocytogenes* isolation sources

Source		Tested samples	*L. monocytogenes* positive (per sent)
Wild animals	Wild mice	654	7 (1,1)
	Marine organisms	986	14 (1,4)
Environments	River waters	61	3 (4,9)
	Soil	76	0
	Oceanic waters	162	0
Humans	Aborted fetuses	n.d.[a]	5
	Healthy pregnant carriers	572	4 (0,7)
Food products	Seafood	971	4 (0,4)
	Meat	113	2 (1,4)
	Dairy products	181	1 (0,6)
	Poultry	50	1 (2,0)
	Vegetables	80	0

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320 [a] –data are not available

321 Table 3. Descriptive analysis of the nucleotide sequence diversity

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Gene		Length of fragment	Coverage of complete CDS	π^a	Number of		No. of mutations	
					Polymorphic sites	alleles	Synonymous	Nonsynonymous
prs	n=41	618	66,0 %	0,01184	22	4	22	0
lineage 1	n=32			0,00051	1	2	1	0
lineage 2	n=9			0,00036	1	2	1	0
inlB	n=41	621	32,8%	0,02639	56	13	35	20
lineage 1	n=32			0,00700	24	9	15	9
lineage 2	n=9			0,00420	8	4	4	4

323 [a] – π is the average number of nucleotide differences per site for pairwise comparisons (Nei and
324 Miller, 1990)

Table 4. Descriptive analysis of *inlB* partial nucleotide sequences among lineage 1 isolates

host	Number of			No. of substitutions		Amino acid substitutions[a]
	polymorphic sites	alleles	isolates with allele type 1	Synonymous	Nonsynonymous	
wild rodents n=7	**0**	**1**	**7**	**0**	**0**	**-**
marine organisms n=13	4	4	10	2	2	N251T K259Q (P)
humans n=6	19	5	2	13	6	Q071P (P) N072S (P) V090I (P) I137L (P) P163L (P) T250M (P)

[a] – amino acids found in the allele type 1 *inlB* that is characteristic for rodent isolates, their positions and substitutions found in marine and human isolates are shown; (P) is for parsimony informative sites.

Table 2. *L. monocytogenes* strains used in the study

№	Strain	Region	Source	Year	Serogroup	Lineage[a]	Serovar specific markers[b]				Strain specific markers		*prs* allele	*inlB* allele	LD_{50}
							0737	1118	2819	2110	*inlG*	*inlH*			
1	VIMVR062	Region 1	*Cletrionomus rutilus*	2004	4b	1	-	-	+	+	-	-	1	1	$1,0x10^4$
2	VIMVR081	Region 2	*Cletrionomus rufocanus*	2005	4b	1	-	-	+	+	-	-	1	1	$1,6x10^7$
3	VIMVR084	Region 2	*Apodemus agrarius*	2005	4b	1	-	-	+	+	-	-	1	1	$6,3x10^6$
4	VIMVR088	Region 2	*Cletrionomus rufocanus*	2005	4b	1	-	-	+	+	-	-	1	1	$4,0x10^5$
5	VIMVR087	Region 2	*Apodemus agrarius*	2005	4b	1	-	-	+	+	-	-	1	1	$1,6x10^7$
6	VIMVR090	Region 2	*Apodemus agrarius*	2005	4b	1	-	-	+	+	-	-	1	1	$6,3x10^6$
7	VIMVR092	Region 2	*Apodemus peninsulae*	2005	4b	1	-	-	+	+	-	-	1	1	$2,5x10^4$
8	VIMVG047	Bay R	*Pectinidae* sp	1993	1/2a	2	+	-	-	-	+	+	3	10	n.d.
9	VIMVG061	Bay R	*Pectinidae* sp	1993	4b	1	-	-	+	+	-	-	1	1	n.d.
10	VIMVG062	Bay T	*Pectinidae* sp	1993	4b	1	-	-	+	+	-	-	1	1	n.d.
11	VIMVG064	Bay T	*Pectinidae* sp	1993	4b	1	-	-	+	+	-	-	1	1	n.d.
12	VIMVG065	Bay R	*Pectinidae* sp	1993	4b	1	-	-	+	+	-	-	1	4	n.d.
13	VIMVG067	Bay R	*Pectinidae* sp	1993	4b	1	-	-	+	+	-	-	1	1	n.d.
14	VIMVG077	Bay T	*Pectinidae* sp	1993	4b	1	-	-	+	+	-	-	1	1	n.d.
15	VIMVG086	Bay R	*Pectinidae* sp	1993	4b	1	-	-	+	+	-	-	1	1	n.d.
16	VIMVG098	Bay R	*Pectinidae* sp	1993	4b	1	-	-	+	+	-	-	1	1	n.d.
17	VIMVG100	Bay R	*Pleuronectes* sp	1993	4b	1	-	-	+	+	-	-	1	5	n.d.
18	VIMVG102	Bay R	Sea-urchin sp	1993	4b	1	-	-	+	+	-	-	1	2	n.d.
19	VIMVG104	Bay A	Starfish sp	1993	4b	1	-	-	+	+	-	-	1	1	n.d.
20	VIMVG106	Bay A	Starfish sp	1993	4b	1	-	-	+	+	-	-	1	1	n.d.
21	VIMVG108	Bay A	Starfish sp	1993	4b	1	-	-	+	+	-	-	1	1	n.d.
22	VIMHA004	Khabarovsk	Aborted fetus	2005	4b	1	-	-	+	+	-	-	1	1	n.d.
23	VIMHA007	Khabarovsk	Aborted fetus	2005	4b	1	-	-	+	+	-	-	1	3	n.d.
24	VIMHA009	Khabarovsk	Aborted fetus	2005	4b	1	-	-	+	+	-	-	2	7	n.d.
25	VIMHA010	Khabarovsk	Aborted fetus	2005	4b	1	-	-	+	+	-	-	2	8	n.d.
26	VIMHA034	Khabarovsk	Aborted fetus	2005	1/2a	2	+	-	-	-	+	-	3	10	n.d.
27	VIMVH234	Vladivostok	Healthy pregnant	1993	4b	1	-	-	+	+	-	-	1	1	n.d.
28	VIMVH071	Vladivostok	Healthy pregnant	2004	1/2b	1	-	-	+	-	-	-	2	9	n.d.
29	VIMVH325	Vladivostok	Healthy pregnant	1993	1/2a	2	+	-	-	-	+	+	3	10	n.d.
30	VIMVH333	Vladivostok	Healthy pregnant	2004	1/2a	2	+	-	-	-	+	+	3	10	n.d.
31	VIMVW039	river Knevichi	River water	1998	4b	1	-	-	+	+	-	-	1	1	n.d.
32	VIMVW037	river Knevichi	River water	1998	4b	1	-	-	+	+	-	-	1	6	n.d.
33	VIMVW048	river Knevichi	River water	1999	4b	1	-	-	+	+	-	-	1	1	n.d.
34	VIMVF038	Vladivostok	Beaf, imported	2005	1/2c	2	+	+	-	-	+	-	3	12	n.d.
35	VIMVF047	Vladivostok	Seafood, local	1999	1/2a	2	+	-	-	-	+	+	3	10	n.d.
36	VIMVF064	Vladivostok	Chicken, imported	2004	1/2b	1	-	-	+	-	-	-	2	9	n.d.
37	VIMVF067	Vladivostok	Pork, imported	2004	1/2a	2	+	-	-	-	+	-	3	11	n.d.
38	VIMVF280	Vladivostok	Cheese, imported	2005	1/2a	2	+	-	-	-	+	-	4	13	n.d.

Отформатировано: Шрифт: 10 пт, не полужирный, курсив

Отформатировано: Отступ: Первая строка: 0 см

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39	VIMVF553	Vladivostok	Seafood, local	2001	4b	1	-	-	+	+	-	-	1	1	n.d.
40	VIMVF549	Vladivostok	Seafood, local	2001	4b	1	-	-	+	+	-	-	1	1	n.d.
41	VIMVF870	Vladivostok	Seafood, local	1999	1/2a	2	+	-	-	-	+	+	3	10	n.d.

[a] – phylogenetic lineages are designated as in (Wiedmann et al., 1997)
[b] – (Doumith et al., 2004a)
[c] – was not done