Available online at www.sciencedirect.com
ScienceDirect
Infection, Genetics and Evolution 7 (2007) 736–742
Infection, Genetics and Evolution
www.elsevier.com/locate/meegid

Short communication

Low genetic diversity and epidemiological significance of *Listeria monocytogenes* isolated from wild animals in the far east of Russia

Elena Zaytseva [a,b], Svetlana Ermolaeva [b,*], Georgy P. Somov [a]

[a] *Research Institute of Epidemiology and Microbiology, Vladivostok, Russia*
[b] *Gamaleya Institute of Epidemiology and Microbiology, Gamaleya Street 18, Moscow 123098, Russia*

Received 17 October 2006; received in revised form 11 July 2007; accepted 13 July 2007
Available online 20 July 2007

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 7 and 14 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. Twenty 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 between 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 (two of three), food (two of eight) and human (two of nine) isolates.

Keywords: Pristine environment; *Listeria monocytogenes*; *inlB*; *prs*; Wild animals; Small rodents

1. Introduction

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 is responsible for infections in domestic animals were demonstrated by the multilocus enzyme electrophoresis, ribotyping and analysis of virulence gene alleles. Some clones

* Corresponding author. Tel.: +7 499 190 4375; fax: +7 499 193 6183.
E-mail address: sveta@ermolaeva.msk.su (S. Ermolaeva).

1567-1348/$ – see front matter
doi:10.1016/j.meegid.2007.07.006

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; Wiedman et al., 1997; Vela et al., 2001; Jeffers et al., 2001).

Many authors link the initial *L. monocytogenes* transmission on farms with the wild environment, which might harbor highly virulent clones. In the mid 1970s 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 might 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., 1989; 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 and 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 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.

Table 1
Combined data on *Listeria monocytogenes* isolation sources

Source	Tested samples	*L. monocytogenes* positive (%)
Wild animals		
Wild rodents	654	7 (1.1)
Marine organisms	986	14 (1.4)
Environments		
River water	61	3 (4.9)
Soil	76	0
Oceanic water	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

[a] Data are not available.

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.

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 to 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. Fifty milliliter of river water and 25–50 g of 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 to 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