

Prevalence of *Listeria monocytogenes* in ready – to – eat food of animal origin

Borović Branka¹, Baltić Tatjana¹, Lakićević Brankica¹, Janković Vesna¹, Mitrović Radmila¹, Jovanović Jelena¹, Lilić Slobodan¹

A b s t r a c t: In this study, the presence of *Listeria monocytogenes* in ready– to– eat meat, milk and fish products has been investigated. In addition, the presence of *L. monocytogenes* on food – contact surfaces, as a potential source of food contamination, has been investigated as well. Samples were analyzed by fluorescent immunoenzyme assay on miniVidas® device and by standard microbiological SRPS EN ISO 11290-1: 2010 and SRPS EN ISO 11290-2: 2010 methods. Out of 881 food samples tested, 12,25% were *Listeria* spp. positive, out of which 8,4% were positive for *L. monocytogenes*. *L. monocytogenes* was most commonly found in smoked salmon, which confirmed fact that smoked fish is a high risk food for the *L. monocytogenes* growth and survival. Out of 512 samples from the food – contact surfaces, *L. monocytogenes* was found in 8,78% of swab samples. This paper highlights the importance of implementing appropriate prevention and control measures, verification procedures, and monitoring and maintenance programs that will help to prevent *L. monocytogenes* food contamination.

Key words: *Listeria monocytogenes*, ready– to– eat food, prevalence, control.

Introduction

Listeria monocytogenes is a Gram – positive, non – spore – forming, facultative intracellular microorganism, ubiquitous in natural environment. It is a significant foodborne pathogen that causes listeriosis in both, humans and animals. Listeriosis in humans occurs infrequently, but it has severity of serious manifestations (including septicaemia, meningitis and fetal death), with a case fatality rate between 20% and 50% (Vazquez-Boland *et al.*, 2001). Although listeriosis can occur in apparently healthy individuals, it is primarily pregnant women and their neonates, elderly people, and immunocompromised individuals who are considered to be at the highest risk (Slutsker and Schuchat, 1999). *Listeria monocytogenes* is widely disseminated throughout the natural environment (Fenlon *et al.*, 1996) and consequently, it is present in many animal and plant food products. The primary mode of transmission of *L. monocytogenes* to humans is the consumption of contaminated minimally processed food (Lakićević *et al.*, 2011; Kathariou, 2002;

Shen *et al.*, 2006; Schlech, 2000) and contaminated ready– to–eat foods (RTE) (Gombas *et al.*, 2003), as well. Its extended distribution in the environment, combined with the specific growth conditions of the pathogen, appear to be the main cause of its high prevalence in different kinds of food products. Studies conducted by several authors (Pan *et al.*, 2006; Kathariou, 2002; Tompkin, 2002) have indicated that certain strains of *L. monocytogenes* survive well within the food – processing environment and the persistence of such strains is of concern as they have the potential to act as a continual source of contamination (Lakićević *et al.*, 2010; Pan *et al.*, 2006). In addition, physical and chemical characteristics of the product and their storage allow us to classify foodstuffs as high and low risk foods (Vitas *et al.*, 2004) for *L. monocytogenes* occurrence/growth. Various RTE food such as dairy products, meat products, fish products, vegetables and complex food were associated with transmission of listeriosis. This confirms the fact that *L. monocytogenes* is a highly resistant organism with the ability to grow under harsh environmental conditions

Acknowledgment: This work was funded by the Ministry of Education, Science and Technological Development of Republic of Serbia, grant No. III 46009 and by the Ministry of Education, Science and Technological Development of Republic of Serbia, grant No. 31083.

¹Institute of Meat Hygiene and Technology, Kačanskog 13, 11000 Belgrade, Republic of Serbia.

Corresponding author: Borović Branka, branka@inmesbgd.com

such as extreme temperature (–0.1 to 45°C), pH (3.0 – 9.5) and salt (up to 10%) ranges (Wagner and McLauchin, 2008). Some products that support the growth of *L. monocytogenes* include: pre-packed sliced meat products, fermented sausages, pâté, cured meat products, cooked sausages, smoked meats, prepared roast meat and grill meat, meat salads and related products, pasteurized cans, cold smoked packaged meat products and marinated meat products, soft cheese and cream. Products that do not support the growth of *L. monocytogenes* include: products with a shelf life of less than 5 days, ice cream, fermented milk products, dairy desserts, dairy spreads, semi-hard and hard cheese and products with the pH value less than 4.4. Vitas et al. (2004) have reported that 19.4% of 3685 fresh and processed food analyzed during 4 years were *Listeria* positive, of which 8.3% were *L. monocytogenes* positive. In study conducted by Gusman et al. (2014), the prevalence of *L. monocytogenes* in examined samples of RTE foods was 1.97%, and the count of *L. monocytogenes* in all positive samples exceeded the limit of 100 colony forming units (CFUs) per gram. According to the data reported by the European Food Safety Authority (2009), prevalence rate of *L. monocytogenes* in RTE foods was 4.4%.

In Serbia, there are still not enough data on the presence of *L. monocytogenes* in RTE products.

The aim of this study was to determine prevalence of *L. monocytogenes* in RTE food of animal origin, before the food has left the immediate control of the food business operator who has produced it. In addition, environmental samples were examined for *L. monocytogenes* presence, in order to perceive the risk of additional food contamination.

Material and methods

Samples

A total of 1393 samples were analyzed over 1– year period (2013). The samples included RTE meat, fish and seafood products (cooked and cured meats, smoked fish, seafood salads), RTE milk products (yogurt, sour cream, butter, cheeses, cheese spreads, dairy desserts) and environmental samples (swabs from the surfaces in food processing facilities and retail establishments).

Environmental, equipment surfaces and working surfaces were sampled according to reference SRPS ISO 18593:2010 method.

The samples were kept refrigerated and analyzed within 2h.

Microbiological and immunoassay analysis

Comparative analysis of *L. monocytogenes* presence in food and environmental samples was performed by standard microbiological SRPS EN ISO 11290-1:2010 method, as well as by enzyme-linked fluorescent immunoassay. Also, enumeration of *L. monocytogenes* in food samples was performed according to standard SRPS EN ISO 11290-2:2010 method. Immunoassay was performed by fully automated miniVIDAS® system (bioMérieux, France) using VIDAS® LMX test kit (REF. 30123, bioMérieux, France) for detection of *L. monocytogenes* antigens in food. According to manufacturers' protocol, 25 g/mL of food sample or 1:10 dilution of environmental sample was enriched with 225 mL of LMX broth (REF. 42647, bioMérieux, France) and subsequently incubated during 26–30 h at 37°C ± 1°C. After incubation, 1–2 ml of enrichment was heated 5±1 min at 95°C, than the tube was cooled down and 250 µL of the enriched sample was taken to test and analyzed according to manufacturers' instruction.

Results and discussion

The overview of analyzed samples and prevalence of *L. monocytogenes* in RTE food of animal origin are presented in Table 1.

Results obtained by immunoassay, as well as from the standard microbiological method used in this study, were in compliance. From the total of 881 food samples examined, 74 samples (8.40%) were positive for *L. monocytogenes*, nevertheless count of *L. monocytogenes* in all positive samples was lower than 100 CFUs/g (data not shown).

In our study, the highest prevalence of *L. monocytogenes* was found in smoked fish, especially smoked salmon, where 29.54% of analyzed fish samples were positive. This could be the reason for higher prevalence of *L. monocytogenes* obtained in our study, with regard to the study by Gusman et al. (2014) who has not examined this type of food. Garrido et al. (2009) have reported that from 783 different food samples being analyzed, RTE smoked fish was the most frequently contaminated food category (25% positive). The similar prevalence of *L. monocytogenes* in smoked fish was reported by Beaufort et al. (2007) and Lončarević et al. (1996). In addition to smoked fish, a relatively high prevalence was observed in seafood salads samples. Out of 86 seafood salads examined, 13.95% were positive for *L. monocytogenes*.

Table 1. Overview of analyzed samples and prevalence of *Listeria monocytogenes* in RTE foods of animal origin**Tabela 1.** Pregled ispitanih uzoraka i prevalenca *L. monocytogenes* u hrani animalnog porekla spremnoj za konzumiranje

Food group/Grupa proizvoda	Type of product/ Tip proizvoda	Samples analyzed/ Analizirani uzorci	<i>L. monocytogenes</i> positive samples/ <i>L. monocytogenes</i> pozitivni uzorci	<i>Listeria</i> spp. positive samples/ <i>Listeria</i> spp. pozitivni uzorci
RTE meat products/ Proizvodi od mesa spremni za konzumiranje	Cooked meat products/ Kuvani proizvodi od mesa	311	3(0.96%)	5(1.61%)
	Cured meat products/ Suhomesnati proizvodi	111	5(4.50%)	10(9.01%)
RTE fish products and seafood products/ Proizvodi od ribe i plodova mora spremni za konzumiranje	Smoked fish/ Dimljena riba	176	52(29.54%)	72(40.91%)
	Seafood salads/ Salate od morskih plodova	86	12(13.95%)	10(11.63%)
RTE milk products/ Proizvodi od mleka spremni za konzumiranje	Cheese spreads/ Sirni namazi	68	0	0
	Fermented milk products/ Fermentisani proizvodi od mleka	25	0	0
	Cheeses/ Sirevi	91	2(2.20%)	8(8.79%)
	Dairy desserts/ Mlečni dezerti	13	0	0
Total/Ukupno		881	74 (8.40%)	108 (12.25%)

Among RTE meat products, *L. monocytogenes* was detected in 0,96% of cooked meat products and 4,50% of cured meat products. Out of 311 cooked meat products examined, *L. monocytogenes* was detected in three frankfurter samples. Čaklovica *et al.* (2011) examined the survival of *L. monocytogenes* in frankfurters cooked at 65 and 72°C, and stored at 0.5°C for 45 days. They concluded that *L. monocytogenes*, compared to other foodborne pathogens, is highly resistant to different heat treatments (65 and 72°C), and that storage temperature of 0,5°C does not inhibit the growth of *L. monocytogenes* in frankfurters, which may explain our findings. Understanding the factors that impact positively and negatively on the ability of *L. monocytogenes* to survive and proliferate in food and in the food processing environment is essential to the development and management of effective *L. monocytogenes* control measures (Lakićević *et al.*, 2014).

The smoking, cooking and drying processes can be considered as antimicrobial processes. Nevertheless, we have detected *L. monocytogenes* in five samples of cured meats (two fermented sausages, one smoked ham and two smoked bacons). The principal factors that influence the survival and growth of *L. monocytogenes* in food are temperature, pH and water activity. As similar to other bacteria, the tolerance of *L. monocytogenes* to particular environmental constraints (processing and/or storage conditions) is greatest when all other conditions are optimal for growth (Lakićević *et al.*, 2014). The growth of *L. monocytogenes* in cured meats should be limited by a_w and pH value, and water phase-salt content. Besides that, contamination level of raw meat, process hygiene, as well as storage conditions can significantly affect the growth of *L. monocytogenes* in these products. Ingham *et al.* (2004) evaluated survival of *L. monocytogenes* on 15 ready – to – eat meat products made using drying,

Table 2. Prevalence of *Listeria monocytogenes* on surfaces and equipment in food processing establishments and retail stores

Tabela 2. Prevalenca *L. monocytogenes* na površini opreme u industrijskim pogonima i maloprodajnim objektima

Sample/Uzorak	Samples analyzed/ Analizirani uzorci	<i>L. monocytogenes</i> positive samples/ <i>L. monocytogenes</i> pozitivni uzorci
Knives/Noževi	47	4 (8.51%)
Slicing machines/Mašine za narezivanje	35	3 (8.57%)
Worktables/Radni stolovi	19	4 (21.05%)
Floor/Podovi	7	3 (42.86%)
Dishes/Posuđe	10	6 (60.0%)
Other surfaces/Ostale površine	394	25 (6.34%)
Total/Ukupno	512	45 (8.78%)

fermentation and/or smoking. They found that numbers of *L. monocytogenes* decreased for all products during storage ranging from a decrease of 0.8 log CFU on smoked cured beef slices during 11 weeks under vacuum at 5 °C to a decrease of 3.3 log CFU on a pork rind product stored 5 weeks under air at 21°C.

Authors suggested that 1– week post– packaging room-temperature storage prior to shipment could act as an effective post-lethality treatment for *L. monocytogenes* occurrence in meat products.

Among milk products examined, *L. monocytogenes* was detected in two soft cheeses, while in cheese spreads and fermented milk products this pathogen has not been detected. In the dairy industry, many problems associated with *L. monocytogenes* contamination are related to post-pasteurization contamination.

The second part of our study was related to the detection of *L. monocytogenes* on food contact surfaces in production facilities and retail stores, as significant sources of food contamination. Several studies have focused on the sources and contamination routes of *L. monocytogenes* in food-processing environments. These studies concluded that raw materials were not a major source of contamination, but that contamination occurred during processing and that the food-processing equipment can act as a reservoir of *L. monocytogenes* (Mörettrö and Langsrud, 2004). It is well known that some serotypes of *L. monocytogenes* have the ability to form biofilms on food contact surfaces, and thus represent a continuous source of contamination. *L. monocytogenes* can survive for long period at low temperatures on process equipment, and the ability of bacteria to survive on the equipment used in production is often cause

of the outbreaks described in the literature (Conly and Johnston, 2008).

Out of 512 environmental samples (swabs) analyzed, 45 samples (8,78%) were positive for *L. monocytogenes*. These results reflect the need to improve hygiene and disinfection programs by addressing more accurate cleaning practices and continuous education of food workers in order to obtain microbiologically safe environment.

Listeria monocytogenes should be considered a serious hazard in retail and food processing establishments. To protect customers and to protect the business, operators should implement a program to control *L. monocytogenes*. Understanding the sources of the pathogen and factors that contribute to the risk of contamination, growth and spread of the pathogen are important building blocks to an effective control program.

Conclusions

Relatively low prevalence of *L. monocytogenes* was found in RTE meat and milk products, and the count of the pathogen in positive samples was below the acceptable limit of 100 CFU/g or mL. The obtained data highlighted the importance of good manufacturing and hygiene practices to improve the microbiological safety of the product. Extension of the storage period, temperature variations during storage and handling, and poor hygiene during handling of RTE products, increase the risk of creating favorable conditions for the growth of *L. monocytogenes* and consequently increase the risk for consumers' health. Out of all tested RTE foods, the highest prevalence of *L. monocytogenes* was found in smoked

fish and seafood salads. Results of our study suggest that this food category carries a high risk for *L. monocytogenes* contamination. We have also found *L. monocytogenes* in heat-treated meat products, as well as in cured meats, which confirms the fact that this pathogen is highly resistant and adaptable

to different environmental conditions. Findings of *L. monocytogenes* in environmental samples indicate poor hygiene and indices equipment as possible source of contamination of the final product. An effective control program is the best defense against this pathogen.

References

- Beaufort A., Rudelle S., Gnanou-Besse N., Toquin M.T., Kerouanton A., Bergis H., Salvat G., Cornu M., 2007.** Prevalence and growth of *Listeria monocytogenes* in naturally contaminated cold-smoked salmon. *Letters in Applied Microbiology*, 44, 406–411.
- Conly M. J., Johnston M. D., 2008.** *Listeria*: A persistent foodborne pathogen. *Canadian Journal of Infectious Diseases and Medical Microbiology*, 19, 327–328.
- Čaklović K., Smajlović M., Čaklović F., Alagić D., Članjak E., 2011.** Utjecaj toplinske obrade barenjem i skladištenja na održivost bakterije *Listeria monocytogenes* u hrenovkama. *Meso*, 13, 3, 148–154.
- Fenlon D. R., Wilson J., Donachie W., 1996.** The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. *Journal of Applied Bacteriology*, 81, 641–650.
- Garrido V., Vitas A. I., García-Jalón I., 2009.** Survey of *Listeria monocytogenes* in ready-to-eat products: Prevalence by brands and retail establishments for exposure assessment of listeriosis in Northern Spain. *Food Control*, 20, 986–991.
- Gombas D. E., Chen Y., Clavero R. S., Scott V. N., 2003.** Survey of *Listeria monocytogenes* in ready-to-eat foods. *Journal of Food Protection*, 66, 559–569.
- Gusman V., Medić D., Jelesić Z., Mihajlović-Ukropina M., Milošević V., Považan A., 2014.** *Listeria monocytogenes* isolated in ready-to-eat food in South Bačka region of Vojvodina province, Serbia. *Archieve of Biological Science*, 66, 11–14.
- Kathariou S., 2002.** *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. *Journal of Food Protection*, 65, 1811–1829.
- Lakićević B., Stjepanović A., Milijašević M., Terzić-Vidojević A., Golić N., Topisirović L., 2010.** The presence of *Listeria* spp. and *Listeria monocytogenes* in a chosen food processing establishment in Serbia. *Archieve of Biological Science*, 62, 881–887.
- Lakićević B., Stjepanović A., Tolinački M., Golić N., Topisirović L., 2011.** Improved sensitivity and reproducibility of the PCR method for detection of *Listeria* spp. and *L. monocytogenes* in milk. *Acta Veterinaria*, 61, 239–245.
- Lakićević B., Katić V., Lepšanović Z., Janković V., Rašeta M., 2014.** *Listeria monocytogenes* important foodborne pathogen and causative agent for listeriosis in humans and animals. *Medical Data*, 6, 171–176.
- Lončarević S., Tham W., Danielsson-Tham M. L., 1996.** Prevalence of *Listeria monocytogenes* and other *Listeria* spp. in smoked and 'gravad' fish. *Acta Veterinaria Scandinavica*, 37, 13–18.
- Mörettrö T., Langsrud S., 2004.** *Listeria monocytogenes*: biofilm formation and persistence in food processing environments. *Biofilms*, 1, 107–121.
- Pan Y., Breidt J F., Kathariou S., 2006.** Resistance of *Listeria monocytogenes* biofilms to sanitizing agents in a simulated food processing environment. *Applied and Environmental Microbiology*, 72, 7711–7717.
- Shen Y., Liu Y., Zhang Y., Cripe J., Conway W., Meng J., Hall G., Bhagwat A. A., 2006.** Isolation and characterization of *Listeria monocytogenes* isolates from ready-to-eat foods in Florida. *Applied and Environmental Microbiology*, 72, 5073–5076.
- Schlech W.F., 2000.** Foodborne listeriosis. *Clinical Infectious Diseases*, 31, 770–775.
- Slutsker L., Schuchat A., 1999.** Listeriosis in humans, In E. T. Ryser and E. H. Marth (ed.), *Listeria, listeriosis and food safety*, 2nd ed., New York, Marcel Dekker 75–95.
- Tompkin R. B., 2002.** Control of *Listeria monocytogenes* in the food-processing environment. *Journal of Food Protection*, 65, 709–725.
- Vazquez-Boland J. A., Kuhn M., Berche P., 2001.** *Listeria* pathogenesis and molecular virulence determinants. *Clinical Microbiology Reviews*, 14, 584–640.
- Vitas A. I., Aguado V., Garcia-Jalon I., 2004.** Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain). *International Journal of Food Microbiology* 90, 349–356.
- Wagner M., McLauchin J., 2008.** Biology and Pathogenicity. In: *Handbook of Listeria monocytogenes*. Dongyou L., Ed. CRC Press. Boca Raton, FL, CRC Press, 3–25.
- Ingham S. C., Buege D. R., Droop B. K., Losinski J. A., 2004.** Survival of *Listeria monocytogenes* during storage of ready to eat meat products processed by drying, fermentation and/or smoking. *Journal of Food Protection*, 67, 12, 2698–2702.

Učestalost nalaza *Listeria monocytogenes* u hrani animalnog porekla spremnoj za konzumiranje

Borović Branka, Baltić Tatjana, Lakićević Brankica, Janković Vesna, Mitrović Radmila, Jovanović Jelena, Lilić Slobodan

Rezime: U okviru ovog istraživanja ispitivano je prisustvo *L. monocytogenes* u proizvodima od mesa, mleka i ribe, spremnim za konzumiranje. Pored toga, ispitivano je i prisustvo *L. monocytogenes* na površinama koje dolaze u kontakt sa hranom, kao mogućeg izvora kontaminacije hrane. Uzorci su ispitivani imunoenzimskom metodom na miniVidas® uređaju i standardnim mikrobiološkim metodama SRPS EN ISO 11290-1: 2010 i SRPS EN ISO 11290-2: 2010. Ispitan je 881 uzorak hrane, od čega je 12,25% bilo *Listeria* spp. pozitivno, a 8,4% pozitivno na *L. monocytogenes*. Najčešći nalaz *L. monocytogenes* utvrđen je kod uzoraka dimljenog lososa, što je potvrdilo činjenicu da je dimljena riba hrana sa visokim rizikom za rast i preživljavanje *L. monocytogenes*. Od 512 ispitanih uzoraka sa površina koje dolaze u kontakt sa hranom, *L. monocytogenes* je utvrđena kod 8,78% uzoraka briseva. Ovim radom istaknut je značaj sprovođenja odgovarajućih mera prevencije i kontrole, procedura verifikacije i praćenja, i programa održavanja koji će pomoći da se spreči kontaminacija hrane *L. monocytogenes*.

Cljučne reči: *Listeria monocytogenes*, hrana spremna za konzumiranje, prevalenca, kontrola.

Paper received: 10.10.2014.

Paper corrected: 31.10.2014.

Paper accepted: 18.11.2014.