

Review of the contamination and health risks related to *Campylobacter* spp. and *Listeria monocytogenes* in the food supply with special reference to Estonia and Latvia

M. Roasto¹, K. Kovalenko², K. Praakle-Amin³,
K. Meremäe¹, T. Tamme¹, T. Kramarenko⁴

¹Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, Department of Food Hygiene, Kreutzwaldi 58A, Tartu, Estonia, 51014; e-mail: mati.roasto@emu.ee

²Latvia University of Agriculture, Faculty of Veterinary Medicine, Department of Food and Environmental Hygiene, K. Helmana iela 8, Jelgava, Latvia, LV-3004; e-mail: ch@no.lv

³University of Helsinki, Faculty of Veterinary Medicine, Department of Food Hygiene and Environmental Health, P.O. Box 66, FI-00014, Finland; e-mail: kristi.praakle@helsinki.fi

⁴Estonian State Veterinary and Food Laboratory, Kreutzwaldi 30, Tartu, Estonia, 51006; e-mail: toomas.kramarenko@vetlab.ee

Abstract. The present study gives a review of the contamination and health risks related to *Campylobacter* spp. and *L. monocytogenes* in the Estonian and Latvian food supply. Campylobacteriosis and human listeriosis are important zoonotic diseases. Therefore, biosecurity measures at the farm level and Good Hygiene Practices at the industry level must be strictly followed. Further improvements in the state surveillance and food industry self-control systems are needed both in Estonia and Latvia.

Key words: *Campylobacter* spp., *Listeria monocytogenes*, contamination, health risks

INTRODUCTION

Campylobacteriosis remains the most frequently reported zoonotic disease in humans in the EU with an incidence rate of approximately 50 confirmed cases per 100,000 population in over 17 countries (EFSA, 2009a). High to extremely high levels of resistance to ciprofloxacin were reported in *Campylobacter* isolates which is likely to limit the therapeutic options for and effectiveness of the treatment of human campylobacteriosis cases (Roasto et al., 2007).

Human listeriosis is a relatively rare but serious zoonotic disease, with high morbidity and mortality in vulnerable populations (EFSA, 2009b). The findings of *L. monocytogenes* exceeding 100 cfu g⁻¹ in RTE-foods indicate a direct risk for human health (Roasto, 2009). There is very limited scientific information on

L. monocytogenes in the Estonian food production chain. *L. monocytogenes* research in Estonia has been previously focused on limited foodstuffs e.g. poultry meat and raw milk and to narrow segments of the food chain. The concept which includes the whole food production chain to determine the presence of *Listeria* and *Campylobacter* risks need to be followed.

The present study gives a review of the health risks related to *Campylobacter* spp. and *L. monocytogenes* in the food supply with special reference to Estonia and Latvia.

CONTAMINATION AND HEALTH RISKS OF CAMPYLOBACTER SPP.

Campylobacter jejuni is the leading cause of human bacterial food-borne illness worldwide (Wesley, 2009). Over the past years *Campylobacter* has overtaken *Salmonella* as the most frequently reported zoonoses in the European Union (EU). *Campylobacter* is also the second most common causative agent of food-borne outbreaks in EU, even though most reported cases of campylobacteriosis are sporadic in nature. The genus *Campylobacter* spp. consists of 17 species and 6 subspecies (Euzéby, 2006). The most important species of *Campylobacter* are the thermophilic species: *C. jejuni* subsp. *jejuni*, *C. coli* and *C. lari*. *Campylobacter jejuni* and *C. coli* are the main cause of *Campylobacter* enteritis in humans (Hänninen et al., 2003). *C. jejuni* is responsible for 80–90% of campylobacteriosis. In 2007 a total of 200 507 human cases of campylobacteriosis were reported in EU member states. An increase of almost 25,000 campylobacteriosis cases in 2007 compared to 2006 was registered in the EU. The community incidence was 45.2 cases per 100,000 population (EFSA, 2009a). The high *Campylobacter* contamination of raw poultry products observed in the Praakle-Amin et al. (2007) study in retail outlets of Estonia may indicate that the prevalence of human campylobacteriosis in Estonia is greater than the 154 cases (11.5 cases per 100 000 inhabitant) reported by the Estonian Health Protection Inspectorate in 2008 (Health Protection Inspectorate, 2009).

Campylobacter have been isolated from food items such as poultry meat, raw milk, pork, beef, lamb, and seafood (Duffy et al., 2001). Most research in Estonia on *Campylobacter* spp. has been previously conducted by the researchers and authors of the present article. Materials and methods of Estonian *Campylobacter* studies are previously described in research articles published by Roasto et al. (2005); Praakle-Amin et al. (2007) and Meremäe et al. (2009). We found that effective quality-control programs in an Estonian large-scale poultry processing plant accounted for the lower contamination levels of fresh chicken meat compared to the contamination level with the same type of products of a small-scale plant (Roasto et al., 2005). Altogether, 279 samples of Estonian raw chicken meat (breasts, carcasses, legs, minced meat, thighs and wings) were analyzed during 2000 and 2002 (Roasto et al., 2005). Of these, 90 were collected directly from the end of the slaughter line of a small-scale poultry meat plant and

189 from traditional market halls of Tartu, Estonia. All chicken meat samples from market halls were sold fresh and unpacked. Of the raw chicken products of Estonian origin, 15.8% were positive for *Campylobacter*. The prevalence of *Campylobacter* in the products (breasts, carcasses, thighs and wings) of the small-scale poultry meat plant (35.6%) was significantly higher than in those originating from the large-scale company (6.3%) ($P < 0.001$). The occurrence of *Campylobacter* spp. in broiler chicken production in Estonia from 2002–07 was reported by Meremäe et al. (2009). *Campylobacter* spp. was isolated in 163 (12.3%) of 1320 chicken meat samples in 2002–07 and in 115 (6.3%) of 1819 fecal samples taken directly from caecum in a slaughterhouse during 2005–07. At the farm level, all 1254 fresh fecal samples collected in 2005 and 2006 from a total of 60 flocks (each containing 20 000 birds) were negative. *C. jejuni* was the most commonly isolated species (98.2%), followed by *C. coli* (1.4%) and *C. lari* (0.4%). The seasonal peak of *Campylobacter* contamination was from July to September.

The Latvian pilot *Campylobacter* study was performed in 2007 and 2008. Sampling was performed at the slaughterhouse level in Latvia where both pig carcass and fecal swab samples were collected. A detailed description of the Latvian pilot study of *Campylobacter* spp. is given in an article of Kovalenko et al. (2009).

During the year 2007, from a total of 129 fecal samples 51 (39.5%) were positive for *Campylobacter* spp. In the year 2008, 21 (9.7%) from a total of 217 fecal and 12 (13.2%) from a total of 91 carcass samples were positive for *Campylobacter* spp. The testing of antimicrobial susceptibility was performed with the VetMICTM Camp test in the Department of Food Hygiene of the Estonian University of Life Sciences in 2009. Among 17 randomly selected *Campylobacter* spp. isolates from pig fecal and carcass samples of Latvian origin 59% of *Campylobacter* strains were resistant to enrofloxacin, 29% to gentamicin, 29% to nalidixic acid, 24% to oxytetracycline, 18% to erythromycin and only one strain showed resistance to ampicillin (Kovalenko et al., 2009).

CONTAMINATION AND HEALTH RISKS OF *LISTERIA MONOCYTOGENES*

In EU member states 1558 human cases of listeriosis were reported in 2007 (EFSA, 2009b). *Listeria monocytogenes* accounts for about 2500 cases, 2289 hospitalizations, and 449 deaths each year in the United States. The mortality rate of *L. monocytogenes* (ca. 28%) remains the highest of all food-borne pathogens (Wesley, 2009). In humans severe illness mainly occurs in unborn children, infants, the elderly and those with compromised immune systems. Symptoms vary, ranging from mild flu-like symptoms and diarrhoea to life threatening infections characterized by septicaemia and meningoenphalitis. In pregnant women the infection can spread to the foetus, which may either be born severely ill or die in the uterus and result in abortion.

L. monocytogenes is a Gram-positive and motile bacterium that is commonly present in the environment and occurs in almost all raw food materials sporadically. Some studies suggest that 1–10% of humans may be intestinal carriers of *L. monocytogenes*. It has been found in at least 37 mammalian species, both domestic and feral, as well as from 17 species of birds and possibly some species of fish and shellfish. *L. monocytogenes* is quite hardy and resists the detrimental effects of freezing, drying and heat remarkably well for a non spore-forming bacterium (Johansson, 1999). *L. monocytogenes* is transmitted via three main routes: contact with animals, cross-infection of newborn babies in hospital and food-borne infection. The latter two sources result in the majority of listeriosis cases in humans. Listeriosis is an uncommon but serious food-borne disease that can be life-threatening to the elderly, people with weakened immune system and pregnant women (Frye et al., 2002).

L. monocytogenes has been associated with food sources such as raw milk, unreliable pasteurized milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (all types) as well as raw and smoked fish. *Listeria* is able to grow at temperatures as low as 3°C and this permits its multiplication in refrigerated foods (Berziņš et al., 2007). It can survive or even grow at pH values as low as 4.4 and at salt concentrations of up to 14% (Roasto et al., 2004). In our previous study by Praakle-Amin et al. (2006) a total of 240 raw broiler legs (120 of Estonian and 120 of foreign origin) from 12 retail stores in the two biggest cities (Tallinn and Tartu) of Estonia were investigated from January to December 2002. Of the raw broiler legs, 70% were positive for *L. monocytogenes*. The prevalence of *L. monocytogenes* in broiler meat of Estonian origin (88%) was significantly higher than in broiler meat of foreign origin (53%) ($P < 0.001$). Praakle-Amin et al. (2006) concluded that the high prevalence of *L. monocytogenes* showing various PFGE types in the broiler meat could be caused by cross-contamination at the retail level. RTE-meat products with a long shelf-life are associated with a risk of transmission of *L. monocytogenes* (Farber & Peterkin, 1991). More extensive studies have been performed in our neighbour country Latvia. In Latvia, where cold-smoked meat products are very popular among consumers, the prevalence of *L. monocytogenes* in cold-smoked, sliced, vacuum packaged pork products during the 15-month period from 2003 until 2004 was studied by Berziņš et al. (2007). Samples originated from 8 Latvian and 7 Lithuanian manufacturers. The prevalences of *L. monocytogenes* in cold-smoked pork varied from 0 to 67% in Latvian products and from 10 to 73% in Lithuanian products. In order to identify the main risk factors associated with *L. monocytogenes* contamination, all production steps were studied separately in each meat processing plant. Berziņš et al. (2007) suggested that brining by injection was a significant ($P < 0.05$) factor in contamination. Moreover, long cold-smoking times (12 h) had a significant ($P < 0.014$) predictive value for a sample to be positive for *L. monocytogenes*. The cold-smoking temperatures between 24 and 30 °C can provide an inhibitory effect on

the presence of *L. monocytogenes* (Berziņš et al., 2007). Materials and methods of Estonian and Latvian *Listeria monocytogenes* studies are previously described in research articles published by Praakle–Amin et al. (2006) and Berziņš et al. (2007).

It is recognized that the presence of *L. monocytogenes* in raw foods cannot be completely eliminated, but through the application of effective hygienic measures, it is possible to reduce its occurrence and level in food products. In order to ensure the safety of food products, growing, harvesting, handling, storage, processing and food supply systems must be managed by food handlers in a manner that can reliably control the growth of *L. monocytogenes* and prevent its multiplication to the potentially harmful level of >100 cfu g⁻¹ (Roasto, 2009).

CONCLUSIONS

It is necessary to follow strict hygienic conditions, HACCP principles and Good Hygiene Practices within the whole food production chain to keep the occurrence of *Campylobacter* spp. and *Listeria monocytogenes* in Estonia and Latvia as low as possible.

Compared to Latvia, *Listeria monocytogenes* studies in Estonia have been very limited. However, according to the self-control results of the meat industries, there are many problems related with production hygiene management with regard to *L. monocytogenes*. The rising popularity of cold-smoked meat (fish, pork, cattle) products among Estonian and Latvian population makes it more problematic.

The present surveillance system of human listeriosis and campylobacteriosis in Estonia and Latvia does not include molecular characterization of pathogen strains and, therefore, it limits the capacity of the epidemiological investigations.

Further improvements in *Campylobacter* and *Listeria* control systems are necessary for proper epidemiological investigations and early outbreak detection.

ACKNOWLEDGEMENTS. This study was supported by the financing project of Estonian University of Life Sciences 'Possibilities for minimizing biological and chemical hazards in food chain', P 5081 VLVL05.

REFERENCES

- Berziņš, A., Hörman, A., Lunden, J., Korkeala, H. 2007. Factors associated with *Listeria monocytogenes* contamination of cold-smoked pork products produced in Latvia and Lithuania. *International Journal of Food Microbiology*, **115**(2), 173–179.
- Duffy, E.A., K.E. Belk, J.N. Sofos, G.R. Bellinger, A. Pape, and G.C. Smith. 2001. Extent of microbial contamination in United States pork retail products. *Journal of Food Protection*, **64**, 172–178.
- EFSA. 2009a. *Campylobacter* 3.2. In *The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007. The*

- EFSA Journal (2009), **223**, 109–133.
- EFSA. 2009b. *Listeria* 3.3. In *The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007. The EFSA Journal* (2009), **223**, 135–159.
- Euzeby, J.P. 2006. Dictionnaire de bacteriologie veterinaire. <http://www.bacdico.net>.
- Farber, J.M., Peterkin, P. 1991. *Listeria monocytogenes*, a foodborne pathogen. *Clinical Microbiology Reviews*, **55**, 476–511.
- Frye, D.M., Zweig, R., Sturgeon, J., Tormey, M., LeCavalier, M., Lee, I., Lawani, L., Mascola, L. 2002. An outbreak of febrile gastroenteritis associated with delicatessen meat contaminated with *Listeria monocytogenes*. *Clinical Infectious Diseases*, **35**, 943–949.
- Health Protection Inspectorate, Republic of Estonia. Estonian Communicable Disease Bulletin 2009. 46 pp.
- Hänninen, M.-L., Haajanen, H., Pummi, T., Wermundsen, K., Katila, M.-L., Sarkkinen, H., Miettinen, I., Rautelin, H. 2003. Detection and typing of *Campylobacter jejuni* and *Campylobacter coli* and analysis of indicator organisms in three waterborne outbreaks in Finland. *Applied Environmental Microbiology* **69**, 1391–1396.
- Johansson, T. 1999. *Tracking of Listeria monocytogenes in foods and industrial environments*. Academic Dissertation. Department of Food Microbiology. National Veterinary and Food Research Institute. Helsinki. Finland. 119 pp.
- Kovaļenko, K., Terentjeva, M., Roasto, M. 2009. *Occurrence and antimicrobial susceptibility of Campylobacter spp. isolated from pigs in Latvia*. Starptautiskās veterinārmedicīnas studetu zinātniskās konferences tēzes. Faculty of Veterinary medicine. Latvian University of Agriculture. Jelgava. Latvia. 23 pp.
- Meremäe, K., Elias, P., Tamme, T., Kramarenko, T., Lillenberg, M., Karus, A., Hänninen, M.-L., Roasto, M. 2009. The occurrence of *Campylobacter* spp. in Estonian broiler chicken production in 2002–2007. *Food Control*, **21**, 272–275.
- Praakle-Amin, K., Hänninen, M.-L., Korkeala, H. 2006. Prevalence and genetic characterization of *Listeria monocytogenes* in retail broiler meat in Estonia. *Journal of Food Protection*, **69**, 436–440.
- Praakle-Amin, K., Roasto, M., Korkeala, H., Hänninen, M.-L. 2007. PFGE genotyping and antimicrobial susceptibility of *Campylobacter* in retail poultry meat in Estonia. *International Journal of Food Microbiology*, **114**, 105–112.
- Roasto, M. *Listeria monocytogenes*. In Roasto, M., Tamme, T., Juhkam, K (eds.), *Toiduhügieen ja -ohutus*. 2004. Halo Kirjastus, Tartu, pp. 49–54 (in Estonian).
- Roasto, M., Praakle, K., Korkeala, H., Elias, P. Hänninen, M.-L. 2005. Prevalence of *Campylobacter* in raw chicken meat of Estonian origin. *Archiv für Lebensmittelhygiene* **56**, (49–72). 61–62.
- Roasto, M., Juhkam, K., Tamme, T., Hörman, A., Häkkinen, L., Reinik, M., Karus, A., Hänninen, M.-L. 2007. High level of antimicrobial resistance in *Campylobacter jejuni* isolated from broiler chickens in Estonia in 2005–2006. *Journal of Food Protection*, **70(8)**, 1940–1944.
- Roasto, M. 2009. *Listeria monocytogenes* toidupatogeenina ja tootmishügieeni mõjutava faktorina. *Veterinaarmeditsiin 2009*, 68–72 (in Estonian).
- Wesley, I. 2009. Public health impact of food-borne illness: impetus for the international food safety effort. In: Norma Heredia, Irene Wesley and Santos Garcia (eds.) *Microbiologically Safe Foods*. Wiley, A John Wiley & Sons, Inc., Publications, United States of America, pp 3–13.