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# **CONTAMINATION ROUTES AND CONTROL OF *LISTERIA* *MONOCYTOGENES* IN FOOD PRODUCTION**

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ACADEMIC DISSERTATION

To be presented, with permission of the Faculty of Veterinary Medicine of the University of Helsinki, for public examination in Auditorium Arpeanum, Snellmaninkatu 3, Helsinki, on 9<sup>th</sup> September 2011, at 12 noon.

Helsinki 2011

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ISBN 978-952-10-7108-9 (paperback)

ISBN 978-952-10-7109-6 (PDF)

Helsinki University Print  
Helsinki 2011

Cover illustration: Gram positive rods. Kimmo Hellström, 2011.

**IN MEMORY OF MY MUM AND DAD**

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## ACKNOWLEDGEMENTS

This study was carried out at the Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki and Center of Excellence on Microbiological Food Safety Research, Academy of Finland (118602). The financial support of the Walter Ehrström Foundation, the Finnish Ministry of Agriculture and Forestry (3629/501/2002), and the Latvian Ministry of Education and Science is gratefully acknowledged.

I express my deepest gratitude to my supervising professor, Hannu Korkeala, for getting me interested in the field of food hygiene and research, and for all the patience and support during this project. Thanks for all the interesting discussions about research questions among numerous other topics. Without you, this work would never have been done.

I want to thank all the co-authors as well as colleagues at the Department of Food Hygiene and Environmental Health for a rewarding collaboration and the inspiring and warm atmosphere at work. Special thanks to Tiina Autio who got me started with this project by supervising my licentiate theses. Thanks belong also to other *Listeria* persons I have had the pleasure to work with: Aivars Bērziņš, Janne Lundén, Annukka Markkula and Riina Tolvanen. I warmly thank Riikka Laukkanen for not only doing research project with me, but also helping in a number of ICT and statistical things and remembering all the details. I am grateful to Johanna Björkroth, Maria Fredriksson-Ahomaa, Marja-Liisa Hänninen, and Riitta Maijala for their enthusiasm and valuable discussions. I want to thank Miia Lindström, Annamari Heikinheimo and Päivi Lahti for being great colleagues as well as being good friends outside the working hours. Thank you Yagmur Derman, Saija Kalenius, Riikka Keto-Timonen, Mari Nevas and my roommate Marjo Ruusunen for good company at work. Thanks belong to Saila Savolainen and Katri Kiviniemi, who did their licentiate theses as part of this project.

My warm thanks go to Johanna Seppälä who has taken care of bureaucracy and known all the answers to administrative questions. I thank Jari Aho, Hanna Korpunen, Anneli Luoti, Erja Merivirta, Kirsi Ristkari, Anu Seppänen, Maria Stark and Heimo Tasanen for excellent technical support and practical advice in the laboratory. I also wish to thank farmers, slaughterhouses, the Finnish Association for Organic Farming and meat inspection veterinarians, who enabled the extensive sampling.

Professor Miguel Prieto and Professor Andreas Stolle are acknowledged for reviewing the theses and Dr. Fred Stoddard for revising the language.

I want to thank all my friends and relatives for the taking care of kids and dog on numerous occasions and providing quality time during these years. Special thanks go to Milko, Katja and Mimmo along with Esko and Varpu for providing excellent surroundings for writing. Thanks to Anne, Ari, Janne, Kimmo, Katja B., Katja R., Leila, Marja-Liisa, Minna and Yrjö as well as the whole Green group. Thanks to the SELL office and board, with whom I had the pleasure to work during these years. Thanks to Wilma for taking me out regularly.

Last but not least I want to thank my beloved wild boys Eelis and Lauri for filling my life with all the joy and happiness – mum will get the sword!

## ABSTRACT

*Listeria monocytogenes* is the causative agent of the severe foodborne infection listeriosis. The number of listeriosis cases in recent years has increased in many European countries, including Finland. Total elimination of the pathogen from the food chain is hardly possible, but contamination needs to be minimized and growth to high numbers in foods prevented in order to reduce the incidence of cases in humans. The aim of this study was to evaluate contamination routes of *L. monocytogenes* in the food chain and to investigate methods for control of the pathogen in food processing.

*L. monocytogenes* was commonly found in wild birds, the pig production chain and in pork production plants. The bacterium was not evenly distributed, but it was found most frequently in certain sites, such as birds feeding at landfill site, organic farms, tonsil samples, and sites and products associated with brining. *L. monocytogenes* in birds, farms, food processing plant or foods did not form distinct genetic groups, but populations overlapped, although some genotypes seemed to be overall more common in the food chain than others.

The majority of genotypes recovered from birds were also detected in other sources such as a variety of foods, food processing environments and other animal species. Clearly, birds do not harbour a distinct group of *L. monocytogenes* of their own and they may disseminate *L. monocytogenes* into food processing environments or directly into foods. Similar genotypes were frequently found in different pigs on the same farm, and rectal swabs collected on farms had similar strains to those later isolated from pigs in the slaughterhouse. *L. monocytogenes* contamination spreads at farm level and may be a contamination source into slaughterhouses and further into carcasses and meat. Incoming raw pork in the processing plant was frequently contaminated with *L. monocytogenes* and genotypes in raw meat were also found in processing environment and in RTE products. Thus, raw material seems to be a considerable source of contamination into processing facilities. In the pork processing plant, the number of *L. monocytogenes*-positive environmental samples and prevalence in pork significantly increased in the brining area, showing that the brining machine and personnel working with brining procedures were an important contamination site.

Recovery of the inoculated *L. monocytogenes* strains clearly showed that there were strain-specific differences in the ability to survive in lettuce and dry sausage. The ability of some *L. monocytogenes* strains to survive well in food production raises a challenge for industry, because these strains can be especially difficult to remove from the products. Possible variation in susceptibility and development of resistance in *L. monocytogenes* strains can create problems and raises a need to use an appropriate hurdle concept to control most resistant strains.

Control of *L. monocytogenes* can be implemented throughout the food chain. Farm-specific factors such as large group size, contact of pigs with pets and pest animals, treatment of manure, hygiene practices, and drinking from a trough affected the prevalence of *L. monocytogenes*. Good farm-level practices can therefore be utilized to reduce the prevalence of this pathogen on the farm and possibly further in the food chain. Since birds harbor *L. monocytogenes*, preventing access of birds into food processing environments is important. Well separated areas in a pork production plant had low prevalences of *L. monocytogenes*, thus showing that compartmentalization controls the pathogen in the processing line. The food processing plant, especially the brining area, should be subjected to disassembling, extensive cleaning and disinfection to eliminate persistent

contamination by *L. monocytogenes*, and replacing brining with dry-salting should be considered. All of the evaluated washing solutions decreased the populations of *L. monocytogenes* on precut lettuce, but did not eliminate the pathogen. Thus, the safety of fresh-cut produce cannot rely on washing with disinfectants, and high-quality raw material and good manufacturing practices remain important. *L. monocytogenes* was detected in higher levels in sausages without the pediocin-producing protective culture than in sausages with this protective strain, although numbers of *L. monocytogenes* by the end of the ripening decreased to the level of <100 MPN/g in all sausages. Protective starter cultures, such as *Lactobacillus plantarum*, provide an appealing hurdle in dry sausage processing and assist in the control of *L. monocytogenes*.

## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers referred to in the text by Roman numerals I to V:

- I Hellström, S., K. Kiviniemi, T. Autio & H. Korkeala. 2008. *Listeria monocytogenes* is common in birds and genotypes are frequently similar with those found along the food chain. J. Appl. Microbiol. 104: 883–888.
  
- II Hellström, S, R. Laukkanen, K.-M. Siekkinen, J. Ranta, R. Majjala & H. Korkeala. 2010. *Listeria monocytogenes* contamination in pork can originate from farms. J. Food Prot. 73: 641–648.
  
- III Bērziņš, A., S. Hellström, I. Siliņš, & H. Korkeala. 2010. Contamination patterns of *Listeria monocytogenes* in cold-smoked pork processing environment. J. Food Prot. 73: 2103–9
  
- IV Hellström, S., R. Kervinen, M. Lyly, R. Ahvenainen-Rantala & H. Korkeala. 2006. Efficacy of disinfectants to reduce *Listeria monocytogenes* on precut iceberg lettuce. J. Food Prot. 69: 1565–1570.
  
- V Tolvanen, R., S. Hellström, D. Elsser, J. Björkroth & H. Korkeala. 2008. Survival of *Listeria monocytogenes* strains in a dry sausage model. J. Food Prot. 71: 1550–1555.

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## ABBREVIATIONS

$a_w$	Water activity
AFLP	Amplified fragment length polymorphism
CFU	Colony forming unit
CAMP	Christine, Atkins, Munch-Petersen test
DFEH	Department of Food Hygiene and Environmental Health
FBO	Food business operator
GAP	Good agricultural practice
GHP	Good hygiene practice
HACCP	Hazard analysis critical control point
MAP	Modified atmosphere packaging
MEE	Multilocus enzyme electrophoresis
MLST	Multilocus sequence typing
MPN	Most probable number
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
rRNA	Ribosomal RNA
RTE	Ready-to-eat

# 1 INTRODUCTION

The history of the bacterium *Listeria monocytogenes* is hundred of years old, but its predominantly foodborne nature, for which it is most known nowadays, was not truly recognized until the 1980s. Since then, *L. monocytogenes* has been recognized as a major cause of foodborne infections and the knowledge of the pathogen and the methodological possibilities in research has taken giant leaps, but still there remain things to be discovered.

The state of *L. monocytogenes* as a causative agent of human infections has changed over times. When it was found to be a human pathogen in the 1920s, it was first thought to be mainly a disease of farmers and veterinarians getting infected straight from the farm animals. After the discovery of the foodborne nature of *L. monocytogenes*, the picture of the infection changed to one of a disease transmitted by food, initially through raw milk and milk products. Changes in eating habits and demographic structure have again changed the picture of listeriosis. Increased consumption of RTE foods, prolonged shelf life of foods due to cold storage and new packing technologies, international trade of foods, and advances in medical care resulting in a growing number of elderly, have increased the possible sources of *L. monocytogenes*, as well as susceptible individuals advancing the spread of the disease. Further developments in food processing are about to create new niches for the pathogen, and other food related issues such as scarce resources, nutritional demands, socio-economic inequality and future changes in food consumption habits are making the situation with *L. monocytogenes* dynamic.

Listeriosis is a rare but severe disease, with 0.3 cases per 100,000 incidence, 20–30 % mortality and over 90 % hospitalization in Europe. *L. monocytogenes* has marked human and economic impacts in society, because of deaths and hospitalizations as well as product recalls, and even leads to the bankruptcies of the producers. Thus controlling listeriosis has been a topic for national and international food safety authorities. Despite the research and action in food processing, numbers of listeriosis cases have not reduced markedly.

Control of the pathogen demands increasing knowledge of its habitats, contamination pathways and control strategies. Moreover, not all the strains of *L. monocytogenes* are the same, and they have differences in adaptation to environments, resistance to adverse conditions, and virulence. Important steps in decreasing listeriosis include preventing contamination and controlling the growth of the pathogen in foods. New practises, technologies and agents for controlling *L. monocytogenes* are developed and their effects have to be studied carefully. Elimination of the pathogen from the food chain is hardly possible, but contamination needs to be minimized and growth to high numbers prevented.

## 2 REVIEW OF LITERATURE

### 2.1 *Listeria* spp. and *Listeria monocytogenes*

*L. monocytogenes* was first discovered by the Swedish veterinary microbiologist Hülphers in 1910 (126) and was named *Bacillus hepatis*, but unfortunately it was not at that time sent to any strain collection. In 1926, Murray et al. published the description of the bacterium with the name of *Bacterium monocytogenes* (213), and this finding is often called the discovery of *L. monocytogenes*. In 1927, Pirie discovered the same organism and named it *Listerella hepatolytica* in honour of Lord Lister (237). The name *Listeria monocytogenes* was given in 1940 (236).

*L. monocytogenes* was found to be pathogenic to humans in 1929 (226). The predominantly foodborne nature of listeriosis was proven in the 1980s (90, 171, 264), although food as one of the infection routes had been recognized earlier, such as in monograph by Seeliger in 1961 (265).

#### 2.1.1 Genus *Listeria*

The genus *Listeria* belongs to the phylum *Firmicutes* (low G+C Gram positive prokaryotes), class *Bacilli*, order *Bacillales* and, together with the genus *Brochotrix*, to the family *Listeriaceae* (178, 216). The genus *Listeria* contains eight species: *L. grayi*, *L. innocua*, *L. ivanovii* (*L. ivanovii* subsp. *ivanovii* and *L. ivanovii* subsp. *londoniensis*), *L. monocytogenes*, *L. seeligeri*, *L. welshimeri* and recently described *L. marthii* and *L. rocourtiae* (111, 165, 201, 249). Two species, *L. monocytogenes* and *L. ivanovii* are pathogenic, *L. monocytogenes* causing disease in humans and animals and *L. ivanovii* causing mainly abortions in animals (177).

#### 2.1.2 Detection and identification

*L. monocytogenes* is a gram-positive, small ( $0.4\text{--}0.5 \times 1\text{--}2 \mu\text{m}$ ) coccoid rod that is motile when cultured  $< 30^\circ\text{C}$  and does not form spores (83, 201). It usually grows on commonly used bacteriological media, forming smooth, bluish gray colonies 0.5–1.5 mm in diameter, and expresses  $\beta$ -hemolysis on blood agar (83, 201). *L. monocytogenes* is catalase positive, oxidase negative and produces acid from rhamnose, but not from xylose (201). It is CAMP (Christine, Atkins, Munch-Petersen test) positive with *Staphylococcus aureus* and negative with *Rhodococcus equi* (201). The optimal growth temperature is  $30\text{--}37^\circ\text{C}$ , but it is able to grow at temperatures from  $<0$  to  $45^\circ\text{C}$  (148, 201) and in aerobic and anaerobic conditions (201).

Traditional isolation and identification of *L. monocytogenes* is based on culture methods and biochemical and phenotypic markers, and these are still common for routine laboratory diagnostics (6, 174), but the current trend is towards the use of DNA-based methods (98, 174). The advantages of DNA-based methods over conventional methods are their high specificity and sensitivity, often rapidity and repeatability, because they rely on the genome and not on expression of certain antigenic structures or enzymes (98, 174). On the other hand, molecular methods often are costly, because of the equipment and reagents needed, and they often require highly trained personnel.

Isolation of *L. monocytogenes* often requires enrichment because of the small numbers of *L. monocytogenes* in food and environmental samples and the presence of sometimes large numbers of competitors. The oldest method is cold enrichment, which is based on the ability of *Listeria* spp. to

grow at low temperatures (113). This enrichment method takes up to several weeks and it is replaced with selective enrichment and plating. In media with selective agents like lithium chloride, acriflavine and antibiotics, incubation is done at optimal growth temperature, but still the procedure takes 5–7 days (62, 136).

Selective plates, such as PALCAM, Oxford, LMBA and ALOA, contain besides selective agents also indicator substrates to enable recognition of *Listeria* spp. among other bacteria. On some plates, e.g. PALCAM and Oxford containing aesculin with ferric ammonium citrate, all *Listeria* spp. appear similar, but can be distinguished from other bacteria (56, 291). On other plates, *Listeria* spp. can be distinguished from each other, e.g., on plates with blood (LMBA), hemolytic *Listeria* strains can be recognized (145). On chromogenic agars, *Listeria* species can be identified by chromogenic substrates that allow identification of colonies by colour, e.g., ALOA agar indicates  $\beta$ -glucosidase enzyme, common to all *Listeria* spp. and, in addition, strains that produce the virulence-associated protein phosphatidylinositol-specific phospholipase C (PI-PLC), namely *L. monocytogenes* and *L. ivanovii* (98, 298).

The identification of *L. monocytogenes* is done by phenotypic markers and biochemical tests such as catalase, Gram staining, motility, hemolysis, carbohydrate utilization and CAMP. Traditional testing for identification is time-consuming and has been widely replaced by commercial test kits such as API *Listeria* (29) or identification by PCR (98).

Enrichment, selective plating and identification are time-consuming, and several rapid methods have been developed to detect *L. monocytogenes* straight from food and environmental samples or enrichment broth: PCR-based methods with species-specific primers can be used to detect *L. monocytogenes* directly from enrichment broth without isolation in 24–48 h and results are comparable with culturing methods (6, 50, 98). Antibody-based commercial test kits, such as Vidas LMO that targets a stable virulence antigen, can be used to detect *L. monocytogenes* straight from food samples in 50 h (153). Bacteriophages, species-specific viruses infecting bacteria, can be used to quickly detect viable *Listeria* cells in foods and food processing environment at a very low level (one cell/g) of contamination (120, 175). Overall, rapid detection can be utilized for timely results when monitoring the presence of *L. monocytogenes* in foods and food processing environments, although isolation of the bacterium is still needed on many occasions, e.g., epidemiological studies.

## 2.2 Subtyping of *L. monocytogenes*

Subtyping is the method to discriminate between different strains that belong to same species. Identification of the bacteria to species level is often not discriminatory enough, so typing is used in epidemiological studies, taxonomy and evolutionary genetics.

Subtyping can be based on phenotypic characteristics, like serotyping, phage typing and multilocus enzyme electrophoresis (MEE). Newer methods, such as pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), ribotyping, random amplified polymorphic DNA (RAPD), multilocus sequence typing (MLST) and microarray typing rely on genotypic characteristics. Genotypic methods can be based on PCR (e.g. RAPD), restriction fragment analysis (e.g. PFGE), hybridization (e.g. microarray) or a combination of the previous (e.g. AFLP and ribotyping). The newest typing methods that rely on sequencing data (e.g. MLST) have been developing rapidly since the sequencing of whole genomes of several *L. monocytogenes* strains (103, 217). Overall, discriminatory power, epidemiological concordance, reproducibility and typeability in genotypic methods are higher than in phenotypic methods (174, 307), and genotypic methods are now mainly used for subtyping of *L. monocytogenes*.

### 2.2.1 Phenotypic methods

Phenotypic methods divide strains according to their phenotypic characteristics, which are a result of gene expression. These methods generally have little use in epidemiology since their discriminatory power is limited and they can mainly be used only in species identification.

Serotyping divides *L. monocytogenes* into 13 serotypes, based on O and H antigens: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7 (266). Serotyping has a limited value in subdividing *L. monocytogenes* strains, because of the finite number of serotypes and the fact that most listeriosis cases are caused by serotypes 1/2a, 1/2b and 4b, and food commonly harbours serotypes 1/2a and 1/2c (294). Despite its disadvantages as a typing method, serotyping is still widely used to characterize bacterial strains and may be a first-level subtyping in epidemiological studies (6, 179). In addition, genotypic methods ought to be concordant with serotyping, in order to be epidemiologically and phylogenetically meaningful (214). *L. monocytogenes* is divided into three genetic lineages, according to antigenic structure, i.e., serotypes: Lineage I contains serotypes 1/2b, 3b, 4b, 4d and 4e, lineage II contains serotypes 1/2a, 1/2c, 3a and 3c, and lineage III contains 4a, 4c and a subset of 4b strains (33, 36, 155, 214, 306).

Bacteriophages are viruses infecting bacteria and hence natural enemies of bacteria. They are extremely host-specific, usually able only to infect an individual species or even strain (120). Phage typing is based on the susceptibility of strains to a set of bacteriophages (8, 248). Phage typing has been used successfully to demonstrate foodborne listeriosis outbreaks (9). The main disadvantage of phage typing is that not all strains are typeable, and it has been widely replaced with other methods (174).

MEE divides strains by major metabolic enzymes that are separated by electrophoresis (174). MEE enhanced the typeability, reproducibility and discriminatory power of phenotypical typing, but its discriminatory power is limited (110) and it has largely been replaced by more discriminatory methods.

### 2.2.2 Genotypic methods

PFGE is based on restriction fragments generated by rare-cutting enzymes of a whole genome, which are separated by electrophoresis in pulsed field. PFGE has high discriminatory power that can be enhanced by using several restriction enzymes (1, 51, 112, 174). PFGE has proven to be a valuable tool in numerous outbreak investigations, contamination studies and surveillances (12, 25, 48, 51, 77, 86, 141, 185, 186, 190, 202, 210, 233) and it is the most widely used method for molecular typing of *L. monocytogenes* in Europe (72). Although it is labour- and time-consuming, PFGE is considered to be the method of choice for subtyping *L. monocytogenes* in epidemiological studies (112, 141). It has also proven to be useful in national and transnational databases (e.g. PulseNet), since results from different laboratories achieved by a standardized protocol are comparable and can be shared electronically (180, 197).

AFLP is based on restriction of the genomic DNA and then further amplification of restriction patterns by PCR (299). In some studies, AFLP has been found to be more discriminatory and reproducible than PFGE, as well as faster and less labour-intensive (13, 155). In contrast to PFGE, it enables automated reading of fragments, which allows accurate measurement and it can also be used for species identification (13, 155). The major disadvantage of the method is that it is technically demanding and it is not as widely used as PFGE, thus lacking the existing database.

Ribotyping is also based on restriction fragments, but it targets only genes encoding ribosomal RNA (rRNA). In addition to total bacterial DNA restriction, a Southern blot step is used to specifically detect genes encoding rRNA. The discriminatory power of ribotyping is not as high as that of PFGE or AFLP, and it is often not enough in epidemiological studies (1, 179). The advantage of ribotyping is that it can be totally automated allowing a high through-put (1, 179).

In RAPD, one or more short random primers are used to anneal to multiple sites in the whole genome and generate several amplicons that can be separated in gel electrophoresis to form specific banding patterns for individual strains (164, 239). The technique requires no prior sequence knowledge of template DNA. RAPD is a rapid and simple method for typing, but its discriminatory power is only moderate and the method is not very reproducible (112, 174).

The disadvantage of all fragment-based methods is that differences in gel patterns do not correlate with genomic changes (307). This problem is overcome with sequence-based methods, where changes that allow distinction of different strains provide an opportunity to identify actual genomic changes (307). Overall, sequence based methods are more reproducible and accurate than fragment-based methods, because of the inherent specificity and high information content of sequences (307). The speed of DNA sequencing as well as its cost efficiency have gone through tremendous advances in recent years and enabled the development of sequence-based methods (192). Sequence data is also more objective than macrorestriction patterns achieved by gel electrophoresis and the results are easily shared via internet (132, 307). These advantages over fragment-based methods have led to a demand to replace PFGE as the standard method for epidemiology and databases (e.g. PulseNet) with a newer method. The advantage of PFGE, however, is the already existing large database of isolates worldwide.

The principle of MLST is the sequencing of small fragments of multiple (6–10) genes selected in different locations from whole genome (193, 194). Target genes are usually housekeeping genes that are essential for cell survival and reproduction. MLST is typically less discriminatory than PFGE (307), but using more rapidly evolving genes like virulence-associated genes, solely or in combination with housekeeping genes as a target, increases the discriminatory power of the method (42, 132).

The microarray is based on hybridization using subtype-specific DNA probes. Thousands of probes based on whole genome sequences are placed onto the small surface of the microarray. With small probes, even single nucleotide mutants can be detected. Microarrays have proved to be a very discriminatory subtyping method for *L. monocytogenes*, and the genetic basis of strain variation can be inferred from hybridization patterns (33, 44). The overall cost of the microarray is still high, although it enables a high-throughput finding of simultaneous variation throughout the entire genome. In addition, the interpretation of microarray results is demanding.

Even whole-genome sequencing is about to become a possible method for subtyping (307). This technique has already been used for *L. monocytogenes* and it revealed a connection between a formerly thought sporadic listeriosis case with subsequent epidemic and a long persistence of the pathogen in processing plant with short term evolutionary changes (231). Besides its use in epidemiological studies, whole genome sequencing is a valuable tool for the identification of core genetic elements and variation between strains to be used for other targeted subtyping methods (132, 307).

## 2.3 Listeriosis

Listeriosis is a disease caused by *Listeria* spp. The vast majority of human cases is caused by *L. monocytogenes*, although rare cases caused by *L. ivanovii* have been reported (55, 118, 168, 273). All *L. monocytogenes* strains are considered pathogenic (200), although most listeriosis cases are caused by serotypes 1/2a, 1/2b or 4b (294), many outbreaks worldwide have been caused by a small, genetically similar group (279, 285), and variation in virulence exists among strain (200, 202, 245, 261, 285, 294).

The main route of transmission to both humans and animals is through the consumption of contaminated food or feed (5, 294) and many listeriosis outbreaks linked to foods have been reported (Tables 1 and 2). In addition, a foetus can get infected from haematogenous spread from the mother (200, 294). In rare cases, infection can also be transmitted directly from infected animals to humans (199).

### 2.3.1 Human listeriosis

Listeriosis appears mainly in two forms: severe invasive listeriosis and non-invasive febrile gastroenteritis (5, 275). Invasive listeriosis manifests as sepsis, meningoenzephalitis, perinatal infection, and abortion (294). In cases associated with pregnancy the mother may have mild flu-like symptoms, while the foetus gets a severe infection or dies in utero (294). Invasive listeriosis has a mean mortality of 20–30 % (73, 294) and a hospitalization rate of over 90 % (203), making it one of the most severe foodborne diseases. Non-invasive gastroenteritis can manifest in immunocompetent adults and it is usually self-resolving (32). *L. monocytogenes* can also produce a wide range of focal infections (26, 104, 176, 251) and occur in cutaneous form mainly as an occupational disease of veterinarians and farmers (199, 245).

Invasive listeriosis is mainly a disease affecting susceptible individuals with underlying predisposing conditions. Susceptible individuals include the elderly, pregnant women and their unborn or newborn infants, and patients with severe underlying diseases such as cancer, AIDS, or organ transplant (82, 135). In Europe, approximately 10–20 % of clinical cases are associated with pregnancy (200) and most cases are reported in the over-65 age group (73). The vast majority of cases in Finland is detected in the elderly (Figure 1). In addition to health status, the risk for listeriosis may be affected by socio-economic determinants: listeriosis cases have been reported to be associated with deprived neighborhoods (101).

The majority of listeriosis cases appear to be sporadic (73). The disease is relatively rare (73), its incubation time may be up to 3 months (171), and contamination in a single factory may continue for a long period of time and spread over a wide geographical area (200, 202, 231). Thus, many times the transmission route remains unknown (73) and outbreaks can be unnoticed. The rarity of food-borne outbreaks due to *L. monocytogenes* may also reflect difficulties encountered in linking sporadic cases and the isolation of the pathogen from food (73). In addition, listeriosis is most probably underdiagnosed (200). Cases of diarrhoeal disease with fever are rarely investigated for listeriosis and other cases may also remain undiagnosed (200). It has been estimated that twice the number of listeriosis cases occur as compared to the ascertained ones (2).

**Table 1.** Reported outbreaks of human food-borne invasive listeriosis in Europe.

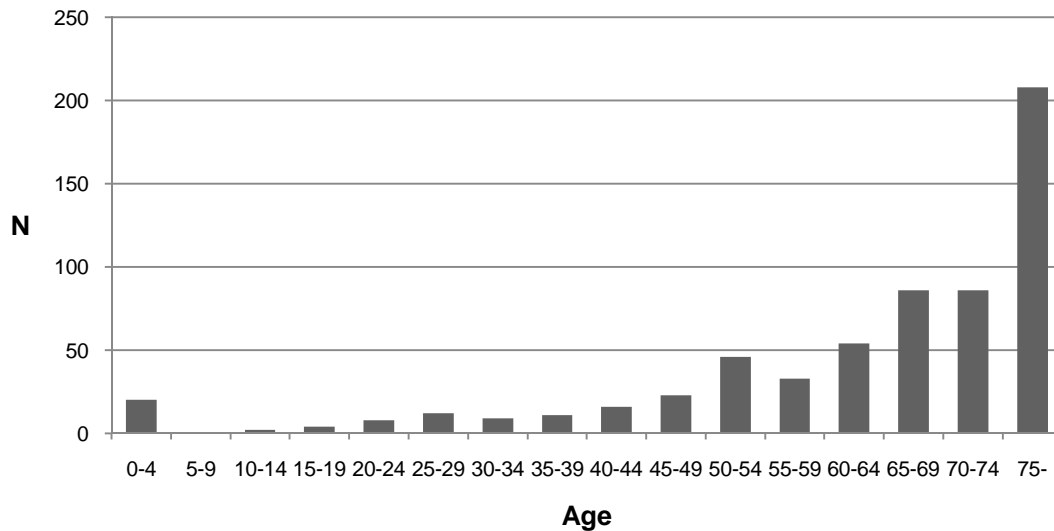
Year	Country	Implicated vehicle	Number of cases (deaths <sup>a</sup> )	Serovar	Reference
1983–87	Switzerland	Soft cheese	122 (33) <sup>b</sup>	4b	27, 38
1986	Austria	Unpasteurized milk or vegetables	28 (5)	1/2a and 4b	4
1987–89	UK	Paté	356 (ND <sup>c</sup> )	4b and 4bX	198
1989–90	Denmark	Blue-mould or hard cheese	26 (ND)	4	142
1992	France	Pork tongue in jelly	279 (63)	4b	108, 141
1993	France	Rillettes (pork meat)	38 (1)	4b	109
1994–95	Sweden	Cold-salted rainbow trout	9 (1)	4b	77
1995	France	Raw-milk soft cheese	20 (0)	ND	107
1998–99	Finland	Butter	25 (6)	3a	190
1999–2000	Finland	Vacuum-packed fish product	10 (4)	1/2	123
1999–2000	France	Rillettes	10 (3)	4b	57
1999–2000	France	Jellied pork tongue	32 (5)	4b	57
2003	UK	Sandwich	2 (2)	1/2a	268
2005	Switzerland	Soft cheese	12(3)	1/2a	28
2006–2008	Germany	Scalded sausage	16 (5)	4b	308
2007	Norway	Soft cheese	21 (5)	ND	70, 146
2009	Denmark	Ready-made meal (beef)	8 (2)	ND	272
2009–2010	Austria, Germany, Czech Republic	Quargel cheese	34 (8)	1/2a	93

<sup>a</sup>Stillbirths not included, <sup>b</sup>Total number of cases in the canton of Vaud, with >80 % of epidemic type, <sup>c</sup>ND = Data not available

**Table 2.** Reported outbreaks of gastroenteritis caused by *L. monocytogenes* in Europe.

Year	Country	Implicated vehicle	Number of cases	Serovar	Reference
1993	Italy	Rice salad	18	ND <sup>a</sup>	258
1997	Finland	Cold-smoked rainbow trout	5	1/2a	210
1997	Italy	Corn and tuna salad	1566	4b	10
2001 <sup>b</sup>	Sweden	On-farm manufactured fresh cheese	48	1/2a	46
2008	Austria	Jellied pork	12	4b	235

<sup>a</sup>ND=Data not available, <sup>b</sup>Mixed etiology possible



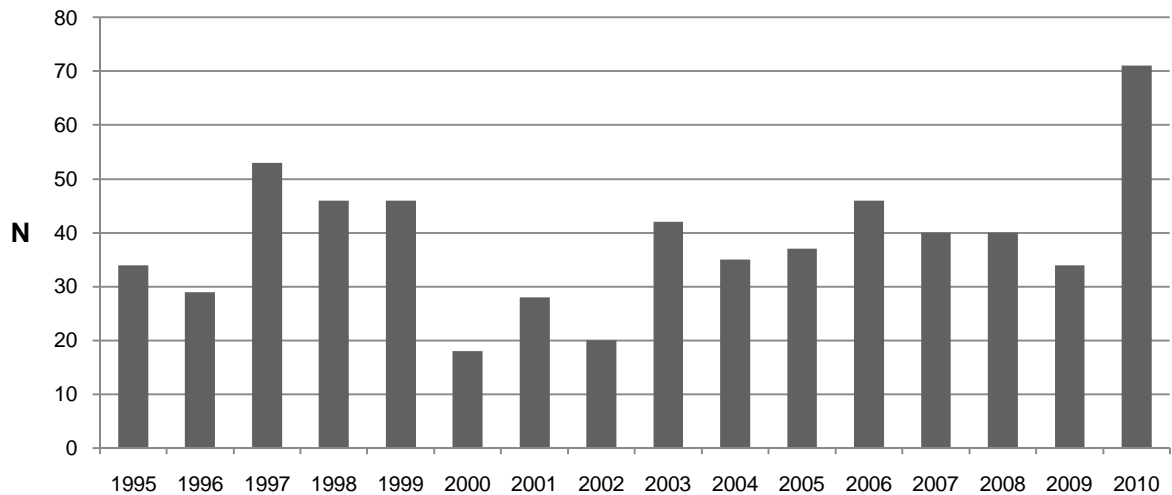
**Figure 1.** Listeriosis cases reported to the National Institute for Health and Welfare (THL) of Finland according to age groups in 1995–2010 (278).

The overall notification rate of confirmed cases of listeriosis in Europe in recent years has been 0.3 cases per 100,000 population (73) summing up to approximately 1,400 to 1,600 ascertained cases annually (67, 68, 71, 73). The highest notification rates have been observed in Denmark, Finland, and Sweden (73). In Finland, approximately 40 cases are reported annually (Figure 2) and the notification rate of confirmed cases in Finland has been 0.8 cases per 100,000 population (73). An increase in cases of listeriosis has been reported in several European countries in recent years (43, 106, 158, 311), with high numbers in 2003–2006, but a decreasing in 2007 and 2008 (71, 73).

The infectious dose of listeriosis remains unclear (200), but according to epidemiological data it is suspected to be high, as the contamination level in foods responsible for listeriosis cases are typically  $>10^4$  CFU/g (230, 294). Consuming foods that contain low levels ( $<10^2$  CFU/g) of *L. monocytogenes* is unlikely to cause clinical disease (49). The size of the infective dose is affected by the virulence of the strain, the susceptibility of the host and the food matrix, so the identification of a single value for an infective dose is unlikely (200, 294). In addition, prolonged daily doses may increase the likelihood of infection (195). Apparently, the infective dose varies markedly between an immunocompetent population and those with impaired immunity, and contamination levels as low as  $10^2$ – $10^4$  CFU/g have been associated with listeriosis in a susceptible population (200, 294). In gastroenteritis, the infectious dose is assumed to be higher than in invasive listeriosis and according to outbreak investigations, contamination level in implicated foods has typically been  $>10^5$  CFU/g (230).

The incubation time of listeriosis is 1–70 days (171). The longest incubation times (average 25 days) are seen in pregnancy-associated cases, whereas in non-pregnant patients the average incubation period has been estimated to be five days (202). In non-invasive gastroenteritis, incubation time is short being about one day (10).

Healthy people may also carry *L. monocytogenes* in their intestines and in the nasal cavity and on their hands. The prevalence of these asymptomatic carriers is usually low (1 % or less) (134, 191, 262), but in certain groups, such as food plant staff, a higher prevalence has been reported (74). People are most likely to be exposed to *L. monocytogenes* for 5 to 9 times per year, but fecal shedding is expected to be short, maximum 4 days (115).



**Figure 2.** Number of human listeriosis cases reported to National Institute for Health and Welfare (THL) of Finland in 1995–2010 (278).

### 2.3.2 Listeriosis in animals

In animals, listeriosis is mainly a disease of ruminants (139, 177). Small ruminants (sheep and goat) seem to be more susceptible to listeriosis than cattle (220). In cattle, cases are mainly sporadic, but high morbidity rates have been reported in sheep and goat flocks (305). The source of infection of animals is in most cases feed, silage being a common source in farm animals (7, 212).

In domestic animals, listeriosis manifests mainly as encephalitis, abortion or septicemia (177). *L. monocytogenes* may also cause eye infections and mastitis (81, 143, 314). Mastitis is usually subclinical, but bacterial shedding into milk is possible (143, 244). Animals commonly are asymptomatic intestinal carriers, frequently shedding the organism and maintaining its populations in the environment (220). Especially, bovine hosts may amplify ingested *L. monocytogenes* and thus serve as a critical factor to maintain a high prevalence of the pathogen on cattle farms (220). According to a surveillance report from 2008, the highest prevalences of *L. monocytogenes* in farm animals in Europe were in goats, sheep and cattle, being 3.6 % in small ruminants and 1.1 % in cattle, whereas the prevalence in poultry and pigs was <0.1 % (73).

## 2.4 *L. monocytogenes* in food chain

*L. monocytogenes* is a ubiquitous organism that is widely distributed in the environment and it has several characteristics that enable its survival in the food chain. The key reservoir for *L. monocytogenes* is soil, and it is frequently found in vegetation, forage, water, sewage and farm environments (34, 64, 89, 97, 263, 304). Domestic and wild animals often harbour *L. monocytogenes* in their intestines and the bacterium is also commonly found in food processing environments and in many types of foods.

### 2.4.1 *L. monocytogenes* as a foodborne pathogen

*L. monocytogenes* can survive and grow over a wide range of temperature, pH and water activity ( $a_w$ ) limits as well as under aerobic and anaerobic conditions. In addition, *L. monocytogenes* can form biofilms and persist in food processing facilities. These characteristics enable the pathogen to survive in food-processing environments and in foods, and make it a great concern for the food industry and a threat for public health.

*L. monocytogenes* has several survival mechanisms for adverse environmental conditions, including changes in membrane composition, changes in gene expression and induction of proteins, accumulation of compatible solutes as cryo- and osmoprotectants, proton transportation across the cell membrane, and utilization of the glutamate decarboxylate system (95). The varied stress responses of the pathogen enable it to adapt to a wide range of environmental conditions and to adapt even better when exposed again to previously experienced sub-lethal stress (65). In addition, cross-adaptation against stress conditions is commonly seen, and the exposure of the pathogen to one kind of sub-lethal stress can provide tolerance to other lethal stresses (95). Growth and survival limits are dependent on overall prevailing conditions. For example, growth limits in refrigerated temperatures depend strongly on pH (282). In addition, differences exist between strains in survival in adverse conditions and persistence in food processing facilities, e.g., strains have various abilities to attach to surfaces, resist disinfectants, and thus persist (13, 182, 224).

*L. monocytogenes* is psychrotrophic and can usually grow at temperatures from 1 to 45°C, although growth of some strains at even lower temperatures (down to -0.4°C) has been observed (148, 303). The optimum growth temperature is 30–37°C and growth rate is slowed in refrigerated temperatures (148). *L. monocytogenes* can grow from less than 100 to more than 10<sup>5</sup> cells per gram over three weeks at 10°C (210). The psychrotrophic nature of *L. monocytogenes* is one of its main traits affecting food safety, because refrigeration is widely used to ensure safety and extended shelf-life (95). Refrigerated foods provide an ideal environment for *L. monocytogenes*, because it can grow at low temperatures, whereas most competitors cannot. In addition, many RTE foods have extended shelf-lives, providing time for *L. monocytogenes* to grow to high numbers. Adequate cooking (internal temperature > 65°C) and pasteurization (71°C, 15 s) eliminates *L. monocytogenes* from foods (65).

The optimum pH range for *L. monocytogenes* is 6 to 8, but it can grow in pH range from 4.0 to 9.6 (83, 159). This pH tolerance is of special concern with regard to low-pH foods, such as fermented meat and dairy products, as well as poor quality silage. Furthermore, acidic conditions in the gastrointestinal tract and in macrophages following phagocytosis can be encountered by the pathogen and acid tolerance thus enhances the virulence (54).

*L. monocytogenes* is able to grow at an  $a_w$  level that is usually lethal to other bacteria i.e.  $a_w$  0.90 (221), and survive even lower  $a_w$  values than that (159). The lowering of  $a_w$  by adding salt to foods

is a widely used strategy for controlling food-borne pathogens, but it poorly controls the growth of *L. monocytogenes*. *L. monocytogenes* may even get an advantage on salted products, when the growth of the competing microbial population is decreased (169).

*L. monocytogenes* can grow in aerobic and anaerobic atmosphere as well as in modified atmosphere packages (MAP) (159). That extend the shelf lives of foods, so *L. monocytogenes* may multiply to high numbers during a product's shelf-life.

*L. monocytogenes* can form biofilms and persist in a food processing plant for several months or years (48, 102, 124, 185, 186, 209, 218, 223, 231). In biofilms, microorganisms are attached to a surface and enclosed in a matrix made up of polysaccharide material, thereby gaining enhanced resistance to sanitizers, disinfectants and antimicrobial agents (246). Persistent *L. monocytogenes* strains can adhere rapidly to the food contact surface and can thus attach in high numbers before sanitizing (184). Moreover, some *L. monocytogenes* strains have found to have shown relatively low sensitivity to some sanitizers used in the food industry (181, 204).

#### 2.4.2 *L. monocytogenes* in nature and in animals

*L. monocytogenes* is commonly found in soil, water and feed (Table 3), but numbers are often low (3, 22, 64, 89). *L. monocytogenes* can survive in the soil for months and even grow in favourable conditions (34, 64, 263). Soil type influences the survival of *L. monocytogenes* (64) and it is more often found in uncultivated than in cultivated fields (64, 304). Sewage is often contaminated by *L. monocytogenes*, even after treatment (3, 22, 97). The occurrence of *L. monocytogenes* in surface waters seems to be related to direct upstream land use, specifically, crop land and proximity to a dairy farms (188, 300).

Silage is the most common feed to harbor *L. monocytogenes*. Chemical quality of silage, i.e. its pH and aerobic deterioration, affects the presence of *L. monocytogenes* and the pathogen is commonly found on poor quality silage, whereas the pathogen is unable to survive when the quality of ensilaged forage is good (66, 129).

Many domestic and wild animals harbor *L. monocytogenes* in their intestines (Table 4). The prevalence of *L. monocytogenes* correlates with the feed eaten (89). The farm environment is frequently contaminated by *L. monocytogenes*, and especially ruminant farms may represent an important natural reservoir (220).

**Table 3.** Prevalence of *L. monocytogenes* in environment and feed.

Sampling site	Numner of samples	Number positive	Prevalence %	Reference
Water				
River	15	6	40	22
River	36	17	47	89
Estuary	10	0	0	22
Ground	15	1	5	292
Surface	314	32	10	188
Sewage				
Untreated	12	12	100	22
Untreated + Treated	115	69	60	191
Treated	12	10	83	22
Soil				
Cultivated field	13	1	8	64
Cultivated field	17	0	0	292
Uncultivated field	13	6	31	64
Garden	136	1	1	191
Farmyard	36	3	8	96
Farm <sup>a</sup>	504	120	24	220
Farm				
Farm environment <sup>b</sup>	66	3	5	85
Trough water	51	4	8	96
Milking equipment	38	0	0	96
Bedding	44	5	11	96
Drinking water <sup>c</sup>	508	100	20	220
Manure	10	0	0	292
Feed				
Silage	225	36	16	129
Silage	51	4	8	85
Silage	11	0	0	96
Silage	10	7	70	89
Silage	39	24	62	270
Pasture grass	68	26	38	129
Alfalfa	27	0	0	96
Concentrate	22	0	0	96
Grain	24	1	4	96
Feedstuff <sup>d</sup>	516	87	17	220

<sup>a</sup>Including grazing pastures, crop fields and farmyard

<sup>b</sup>including manure, soil, straw and swabs

<sup>c</sup>including troughs, water buckets in barn, and ponds in pasture

<sup>d</sup>Including silage, haylage, corn, mixed ration and pasture grass

**Table 4.** Prevalence of *L. monocytogenes* in faeces of wild, farmed and pet animals.

<b>Animal</b>	<b>Number of samples</b>	<b>Number positive</b>	<b>Prevalence %</b>	<b>Reference</b>
<b>Wild animals</b>				
Birds	451	35	8	87
Birds	264	25	10	242
Birds	112	37	33	35
Birds	100	0	0	47
Birds	46	8	17	304
Rat	199	13	7	133
Deer	34	2	6	187
Beaver	24	0	0	187
Elk	22	1	5	187
Muskrat	9	0	0	187
Coyote	9	0	0	187
Hare	8	0	0	187
<b>Farm animals</b>				
Cattle	9539	189	2	134
Cattle	3878	258	7	128
Cattle	323	58	18	220
Cattle	183	25	14	85
Cattle	75	39	52	270
Cattle	25	5	20	292
Cattle	15	8	53	89
Pig	5975	46	1	134
Pig	50	2	4	92
Pig	25	4	16	292
Pig	24	1	4	89
Small ruminants	205	19	9	220
Ewe	37	1	3	96
Poultry	25	0	0	187
Chicken	150	0	0	133
Chicken	11	8	72	96
Goose	18	0	0	187
<b>Pet animals</b>				
Dog	540	5	1	133
Dog	15	0	0	187
Cat	161	0	0	133
Horse	7	0	0	187

### 2.4.3 *L. monocytogenes* in food processing environments

*L. monocytogenes* is frequently found in dairy, fish and meat processing plants as well as from slaughterhouse (11, 48, 74, 116, 169, 183, 206). The prevalence of *L. monocytogenes* in food plant environmental samples is affected by sampling sites, time of processing and type of food processed (74, 116, 183). A processing plant may be free of *L. monocytogenes* at the time of monitoring (116, 149), but the prevalence of positive environmental samples at another may exceed 50 % during processing (281). Prevalence decreases after cleaning, but *L. monocytogenes* is often found even after sanitation, showing the persistence of strains and, on many occasions, the insufficiency of the cleaning (48, 74, 116, 233). Improper cleaning enhances the presence of *L. monocytogenes*, and detection of the organism is associated with organic residues (281). The prevalence of *L. monocytogenes* in foods during processing and before cooking may be 71–100 % (75, 259, 281), and this high prevalence correlates with the complexity of the processing line and machines (185, 281). *L. monocytogenes* is unlikely to be eliminated from food production and it is likely to be found in any processing facility that handles uncooked material at some point, if monitoring extensive enough (285).

*L. monocytogenes* can form biofilms and then persist in plants for several years (86, 181, 209, 231). *L. monocytogenes* has been shown to adhere to many materials commonly used in food-processing facilities such as plastic, rubber and stainless steel (184, 286, 309). These persisting strains are hard to eradicate and they may serve as a continuing source of contamination of foods (181, 184, 286, 309).

Common places to find *L. monocytogenes* in food processing environment are floors and drains (74, 116). In fact, most plants that are contaminated also harbour *L. monocytogenes* in drains, and this site could be used as an indicator of plant contamination (74). In addition, all food contact surfaces, such as conveyer belts and equipment, are prone to contamination (11, 116). Especially, complex machinery is often hard to clean and represents a particular site for persistent contamination (11).

### 2.4.4 *L. monocytogenes* in foods

*L. monocytogenes* has been found in many types of foods, but numbers are usually low and seldom above the European legal safety limit of 100 CFU/g during the shelf life of a product (Table 5). *L. monocytogenes* is frequently found on raw materials and raw products (116, 297), but these are not likely to be a direct vehicle for listeriosis, because of the usual heat treatment or other listericidal process before consumption.

Overall, the prevalence of *L. monocytogenes* is often high in products that are minimally processed or have potential of contamination after heat treatment. Other criteria for risk include support of the growth of *L. monocytogenes* in product, extended storage in chilled temperature and lack of heat treatment before consumption (135). Foods can be divided into risk categories on basis of their possibility of contamination, ability to support the growth of *L. monocytogenes* and previous association with listeriosis outbreaks (302). The majority of listeriosis cases are linked to refrigerated, RTE foods that are consumed without reheating. Generally, the increased availability and demand of RTE foods and extended shelf-lives has given *L. monocytogenes* more opportunities to prevail in foods (174).

**Table 5.** Prevalence of *L. monocytogenes* in raw and processed foods in Europe.

Product	Country	Number samples / number positive (%)	No with > 100 CFU/g <sup>a</sup> (% of total)	Reference
<b>Meat and poultry</b>				
Raw meat	Denmark	343/106 (31)	12 (4)	222
Raw meat <sup>b</sup>	Nordic countries <sup>c</sup>	80/8 (10)	ND	116
Raw pork meat	France	121/41 (34)	ND	281
Raw poultry <sup>b</sup>	Nordic countries <sup>c</sup>	30/5 (17)	ND	116
Raw poultry meat	Finland	61/38 (61)		211
Raw broiler meat	Estonia	240/169 (70)	ND	240
<b>Preserved meat products</b>				
(not heat-treated)	Denmark	328/77 (24)	2 (< 1)	222
(not heat-treated)	Denmark	357/56 <sup>c</sup> (16)	5 (1)	222
Raw cured meat products	Belgium	824/113 (14)	ND	290
Heat-treated meat products	Denmark	772/45 (6)	11 (1)	222
Heat-treated meat products	Denmark	6809/615 <sup>d</sup> (9)	24 (< 1)	222
Cooked meat products	Belgium	639/7 (1)	0(0)	289
Cooked meat products	Belgium	3405/167 (5)	ND	290
RTE meat products	Spain	501/22 (4)	0 (0)	40
RTE meat products	Austria	553/23 (4)	0 (0)	302
RTE meat products	Nordic countries <sup>c</sup>	43/1 (2)	ND	116
Cold smoked pork	Latvia and Lithuania	312/120 (38)	3 (20) <sup>e</sup>	25
Smoked meat sausage	Portugal	48/28 (58)	14 (29)	86
<b>Seafood</b>				
Raw fish	Denmark	232/33 (14)	1 (1)	222
Raw fish <sup>b</sup>	Nordic countries <sup>c</sup>	115/26 (23)	ND	116
Raw fish	Finland	257/11 (4)	ND	196
Raw rainbow trout	Finland	103/15 (15)	ND	208
Roe	Finland	147/25 (17)	ND	207
<b>Preserved fish products</b>				
(not heat-treated)	Denmark	335/35 (11)	6 (2)	222
(not heat-treated)	Denmark	282/63 <sup>d</sup> (22)	3 (1)	222
Vacuum-packaged fish products	Finland	200/24 (12)	ND	189
Smoked fish	Belgium	90/25 (28)	4 (4)	289
RTE fish products	Spain	140/9 (6)	0 (0)	40
RTE fish products	Austria	96/18 (19)	5 (5)	302
RTE fish products	Nordic countries <sup>c</sup>	63/3 (5)	ND	116

Product	Country	Number samples / number positive (%)	No with > 100 CFU/g <sup>a</sup> (% of total)	Reference
<b>Dairy</b>				
Raw farm tank milk	France	1459/25 (2)	1 (< 1) <sup>f</sup>	205
Raw farm tank milk	Sweden	294/3 (1)	0 (0)	300
Raw dairy silo milk	Sweden	295/58 (20)	ND	300
Cheese or cheese products	Denmark	73/14 (19)	0 (0)	222
RTE dairy products	Spain	462/1 (< 1)	0 (0)	40
RTE dairy products	Austria	321/12 (4)	0 (0)	302
Butter	UK	3229/13 (< 1)	0 (0)	169
<b>Fruits and vegetables</b>				
Fruits and vegetables	Norway	890/3 (< 1)	ND	144
Lettuce	UK	151/0 (0)	0 (0)	172
Lettuce	Spain	72/0 (0)	0 (0)	228
Sprouts or sliced vegetables	Denmark	350/82 <sup>d</sup> (23)	2 (1)	222
RTE fruits and vegetables	Austria	314/0 (0)	0 (0)	302
RTE salad vegetables	UK	2950/88 (3)	1 (< 1)	256
RTE organic vegetables	UK	3200/2 (< 1)	ND	255
<b>Mixed</b>				
Meat or vegetable mayonnaise	Denmark	3731/189 <sup>d</sup> (5)	17 (< 1)	222
Mayonnaise-based salads	Belgium	1187/80 (6,7)	0 (0)	289
Mayonnaise-based salads	Belgium	874/186 (21)	ND	290
Salad with meat	UK	1268/76 (6)	2 (< 1)	173
Salad with seafood	UK	1418/54 (4)	0 (0)	173
RTE meals	Denmark	3830/411 <sup>d</sup> (11)	3 (< 1)	222
RTE meals	Spain	123/11 (9)	1 (1)	40
RTE meals	Belgium	842/94 (11)	ND	290
Vacuum-packed RTE meals	UK	2981/190 (6)	27 (< 1)	254
Mixed RTE foods	Austria	109/3 (3)	0 (0)	302

<sup>a</sup> European food safety limit (79)

<sup>b</sup> Includes raw material and raw product samples

<sup>c</sup> Faroe Islands, Finland, Iceland, Norway and Sweden

<sup>d</sup> Numer of samples with *L. monocytogenes* > 10 CFU/g

<sup>e</sup> Enumeration of *L. monocytogenes* was done on 15 samples from two producers

<sup>f</sup> Enumeration was done on positive samples detected from 168 bulk tank samples.

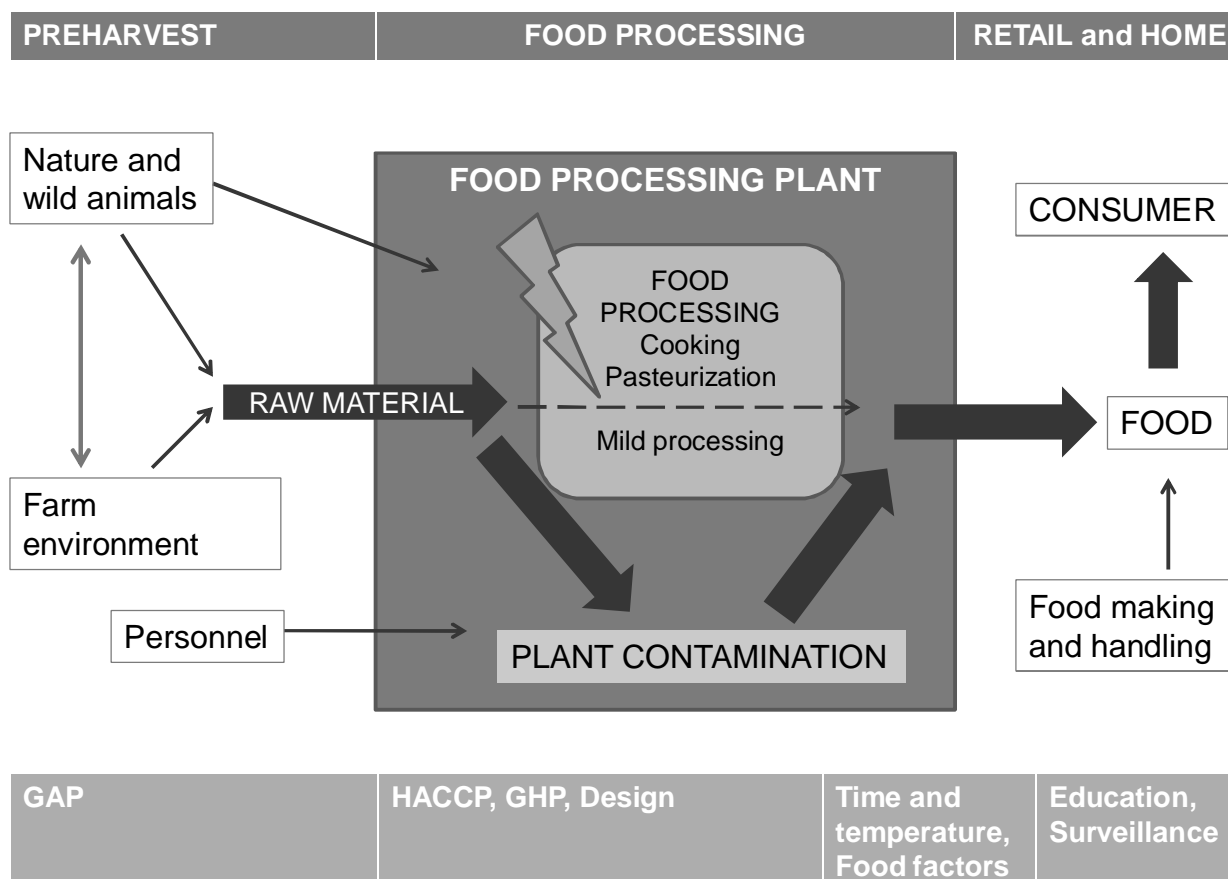
Certain food items are often linked to high prevalence and high numbers of *L. monocytogenes* leading to a risk of listeriosis, such as soft cheese, smoked fish, paté, non re-heated frankfurters, deli meats, and unpasteurized milk (5, 142, 275, 289, 302). The highest proportion of RTE foods that do not meet the microbiological criteria in Europe includes fishery products, particularly smoked fish,

cheeses, RTE meat products, and other RTE products such as sandwiches and RTE salads, although the proportion of samples found to be non-compliant is very low (73). In Finland, high proportions of *L. monocytogenes*-positive fishery products have been reported in packaged gravad and cold-smoked fish (34 %) (73).

Some special local foods may have very high prevalence of *L. monocytogenes*, although they are often not shown in national or transnational surveillances because of the local nature of their consumption (25, 86). Risk assessments may underestimate the public health hazard associated with these kinds of foods (86).

## 2.5 Contamination of foods by *L. monocytogenes*

Contamination by *L. monocytogenes* can occur at all the steps of the food chain from farm to table (Figure 3). The impact of contamination on public health differs at different steps and depends on the type of food. For instance, raw materials can be expected to have small numbers of *L. monocytogenes* and they should be treated accordingly, whereas RTE foods that support the growth of *L. monocytogenes* should be free of the pathogen at the time of production. Because of the ubiquitous nature of *L. monocytogenes*, it is unrealistic to eliminate the organism from the food chain and possible contamination sites will exist throughout the chain.



**Figure 3.** Contamination routes of *L. monocytogenes* from farm to table. Top bar indicates the processing step and bottom bar the control strategies. Thick lines indicate the most important contamination routes.

### 2.5.1 Contamination at preharvest level

The farm environment, especially where there is poor quality silage and animals, often harbours *L. monocytogenes* and the farm environment may be a source of contamination further along the food chain (7, 96, 220, 229, 300). Animals most likely get the pathogen from feed and then may further shed the organism into the natural, and farm environments (89, 220). Wild animals, like birds and rats, may disseminate organism over the natural environment and into forage and the farm environment (87, 133).

The source of *L. monocytogenes* in bulk milk is often environmental, and contamination from within the udder is likely to be rare (85, 205). Fecal carriage can be a source of raw meat contamination into the slaughterhouse (292). Crops in fields can be contaminated by irrigation water or organic fertilizers such as manure and sewage sludge (3). The soil is also a reservoir for *L. monocytogenes* and may be a source of contamination into fruits, vegetables and animal feed (228).

Spread of *L. monocytogenes* from the farm environment into food processing has been recognized. Similar *L. monocytogenes* genotypes have been found on farms and in dairy processing facilities (7) and *L. monocytogenes* populations found on farms overlap with those responsible for human cases (220). One farm bulk tank may contaminate the corresponding tanker truck. Thus, a few cells on the farm may contaminate a large amount of milk, although at a very low level (85, 205, 300).

### 2.5.2 Contamination during processing

Contamination during food processing in a plant can be considered as the major source of contamination into foods (11, 163, 268). All food contact surfaces can be a source of contamination, but concern especially rests on complex machinery that is hard to clean and equipment that has contact with large production lots (11, 181). Contamination in processing plants may originate from persistent strains in the factory, or can be sporadic (25, 181). Persistent strains are of major concern, because of the continuous source of contamination and the possibility to contaminate large amounts of products over long periods of time (209, 231).

Raw materials are frequently contaminated by *L. monocytogenes* and may serve as a source of contamination into the processing plant (116, 196, 229, 233, 280). Other possible sources of contamination into processing facilities are materials or equipment, workers and soil (74, 116, 185). Contamination of processing equipment may also originate from inside the food plant, i.e., persistent contamination sites in factory or personnel by poor hygiene, or movement from raw material sites into clean areas (281).

Cooking and pasteurization are effective processes to eliminate *L. monocytogenes*. Recontamination after heat-treatment, especially when foods are handled (163), is one of the most important sites of contamination concerning public health (285). Sources of contamination may be food contact surfaces, processing machinery and workers (11, 163). In principle, increased handling correlates with increased prevalence of *L. monocytogenes* in foods (163, 169). Some processes may be especially prone to contaminate foods, including brining, dicing and slicing (12, 25, 185). In these processes, materials are in contact with food, touch the whole production lot, and equipment is often complex. Recirculated brine may be contaminated, since food materials may contaminate the solution and *L. monocytogenes* can tolerate the high salt content (12).

Contamination of cooked foods may also occur from ingredients added after heat treatment, such as spices (86). In addition, packaging technique may affect contamination, for example butter

has been found to be more frequently contaminated when packed in plastic tubs (169). Moreover, raw material may be the initial source into foods when no listericidal step is included in the processing, for example RTE packed salads and cold-smoked RTE products (25, 173).

### 2.5.3 Contamination at the retailer and in the home

Contamination of foods can happen at retail level. As in processing, the source of contamination can be surfaces, equipment and workers, and persistence of strains has been detected at retail level (261). In homes, contamination may be common, but because of the limited number of people consuming food and the relatively short storage times of home-made foods, it is not a major public health concern. Nevertheless, for an individual, contamination at home may be a serious health risk (147). Knowledge about contamination levels at home is limited, but some studies report that contamination at household level exists and individual eating habits might affect prevalence (302).

## 2.6 Control of *L. monocytogenes* in the food chain

Contamination of foods by *L. monocytogenes* can occur in all the steps from farm to table, which brings about the need to put forward control measures at every step (Figure 3). While *L. monocytogenes* is unlikely to be eliminated from the food chain, the objective is to decrease the risk of listeriosis infections. Since low numbers of *L. monocytogenes* are unlikely to cause infection, the growth of the pathogen to high numbers in foods has a great impact on food safety. Furthermore, the susceptibility of individuals and public health goals have to be taken into consideration when deciding on the control actions.

### 2.6.1 Preventing contamination of foods by *L. monocytogenes*

Since the farm environment may be a source of contamination, it is important that control of *L. monocytogenes* starts at farm level (7, 96). Following Good agricultural practice (GAP) can minimize the contamination load on farm. Production of high-quality silage with appropriate silage-making technique and crop composition is important, since poor quality silage is often contaminated with *L. monocytogenes* (66). Preventing contamination of raw milk can be achieved with good sanitation and milking practices (85, 122). Vegetables and forage can be contaminated in fields, so manure and sewage used as fertilizer has to be properly composted and irrigating water has to be pathogen free. Intact vegetables usually do not support the growth of *L. monocytogenes* and the produce has to be handled with care to minimize physical damage. Injured surfaces of vegetables may also be resistant to sanitizers (121).

In food processing, a hazard analysis and critical control points (HACCP) program and good hygiene practices (GHP) have to be followed and closely monitored. An effective corrective action plan is needed to reduce the likelihood of contamination of foods. The usage of HACCP in food premises can markedly contribute to the microbiological safety of foods and to less frequent findings of *L. monocytogenes* in food products (135, 169).

Food processing facilities have to be designed to prevent cross contamination. Since raw materials are a potential source of contamination into facilities, proper source and storage of raw materials is essential. Raw product areas have to be segregated from cooked product areas. Segregation includes material flows, employees, tools, and air. Cooking and pasteurization kill *L.*

*monocytogenes* and the protection of the cooked products from recontamination is one of the key elements in controlling this pathogen (163). The most compartmentalized processing lines have been found to be least contaminated by *L. monocytogenes*, and the separation of the raw material area and the post heat-treatment area particularly affects the contamination status of the processing lines (154, 186). Avoiding post-process contamination can also be achieved by post-packaging heat treatment (24).

Regular and sufficient cleaning is important to decrease contamination of equipment and environment in the food processing facility and to prevent the formation of biofilms and persistence (11, 74, 281). Eradication of persistent contamination from processing lines and equipment is difficult (11), and in poorly designed plants recontamination is likely (181). Moreover, cleaning operations have to be monitored in order that any insufficiencies are quickly recognized. Equipment and the processing line should be designed to be easily cleaned, including the appropriate choice of materials. Condition of materials has to be monitored, since *L. monocytogenes* can easily persist in cracks. Co-operation between the suppliers of equipment and cleaning agents and food business operators (FBOs) is needed to solve problems regarding sanitation and design of food processing equipment (116).

Non-food contact surfaces, especially floors and drains, can be a reservoir of *L. monocytogenes* in the factory. Care has to be taken to clean and sanitize these sites, because they may contaminate other sites in the food processing facility. A moist environment favors the survival of *L. monocytogenes* in food processing facilities and therefore keeping the factory as dry as possible is important (116). Raw material sites have to be kept as free from *L. monocytogenes* as possible, to avoid the spread of contamination further into plant (116, 181).

Cross contamination needs to be minimized at retail level and at home. Enabling cross-contamination by leaving food packages open and sharing tools with several food items increases the risk of finding *L. monocytogenes* in food (169).

## 2.6.2 Prevention of the growth and inactivation of *L. monocytogenes* in foods

Cooking and pasteurization are effective methods to eliminate *L. monocytogenes* from foods. Nevertheless, not all foods are suitable for a listericidal thermal process, because of the effect on sensory or nutritional quality, and because consumers increasingly seek fresh-looking and nutritionally healthy foods. Several interventions have been developed and studied to eliminate or reduce the number of *L. monocytogenes* in foods (Table 6). The ideal processing method would improve the shelf life and safety of the product, not compromise organoleptic or nutritional value or leave residues, be convenient and economical to apply, and not cause objections by consumers or legislation (243). Hardly any single treatment can meet all these demands. Most non-thermal treatments reduce the number of *L. monocytogenes* or control the growth, but do not eliminate the organism. Ionizing irradiation is an effective treatment to eliminate *L. monocytogenes* (19, 84, 241), but the use of the method is restricted by legislation as well as lack of consumer acceptance (78).

Probably the greatest impact on preventing listeriosis is to prevent the growth of the pathogen to high numbers in foods (135). Data on the prevalence of *L. monocytogenes* in foods and consumption of foods reveals that people are frequently exposed to small numbers of *L. monocytogenes*, and yet the number of cases is relatively low (135). Growth can be controlled by strict temperature and time limits, modification of foods to prevent the growth, and packaging techniques.

Strict temperature control needs to be established at all points of the food processing chain (163). Although *L. monocytogenes* is able to grow at refrigerator, the growth is slowed, and refrigerators

should be kept at 4°C or less (148, 169). The chill-chain in processing and distribution is in most cases satisfactory, but temperatures in home refrigerators are often 5–7°C (69). In addition, acceptable storage times have to be established for foods that support growth of *L. monocytogenes* to high numbers. For example, butter has a long shelf life that may enable the bacteria to grow to high numbers, although initial contamination is usually low (169). For products that support the growth of *L. monocytogenes* and receive minimal processing, such as mixed salads, temperature is a principal controlling factor (173).

Foods can be reformulated to prevent or retard the growth of *L. monocytogenes*. Traditional preservation methods are fermentation and adding salt, but salt alone is not adequate to control the growth of *L. monocytogenes* (159). Fermentation lowers the product pH, but lowered pH is not sufficient to prevent the survival of *L. monocytogenes* (288). Several chemical agents can be used as food additives to control the growth of the pathogen (Table 6). Listericidal and listeristatic agents can also be added into packaging materials and coating films (41, 119).

Reducing the level of *L. monocytogenes* in the final product and preventing its growth can be achieved in different ways, but generally no single method is effective, so combined preservative factors, i.e. hurdles, have to be used. This is known as multiple hurdle technology (167). For example, refrigeration is a widely used hurdle, but if chilling is insufficient, or in the case of *L. monocytogenes*, when the pathogen is able to grow in chilled temperatures, additional hurdles should be used (167). Although a single method may not be adequate for *L. monocytogenes* control, combining interventions can be listericidal or listeristatic. In dry sausages, the combination of low pH and  $a_w$  is generally listeristatic, but bacteriocin-producing starter cultures can be used as an additional hurdle to control the pathogen (287, 288). Novel nonthermal technologies such as high-pressure processing, pulsed electric fields and ultrasound can be combined with conventional preservation methods, or several non-thermal processes can be combined, to achieve optimal microbiological control (252). Different hurdles in food processing might not have only a combined effect, but they can act synergistically, and thus using multiple hurdles is often worthwhile (167, 252). In addition to food safety, hurdle technology adds to food quality, when multiple gentle preservation techniques are used and none is detrimental to sensory quality (167).

Growth and survival of *L. monocytogenes* in foods are affected by multiple environmental- and food-related factors. Thus, methodology for rapidly evaluating the growth of *L. monocytogenes* in particular products is needed. Predictive modeling can be used to combine mathematical modelling with experimental data on combinations of factors that influence the growth of the pathogen to predict the fate of *L. monocytogenes* in foods (282). Finally, the effects of control interventions on the sensory quality of foods, along with consumer acceptance, have to be taken into consideration.

**Table 6.** Agents and technologies used in food processing to inactivate, inhibit and control the growth of *L. monocytogenes* or prevent contamination.

Agent or technology	Use	Effect on <i>L. monocytogenes</i>	Reference
<b>Physical technologies</b>			
Temperature			
Heating > 70°C	Cooking and pasteurization, cleaning of equipment and food processing environment (steam, air, hot water)	Elimination	12, 99, 190
Heat treatment < 70°C	Mild heat treatment	Elimination, decreasing number, reducing viability	131, 243
Cooling	Refrigeration	Reducing growth rate	130, 148, 169
Freezing	Frozen foods	Preventing growth, decreasing number	159
Dehydration	Dry foods	Preventing growth	159
Smoking	Preserve foods and enhance sensory quality	Decreasing number	131, 159
<b>Novel nonthermal technologies</b>			
Ionizing irradiation <sup>c</sup>	Fresh produce and endproduct decontamination	Elimination	19, 84, 241, 243
Ultraviolet (UV) and intense light pulses	Surface decontamination of foods, post-process decontamination after heat treatment	Decreasing number	243
High-pressure processing (HPP)	Post-process treatment with or without packaging	Elimination, decreasing number	238, 243
Pulsed electric field (PEF)	Substitute for thermal pasteurization of liquid products	Decreasing number	243
Ultrasound	Cleaning of food processing environment (conveyor belt), sanitizing of fresh produce	Decreasing number	253, 283, 284
<b>Chemical agents</b>			
Salt (NaCl)	Food additive for preservation and enhancing sensory quality	Reducing growth rate	131, 159
Organic acids and their salts <sup>a</sup>	Aqueous wash of produce, Surface decontamination by post-process dip or spray, carcass decontamination rinse, food additive	Decreasing number, preventing growth	100, 138, 159, 243, 313
Nitrite	Food additive	Preventing growth	159, 234

Agent or technology	Use	Effect on <i>L. monocytogenes</i>	Reference
Phosphate	Dip	Decreasing number	45
Chlorine and chlorine dioxide	Aqueous wash and spray and gaseous treatment of foods, additive in chiller water to prevent cross-contamination, Sanitizing and cleaning equipment and food processing environments	Decreasing number	121, 157, 160, 166, 182, 243, 246, 313
Quaternary ammonium compounds	Sanitizing and cleaning equipment and food processing environments	Decreasing number	182, 310
Ozone	Aqueous wash of foods, gaseous preservation, sanitizing and cleaning equipment and food processing environments	Decreasing number, reducing viability	225, 246, 301
Hydrogen peroxide	Sanitizing and cleaning equipment and food processing environments, Food additive	Decreasing number	159, 246
<b>Spices, herbs and plant extracts</b>	Food additives	Decreasing number, Preventing growth	58, 94, 159
<b>Biocontrol</b>			
Starter cultures	Fermented foods: preservation and enhancing sensory quality	Reducing growth rate, preventing growth	288
Bacteriocins <sup>b</sup>	Bacteriocin-producing starter cultures, food additives, post-process dip	Decreasing number, preventing growth	17, 23, 37, 39, 52, 76, 91, 100, 140, 162, 232
Bacteriophage	Food additive	Decreasing number	117, 274

<sup>a</sup>For example: Acetic acid, diacetate, benzoate, lactic acid and lactate

<sup>b</sup>For example: nisin, pediocin and enterocin

### 2.6.3 Surveillance, knowledge, education, regulatory framework

Proper control of *L. monocytogenes* requires appropriate monitoring and surveillance, at the food plant as well as at governmental level. Consumer knowledge is of utmost importance in reducing the number of listeriosis cases. In addition, society has to state food safety objectives, which can be followed by FBOs to achieve the appropriate level of control in cost-beneficial ways.

#### Monitoring and surveillance

An intensive environmental sampling program has to be implemented in plants, especially those processing high-risk foods (285). End-product testing has limited value, because a problem might exist long before contamination is detected (285). When sanitation and hygiene procedures are not

effective to eliminate persistent contamination or foods are regularly contaminated, more intensive study of the contamination of food plant may be needed to detect the responsible site or failures in processing.

Better understanding of the transmission of listeriosis, as well as linking food-borne outbreaks and sporadic cases to a source, is critically important for better control of the disease (307). Surveillance networks and databases are needed to gather the information for fast and accurate recognition of outbreaks and to respond to trends in the incidence of the disease (60). Since distribution of food is international, these databases should also be transnational and even worldwide (197). These databases would strongly reinforce the ability to detect international outbreaks (197) and rapid detection of listeriosis outbreaks and their sources, and could be used to limit the number of cases (307). Monitoring and reporting of foodborne diseases by government agencies is needed as well as routine food sampling and testing. Furthermore, harmonizing the reporting of human cases, and combining the information about the clinical cases with microbiological data, is important (60).

The significance of the detection of low concentrations of *L. monocytogenes* in different food categories has to be judged. Foods need to be clearly differentiated into those that can, and those that cannot, support growth of *L. monocytogenes*. Some foods that occasionally harbour *L. monocytogenes*, such as mayonnaise-based salads may not necessarily support the growth of the pathogen (289). On the other hand, foods that have been considered low risk for listeriosis, such as butter, can support the growth of *L. monocytogenes* to high numbers, because of the extended storage time (169, 190). The manner and frequency of consumption of certain foods affects the epidemiological association of that food category with listeriosis (135). Some foods that frequently harbour *L. monocytogenes* may still be consumed rarely or in small portions.

## **Education and consumer knowledge**

Management training in food hygiene contributes to microbiological quality of foods and reduces the prevalence of *L. monocytogenes* (169). Knowledge about hygienic practices has to be taught to all food workers.

Public awareness and knowledge is an important means of controlling listeriosis in particular. General recommendations to avoid listeriosis need to be taught to all people, i.e., preventing cross-contamination in the household kitchen, correct storage times, and knowledge about risk foods (32). Those that belong to risk groups (pregnant, persons with underlying disease, elderly) have to take more severe actions and avoid certain risk foods, including soft cheeses, cold smoked and gravad fish, unpasteurized milk and milk products and pâté. The most susceptible individuals (cancer and organ-transplant patients) should even more carefully avoid all kinds of risk foods, that is, all uncooked products and foods that have been handled after heat-treatment (135). Education messages need to be targeted to susceptible populations as well as their caregivers, and to food-processing personnel (135).

## **Regulatory framework**

The general approach in legislation in Europe is that production of safe foods using HACCP-based approach is the responsibility of FBOs. The EU legislation (79) lays down food safety criteria for *L. monocytogenes* in RTE foods, according to which, *L. monocytogenes* must not be present at levels above 100 CFU/g during the shelf life of a product. In addition, products that support the growth of the pathogen, must not contain *L. monocytogenes* in 25 g when they leave the production plant, unless the producer can demonstrate that the product will not exceed the 100 CFU/g limit

throughout its shelf life.

The acceptable level of risk is ultimately decided by society, but it should be decided on the basis of risk-based measures (49). Some countries, like the USA, practice a zero-tolerance policy on *L. monocytogenes*, but the incidence of listeriosis cases is not generally lower than in countries that allow minimal numbers of *L. monocytogenes* in foods that do not support the growth of the pathogen (61). It is evident that regulatory policies are wise to aim at minimizing the risk of listeriosis and not to eliminate *L. monocytogenes*, which is most probably an impossible goal (285). Furthermore, contamination in food samples does not always correlate with the potential risk to public health: low levels of *L. monocytogenes* in sampled foods were nevertheless associated with a deadly nationwide outbreak in the USA (202). Latest Codex criteria suggest zero tolerance throughout the shelf life for RTE products that can support the growth of *L. monocytogenes* (53). An additional option is to tolerate 100 CFU/g throughout the shelf life, provided that the manufacturer is able to demonstrate that the limit is not exceeded (79).

## Research

Advances in methodology, especially in molecular genetics, have been tremendous and genetic data is relatively easily available (192, 307). At the same time, other demands in research are increasing. New methodology and shared databases are about to recognize more outbreaks than ever, but time-consuming epidemiological follow-up is still needed to identify food vehicles (307). In addition, enormous datasets need analyzing and storing, and microbiology laboratories will be reliant on bioinformatics and information technology (192).

*L. monocytogenes* has been of major concern in food processing for many years, and multiple control measures in preventing contamination and control have been studied and applied. *L. monocytogenes* is regulated by legislation as well as by the food control plans of individual FBOs, but the incidence of listeriosis has not declined remarkably. Some reasons for this are most probably the increasing number of susceptible individuals, changes in food consumption habits and food processing, and better recognition of outbreaks. Apart from this, impact of interventions used and specifically cost-benefit measures need to be considered continuously. The basis for such research is appropriate base-line studies to accurately recognize changes in numbers of food-borne listeriosis.

Food processing is evolving and food consumption habits are changing, creating a constant need to stay in line with the adaptation of the pathogen into new niches (219). In addition to traditional food hygiene and microbiology, food-related issues such as food security, climate change, competition of resources, obesity, and socio-economic inequalities increasingly affect the food-safety issues (219, 267). Control of listeriosis has to be done in this framework and this means that multidisciplinary research needs to involve sciences beyond those in the food safety field (219).

### 3 AIMS OF THE STUDY

*L. monocytogenes* contamination routes and control in food production were investigated. Specific aims of this thesis were as follows:

1. to study the prevalence and the diversity of *L. monocytogenes* in wild birds and throughout the pork production chain (I, II, III).
2. to evaluate the contamination routes of *L. monocytogenes* in foods and in the pork production chain (I, II, III)
3. to determine specific procedures in farm management and in food processing to control *L. monocytogenes* (II, III, IV, V)
4. to observe strain-specific characteristics of *L. monocytogenes* in food processing (IV, V)

## 4 MATERIALS AND METHODS

### 4.1 *L. monocytogenes* strains (I, III, IV, V)

Altogether, 1174 *L. monocytogenes* isolates from animals, foods and food processing environments representing 310 *AscI* genotypes from the collection of the Department of Food Hygiene and Environmental Health (DFEH) were compared with the strains isolated in study I. In study III, previously collected RTE cold-smoked pork samples from the same processing plant were analyzed.

In study IV, the inoculum consisted of a mixture of five *L. monocytogenes* strains: NCTC7973, NCTC5214, ATCC19116, LM206, and LM168. Strains LM206 and LM168 were originally isolated from mixed salads. Inoculated *L. monocytogenes* strains in study V: DCS 31, DCS 184, (Danisco, Niebüll, Germany), AT3E, HT4E, and HR5E (DFEH) were originally isolated from meat and were determined to be sensitive to pediocin by the well diffusion test (277).

### 4.2 Collection and preparation of samples (I–V)

#### 4.2.1 Collection of samples (I, II, III)

Altogether 212 samples of bird faeces were collected, 84 from urban areas of Helsinki, Finland, and 128 in the municipal landfill site of the Helsinki region (I). A total of 1962 samples was collected from the pig production chain originating from 15 farms, 5 organic and 10 conventional. Samples were collected from farms, slaughterhouse, and meat cutting plants (II). From each farm, 21–26 pigs were selected for sampling, and samples from slaughterhouses and meat cutting plants were logistically connected to the corresponding pig or farm. A total of 183 samples was collected for contamination analyses, covering different stages in processing and RTE cold-smoked pork products (III).

The samples of bird droppings (I) and pigs at farms (II) were collected with sterile cotton wool sticks into tubes containing half-Fraser broth (Oxoid, Hampshire, England) or into transport medium. At the slaughterhouse, tonsils were cut for samples, intestinal content was collected, and the pluck sets and carcasses were sampled by surface swabbing (II). Feed and litter were collected at farms and meat samples were delivered for investigations in cold storage directly from the cutting facilities (II). At the processing plant, pork samples and brining solution were collected aseptically, environmental samples were taken by surface swabbing, and plastic boot-covers were collected after use (III).

#### 4.2.2 Preparation of *L. monocytogenes* inoculants (IV, V)

In study IV, *L. monocytogenes* strains were incubated in brain heart infusion (BHI) broth (Difco, Sparks, MD, USA) for 18 h at 37°C. Equal amounts of each culture were mixed in a BHI broth followed by dilution with sterile peptone water (0.1 % w/v) and inoculated in a volume of 0.5 ml / 100 g of lettuce, giving the final number of inoculated bacteria approximately 4.7 log CFU/g. In study V, concentration levels were measured by optical densities, and equal amounts of each strain were inoculated in a total amount of 10 ml peptone (0.1 %) saline (0.85 %) to yield a final concentration of 10<sup>3</sup> CFU/g of *L. monocytogenes* in sausage mass.

#### 4.2.3 Preparation and sampling of lettuce (IV)

Iceberg lettuce was shredded and then washed with potable water (control), chlorinated water (100 ppm), 0.05 % peracetic acid solution (Fluka, Steinheim, Germany), or a 0.25 % solution of commercial citric acid-based produce wash (Fresh Produce Wash, Vegetec International Ltd., Suffolk, UK). After washes, the lettuce was packaged in 100-g lots in plastic bags made of Cryovac (Sealed Air Corporation, Saddle Brook, NJ, USA) packaging film (O<sub>2</sub> permeability 5300 cm<sup>3</sup>/m<sup>2</sup>·d 101.3 kPa, 23°C) and sealed using a heat sealer. Packages were stored at 6°C and sampled on packing day and after 3 and 6 days of storage.

#### 4.2.4 Preparation and sampling of dry sausage (V)

Two different starter cultures and the protective strain *L. plantarum* DDEN 2205 [designated earlier as WHE 92 (76)] were used. The concentration of starter cultures was 10<sup>6</sup> CFU/g and protective strain was used at two different concentrations. Ingredients in the 15 kg batch of dry sausage comprised 5 kg of frozen lean pork, 5 kg of frozen pork back fat, 5 kg of lean pork shoulder without rind (not frozen), 2.8 % salt, 170 ppm sodium nitrite, 1.1 % milk protein, 0.7 % dextrose, 0.35 % spice mix, and 0.04 % sodium ascorbate. All ingredients were mixed in a bowl cutter, vacuum packed, and stuffed into fibrous collagen casings (Naturin, Weinheim, Germany). Sausages were fermented and ripened according to the following processing parameters: the sausages were stored at 24°C and 90 % relative humidity (RH) for 72 h. On the first two days (at 24 h and 48 h), the sausages were smoked for 1 h. After 72 h, the sausages were dried at 14°C and 75 % RH for 25 days to obtain the final product on day 28. Sausages were weighed after stuffing (day 0) and on days 3, 7, 10, 14, 17, and 21, and pH was measured at 0, 24, 48, and 63 or 70 h after stuffing. For microbiological analyses, samples were taken after 4 h and on days 2, 7, 17, and 28 of ripening. In addition, samples of control sausages without *L. plantarum* DDEN 2205 were taken immediately after stuffing. Samples for enrichment of *L. monocytogenes* were also taken from raw materials.

#### 4.3 **Detection, enumeration and identification of *L. monocytogenes* (I–V)**

Detection of *L. monocytogenes* was performed according to ISO method (136), with some modifications. Samples were initially enriched in half-Fraser broth (Oxoid) for 24 h at 30°C and then in Fraser broth (Oxoid) for 48 h at 37°C. In addition, cold enrichment was performed in study I. After each enrichment, samples were streaked onto two (II–V) or three (I) selective agar plates: Palcam (Oxoid) (I–V), Oxford (Oxoid) (I), *Listeria monocytogenes* blood agar (LMBA) plates (LAB M, Lancashire, UK) (I, II, IV, V), and Agar *Listeria* according to Ottaviani and Agosti (ALOA, AES Chemunex, Rennes, France) (III). Five typical colonies from each selective agar plate were cultured on blood agar, and *L. monocytogenes* was identified by a catalase test (I, II, III, V), Gram-staining (I, II, III, V), an API Listeria kit (bioMérieux, Marcy-l’Etoile, France) (I, II, III) and multiplex PCR method (I) with specific primers for the 16S rRNA sequence of the genus (31) and for the virulence gene listeriolysin O sequence of the species (18). One confirmed *L. monocytogenes* isolate was collected from each selective plate, i.e., 1–7 isolates were collected from each positive sample.

Quantitative *L. monocytogenes* analysis was performed according to the ISO method (137), with minor modifications (IV) or by the 9-tube Most Probable Number (MPN) method (V). In the ISO method, the initial suspension was prepared in Fraser broth base with decimal dilutions in buffered peptone water, and samples were plated onto LMBA agar instead of PALCAM agar. For MPN analyses, 10 g of dry sausage, taken from the middle of the sausage, was mixed with 90 ml of peptone saline and blended with a laboratory blender (Stomacher 400, Seward Medical, London, England) for 1 min. Further decimal dilutions were made to obtain sampling sizes of 1, 0.1, 0.01, 0.001, and 0.0001 g. Enrichment, plating and identification were done as in *L. monocytogenes* detection.

#### 4.4 **Serotyping (I–III)**

One to four representative isolates of each genotype were serotyped with commercial antisera (Denka Seiken, Tokyo, Japan) according to the manufacturer’s instructions (I, III). In study II one isolate of each genotype was subjected to multiplex PCR (63), to separate four serovar groups (1/2a [including 1/2a and 3a], 1/2b [1/2b, 3b, and 7], 1/2c [1/2c and 3c], and 4b [4b, 4d and 4e]) and then commercial O-antisera (Denka Seiken) were used to identify each serotype in the serovar group obtained by PCR.

## 4.5 Pulsed-field gel electrophoresis (PFGE) (I–V)

### 4.5.1 Isolation of DNA and PFGE

DNA isolation and PFGE were performed (12, 14) with use of Pronase (Roche Diagnostics GmbH, Mannheim, Germany) instead of proteinase K. A single colony from blood agar was inoculated into Brain heart infusion (Difco) broth and cells were harvested from 2 ml of the broth after overnight incubation. Cells were embedded into 2 % low melting point agarose (InCert agarose; FMC Bioproducts, Rockland, ME, USA), lysed for 3 h at 37°C and washed with for 1 h at 50°C. Digestion of agar-embedded DNA was performed by restriction endonuclease (New England Biolabs, Beverly, Mass., USA) *ApaI* (II) and *AscI*(I–V) as described by the manufacturer. Samples were electrophoresed (14) in a Gene Navigator system with a hexagonal electrode (Pharmacia, Uppsala, Sweden). The pulse time ramped from 1 s to 30 s or 1 s to 35 s for *ApaI* and *AscI*, respectively, for 18 h. Low-Range PFG marker (New England Biolabs) was used for fragment size determination. The gels were stained with ethidium bromide and digitally photographed with an Alpha Imager 2000 documentation system (Alpha Innotech, San Leandro, Calif., USA).

### 4.5.2 PFGE pattern analysis

PFGE patterns were analysed by BioNumerics software (Applied Maths, Sint-Martens-Platen, Kortrijk, Belgium). The similarities among restriction patterns on the basis of band position were expressed as Dice coefficient correlations. Clustering and construction of the dendrogram were performed by using an unweighted pair group method with arithmetic averages (UPGMA). A composite data set was created by averaging the results obtained by both enzymes in study II.

Identification of inoculated *L. monocytogenes* strains with PFGE was done in studies IV and V. Altogether 160 *L. monocytogenes* isolates from the inoculated lettuce samples were collected after 6 days of storage (IV). A total of 107 *L. monocytogenes* isolates were collected during or after ripening of dry sausages (V).

## 4.6 Statistical analyses (II, III, IV)

To assess different features associated with the presence of *L. monocytogenes* in pigs, correlations between farm factors (269) and the prevalence of *L. monocytogenes* were calculated (SPSS 12.0.1, SPSS Inc., Chicago, IL, USA) and a two-level multivariate logistic regression model was constructed (MLwiN 2.02, Centre for Multilevel Modelling, University of Bristol, UK) (II).

The  $\chi^2$  test or the Fisher's exact test was used to compare the differences of *L. monocytogenes*-positive meat or environmental samples at the different stages of the production chain (III).

Data from the inoculation tests of lettuce were subjected to SPSS for Windows for analysis of variance and to Student's *t*-test (IV).

## 5 RESULTS

### 5.1 Prevalence of *L. monocytogenes* (I–III)

Altogether 2358 samples from wild birds, pig production chain and pork processing plant were analysed and the prevalence of *L. monocytogenes* varied from 0 % to 54 % (Table 7). The overall prevalence of *L. monocytogenes* in bird faeces was 36 %, but this figure was significantly higher in birds from the municipal landfill site than from urban areas ( $\chi^2$  test,  $p < 0.001$ ). In the pig production chain, 119 of 1962 samples investigated (6 %) were *L. monocytogenes*-positive. The overall prevalence of *L. monocytogenes* was significantly higher on organic farms than conventional farms both when samples were assumed to be random ( $\chi^2$  test,  $p < 0.001$ ) and when samples were clustered according to farms (Mann-Whitney U-test,  $p < 0.01$ ). In addition, prevalence was significantly higher in tonsil samples than in other samples from pigs ( $\chi^2$  test,  $p < 0.001$ ). In the pork processing plant, 38 (21 %) *L. monocytogenes* positive samples were found from 183 samples collected. In the brining area, 10 samples (55 %) were positive, whereas none of the samples were positive for *L. monocytogenes* in the dry salting area and most of the contaminated sites were associated with brining machine. The number of the *L. monocytogenes*-positive pork samples clearly increased after brining injections and the prevalence of *L. monocytogenes* was significantly higher ( $p < 0.0001$ ) in the finished RTE cold-smoked pork products where brining injections were applied than in RTE cold-smoked pork products where dry salting and ripening alone were used.

**Table 7.** Prevalence of *L. monocytogenes* in wild birds, pig production and a pork processing plant.

Sampling site	No samples	No positive	Prevalence %
Wild birds			
Urban areas	84	8	10
Municipal landfill site	128	69	54
Pig production chain			
Farm			
Pigs at farm	364	4	1
Feed and litter	38	4	11
Pigs at slaughter			
Intestinal content	358	5	1
Tonsil	350	83	24
Pluck set	354	17	5
Carcass	359	2	1
Meat	140	5	4
Pork processing plant			
Raw pork	67	12	18
Cutting area	6	0	0
Salting and brining area	38	16	42
Smoking area	6	0	0
Slicing and packing area	17	1	1
RTE cold-smoked pork	49	9	18

## 5.2 Diversity of *L. monocytogenes* (I–III)

### 5.2.1 PFGE typing

PFGE typing revealed altogether 48, 36 and 17 *L. monocytogenes* genotypes from wild birds (212 isolates), the pig production chain (424) and the pork processing plant (66), respectively. Simpsons index of diversity (127) was for the bird 0.9701, pig production 0.9307, and pork plant 0.9026.

Comparison of *L. monocytogenes* profiles of bird samples with the profiles in the DFEH collection revealed that 34 genotypes (71 %), representing 60 samples (78 %), were previously recovered from other sources. Similar genotypes were previously detected in animals, farms, food processing environments and different foods.

In pig production, the highest number of different genotypes was recovered from the farm that also had the highest prevalence of *L. monocytogenes*. All genotypes detected in feed or litter were also recovered from pig samples. Six of the seven genotypes detected in pluck sets were also found in tonsils, and 16 of the 17 pigs that had *L. monocytogenes* in more than one sample had the bacterium in the tonsils. The two *L. monocytogenes*-positive carcass samples harboured the genotypes that were also found previously from pigs of the same farm.

In the pork processing plant, one genotype was found in all processing stages, whereas seven genotypes were found at only one sampling site. Raw pork contained eight different genotypes of which three were found also in the finished RTE cold-smoked pork and two in latter stages of processing, but not in the finished product. All three genotypes that were found in RTE pork and in the plant environment were detected in the brining area. Moreover, all samples taken from the personnel in the brining area were found to be positive for *L. monocytogenes*. Four genotypes were found only in the meat processing environment and were found neither in incoming raw pork nor in the finished RTE cold-smoked pork products. Of the eight genotypes found in RTE pork, six were found in earlier stages of production, either in raw material or in plant environment.

### 5.2.2 Serotyping

The most common serotype in wild birds, the pig production chain and the pork processing plant was 1/2a, representing 30 (63 %), 35 (97 %), and 13 (76 %) of genotypes, respectively. In birds, 12, 4 and 2 genotypes belonged to serotypes 4b, 1/2c and 1/2b, respectively. In pig production, serotype 1/2c was represented in one genotype. In the pork production plant, 1/2c represented two genotypes and 4b and 4d both one genotype.

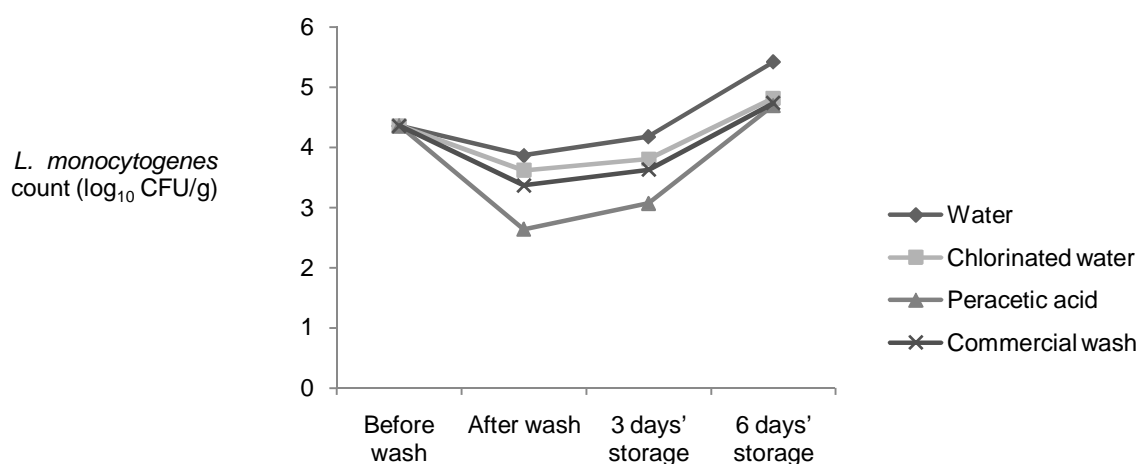
## 5.3 Farm factors affecting on presence of *L. monocytogenes* (II)

In correlation and logistic regression analyses, large group size and contact with pets and pest animals were associated with a high prevalence of *L. monocytogenes* on farms. In correlation analyses, organic production, hygiene conditions, and farm management practices, i.e., management of manure, use of coarse feed, access to outdoor area, and drinking from the trough were also associated with a high prevalence of *L. monocytogenes*.

## 5.4 Survival of *L. monocytogenes* during food processing (IV, V)

### 5.4.1 Survival of *L. monocytogenes* in lettuce after washing (IV)

Mean inoculation level of the mixture of five *L. monocytogenes* strains was 4.36 log CFU/g. Chlorinated water, commercial citric acid-based produce wash, and peracetic acid solution reduced the numbers of *L. monocytogenes* by 0.7, 1.0, and 1.7 log CFU/g, respectively (Figure 4). Water was significantly less effective ( $p < 0.05$ ) than any of the disinfectants in decreasing the number of *L. monocytogenes*. Peracetic acid solution reduced the number of *L. monocytogenes* significantly more ( $p < 0.01$ ) than commercial citric acid-based produce wash or chlorinated water. The number of *L. monocytogenes* remained lower than inoculation levels during 3 days storage at 6°C, being significantly lower ( $p < 0.05$ ) on samples washed with commercial citric acid-based produce wash and peracetic acid than on those washed with water. After 6 days of storage, the number of *L. monocytogenes* had reached the inoculation level on all samples. Samples washed with water had significantly higher numbers ( $p < 0.05$ ) of *L. monocytogenes* at the end of the storage period than the samples washed with disinfectants.



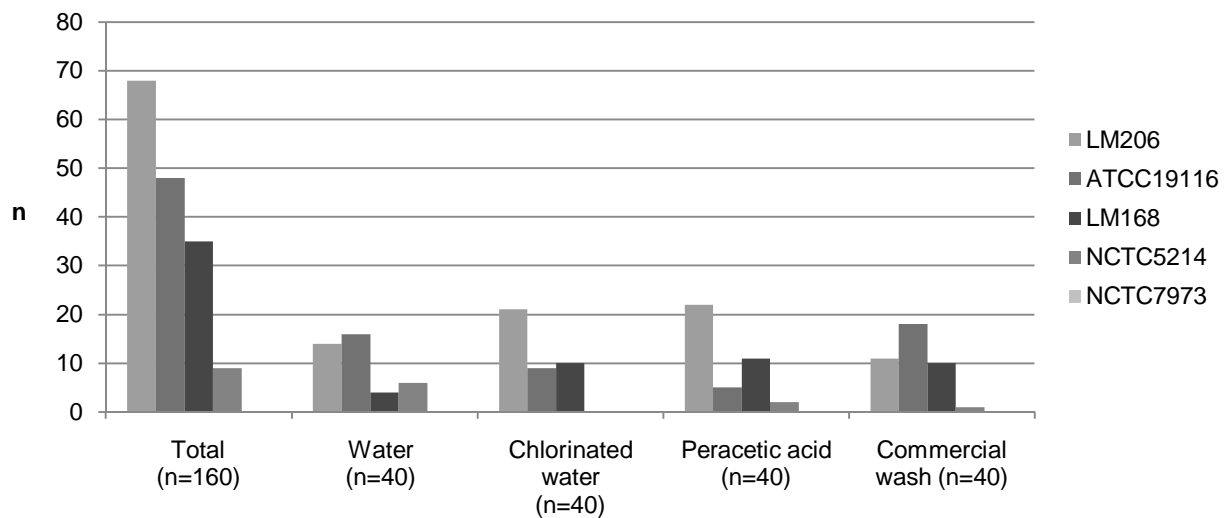
**Figure 4.** *L. monocytogenes* counts on pre-cut lettuce samples inoculated with a mixture (4.7 log CFU/g) of equal amounts of five *L. monocytogenes* strains before and after washing treatment and after 3 and 6 days of storage at 6°C.

### 5.4.2 Survival of *L. monocytogenes* in dry sausage (V)

*L. monocytogenes* was detected at the end of the ripening in sausages without *L. plantarum* DDEN 2205, and with the combination of starter A and low level of *L. plantarum* DDEN 2205, at 12–14 and  $< 0.1$ – $0.1$  MPN/g, respectively. The other two sausage treatments were *Listeria*-free after 17 and 28 days of ripening. All sausages with *L. plantarum* DDEN 2205 contained less than 100 MPN/g at all sampling times. Weight losses over 21 days of ripening were 200–240 g (27–32 %). The pH values of the sausages decreased from 5.6–5.7 to 4.7–5.0 and 4.5–4.7 in 48 h and 63–70 h of fermentation, respectively.

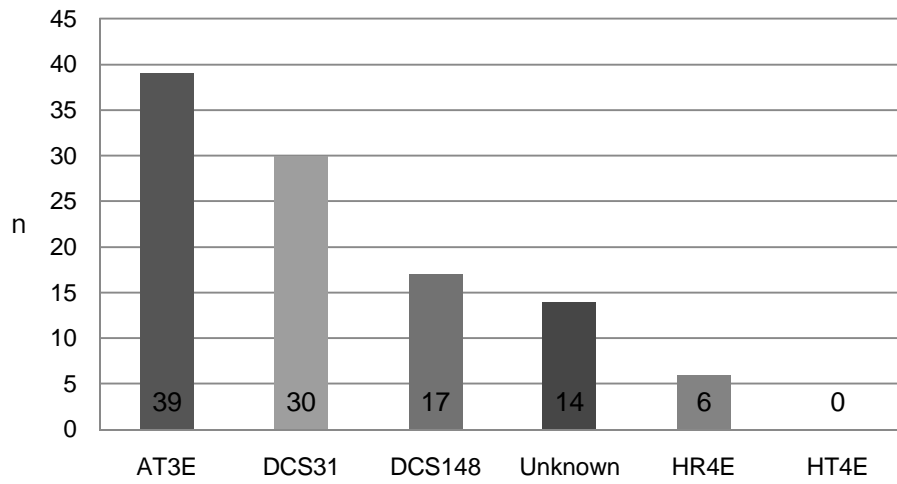
### 5.4.3 Differences in survival between *L. monocytogenes* strains (IV, V)

In lettuce, *L. monocytogenes* strain LM206 was recovered most frequently after 6 days of storage (Figure 5), and it was the most prevalent strain after washes with chlorinated water and peracetic acid, whereas strain ATCC19116 was the most frequent after washes with water and commercial citric acid-based produce wash. Strain NCTC7973 was not present among the 160 strains analyzed after 6 days of storage.



**Figure 5.** Recovery of five *L. monocytogenes* strains in lettuce, inoculated in a mixture of equal amounts of each, after different washes and 6 days of storage at 6°C

In sausage, strain AT3E was recovered most frequently (Figure 6) and was detected in all sausages at some point of ripening. Strain HT4E was not detected in any of the samples, and HR5E was detected only in sausages without *L. plantarum* DDEN 2205. Raw material was contaminated with a *L. monocytogenes* strain before inoculation, and the strain was also detected in sausages during and at the end of ripening.



**Figure 6.** Recovery of *L. monocytogenes* strains, inoculated in equal amounts, in dry sausage during ripening. Unknown = strain originating from raw material

## 6 DISCUSSION

### 6.1 Prevalence of *L. monocytogenes* (I–III)

*L. monocytogenes* was commonly found in wild birds, pigs and the pork production chain. Prevalence varied among sampling sites: the pathogen was found more in wild birds feeding in the landfill site than in urban areas; it was more common in organic pig farms than on conventional ones, and in tonsils rather than in other samples; and it was associated with brining at the pork production plant.

The mean prevalence of *L. monocytogenes* (36 %) in birds was higher than the previously reported figures of 0–33 % (35, 47, 87, 242, 304, 312). The great variation in prevalence in different studies may be due to differences in the living environment and feeding habits of birds. Fenlon (87) reported that seagulls feeding in sewage had a higher prevalence of *L. monocytogenes* than seagulls feeding elsewhere, which is a similar finding with ours in the respect that the feeding environment influenced the prevalence of *L. monocytogenes*. Other researchers (47, 242, 312) have also suggested that living environment has an influence on prevalence of *L. monocytogenes*. The living environment combined with the feeding habits likely affect the fecal carriage of *L. monocytogenes*. Most birds can probably carry *Listeria* spp. asymptotically in their intestines, with prevalence varying according to feeding habits.

The prevalence of *L. monocytogenes* in pigs was consistent with earlier findings, except the high prevalence observed in tonsils and pluck sets of pigs from organic farms. In fecal and carcass samples, the prevalence of *L. monocytogenes* has been reported to be low, 0–2 % (89, 150, 156, 170, 257, 271), but it was higher in tonsils (7–14 %) than in other pig samples (15, 16, 150, 271). The prevalence of *L. monocytogenes* was higher in organic than conventional pig production, and *L. monocytogenes* was isolated from pigs of all organic farms. Certain practices on organic farms, such as large group size, access to outdoor areas, and use of coarse feed, seem to explain this higher prevalence. Large numbers of pigs in one pen enable contact with more pigs, thus spreading the bacterium. Moreover, because *L. monocytogenes* is common in the environment (220, 247), outdoor areas may be a source of contamination. Finally, coarse feed is frequently contaminated with *L. monocytogenes* (88, 129). Although these practices appear to be associated with the prevalence of *L. monocytogenes*, they may otherwise be advantageous with regard to pig welfare, which is one aim in organic production, and some of these practices are also required by the EU's organic production regulations (80), under which pigs must have permanent access to pasture or roughage.

Prevalences of *L. monocytogenes* among other animal species and humans have varied in different studies and in different populations. Point prevalences of *L. monocytogenes* in faeces among healthy humans, pigs and cattle have been about 1 %, 1–16 %, and 2–53 %, respectively, and they have been affected by type of food, feed and environment (89, 134, 262, 292).

In the pork processing plant, the prevalence of *L. monocytogenes* was significantly higher in the finished RTE cold-smoked pork products when brining injections were used than in those that were dry salted. The brining area was the most contaminated site with *L. monocytogenes*. In some food processing plants, the presence of *L. monocytogenes* has been reported to strongly correlate with the use of brining injections and cold-smoking time (12, 25). Moreover, brining injection has been observed as a significant factor in contamination of cold-smoked meat with *L. monocytogenes* (25).

## 6.2 Diversity of *L. monocytogenes* (I–III)

### 6.2.1 Genotypes

*L. monocytogenes* isolates collected from wild birds, pigs and pork production plant showed a high level of diversity. Marked diversity of *L. monocytogenes* in many environments has been reported previously (14, 260, 295), and was confirmed in our study. Because of the high diversity of *L. monocytogenes* in the environment, feed and food, animals such as pigs and birds may harbour the strains present in their living environment and feed.

The high diversity of the pathogen recovered from birds may be due to their eating a variety of waste foods from the ground; they may indeed harbour in their intestines the entire range of *L. monocytogenes* from their living environment. This hypothesis is supported by the fact that although the overall diversity was high nearly half (44 %) of the genotypes were recovered from more than one bird, suggesting a common origin for these strains. In addition, the prevailing genotypes in birds were also often detected in other sources, which may simply mean that some *L. monocytogenes* genotypes are, overall, more common than others.

A large variety of genotypes was recognized in incoming raw pork in the pork processing plant, but only three out of eight genotypes were found later in the production line. This finding shows that a genetically diverse population of *L. monocytogenes* entered the meat processing plant with raw material, whereas only some of the strains colonized the establishment. Moreover, two strains were found to persist in the production plant over a period of five years. Thus, *L. monocytogenes* population in incoming raw material and in the food processing plants may be separate and the population in the plant may include strains persisting, possibly for years, within plants (12, 25, 124).

### 6.2.2 Serotypes

Serotype 1/2a represented over two thirds of genotypes in wild birds, pigs and a pig production plant, and other detected serotypes were 1/2b, 1/2c, 4b and 4d. Serotypes 1/2a, 1/2b and 4b are most commonly detected in human listeriosis cases and predominant serotypes in foods have been reported to be 1/2a, 1/2b and 1/2c (294), but serotype 4b is not rare. This shows that serotypes causing human listeriosis and found in foods are also common in the environment and the food processing chain.

The prevalence of serotype 4b in birds seems to be higher than in pigs or the pork production plant and in foods in earlier reports (14, 179). This may reflect longer persistence in birds of 4b strains than other serotypes or better adaptation of serotype 4b to waste foods than foods in other environments, i.e., refrigerated temperatures. In pigs, nearly all isolated *L. monocytogenes* strains were serotype 1/2a, indicating that some *L. monocytogenes* types may be better adapted to pig production environments than others. Adaptation of a certain group of *L. monocytogenes*, including serotype 1/2a, to that niche has also been discussed previously (102, 125). Nevertheless, the pork production plant harboured a higher diversity of serotypes than primary production. Persistent strains were found in a pork production plant and this may reflect serotype diversity.

### 6.3 Contamination by *L. monocytogenes* in food production chain

The majority of genotypes recovered from birds were also detected in other sources such as in a variety of foods, along the food processing chain and in other animal species. The probable explanation is that the origin of the *L. monocytogenes* in birds is the foods that they eat; conversely birds might also disseminate bacteria in their droppings into the food chain when they enter food processing plants, or when vegetables are grown in open fields, or foods are sold in outdoor marketplaces. Clearly, birds do not harbour a distinct population of *L. monocytogenes* of their own and in this respect they probably have a role in disseminating *L. monocytogenes* in nature and might also serve as a vehicle in contaminating foods and food processing plants.

Similar genotypes were frequently found in different pigs of the same farm, implying a common origin for the *L. monocytogenes* in the pigs of one farm. Feed and litter were found to be contaminated with *L. monocytogenes*, and *L. monocytogenes* genotypes found in feed or litter were also detected in pig samples. Animal feeds and the farm environment commonly harbour *L. monocytogenes* (88, 129, 220), thus serving as a contamination source. This contamination may be the origin of the bacterium further along in the food chain. In addition to a common origin, bacterial spreading from pig to pig on farms is possible since pigs are reared in close contact with each other. *L. monocytogenes* genotypes found in pluck sets were similar to those in tonsils, indicating direct contact and contamination during slaughter between tonsils and pluck sets. Contamination of tonsils and pluck sets could also spread from pig to pig since the same equipment is used in the slaughtering line (16, 233). However, rectal swabs collected already from farms had similar strains to those later isolated from pigs in the slaughterhouse, and thus, at least part of the contamination detected at the slaughterhouse originates from farms. Carcass samples contaminated with *L. monocytogenes* harboured the same genotypes as pluck sets, and thus, the carcass likely becomes contaminated during slaughter, although this is not as common as contamination of pluck sets. Furthermore, the same genotype as in carcasses was detected in cut meats, indicating that contamination of meats may originate from carcasses. Transmission of *L. monocytogenes* has been suspected to occur mainly via the slaughterhouse environment, not primarily via animals (30). As shown in this study, direct contamination during slaughter from tonsils to pluck sets and carcasses is also possible. Further, the present study demonstrated that *L. monocytogenes* in pigs may spread all the way from the farm to meat cuts, as similar genotypes were found in samples from pigs, carcass and meat. In addition, *L. monocytogenes* was also detected in meats from farms where no contaminated carcasses were detected and those strains were not found in other samples. This indicates that contamination has most likely occurred from environment in cutting facilities, which is known to be often a source of contamination (151).

Incoming raw pork in the processing plant was frequently contaminated with *L. monocytogenes* and genotypes in raw meat were also found in processing environment and in RTE products. Thus, raw material seems to be a considerable source of contamination into processing facilities and may also be an important source of contamination of finished products (12, 21, 22, 25), particularly when the processing does not involve sufficient heat treatment to inactivate *L. monocytogenes*. Further, strains showed to persist in the plant over many years and environmental contamination has been shown to be a major source of contamination of finished products (12, 25, 124). In the pork processing plant, the number of *L. monocytogenes*-positive environmental samples significantly increased in the brining area, revealing the brining machine and personnel working with brining procedures to be the most contaminated sites. Thus, the overall prevalence of *L. monocytogenes* in raw pork increased after brining injections. Brining has been associated with the contamination by *L. monocytogenes*, especially when the brine is recirculated (12, 114). The complexity and poor hygienic design of the brining machines could facilitate further spread of the *L. monocytogenes* in

the meat processing environment, especially when improper cleaning and disinfection procedures are applied (12, 114, 184, 185). Contamination may also spread via personnel in processing plant facilities, especially when plant design and traffic are poor, or when rotating assigned duties (12, 250).

## 6.4 Control of *L. monocytogenes* in food production

### 6.4.1 Farm

Even within the same production system, a wide range of prevalence existed between farms, suggesting that some farm-specific factors affect the presence of *L. monocytogenes*. These farm factors include large group size, contact of pigs with pets and pest animals, treatment of manure, hygiene practices, and drinking from a trough. In large groups, the bacterium may spread from one pig to many others, and thus, smaller groups in pens may be advantageous. Pet and pest animals may spread the bacterium into the farm environment or contaminate feeds, so controlling pest animals and restricting the entrance of pets and birds into piggeries reduces the prevalence of *L. monocytogenes*. Drinking water can easily be contaminated when pigs drink from a common trough; use of nipple drinkers may be recommended. In addition, liquid manure compared with solid manure as well as mechanical removal of manure reduce the prevalence of *L. monocytogenes*. Some earlier studies have reported similar results, showing that farm management practices, such as specific pathogen-free herds (271) and type of feed (20, 21), influence the prevalence of *L. monocytogenes*.

Farms with the highest prevalence of *L. monocytogenes* had no contaminated carcasses. This shows that a high prevalence of *L. monocytogenes* in pigs does not inevitably lead to highly contaminated meats. Several preventive actions can be utilized in the slaughtering process to reduce contamination of pathogenic bacteria, including good slaughtering technique and hygiene, proper cleaning and disinfection of equipment and good operating protocols (30, 161). With good manufacturing practices, contamination from pigs to the food chain may be substantially reduced, and thus, solid hygienic practices are of the utmost importance during slaughter.

## 6.4.2 Manufacturing practices

Only one *L. monocytogenes*-positive sample was found in the slicing and packaging area of the meat processing plant and none of the positive samples was found in the smoking area. Both areas of the meat processing plant were well separated from the other facilities of the raw and processed meats, thus maintaining good manufacturing and hygienic procedures to minimize *L. monocytogenes* contamination during production. The degree of compartmentalization had an impact on *L. monocytogenes* contamination status in the meat processing plants. Thus, less compartmentalized areas of the processing line were more frequently contaminated and for longer periods of time than the processing lines with a well separated processing areas (48, 186, 285).

## 6.4.3 Washes

All of the tested washing solutions decreased the populations of *L. monocytogenes* on precut iceberg lettuce, peracetic acid being the most effective. Reduction by peracetic acid compared to water was higher in this study than in earlier reports (313). The reduction of *L. monocytogenes* with chlorine wash was consistent with the results of previous studies (59, 276). The commercial citric acid-based produce wash was as effective as chlorinated water against *L. monocytogenes*. Regardless of the statistically significant differences, the total reduction of *L. monocytogenes* was at maximum 1.7 log CFU/g and none of the sanitizers eliminated *L. monocytogenes* from the produce. The number of *L. monocytogenes* rose during storage, reaching the initial inoculation level prior to 6 days of storage at 6°C. *L. monocytogenes* is reported to be able to grow on lettuce (59, 276), with growth increasing at higher temperatures (227). The optimal upper limit for refrigerated products is lower than in our study, being about 4°C, but in practice the optimal temperature limits are frequently abused before consumption of the produce (293). Our results show that *L. monocytogenes* is able to grow, regardless of the disinfectant used, at typical household refrigerator temperatures. This stresses the importance to allowing only good-quality products into the market to ensure the quality and safety of fresh-cut lettuce for the consumer. The initial inoculation levels of *L. monocytogenes* were achieved during storage and, although the counts of *L. monocytogenes* were lower on samples washed with disinfectants than on those washed with water, the differences reduced during the storage. Therefore, using disinfectants to wash lettuce is not enough to assure the safety of the produce. Nevertheless, disinfectants prevent the build-up of pathogens in washing solution (296), and thus, the use of sanitizers may be advisable.

## 6.4.4 Protective cultures

The number of *L. monocytogenes* by the end of the ripening decreased to the level of <100 MPN/g, which is the European regulation for RTE foods like dry sausage during their self-life (79), in all sausages. The decrease in pH during ripening was not in itself sufficient to eliminate *Listeria*, since the bacterium is capable of multiplying at pH values from 4.0 to 9.6 (83, 159), but other hurdles, such as low water activity ( $a_w$ ) and pediocin produced by *L. plantarum* DDEN 2205 inactivated the bacterium. *L. monocytogenes* was detected in higher levels in sausages without pediocin-producing *L. plantarum* DDEN 2205 than in sausages with this protective strain. Starter A with high concentration of protective culture DDEN 2205 and starter B with low concentration of DDEN 2205 eliminated *L. monocytogenes* from dry sausage during ripening and they appeared to be effective combinations to control *L. monocytogenes* in dry sausages.

## 6.5 Strain-specific characteristics of *L. monocytogenes*

Recovery of the inoculated *L. monocytogenes* strains clearly showed that there were strain-specific differences in their ability to survive in lettuce and dry sausage, and clearly some strains are harder to eliminate from products than others. Some strains may be better adapted to certain conditions in food processing or foods. In addition, these detected differences between strains provide evidence that observations on single strain are inadequate.

In lettuce, the differences may be due to variable ability to tolerate different disinfectants, or to differences in attachment to the lettuce surface, or to a combination of these. Different *L. monocytogenes* strains are known to have variable resistance to disinfectants (215), and this may lead to better survival of certain strains during the washing of the lettuce. In addition, strain-specific differences have been observed in the ability of *L. monocytogenes* to attach to the surface of alfalfa sprouts, and the poorest colonizing strain was unable to attach to the sprout (105). In our study, one strain (NCTC7973) could not be detected on lettuce after storage, and this strain may not have been able to attach to the lettuce surface, thus being easily removed with all of the washing methods. Moreover, the strain originally isolated from salads was recovered most often overall and another was the second most recovered strain after washes with chlorinated water and peracetic acid. This may indicate that strains originating from salads have adapted to their environments.

In sausage, two of the strains, HT4E and HR5E, were inhibited in all sausages with the protective *L. plantarum* strain, and HT4E was not detected in any of the sausages. One strain, AT3E, was detected in all sausages at some point of ripening, and two of the strains, DCS148 and DCS31, were detected in all sausages other than the one with the high concentration of protective culture. Overall, strains DCS31 and AT3E were detected more often than other strains. Strain-specific difference in survival of *Listeria* in dry sausage between strains isolated from sausages and sausage-making environments and strains isolated from other sources have been observed previously (280). In the present study, all strains were originally isolated from meats. The natural *L. monocytogenes* contaminant was isolated in all sausages except the ones without pediocin producing protective culture. The probable explanation is that this strain was more resistant to pediocin than inoculated strains and it was a better competitor in sausages with *L. plantarum* DDEN 2205 than other *L. monocytogenes* strains. *L. monocytogenes* strains have been found to vary in their susceptibility to class IIa bacteriocins *in vitro* (75, 76, 152). The observed survival of the natural contaminant shows that raw material may contain strains, possibly originating from meat processing environments, that are well adapted to conditions during fermentation. Overall, the hurdle concept in dry sausage making should be effective against the most resistant strains of *L. monocytogenes*.

## 7 CONCLUSIONS

1. *L. monocytogenes* is commonly found in wild birds, the pig production chain and in pork production plants, and a high level of genetic diversity was demonstrated among strains. The bacterium is not evenly distributed, but is found most frequently in certain sites, i.e., birds feeding in a landfill site, organic farms, tonsil samples and sites and products associated with brining. *L. monocytogenes* in birds, farms, food processing plant or foods do not form distinct genetic groups, rather, populations overlap, although some genotypes were more common in the food chain than others. In addition, some serotypes seem to be more frequent in certain niches than in others.
2. Contamination of *L. monocytogenes* may originate from several sources. Contamination by *L. monocytogenes* in pork production may originate from farm. Birds may disseminate *L. monocytogenes* into food processing environments or directly into foods. Raw material can be a source of contamination into the food processing plant and foods. *L. monocytogenes* can persist in food processing plants and further contaminate foods. Especially, brining in production of RTE pork is an important source of contamination.
3. Control of *L. monocytogenes* can be implemented throughout the food chain. Reduction of the prevalence of *L. monocytogenes* on a farm can be achieved by specific farm management practices and this can contribute to the overall reduction of *L. monocytogenes* in the food chain. Since birds harbour *L. monocytogenes*, preventing access of birds to food processing environments is important. In the food processing plant, especially the brining area should be subjected to disassembling, extensive cleaning and disinfection to eliminate persistent contamination with *L. monocytogenes*. In addition, the removal of the brining step from the production of cold-smoked meat products and using dry-salting instead has to be taken into consideration. With regard to the production of pre-cut lettuce, the present work demonstrates that the number of *L. monocytogenes* is reduced by the sanitizers, but none of the washing solutions eliminated *L. monocytogenes* from produce, so high-quality raw material and good manufacturing practices remain important. Protective starter cultures, such as *L. plantarum*, provide an appealing hurdle in dry sausage processing and assist in the control of *L. monocytogenes*.
4. *L. monocytogenes* show strain-specific differences in survival and growth in lettuce and in dry sausage. The ability of some *L. monocytogenes* strains to survive well in food production raises a challenge for industry because these strains can be especially difficult to remove from the products. Possible variations in susceptibility and development of resistance in *L. monocytogenes* strains can create problems and raises a need to use the appropriate hurdle concept to control most resistant strains.

## 8 REFERENCES

1. Aarnisalo, K., T. Autio, A.-M. Sjöberg, J. Lundén, H. Korkeala, and M.-L. Suihko. 2003. Typing of *Listeria monocytogenes* isolates originating from the food processing industry with automated ribotyping and pulsed-field gel electrophoresis. *J. Food Prot.* 66:249-255.
2. Adak, G. K., S. M. Long, and S. J. O'Brien. 2002. Trends in indigenous foodborne disease and deaths, England and Wales: 1992 to 2000. *Gut.* 51:832-841.
3. Al-Ghazali, M. R., and S. K. Al-Azawi. 1990. *Listeria monocytogenes* contamination of crops grown on soil treated with sewage sludge cake. *J. Appl. Bacteriol.* 69:642-647.
4. Allerberger, F., and J. P. Guggenbichler. 1989. Listeriosis in Austria – report of an outbreak in 1986. *Acta Microbiol. Hung.* 36:149-152.
5. Allerberger, F., and M. Wagner. 2010. Listeriosis: a resurgent foodborne infection. *Clin. Microbiol. Infect.* 16:16-23.
6. Allerberger, F. 2003. *Listeria*: growth, phenotypic differentiation and molecular microbiology. *FEMS Immunology & Medical Microbiology.* 35:183-189.
7. Arimi, S. M., E. T. Ryser, T. J. Pritchard, and C. W. Donnelly. 1997. Diversity of *Listeria* Ribotypes Recovered from Dairy Cattle, Silage, and Dairy Processing Environments. *J. Food Prot.* 60:811-816.
8. Audurier, A., and C. Martin. 1989. Phage typing of *Listeria monocytogenes*. *Int. J. Food Microbiol.* 8:251-257.
9. Audurier, A., A. G. Taylor, B. Carbonnelle, and J. McLauchlin. 1984. A phage typing system for *Listeria monocytogenes* and its use in epidemiological studies. *Clin. Invest. Med.* 7:229-232.
10. Aureli, P., G. C. Fiorucci, D. Caroli, G. Marchiaro, O. Novara, L. Leone, and S. Salmaso. 2000. An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria monocytogenes*. *N. Engl. J. Med.* 342:1236-1241.
11. Autio, T. 2003. Tracing the sources of *Listeria monocytogenes* contamination and listeriosis using molecular tools. Academic dissertation. Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki, Finland.
12. Autio, T., S. Hielm, M. Miettinen, A.-M. Sjöberg, K. Aarnisalo, J. Björkroth, T. Mattila-Sandholm, and H. Korkeala. 1999. Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. *Appl. Environ. Microbiol.* 65:150-155.
13. Autio, T., R. Keto-Timonen, J. Lundén, J. Björkroth, and H. Korkeala. 2003. Characterisation of persistent and sporadic *Listeria monocytogenes* strains by pulsed-field gel electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP). *Syst. Appl. Microbiol.* 26:539-545.
14. Autio, T., J. Lundén, M. Fredriksson-Ahomaa, J. Björkroth, A.-M. Sjöberg, and H. Korkeala. 2002. Similar *Listeria monocytogenes* pulsotypes detected in several foods originating from different sources. *Int. J. Food Microbiol.* 77:83-90.
15. Autio, T., A. Markkula, S. Hellström, T. Niskanen, J. Lundén, and H. Korkeala. 2004. Prevalence and genetic diversity of *Listeria monocytogenes* in the tonsils of pigs. *J. Food Prot.* 67:805-808.
16. Autio, T., T. Säteri, M. Fredriksson-Ahomaa, M. Rahkio, J. Lundén, and H. Korkeala. 2000. *Listeria monocytogenes* contamination pattern in pig slaughterhouses. *J. Food Prot.* 63:1438-1442.
17. Aymerich, T., M. G. Artigas, M. Garriga, J. M. Monfort, and M. Hugas. 2000. Effect of sausage ingredients and additives on the production of enterocin A and B by *Enterococcus faecium* CTC492. Optimization of *in vitro* production and anti-listerial effect in dry fermented sausages. *J. Appl. Microbiol.* 88:686-694.
18. Bansal, N. S. 1996. Development of a polymerase chain reaction assay for the detection of *Listeria monocytogenes* in foods. *Lett. Appl. Microbiol.* 22:353-356.
19. Bari, M. L., M. Nakauma, S. Todoriki, V. K. Juneja, K. Isshiki, and S. Kawamoto. 2005. Effectiveness of irradiation treatments in inactivating *Listeria monocytogenes* on fresh vegetables at refrigeration temperature. *J. Food Prot.* 68:318-323.
20. Belœil, P.-A., C. Chauvin, M. T. Toquin, C. Fablet, Y. Le Nôtre, G. Salvat, F. Madec, and P. Fravallo. 2003. *Listeria monocytogenes* contamination of finishing pigs: an exploratory epidemiological survey in France. *Vet. Res.* 34:737-748.

21. Belœil, P.-A., P. Fravalo, C. Chauvin, C. Fablet, G. Salvat, and F. Madec. 2003. *Listeria* spp. contamination in piggeries: comparison of three sites of environmental swabbing for detection and risk factor hypothesis. *J. Vet. Med.* 50:155-160.
22. Bernagozzi, M., F. Bianucci, R. Sacchetti, and P. Bisbini. 1994. Study of the prevalence of *Listeria* spp. in surface water. *Zentralbl. Hyg. Umweltmed.* 196:237-244.
23. Berry, E. D., M. B. Liewen, R. W. Mandigo, and R. W. Hutkins. 1990. Inhibition of *Listeria monocytogenes* by bacteriocin-producing *Pediococcus* during the manufacture of fermented semidry sausage. *J. Food Prot.* 194-197.
24. Bērziņš, A. 2010. Molecular epidemiology and heat resistance of *Listeria monocytogenes* in meat products and meat-processing plants and listeriosis in Latvia. Academic dissertation. Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland.
25. Bērziņš, A., A. Hörman, J. Lundén, and H. Korkeala. 2007. Factors associated with *Listeria monocytogenes* contamination of cold-smoked pork products produced in Latvia and Lithuania. *Int. J. Food Microbiol.* 115:173-179.
26. Betriu, C., S. Fuentesmilla, R. Méndez, J. J. Picazo, and J. García-Sánchez. 2001. Endophthalmitis Caused by *Listeria monocytogenes*. *J. Clin. Microbiol.* 39:2742-2744.
27. Bille, J. and Glauser, M.-P. 1988. Listériose en Suisse/Zur Listeriose-Situation in der Schweiz. *Bulletin de l'Office Fédéral de la Santé Publique/Bulletin des Bundesamtes für Gesundheitswesen.* 3:28-29.
28. Bille, J., D. S. Blanc, H. Schmid, K. Boubaker, A. Baumgartner, H. H. Siegrist, M. L. Tritten, R. Lienhard, D. Berner, R. Anderau, M. Treboux, J. M. Ducommun, R. Malinverni, D. Genné, P. H. Erard, and U. Waespi. 2006. Outbreak of human listeriosis associated with tomme cheese in northwest Switzerland, 2005. *Euro Surveill.* 11:91-93.
29. Bille, J., B. Catimel, E. Bannerman, C. Jacquet, M.-N. Yersin, I. Caniaux, D. Monget, and J. Rocourt. 1992. API *Listeria*, a new and promising one-day system to identify *Listeria* isolates. *Appl. Environ. Microbiol.* 58:1857-1860.
30. Borch, E., T. Nesbakken, and H. Christensen. 1996. Hazard identification in swine slaughter with respect to foodborne bacteria. *Int. J. Food Microbiol.* 30:9-25.
31. Border, P. M., J. J. Howard, G. S. Plastow, and K. W. Siggins. 1990. Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. *Lett. Appl. Microbiol.* 11:158-162.
32. Bortolussi, R. 2008. Listeriosis: a primer. *CMAJ.* 179:795-797.
33. Borucki, M. K., S. H. Kim, D. R. Call, S. C. Smole, and F. Pagotto. 2004. Selective discrimination of *Listeria monocytogenes* epidemic strains by a mixed-genome DNA microarray compared to discrimination by pulsed-field gel electrophoresis, ribotyping, and multilocus sequence typing. *J. Clin. Microbiol.* 42:5270-5276.
34. Botzler, R. G., A. B. Cowan, and T. F. Wetzler. 1974. Survival of *Listeria monocytogenes* in soil and water. *J. Wildl. Dis.* 10:204-212.
35. Bouttefroy, A., J. P. Lemaître, and A. Rousset. 1997. Prevalence of *Listeria* sp. in droppings from urban rooks (*Corvus frugilegus*). *J. Appl. Microbiol.* 82:641-647.
36. Brosch, R., J. Chen, and J. B. Luchansky. 1994. Pulsed-field fingerprinting of *Listeria* spp.: Identification of genomic divisions for *Listeria monocytogenes* and their correlation with serovar. *Appl. Environ. Microbiol.* 60:2584-2592.
37. Budde, B. B., T. Hornbæk, T. Jacobsen, V. Barkholt, and A. G. Koch. 2003. *Leuconostoc carnosum* 4010 has the potential for use as a protective culture for vacuum-packed meats: culture isolation, bacteriocin identification, and meat application experiments. *Int. J. Food Microbiol.* 83:171-184.
38. Büla, C. J., J. Bille, and M. P. Glauser. 1995. An epidemic of food-borne listeriosis in western Switzerland: description of 57 cases involving adults. *Clin. Infect. Dis.* 20:66-72.
39. Buyong, N., J. Kok, and J. B. Luchansky. 1998. Use of a genetically enhanced, pediocin-producing starter culture, *Lactococcus lactis* subsp. *lactis* MM217, To control *Listeria monocytogenes* in cheddar cheese. *Appl. Environ. Microbiol.* 64:4842-4845.
40. Cabedo, L., L. Picart i Barrot, and A. Teixidó i Canelles. 2008. Prevalence of *Listeria monocytogenes* and *Salmonella* in ready-to-eat food in Catalonia, Spain. *J. Food Prot.* 71:855-859.
41. Cagri, A., Z. Ustunol, and E. T. Ryser. 2004. Antimicrobial edible films and coatings *J. Food Prot.* 67:833-848.
42. Cai, S., D. Y. Kabuki, A. Y. Kuaye, T. G. Cargioli, M. S. Chung, R. Nielsen, and M. Wiedmann.

2002. Rational design of DNA sequence-based strategies for subtyping *Listeria monocytogenes*. *J. Clin. Microbiol.* 40:3319-3325.
43. Cairns, B. J., and R. J. H. Payne. 2009. Sudden increases in listeriosis rates in England and Wales, 2001 and 2003. *Emerg. Infect. Dis.* 15:465-468.
44. Call, D. R., M. K. Borucki, and T. E. Besser. 2003. Mixed-genome microarrays reveal multiple serotype and lineage-specific differences among strains of *Listeria monocytogenes*. *J. Clin. Microbiol.* 41:632-639.
45. Capita, R., C. Alonso-Calleja, C. García-Fernández, and B. Moreno. 2001. Efficacy of trisodium phosphate solutions in reducing *Listeria monocytogenes* populations on chicken skin during refrigerated storage. *J. Food Prot.* 64:1627-1630.
46. Carrique-Mas, J. J., I. Hökeberg, Y. Andersson, M. Arneborn, W. Tham, M.-L. Danielsson-Tham, B. Osterman, M. Leffler, M. Steen, E. Eriksson, G. Hedin, and J. Giesecke. 2003. Febrile gastroenteritis after eating on-farm manufactured fresh cheese – an outbreak of listeriosis? *Epidemiol. Infect.* 130:79-86.
47. Casanovas, L., M. de Simón, M. D. Ferrer, J. Arqués, and G. Monzón. 1995. Intestinal carriage of campylobacters, salmonellas, yersinias and listerias in pigeons in the city of Barcelona. *J. Appl. Bacteriol.* 78:11-13.
48. Chasseignaux, E., M.-T. Toquin, C. Ragimbeau, G. Salvat, P. Colin, and G. Ermel. 2001. Molecular epidemiology of *Listeria monocytogenes* isolates collected from the environment, raw meat and raw products in two poultry- and pork-processing plants. *J. Appl. Microbiol.* 91:888-899.
49. Chen, Y., W. H. Ross, V. N. Scott, and D. E. Gombas. 2003. *Listeria monocytogenes*: low levels equal low risk. *J. Food Prot.* 66:570-577.
50. Churchill, R. L. T., H. Lee, and J. C. Hall. 2006. Detection of *Listeria monocytogenes* and the toxin listeriolysin O in food. *J. Microbiol. Methods.* 64:141-170.
51. Clark, C. G., J. Farber, F. Pagotto, N. Ciampa, K. Doré, C. Nadon, K. Bernard, L.-K. Ng, and the CPHLN. 2010. Surveillance for *Listeria monocytogenes* and listeriosis, 1995-2004. *Epidemiol. Infect.* 138:559-572.
52. Cleveland, J., T. J. Montville, I. F. Nes, and M. L. Chikindas. 2001. Bacteriocins: safe, natural antimicrobials for food preservation. *Int. J. Food Microbiol.* 71:1-20.
53. Codex Alimentarius Commission (CAC). 2007. Guidelines on the application of general principles of food hygiene to the control of *Listeria monocytogenes* in foods. CAC/GL 61, 28 pp.
54. Cotter, P. D., and C. Hill. 2003. Surviving the Acid Test: Responses of Gram-Positive Bacteria to Low pH. *Microbiol. Mol. Biol. Rev.* 67:429-453.
55. Cummins, A. J., A. K. Fielding, and J. McLauchlin. 1994. *Listeria ivanovii* infection in a patient with AIDS. *J. Infect.* 28:89-91.
56. Curtis, G. D. W., R. G. Mitchell, A. F. King, and E. J. Griffin. 1989. A selective differential medium for the isolation of *Listeria monocytogenes*. *Lett. Appl. Microbiol.* 8:95-98.
57. de Valk, H., V. Vaillant, C. Jacquet, J. Rocourt, F. Le Querrec, F. Stainer, N. Quelquejeu, O. Pierre, V. Pierre, J.-C. Desenclos, and V. Goulet. 2001. Two consecutive nationwide outbreaks of listeriosis in France, October 1999-February 2000. *Am. J. Epidemiol.* 154:944-950.
58. Del Campo, J., M.-J. Amiot, and C. Nguyen-The. 2000. Antimicrobial effect of rosemary extracts. *J. Food Prot.* 63:1359-1368.
59. Delaquis, S., S. Stewart, S. Cazaux, and P. Toivonen. 2002. Survival and growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in ready-to-eat iceberg lettuce washed in warm chlorinated water. *J. Food Prot.* 65:459-464.
60. Denny, J., and J. McLauchlin. 2008. Human *Listeria monocytogenes* infections in Europe – an opportunity for improved European surveillance. *Euro Surveill.* 13:8082.
61. DG SANCO. 2009. Listeriosis. [http://ec.europa.eu/health/ph\\_information/dissemination/echi/docs/listeriosis\\_en.pdf](http://ec.europa.eu/health/ph_information/dissemination/echi/docs/listeriosis_en.pdf) Accessed 24.02.2011.
62. Donnelly, C. W., and D. G. Nyachuba. 2007. Conventional methods to detect and isolate *Listeria monocytogenes*, p. 215-282. *In* E. T. Ryser and E. H. Marth (eds.), *Listeria, listeriosis, and Food Safety*, 3rd ed., CRC Press, Boca Raton, FL.
63. Doumith, M., C. Buchrieser, P. Glaser, C. Jacquet, and P. Martin. 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.* 42:3819-3822.
64. Dowe, M., J., E. Jackson D., J. Mori G., and C. Bell. 1997. *Listeria monocytogenes* Survival in Soil and Incidence in Agricultural Soils. *J. Food Prot.* 60:1201-1207.

65. Doyle, M. E., A. S. Mazzotta, T. Wang, D. W. Wiseman, and V. N. Scott. 2001. Heat resistance of *Listeria monocytogenes*. *J. Food Prot.* 64:410-429.
66. Driehuis, F., and S. J. Oude Elferink. 2000. The impact of the quality of silage on animal health and food safety: a review. *Vet. Q.* 22:212-216.
67. EFSA. 2006. The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2005. *The EFSA Journal.* 94.
68. EFSA. 2007. The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. *The EFSA Journal.* 130.
69. EFSA. 2007. Scientific opinion of the panel on Biological hazards on a request from the European Commission on request for updating the former SCVPH opinion on *Listeria monocytogenes* in ready-to-eat foods and scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat foods and the related risk for human illness. *The EFSA Journal.* 599:1-42.
70. EFSA. 2009. The community summary report on food-borne outbreaks in the European Union in 2007. *The EFSA Journal.* 271.
71. EFSA. 2009. The community summary report on trends and sources of zoonoses and zoonotic agents in the European Union in 2007. *The EFSA Journal.* 223.
72. EFSA. 2009. Reports on the availability of molecular typing methods for *Salmonella*, *Campylobacter*, verotoxigenic *Esherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* isolates from food, animals and feedstuffs in European Union Member States (and in some other reporting countries). *The EFSA Journal.* 272:1-52.
73. EFSA. 2010. The community summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. *The EFSA Journal.* 8:1496.
74. El-Shenawy, M. A. 1998. Sources of *Listeria* spp. in domestic food processing environment. *Int. J. Environ. Health Res.* 8:241-251.
75. Encinas, J. P., J.-J. Sanz, M.-L. García-López, and A. Otero. 1999. Behaviour of *Listeria* spp. in naturally contaminated chorizo (Spanish fermented sausage). *Int. J. Food Microbiol.* 46:167-171.
76. Ennahar, S., D. Aoude-Werner, O. Sorokine, A. Van Dorsselaer, F. Bringel, J.-C. Hubert, and C. Hasselmann. 1996. Production of pediocin AcH by *Lactobacillus plantarum* WHE 92 isolated from cheese. *Appl. Environ. Microbiol.* 62:4381-4387.
77. Ericsson, H., A. Eklöv, M. L. Danielsson-Tham, S. Loncarevic, L. O. Mentzing, I. Persson, H. Unnerstad, and W. Tham. 1997. An outbreak of listeriosis suspected to have been caused by rainbow trout. *J. Clin. Microbiol.* 35:2904-2907.
78. The European Union (EU). 1999. Directive 1999/3/EC of European Parliament and of the Council on the establishment of a Community list of foods and food ingredients treated with ionising radiation. *Off. J. Eur. Communities. L* 66:24-25.
79. The European Union (EU). 2005. Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Union. L* 338:1-25.
80. The European Union (EU). 2007. Council regulation (EC) No 834/2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. *Off. J. Eur. Union. L* 189:1-23.
81. Evans, K., M. Smith, P. McDonough, and M. Wiedmann. 2004. Eye infections due to *Listeria monocytogenes* in three cows and one horse. *J. Vet. Diagn. Invest.* 16:464-469.
82. FAO/WHO. 2004. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: interpretative summary. *Microbiological Risk Assessment Series*; no. 4. 4:48 pp.
83. Farber, J. M., and P. I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Rev.* 55:476-511.
84. Farkas, J. 1998. Irradiation as a method for decontaminating food: A review. *Int. J. Food Microbiol.* 44:189-204.
85. Fedio, W. M., and H. Jackson. 1992. On the Origin of *Listeria monocytogenes* in Raw Bulk-Tank Milk. *Int. Dairy J.* 2:197-208.
86. Felício, M. T., T. Hogg, P. Gibbs, P. Teixeira, and M. Wiedmann. 2007. Recurrent and sporadic *Listeria monocytogenes* contamination in alheiras represents considerable diversity, including virulence-attenuated isolates. *Appl. Environ. Microbiol.* 73:3887-3895.
87. Fenlon, D. R. 1985. Wild birds and silage as reservoirs of *Listeria* in the agricultural environment. *J. Appl. Bacteriol.* 59:537-543.

88. Fenlon, D. R. 1989. Silage as a potential source of *Listeria monocytogenes* in the food chain. *Microbiologie - Aliments - Nutrition*. 7:171-173.
89. Fenlon, D. R., J. Wilson, and W. Donachie. 1996. The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. *J. Appl. Bacteriol.* 81:641-650.
90. Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *The New England Journal of Medicine*. 14:404-407.
91. Foegeding, P. M., A. B. Thomas, D. H. Pilkington, and T. R. Klaenhammer. 1992. Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. *Appl. Environ. Microbiol.* 58:884-890.
92. Fredriksson-Ahomaa, M., M. Gerhardt, and A. Stolle. 2009. High bacterial contamination of pig tonsils at slaughter. *Meat Sci.* 83:334-336.
93. Fretz, R., J. Pichler, U. Sagel, P. Much, W. Ruppitsch, A. T. Pietzka, A. Stöger, S. Huhulescu, S. Heuberger, G. Appl, D. Werber, K. Stark, R. Prager, A. Flieger, R. Karpíšková, G. Pfaff, and F. Allerberger. 2010. Update: Multinational listeriosis outbreak due to 'Quargel', a sour milk curd cheese, caused by two different *L. monocytogenes* serotype 1/2a strains, 2009-2010. *Euro Surveill.* 15:19543.
94. Friedman, M., P. R. Henika, and R. E. Mandrell. 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *J. Food Prot.* 65:1545-1560.
95. Gandhi, M., and M. L. Chikindas. 2007. *Listeria*: A foodborne pathogen that knows how to survive. *Int. J. Food Microbiol.* 113:1-15.
96. Garcia, E., M. De Paz, J. L. Rodriguez, P. Gaya, M. Medina, and M. Nuñez. 1996. Exogenous sources of *Listeria* contamination in raw ewe's Milk. *J. Food Prot.* 59:950-954.
97. Garrec, N., F. Picard-Bonnaud, and A. M. Pourcher. 2003. Occurrence of *Listeria* sp. and *L. monocytogenes* in sewage sludge used for land application: effect of dewatering, liming and storage in tank on survival of *Listeria* species. *FEMS Immunol. Med. Microbiol.* 35:275-283.
98. Gasanov, U., D. Hughes, and P. M. Hansbro. 2005. Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: a review. *FEMS Microbiol. Rev.* 29:851-875.
99. Gaze, J. E., G. D. Brown, D. E. Gaskell, and J. G. Banks. 1989. Heat resistance of *Listeria monocytogenes* in homogenates of chicken, beef steak and carrot. *Food Microbiol.* 6:251-259.
100. Geornaras, I., P. N. Skandamis, K. E. Belk, J. A. Scanga, P. A. Kendall, G. C. Smith, and J. N. Sofos. 2006. Post-processing application of chemical solutions for control of *Listeria monocytogenes*, cultured under different conditions, on commercial smoked sausage formulated with and without potassium lactate-sodium diacetate. *Food Microbiol.* 23:762-771.
101. Gillespie, I., P. Mook, C. Little, K. Grant, and J. McLauchlin. 2010. Human listeriosis in England, 2001-2007: association with neighbourhood deprivation. *Euro Surveill.* 15:19609.
102. Giovannacci, I., C. Ragimbeau, S. Queguiner, G. Salvat, J.-L. Vendeuvre, V. Carlier, and G. Ermel. 1999. *Listeria monocytogenes* in pork slaughtering and cutting plants. Use of RAPD, PFGE and PCR-REA for tracing and molecular epidemiology. *Int. J. Food Microbiol.* 53:127-140.
103. Glaser, P., L. Frangeul, C. Buchrieser, C. Rusniok, A. Amend, F. Baquero, P. Berche, H. Bloecker, P. Brandt, T. Chakraborty, A. Charbit, F. Chetouani, E. Couvé, A. de Daruvar, P. Dehoux, E. Domann, G. Domínguez-Bernal, E. Duchaud, L. Durant, O. Dussurget, K.-D. Entian, H. Fsihi, F. Garcia-Del Portillo, P. Garrido, L. Gautier, W. Goebel, N. Gómez-López, T. Hain, J. Hauf, D. Jackson, L.-M. Jones, U. Kaerst, J. Kreft, M. Kuhn, F. Kunst, G. Kurapkat, E. Madueño, A. Maitournam, J. Mata Vicente, E. Ng, H. Nedjari, G. Nordsiek, S. Novella, B. de Pablos, J.-C. Pérez-Díaz, R. Purcell, B. Rimmel, M. Rose, T. Schlueter, N. Simoes, A. Tierrez, J.-A. Vázquez-Boland, H. Voss, J. Wehland, and P. Cossart. 2001. Comparative genomics of *Listeria* species. *Science.* 294:849-852.
104. Gomber, S., G. Revathi, A. Krishna, and A. Gupta. 1998. Perinephric abscess (presenting as abdominal pain) due to *Listeria monocytogenes*. *Ann. Trop. Paediatr.* 18:61-62.
105. Gorski, L., J. D. Palumbo, and K. D. Nguyen. 2004. Strain-specific differences in the attachment of *Listeria monocytogenes* to alfalfa sprouts. *J. Food Prot.* 67:2488-2495.
106. Goulet, V., C. Hedberg, A. Le Monnier, and H. de Valk. 2008. Increasing incidence of listeriosis in

- France and other European countries. *Emerg. Infect. Dis.* 14:734-740.
107. Goulet, V., C. Jacquet, V. Vaillant, I. Rebière, E. Mouret, C. Lorente, E. Maillot, F. Staïner, and J. Rocourt. 1995. Listeriosis from consumption of raw-milk cheese. *Lancet.* 345:1581-1582.
108. Goulet, V., A. Lepoutre, J. Rocourt, A.-L. Courtieu, P. Dehaumont, and P. Veit. 1993. Épidémie de listériose en France; bilan final et résultats de l'enquête épidémiologique. *Bull. Épidémiol. Hebdomadaire* 39:13-14.
109. Goulet, V., J. Rocourt, I. Rebiere, C. Jacquet, C. Moyse, P. Dehaumont, G. Salvat, and P. Veit. 1998. Listeriosis outbreak associated with the consumption of rillettes in France in 1993. *J. Infect. Dis.* 177:155-160.
110. Graves, L. M., B. Swaminathan, M. W. Reeves, S. B. Hunter, R. E. Weaver, B. D. Plikaytis, and A. Schuchat. 1994. Comparison of ribotyping and multilocus enzyme electrophoresis for subtyping of *Listeria monocytogenes* isolates. *J. Clin. Microbiol.* 32:2936-2943.
111. Graves, L. M., L. O. Hesel, A. G. Steigerwalt, R. E. Morey, M. I. Daneshvar, S. E. Roof, R. H. Orsi, E. D. Fortes, S. R. Milillo, H. C. den Bakker, M. Wiedmann, B. Swaminathan, and B. D. Saunders. 2010. *Listeria marthii* sp. nov., isolated from the natural environment, Finger Lakes National Forest. *Int. J. Syst. Evol. Microbiol.* 60:1280-1288.
112. Gravesen, A., T. Jacobsen, P. L. Møller, F. Hansen, A. G. Larsen, and S. Knøchel. 2000. Genotyping of *Listeria monocytogenes*: comparison of RAPD, ITS, and PFGE. *Int. J. Food. Microbiol.* 43-51.
113. Gray, M. L., H. J. Stafseth, F. Thorp Jr, L. B. Sholl, and W. F. Riley Jr. 1947. A new technique for isolating *Listerellae* from the bovine brain. 471-476.
114. Greer, G. G., F. Nattress, B. Dilts, and L. Baker. 2004. Bacterial contamination of recirculating brine used in the commercial production of moisture-enhanced pork. *J. Food Prot.* 67:185-188.
115. Grif, K., G. Patscheider, M. P. Dierich, and F. Allerberger. 2003. Incidence of fecal carriage of *Listeria monocytogenes* in three healthy volunteers: a one-year prospective stool survey. *Eur. J. Clin. Microbiol. Infect. Dis.* 22:16-20.
116. Gudbjörnsdóttir, B., M.-L. Suihko, P. Gustavsson, G. Thorkelsson, S. Salo, A.-M. Sjöberg, O. Niclasen, and S. Bredholt. 2004. The incidence of *Listeria monocytogenes* in meat, poultry and seafood plants in the Nordic countries. *Food Microbiology.* 21:214-225.
117. Guenther, S., D. Huwyler, S. Richard, and M. J. Loessner. 2009. Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. *Appl. Environ. Microbiol.* 75:93-100.
118. Guillet, C., O. Join-Lambert, A. Le Monnier, A. Leclercq, F. Mechai, M.-F. Mamzer-Bruneel, M. K. Bielecka, M. Scortti, O. Disson, P. Berche, J. Vazquez-Boland, O. Lortholary, and M. Lecuit. 2010. Human listeriosis caused by *Listeria ivanovii*. *Emerg. Infect. Dis.* 16:136-138.
119. Gutiérrez, L., R. Batlle, C. Sánchez, and C. Nerín. 2010. New approach to study the mechanism of antimicrobial protection of an active packaging. *Foodborne Pathog. Dis.* 7:1063-1069.
120. Hagens, S., and M. J. Loessner. 2007. Application of bacteriophages for detection and control of foodborne pathogens. *Appl. Microbiol. Biotechnol.* 76:513-519.
121. Han, Y., R. H. Linton, S. S. Nielsen, and P. E. Nelson. 2001. Reduction of *Listeria monocytogenes* on green peppers (*Capsicum annuum* L.) by gaseous and aqueous chlorine dioxide and water washing and its growth at 7 degrees C. *J. Food Prot.* 64:1730-1738.
122. Hassan, L., H. O. Mohammed, and P. L. McDonough. 2001. Farm-management and milking practices associated with the presence of *Listeria monocytogenes* in New York state dairy herds. *Prev. Vet. Med.* 51:63-73.
123. Hatakka, M., T. Johansson, O. Lyytikäinen, and A. Siitonen. 2000. Listeriosis cases suspected to have been caused by vacuum-packed fish products in Finland. *Euro Surveill.* 4:pii=1623.
124. Hoffman, A. D., K. L. Gall, D. M. Norton, and M. Wiedmann. 2003. *Listeria monocytogenes* contamination patterns for the smoked fish processing environment and for raw fish. *J. Food Prot.* 66:52-60.
125. Hong, E., M. Doumith, S. Duperrier, I. Giovannacci, A. Morvan, P. Glaser, C. Buchrieser, C. Jacquet, and P. Martin. 2007. Genetic diversity of *Listeria monocytogenes* recovered from infected persons and pork, seafood and dairy products on retail sale in France during 2000 and 2001. *Int. J. Food Microbiol.* 114:187-194.
126. Hülphers, G. 1911. Lefvernekros hos kanin orsakad af en ej förut beskriven bakterie. *Svensk Veterinärtidskrift.* 16:265-273.
127. Hunter, P. R., and M. A. Gaston. 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J. Clin. Microbiol.* 26:2465-2466.

128. Husu, J. R. 1990. Epidemiological studies on the occurrence of *Listeria monocytogenes* in the feces of dairy cattle. *J. Vet. Med.* 37:276-282.
129. Husu, J. R., S. K. Sivelä, and A. L. Rauramaa. 1990. Prevalence of *Listeria* species as related to chemical quality of farm-ensiled grass. *Grass and Forage Science.* 45:309-314.
130. Hwang, C.-A., and B. S. Marmer. 2007. Growth of *Listeria monocytogenes* in egg salad and pasta salad formulated with mayonnaise of various pH and stored at refrigerated and abuse temperatures. *Food Microbiol.* 24:211-218.
131. Hwang, C.-A., S. Sheen, and V. K. Juneja. 2009. Effect of salt, smoke compound, and temperature on the survival of *Listeria monocytogenes* in salmon during simulated smoking processes. *J. Food Sci.* 74:M522-529.
132. Hyytiä-Trees, E. K., K. Cooper, E. M. Ribot, and P. Gerner-Smidt. 2007. Recent developments and future prospects in subtyping of foodborne bacterial pathogens. *Future Microbiol.* 2:175-185.
133. Iida, T., M. Kanzaki, T. Maruyama, S. Inoue, and C. Kaneuchi. 1991. Prevalence of *Listeria monocytogenes* in intestinal contents of healthy animals in Japan. *J. Vet. Med. Sci.* 53:873-875.
134. Iida, T., M. Kanzaki, A. Nakama, Y. Kokubo, T. Maruyama, and C. Kaneuchi. 1998. Detection of *Listeria monocytogenes* in humans, animals and foods. *J. Vet. Med. Sci.* 60:1341-1343.
135. ILSI Research Foundation/Risk Science Institute, Expert Panel on *Listeria monocytogenes* in Foods. 2005. Achieving continuous improvement in reductions in foodborne listeriosis – a risk-based approach. *J. Food Prot.* 68:1932-1994.
136. International Organization for Standardization. 1996. ISO 11290-1 Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method. International Standard ISO 11290-1:.
137. International Organization for Standardization. 1998. ISO 11290-2 Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration method. .
138. Islam, M., J. Chen, M. P. Doyle, and M. Chinnan. 2002. Control of *Listeria monocytogenes* on turkey frankfurters by generally-recognized-as-safe preservatives. *J. Food Prot.* 65:1411-1416.
139. Ivanek, R., Y. T. Gröhn, and M. Wiedmann. 2006. *Listeria monocytogenes* in multiple habitats and host populations: review of available data for mathematical modeling. *Foodborne Pathog. Dis.* 3:319-336.
140. Jacobsen, T., B. B. Budde, and A. G. Koch. 2003. Application of *Leuconostoc carnosum* for biopreservation of cooked meat products. *J. Appl. Microbiol.* 95:242-249.
141. Jacquet, C., B. Catimel, R. Brosch, C. Buchrieser, P. Dehaumont, V. Goulet, A. Lepoutre, P. Veit, and J. Rocourt. 1995. Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992. *Appl. Environ. Microbiol.* 61:2242-2246.
142. Jensen, A., W. Frederiksen, and P. Gerner-Smidt. 1994. Risk factors for listeriosis in Denmark, 1989-1990. *Scand. J. Infect. Dis.* 26:171-178.
143. Jensen, N. E., F. M. Aarestrup, J. Jensen, and H. C. Wegener. 1996. *Listeria monocytogenes* in bovine mastitis. Possible implication for human health. *Int. J. Food Microbiol.* 32:209-216.
144. Johannessen, G. S., S. Loncarevic, and H. Kruse. 2002. Bacteriological analysis of fresh produce in Norway. *Int. J. Food Microbiol.* 77:199-204.
145. Johansson, T. 1998. Enhanced detection and enumeration of *Listeria monocytogenes* from foodstuffs and food-processing environments. *Int. J. Food Microbiol.* 40:77-85.
146. Johnsen, B. O., E. Lingaas, D. Torfoss, E. H. Strøm, and I. Nordøy. 2010. A large outbreak of *Listeria monocytogenes* infection with short incubation period in a tertiary care hospital. *J. Infect.* 61:465-470.
147. Junttila, J., and M. Brander. 1989. *Listeria monocytogenes* septicemia associated with consumption of salted mushrooms. *Scand. J. Infect. Dis.* 21:339-342.
148. Junttila, J. R., S. I. Niemelä, and J. Hirn. 1988. Minimum growth temperatures of *Listeria monocytogenes* and non-haemolytic listeria. *J. Appl. Bacteriol.* 65:321-327.
149. Kaneko, K., H. Hayashidani, K. Takahashi, Y. Shiraki, S. Limawongpranee, and M. Ogawa. 1999. Bacterial contamination in the environment of food factories processing ready-to-eat fresh vegetables. *J. Food Prot.* 62:800-804.
150. Kanuganti, S. R., I. V. Wesley, P. G. Reddy, J. McKean, and H. S. Hurd. 2002. Detection of *Listeria*

*monocytogenes* in pigs and pork. J. Food Prot. 65:1470-1474.

151. Kathariou, S. 2002. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. J. Food Prot. 65:1811-1829.

152. Katla, T., K. Naterstad, M. Vancanneyt, J. Swings, and L. Axelsson. 2003. Differences in susceptibility of *Listeria monocytogenes* strains to sakacin P, sakacin A, pediocin PA-1, and nisin. Appl. Environ. Microbiol. 69:4431-4437.

153. Kerdahi, K. F., and P. F. Istafanos. 2000. Rapid determination of *Listeria monocytogenes* by automated enzyme-linked immunoassay and nonradioactive DNA probe. J. AOAC Int. 83:86-88.

154. Keto-Timonen, R., R. Tolvanen, J. Lundén, and H. Korkeala. 2007. An 8-year surveillance of the diversity and persistence of *Listeria monocytogenes* in a chilled food processing plant analyzed by amplified fragment length polymorphism. J. Food Prot. 70:1866-1873.

155. Keto-Timonen, R. O., T. J. Autio, and H. J. Korkeala. 2003. An improved amplified fragment length polymorphism (AFLP) protocol for discrimination of *Listeria* isolates. Syst. Appl. Microbiol. 26:236-244.

156. Korsak, N., G. Daube, Y. Ghafir, A. Chahed, S. Jolly, and H. Vindevogel. 1998. An efficient sampling technique used to detect four foodborne pathogens on pork and beef carcasses in nine Belgian abattoirs. J. Food Prot. 61:535-541.

157. Koseki, S., S. Isobe, and K. Itoh. 2004. Efficacy of acidic electrolyzed water ice for pathogen control on lettuce. J. Food Prot. 67:2544-2549.

158. Kvistholm Jensen, A., S. Ethelberg, B. Smith, E. Møller Nielsen, J. Larsson, K. Mølbak, J. J. Christensen, and M. Kemp. 2010. Substantial increase in listeriosis, Denmark 2009. Euro Surveill. 15:19522.

159. Lado, B. H., and A. E. Yousef. 2007. Characteristics of *Listeria monocytogenes* important to food processors, p. 157-213. In E. T. Ryser and E. H. Marth (eds.), *Listeria*, Listeriosis, and Food Safety, 3rd ed., CRC Press, Boca Raton, FL.

160. Lang, M. M., L. J. Harris, and L. R. Beuchat. 2004. Survival and recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on lettuce and parsley as affected by method of inoculation, time between inoculation and analysis, and treatment with chlorinated water. J. Food Prot. 67:1092-1103.

161. Laukkanen, R., J. Ranta, X. Dong, M. Hakkinen, P. O. Martínez, J. Lundén, T. Johansson, and H. Korkeala. 2010. Reduction of enteropathogenic *Yersinia* in the pig slaughterhouse by using bagging of the rectum. J. Food Prot. 73:2161-2168.

162. Lauková, A., S. Czikková, S. Laczková, and P. Turek. 1999. Use of enterocin CCM 4231 to control *Listeria monocytogenes* in experimentally contaminated dry fermented Hornád salami. Int. J. Food Microbiol. 52:115-119.

163. Lawrence, L. M., and A. Gilmour. 1994. Incidence of *Listeria* spp. and *Listeria monocytogenes* in a poultry processing environment and in poultry products and their rapid confirmation by multiplex PCR. Appl. Environ. Microbiol. 60:4600-4604.

164. Lawrence, L. M., J. Harvey, and A. Gilmour. 1993. Development of a random amplification of polymorphic DNA typing method for *Listeria monocytogenes*. Appl. Environ. Microbiol. 59:3117-3119.

165. Leclercq, A., D. Clermont, C. Bizet, P. A. D. Grimont, A. Le Flèche-Matéos, S. M. Roche, C. Buchrieser, V. Cadet-Daniel, A. Le Monnier, M. Lecuit, and F. Allerberger. 2009. *Listeria rocourtiae* sp. nov. Int. J. Syst. Evol. Microbiol. 60:2210-2214.

166. Lee, S.-Y., M. Costello, and D.-H. Kang. 2004. Efficacy of chlorine dioxide gas as a sanitizer of lettuce leaves. J. Food Prot. 67:1371-1376.

167. Leistner, L. 2000. Basic aspects of food preservation by hurdle technology. Int. J. Food Microbiol. 55:181-186.

168. Lessing, M. P. A., G. D. W. Curtis, and I. C. J. Bowler. 1994. *Listeria ivanovii* infection. J. Infect. 29:230-231.

169. Lewis, H. C., C. L. Little, R. Elson, M. Greenwood, K. A. Grant, and J. McLaughlin. 2006. Prevalence of *Listeria monocytogenes* and other *Listeria* species in butter from United Kingdom production, retail, and catering premises. J. Food Prot. 69:1518-1526.

170. Lindblad, M., H. Lindmark, S. T. Lambertz, and R. Lindqvist. 2007. Microbiological baseline study of swine carcasses at Swedish slaughterhouses. J. Food Prot. 70:1790-1797.

171. Linnan, M. J., L. Mascola, X. D. Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, M. L. Yonekura, P. Hayes, and R. Weaver. 1988. Epidemic listeriosis associated with Mexican-style cheese. N. Engl. J. Med. 319:823-828.

172. Little, C., D. Roberts, E. Youngs, and J. de Louvois. 1999. Microbiological quality of retail imported unprepared whole lettuces: a PHLS Food Working Group Study. Public Health Laboratory Service. *J. Food Prot.* 62:325-328.
173. Little, C. L., F. C. Taylor, S. K. Sagoo, I. A. Gillespie, K. Grant, and J. McLauchlin. 2007. Prevalence and level of *Listeria monocytogenes* and other *Listeria* species in retail pre-packaged mixed vegetable salads in the UK. *Food Microbiol.* 24:711-717.
174. Liu, D. 2006. Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *J. Med. Microbiol.* 55:645-659.
175. Loessner, M. J., C. E. Rees, G. S. Stewart, and S. Scherer. 1996. Construction of luciferase reporter bacteriophage A511::luxAB for rapid and sensitive detection of viable *Listeria* cells. *Appl. Environ. Microbiol.* 62:1133-1140.
176. López-Prieto, M. D., A. I. Aller García, S. Alcaraz García, and J. López Cepero. 2000. Liver abscess due to *Listeria monocytogenes*. *Clin. Microbiol. Infect.* 6:226-227.
177. Low, J. C., and W. Donachie. 1997. A review of *Listeria monocytogenes* and listeriosis. *Vet. J.* 153:9-29.
178. Ludwig, W., K.-H. Schleifer, and W. B. Whitman. 2009. Family III. Listeriaceae, p. 244. *In* Bergey's Manual of Systemic Bacteriology, 2nd ed., vol. 3. Springer, New York, NY.
179. Lukinmaa, S., K. Aarnisalo, M.-L. Suihko, and A. Siitonen. 2004. Diversity of *Listeria monocytogenes* isolates of human and food origin studied by serotyping, automated ribotyping and pulsed-field gel electrophoresis. *Clin. Microbiol. Infect.* 10:562-568.
180. Lukinmaa, S., U. M. Nakari, M. Eklund, and A. Siitonen. 2004. Application of molecular genetic methods in diagnostics and epidemiology of food-borne bacterial pathogens. *APMIS.* 112:908-929.
181. Lundén, J. 2004. Persistent *Listeria monocytogenes* contamination in food processing plants. Academic dissertation. Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland.
182. Lundén, J., T. Autio, A. Markkula, S. Hellström, and H. Korkeala. 2003. Adaptive and cross-adaptive responses of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants. *Int. J. Food Microbiol.* 82:265-272.
183. Lundén, J., J. Björkroth, and H. Korkeala. 2005. Contamination routes and analysis in food processing environments, p. 539-555. *In* H. L. M. Lelieveld, M. A. Mostert, and J. Holah (eds.), Handbook of hygiene control in the food industry. Woodhead Publishing Limited, Cambridge, England.
184. Lundén, J., M. Miettinen, T. Autio, and H. Korkeala. 2000. Persistent *Listeria monocytogenes* strains show enhanced adherence to food contact surface after short contact times. *J. Food Prot.* 63:1204-1207.
185. Lundén, J. M., T. J. Autio, and H. J. Korkeala. 2002. Transfer of persistent *Listeria monocytogenes* contamination between food-processing plants associated with a dicing machine. *J. Food Prot.* 65:1129-1133.
186. Lundén, J. M., T. J. Autio, A. M. Sjöberg, and H. J. Korkeala. 2003. Persistent and nonpersistent *Listeria monocytogenes* contamination in meat and poultry processing plants. *J. Food Prot.* 66:2062-2069.
187. Lyautey, E., A. Hartmann, F. Pagotto, K. Tyler, D. R. Lapen, G. Wilkes, P. Piveteau, A. Rieu, W. J. Robertson, D. T. Medeiros, T. A. Edge, V. Gannon, and E. Topp. 2007. Characteristics and frequency of detection of fecal *Listeria monocytogenes* shed by livestock, wildlife, and humans. *Can. J. Microbiol.* 53:1158-1167.
188. Lyautey, E., D. R. Lapen, G. Wilkes, K. McCleary, F. Pagotto, K. Tyler, A. Hartmann, P. Piveteau, A. Rieu, W. J. Robertson, D. T. Medeiros, T. A. Edge, V. Gannon, and E. Topp. 2007. Distribution and characteristics of *Listeria monocytogenes* isolates from surface waters of the South Nation River watershed, Ontario, Canada. *Appl. Environ. Microbiol.* 73:5401-5410.
189. Lyhs, U., M. Hatakka, N. Mäki-Petäys, E. Hyytiä, and H. Korkeala. 1998. Microbiological quality of Finnish vacuum-packaged fishery products at retail level. *Arch. Lebensmittelhyg.* 49:146-150.
190. Lyytikäinen, O., T. Autio, R. Maijala, P. Ruutu, T. Honkanen-Buzalski, M. Miettinen, M. Hatakka, J. Mikkola, V. J. Anttila, T. Johansson, L. Rantala, T. Aalto, H. Korkeala, and A. Siitonen. 2000. An outbreak of *Listeria monocytogenes* serotype 3a infections from butter in Finland. *J. Infect. Dis.* 181:1838-1841.
191. MacGowan, A. P., K. Bowker, J. McLauchlin, P. M. Bennett, and D. S. Reeves. 1994. The occurrence and seasonal changes in the isolation of *Listeria* spp. in shop bought food stuffs, human faeces, sewage and soil from urban sources. *Int. J. Food Microbiol.* 21:325-334.

192. MacLean, D., J. D. Jones, and D. J. Studholme. 2009. Application of 'next-generation' sequencing technologies to microbial genetics. *Nat. Rev. Microbiol.* 7:287-296.
193. Maiden, M. C. 2006. Multilocus sequence typing of bacteria *Annu. Rev. Microbiol.* 60:561-588.
194. Maiden, M. C., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt. 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms *Proc. Natl. Acad. Sci. U. S. A.* 95:3140-3145.
195. Maijala, R., O. Lyytikäinen, T. Johansson, T. Autio, T. Aalto, L. Haavisto, and T. Honkanen-Buzalski. 2001. Exposure of *Listeria monocytogenes* within an epidemic caused by butter in Finland. *Int. J. Food Microbiol.* 70:97-109.
196. Markkula, A., T. Autio, J. Lundén, and H. Korkeala. 2005. Raw and processed fish show identical *Listeria monocytogenes* genotypes with pulsed-field gel electrophoresis. *J. Food Prot.* 68:1228-1231.
197. Martin, P., C. Jacquet, V. Goulet, V. Vaillant, and H. De Valk. 2006. Pulsed-field gel electrophoresis of *Listeria monocytogenes* strains: the PulseNet Europe Feasibility Study. *Foodborne Pathog. Dis.* 3:303-308.
198. McLauchlin, J., S. M. Hall, S. K. Velani, and R. J. Gilbert. 1991. Human listeriosis and paté: a possible association. *BMJ.* 303:773-775.
199. McLauchlin, J., and J. C. Low. 1994. Primary cutaneous listeriosis in adults: an occupational disease of veterinarians and farmers. *Vet. Rec.* 135:615-617.
200. McLauchlin, J., R. T. Mitchell, W. J. Smerdon, and K. Jewell. 2004. *Listeria monocytogenes* and listeriosis: a review of hazard characterisation for use in microbiological risk assessment of foods. *Int. J. Food Microbiol.* 92:15-33.
201. McLauchlin, J., and C. E. D. Rees. 2009. Genus I. *Listeria*, p. 244-257. *In* *Bergey's Manual of Systematic Bacteriology*, second ed., vol. 3. Springer, New York, NY.
202. Mead, P. S., E. F. Dunne, L. Graves, M. Wiedmann, M. Patrick, S. Hunter, E. Salehi, F. Mostashari, A. Craig, P. Mshar, T. Bannerman, B. D. Sauters, P. Hayes, W. Dewitt, P. Sparling, P. Griffin, D. Morse, L. Slutsker, and B. Swaminathan. 2006. Nationwide outbreak of listeriosis due to contaminated meat. *Epidemiol. Infect.* 134:744-751.
203. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607-625.
204. Mereghetti, L., R. Quentin, N. Marquet-Van Der Mee, and A. Audurier. 2000. Low sensitivity of *Listeria monocytogenes* to quaternary ammonium compounds. *Appl. Environ. Microbiol.* 66:5083-5086.
205. Meyer-Broseta, S., A. Diot, S. Bastian, J. Rivière, and O. Cerf. 2003. Estimation of low bacterial concentration: *Listeria monocytogenes* in raw milk. *Int. J. Food Microbiol.* 80:1-15.
206. Miettinen, H. 2006. *Listeria monocytogenes* in fish farming and processing. Academic dissertation. Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki, Finland.
207. Miettinen, H., A. Arvola, T. Luoma, and G. Wirtanen. 2003. Prevalence of *Listeria monocytogenes* in, and microbiological and sensory quality of, rainbow trout, whitefish, and vendace roes from Finnish retail markets. *J. Food Prot.* 66:1832-1839.
208. Miettinen, H., and G. Wirtanen. 2005. Prevalence and location of *Listeria monocytogenes* in farmed rainbow trout. *Int. J. Food Microbiol.* 104:135-143.
209. Miettinen, M., K. Björkroth, and H. Korkeala. 1999. Characterization of *Listeria monocytogenes* from an ice cream plant by serotyping and pulsed-field gel electrophoresis. *Int. J. Food Microbiol.* 46:187-192.
210. Miettinen, M., A. Siitonen, P. Heiskanen, H. Haajanen, K. Björkroth, and H. Korkeala. 1999. Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold-smoked rainbow trout. *J. Clin. Microbiol.* 37:2358-2360.
211. Miettinen, M. K., L. Palmu, K. J. Björkroth, and H. Korkeala. 2001. Prevalence of *Listeria monocytogenes* in broilers at the abattoir, processing plant, and retail level. *J. Food Prot.* 64:994-999.
212. Moisan, P., G. Andrews A., B. DeBey M., and J. Nietfeld C. 1998. Listeriosis associated with silage feeding in six midwestern cattle herds. *Large Animal Practice.* 19:40-46.
213. Murray, E. G. D., R. A. Webb, and M. B. R. Swann. 1926. A disease of rabbits characterised by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacillus monocytogenes* (n.sp.). *J. Pathol. Bacteriol.* 29:407-439.

214. Nadon, C. A., D. L. Woodward, C. Young, F. G. Rodgers, and M. Wiedmann. 2001. Correlations between molecular subtyping and serotyping of *Listeria monocytogenes*. *J. Clin. Microbiol.* 39:2704-2707.
215. Nascimento, M. S., N. Silva, M. P. Catanozi, and K. C. Silva. 2003. Effects of different disinfection treatments on the natural microbiota of lettuce. *J. Food Prot.* 66:1697-1700.
216. National Center for Biotechnology Information (NCBI). Taxonomy Browser. <http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wvwtax.cgi?name=Eubacteria> Accessed at 29.03.2011.
217. Nelson, K. E., D. E. Fouts, E. F. Mongodin, J. Ravel, R. T. DeBoy, J. F. Kolonay, D. A. Rasko, S. V. Angiuoli, S. R. Gill, I. T. Paulsen, J. Peterson, O. White, W. C. Nelson, W. Nierman, M. J. Beanan, L. M. Brinkac, S. C. Daugherty, R. J. Dodson, A. S. Durkin, R. Madupu, D. H. Haft, J. Selengut, S. Van Aken, H. Khouri, N. Fedorova, H. Forberger, B. Tran, S. Kathariou, L. D. Wonderling, G. A. Uhlich, D. O. Bayles, J. B. Luchansky, and C. M. Fraser. 2004. Whole genome comparisons of serotype 4b and 1/2a strains of the food-borne pathogen *Listeria monocytogenes* reveal new insights into the core genome components of this species. *Nucleic Acids Res.* 32:2386-2395.
218. Nesbakken, T., G. Kapperud, and D. A. Caugant. 1996. Pathways of *Listeria monocytogenes* contamination in the meat processing industry. *Int. J. Food Microbiol.* 31:161-171.
219. Newell, D. G., M. Koopmans, L. Verhoef, E. Duizer, A. Aidara-Kane, H. Sprong, M. Opsteegh, M. Langelaar, J. Threfall, F. Scheutz, J. v. der Giessen, and H. Kruse. 2010. Food-borne diseases — The challenges of 20 years ago still persist while new ones continue to emerge. *Int. J. Food Microbiol.* 139:S3-S15.
220. Nightingale, K. K., Y. H. Schukken, C. R. Nightingale, E. D. Fortes, A. J. Ho, Z. Her, Y. T. Grohn, P. L. McDonough, and M. Wiedmann. 2004. Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. *Appl. Environ. Microbiol.* 70:4458-4467.
221. Nolan, D. A., D. C. Chamblin, and J. A. Troller. 1992. Minimal water activity levels for growth and survival of *Listeria monocytogenes* and *Listeria innocua*. *Int. J. Food Microbiol.* 16:323-335.
222. Nørrung, B., J. K. Andersen, and J. Schlundt. 1999. Incidence and control of *Listeria monocytogenes* in foods in Denmark. *Int. J. Food Microbiol.* 53:195-203.
223. Norton, D. M., J. M. Scarlett, K. Horton, D. Sue, J. Thimothe, K. J. Boor, and M. Wiedmann. 2001. Characterization and pathogenic potential of *Listeria monocytogenes* isolates from the smoked fish industry. *Appl. Environ. Microbiol.* 67:646-653.
224. Norwood, D. E., and A. Gilmour. 1999. Adherence of *Listeria monocytogenes* strains to stainless steel coupons. *J. Appl. Microbiol.* 86:576-582.
225. Novak, J. S., and J. T. Yuan. 2003. Viability of *Clostridium perfringens*, *Escherichia coli*, and *Listeria monocytogenes* surviving mild heat or aqueous ozone treatment on beef followed by heat, alkali, or salt stress. *J. Food Prot.* 66:382-389.
226. Nyfeldt, A. 1929. Etiologie de la mononucléose infectieuse. *Comptes rendus hebdomadaires des séances et mémoires de la Société de biologie et de ses filiales.* (Soc. Biol.) 101:590-592.
227. Odumeru, J. A., S. J. Mitchell, D. M. Alves, J. A. Lynch, A. J. Yee, S. L. Wang, S. Styliadis, and J. M. Farber. 1997. Assessment of the microbiological quality of ready-to-use vegetables for health-care food services. *J. Food. Prot.* 60:954-960.
228. Oliveira, M., J. Usall, I. Viñas, M. Anguera, F. Gatiús, and M. Abadias. 2010. Microbiological quality of fresh lettuce from organic and conventional production. *Food Microbiol.* 27:679-684.
229. Oliver, S. P., B. M. Jayarao, and R. A. Almeida. 2005. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. *Foodborne Pathog. Dis.* 2:115-129.
230. Ooi, S. T., and B. Lorber. 2005. Gastroenteritis due to *Listeria monocytogenes*. *Clin. Infect. Dis.* 40:1327-1332.
231. Orsi, R. H., M. L. Borowsky, P. Lauer, S. K. Young, C. Nusbaum, J. E. Galagan, B. W. Birren, R. A. Ivy, Q. Sun, L. M. Graves, B. Swaminathan, and M. Wiedmann. 2008. Short-term genome evolution of *Listeria monocytogenes* in a non-controlled environment. *BMC Genomics.* 9:539.
232. Papagianni, M. 2003. Ribosomally synthesized peptides with antimicrobial properties: biosynthesis, structure, function, and applications. *Biotechnol. Adv.* 21:465-499.
233. Peccio, A., T. Autio, H. Korkeala, R. Rosmini, and M. Trevisani. 2003. *Listeria monocytogenes* occurrence and characterization in meat-producing plants. *Lett. Appl. Microbiol.* 37:234-238.
234. Pelroy, G., M. Peterson, R. Paranjpye, J. Almond, and M. Eklund. 1994. Inhibition of *Listeria*

- monocytogenes* in cold-process (smoked) salmon by sodium nitrite and packaging method. J. Food Prot. 57:114-119.
235. Pichler, J., P. Much, S. Kasper, R. Fretz, B. Auer, J. Kathan, M. Mann, S. Huhulescu, W. Ruppitsch, A. Pietzka, K. Silberbauer, C. Neumann, E. Gschiel, A. de Martin, A. Schuetz, J. Gindl, E. Neugschwandtner, and F. Allerberger. 2009. An outbreak of febrile gastroenteritis associated with jellied pork contaminated with *Listeria monocytogenes*. Wien. Klin. Wochenschr. 121:149-156.
236. Pirie, J. H. 1940. The genus *Listerella* Pirie. Science. 91:383.
237. Pirie, J. H. H. 1927. A new disease of veld rodents. "Tiger river disease". Publ. S. Afr. Inst. Med. Res. 3:163-186.
238. Porto-Fett, A. C. S., J. E. Call, B. E. Shoyer, D. E. Hill, C. Pshebniski, G. J. Cocoma, and J. B. Luchansky. 2010. Evaluation of fermentation, drying, and/or high pressure processing on viability of *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* spp., and *Trichinella spiralis* in raw pork and Genoa salami. Int. J. Food Microbiol. 140:61-75.
239. Power, E. G. 1996. RAPD typing in microbiology – a technical review. J. Hosp. Infect. 34:247-265.
240. Praakle-Amin, K., M.-L. Hänninen, and H. Korkeala. 2006. Prevalence and genetic characterization of *Listeria monocytogenes* in retail broiler meat in Estonia. J. Food Prot. 69:436-440.
241. Prakash, A., P. Inthajak, H. Huibregtse, F. Caporaso, and D. M. Foley. 2000. Effects of low-dose gamma irradiation and conventional treatments on shelf life and quality characteristics of diced celery. J. Food Sci. 65:1070-1075.
242. Quessy, S., and S. Messier. 1992. Prevalence of *Salmonella* spp., *Campylobacter* spp. and *Listeria* spp. in ring-billed gulls (*Larus delawarensis*). J. Wildl. Dis. 28:526-531.
243. Rajkovic, A., N. Smigic, and F. Devlieghere. 2010. Contemporary strategies in combating microbial contamination in food chain. Int. J. Food Microbiol. 141:S29-42.
244. Rawool, D. B., S. V. S. Malik, I. Shakuntala, A. M. Sahare, and S. B. Barbuddhe. 2007. Detection of multiple virulence-associated genes in *Listeria monocytogenes* isolated from bovine mastitis cases. Int. J. Food Microbiol. 113:201-207.
245. Regan, E. J., G. A. J. Harrison, S. Butler, J. McLauchlin, M. Thomas, and S. Mitchell. 2005. Primary cutaneous listeriosis in a veterinarian. Vet. Rec. 157:207.
246. Robbins, J. B., C. W. Fisher, A. G. Moltz, and S. E. Martin. 2005. Elimination of *Listeria monocytogenes* biofilms by ozone, chlorine, and hydrogen peroxide. J. Food Prot. 68:494-498.
247. Rocourt, J. 1994. *Listeria monocytogenes*: The State of the Science. Dairy, Food and Environmental Sanitation. 14:70-82.
248. Rocourt, J., A. Audurier, A. L. Courtieu, J. Durst, S. Ortel, A. Schrettenbrunner, and A. G. Taylor. 1985. A multi-centre study on the phage typing of *Listeria monocytogenes*. Zentralbl. Bakteriol. Mikrobiol. Hyg. A. 259:489-497.
249. Rocourt, J., and C. Buchrieser. 2007. The genus *Listeria* and *Listeria monocytogenes*: phylogenetic position, taxonomy, and identification, p. 1-20. In E. T. Ryser and E. H. Marth (eds.), *Listeria*, Listeriosis, and Food Safety, 3rd ed., CRC Press, Boca Raton, FL.
250. Rørvik, L. M., E. Skjerve, B. R. Knudsen, and M. Yndestad. 1997. Risk factors for contamination of smoked salmon with *Listeria monocytogenes* during processing. Int. J. Food Microbiol. 37:215-219.
251. Rosenthal, R., P. Vogelbach, M. Gasser, and W. Zimmerli. 2001. Cervical lymphadenitis – a rare case of focal listeriosis. Infection. 29:170-172.
252. Ross, A. I., M. W. Griffiths, G. S. Mittal, and H. C. Deeth. 2003. Combining nonthermal technologies to control foodborne microorganisms. Int. J. Food Microbiol. 89:125-138.
253. Sagong, H.-G., S.-Y. Lee, P.-S. Chang, S. Heu, S. Ryu, Y.-J. Choi, and D.-H. Kang. 2011. Combined effect of ultrasound and organic acids to reduce *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on organic fresh lettuce. Int. J. Food Microbiol. 145:287-292.
254. Sagoo, S. K., C. L. Little, G. Allen, K. Williamson, and K. A. Grant. 2007. Microbiological safety of retail vacuum-packed and modified-atmosphere-packed cooked meats at end of shelf life. J. Food Prot. 70:943-951.
255. Sagoo, S. K., C. L. Little, and R. T. Mitchell. 2001. The microbiological examination of ready-to-eat organic vegetables from retail establishments in the United Kingdom. Lett. Appl. Microbiol. 33:434-439.

256. Sagoo, S. K., C. L. Little, L. Ward, I. A. Gillespie, and R. T. Mitchell. 2003. Microbiological study of ready-to-eat salad vegetables from retail establishments uncovers a national outbreak of salmonellosis. *J. Food Prot.* 66:403-409.
257. Saide-Albornoz, J. J., C. L. Knipe, E. A. Murano, and G. W. Beran. 1995. Contamination of pork carcasses during slaughter, fabrication, and chilling storage. *J. Food Prot.* 58:993-997.
258. Salamina, G., E. Dalle Donne, A. Niccolini, G. Poda, D. Cesaroni, M. Bucci, R. Fini, M. Maldini, A. Schuchat, B. Swaminathan, W. Bibb, J. Rocourt, N. Binkin, and S. Salmaso. 1996. A foodborne outbreak of gastroenteritis involving *Listeria monocytogenes*. *Epidemiol. Infect.* 117:429-436.
259. Samelis, J., A. Kakouri, K. G. Georgiadou, and J. Metaxopoulos. 1998. Evaluation of the extent and type of bacterial contamination at different stages of processing of cooked ham. *J. Appl. Microbiol.* 84:649-660.
260. Sauders, B. D., M. Z. Durak, E. Fortes, K. Windham, Y. Schukken, A. J. Lembo Jr, B. Akey, K. K. Nightingale, and M. Wiedmann. 2006. Molecular characterization of *Listeria monocytogenes* from natural and urban environments. *J. Food Prot.* 69:93-105.
261. Sauders, B. D., K. Mangione, C. Vincent, J. Schermerhorn, C. M. Farchione, N. B. Dumas, D. Bopp, L. Kornstein, E. D. Fortes, K. Windham, and M. Wiedmann. 2004. Distribution of *Listeria monocytogenes* molecular subtypes among human and food isolates from New York State shows persistence of human disease – associated *Listeria monocytogenes* strains in retail environments. *J. Food Prot.* 67:1417-1428.
262. Sauders, B. D., D. Pettit, B. Currie, P. Suits, A. Evans, K. Stellrecht, D. M. Dryja, D. Slate, and M. Wiedmann. 2005. Low prevalence of *Listeria monocytogenes* in human stool. *J. Food Prot.* 68:178-181.
263. Sauders, B. D., and M. Wiedmann. 2007. Ecology of *Listeria* species and *L. monocytogenes* in the natural environment, p. 21-53. In E. T. Ryser and E. H. Marth (eds.), *Listeria, Listeriosis, and Food Safety*. 3rd ed., CRC Press, Boca Raton, FL.
264. Schlech, W. F., 3rd, P. M. Lavigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome. 1983. Epidemic listeriosis – evidence for transmission by food. *N. Engl. J. Med.* 308:203-206.
265. Seeliger, H. P. R. 1961. *Listeriosis*. Hafner Publishing Company, New York. 308 pp.
266. Seeliger, H. P. R., and K. Höhne. 1979. Serotyping of *Listeria monocytogenes* and related species. *Methods Microbiol.* 13:31-49.
267. Semenza, J., J. Suk, and S. Tsoлова. 2010. Social determinants of infectious diseases: a public health priority. *Euro Surveill.* 15:19608.
268. Shetty, A., J. McLauchlin, K. Grant, D. O'Brien, T. Howard, and E. M. Davies. 2009. Outbreak of *Listeria monocytogenes* in an oncology unit associated with sandwiches consumed in hospital. *J. Hosp. Infect.* 72:332-336.
269. Siekkinen, K.-M., L. Nuotio, J. Ranta, R. Laukkanen, S. Hellström, H. Korkeala, and R. Maijala. 2006. Assessing hygiene proficiency on organic and conventional pig farms regarding pork safety: A pilot study in Finland. *Livestock Science.* 104:193-202.
270. Skovgaard, N., and C.-A. Morgan. 1988. Detection of *Listeria* spp. in faeces from animals, in feed, and in raw foods of animal origin. *Int. J. Food Microbiol.* 6:229-242.
271. Skovgaard, N., and B. Nørrung. 1989. The incidence of *Listeria* spp. in faeces of Danish pigs and in minced pork meat. *Int. J. Food Microbiol.* 8:59-63.
272. Smith, B., J. T. Larsson, M. Lisby, L. Muller, S. B. Madsen, J. Engberg, J. Bangsborg, S. Ethelberg, and M. Kemp. 2011. Outbreak of listeriosis caused by infected beef meat from a meals-on-wheels delivery in Denmark 2009. *Clin. Microbiol. Infect.* 17:50-52.
273. Snapir, Y. M., E. Vaisbein, and F. Nassar. 2006. Low virulence but potentially fatal outcome—*Listeria ivanovii*. *Eur. J. Intern. Med.* 17:286-287.
274. Soni, K. A., and R. Nannapaneni. 2010. Bacteriophage significantly reduces *Listeria monocytogenes* on raw salmon fillet tissue. *J. Food Prot.* 73:32-38.
275. Swaminathan, B., and P. Gerner-Smidt. 2007. The epidemiology of human listeriosis. *Microb. Infect.* 9:1236-1243.
276. Szabo, E. A., L. Simons, M. J. Coventry, and M. B. Cole. 2003. Assessment of control measures to achieve a food safety objective of less than 100 CFU of *Listeria monocytogenes* per gram at the point of consumption for fresh pre-cut iceberg lettuce. *J. Food Prot.* 66:256-264.
277. Tagg, J. R., and A. R. McGiven. 1971. Assay system for bacteriocins. *Appl. Microbiol.* 21:943.

278. Terveyden- ja hyvinvoinnin laitos, THL (National institute for health and welfare). 2011. Tartuntatautirekisteri (National infectious diseases register database). <http://www3.ktl.fi/> Accessed at 25.02.2011.
279. Tham, W., H. Ericsson, S. Loncarevic, H. Unnerstad, and M. L. Danielsson-Tham. 2000. Lessons from an outbreak of listeriosis related to vacuum-packed gravad and cold-smoked fish. *Int. J. Food Microbiol.* 62:173-175.
280. Thévenot, D., M. L. Delignette-Muller, S. Christeans, and C. Vernozy-Rozand. 2005. Fate of *Listeria monocytogenes* in experimentally contaminated French sausages. *Int. J. Food Microbiol.* 101:189-200.
281. Thévenot, D., M. L. Delignette-Muller, S. Christeans, and C. Vernozy-Rozand. 2005. Prevalence of *Listeria monocytogenes* in 13 dried sausage processing plants and their products. *Int. J. Food Microbiol.* 102:85-94.
282. Tienungoon, S., D. A. Ratkowsky, T. A. McMeekin, and T. Ross. 2000. Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. *Appl. Environ. Microbiol.* 66:4979-4987.
283. Tolvanen, R., J. Lundén, A. Hörman, and H. Korkeala. 2009. Pilot-scale continuous ultrasonic cleaning equipment reduces *Listeria monocytogenes* levels on conveyor belts. *J. Food Prot.* 72:408-411.
284. Tolvanen, R., J. Lundén, H. Korkeala, and G. Wirtanen. 2007. Ultrasonic cleaning of conveyor belt materials using *Listeria monocytogenes* as a model organism. *J. Food Prot.* 70:758-761.
285. Tompkin, R. B. 2002. Control of *Listeria monocytogenes* in the food-processing environment. *J. Food Prot.* 65:709-725.
286. Truelstrup Hansen, L., and B. F. Vogel. 2011. Desiccation of adhering and biofilm *Listeria monocytogenes* on stainless steel: Survival and transfer to salmon products. *Int. J. Food Microbiol.* 146:88-93.
287. Työppönen, S., A. Markkula, E. Petäjä, M. -. Suihko, and T. Mattila-Sandholm. 2003. Survival of *Listeria monocytogenes* in North European type dry sausages fermented by bioprotective meat starter cultures. *Food Control.* 14:181-185.
288. Työppönen, S., E. Petäjä, and T. Mattila-Sandholm. 2003. Bioprotectives and probiotics for dry sausages. *Int. J. Food Microbiol.* 83:233-244.
289. Uyttendaele, M., P. Busschaert, A. Valero, A. H. Geeraerd, A. Vermeulen, L. Jacxsens, K. K. Goh, A. De Loy, J. F. Van Impe, and F. Devlieghere. 2009. Prevalence and challenge tests of *Listeria monocytogenes* in Belgian produced and retailed mayonnaise-based deli-salads, cooked meat products and smoked fish between 2005 and 2007. *Int. J. Food Microbiol.* 133:94-104.
290. Uyttendaele, M., P. De Troy, and J. Debevere. 1999. Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market. *Int. J. Food Microbiol.* 53:75-80.
291. van Netten, P., I. Perales, A. van de Moosdijk, G. D. Curtis, and D. A. Mossel. 1989. Liquid and solid selective differential media for the detection and enumeration of *L. monocytogenes* and other *Listeria* sp. *Int. J. Food Microbiol.* 8:299-316.
292. Van Renterghem, B., F. Huysman, R. Rygole, and W. Verstraete. 1991. Detection and prevalence of *Listeria monocytogenes* in the agricultural ecosystem. *J. Appl. Bacteriol.* 71:211-217.
293. Vasconcellos, J. A. 2000. Regulatory and Safety Aspects of Refrigerated Minimally Processed Fruits and Vegetables: A Review, p. 319-343. *In* S. M. Alzamora, M. S. Tapia, and A. López-Malo (eds.), *Minimally Processed Fruits and Vegetables*. An Aspen Publication, Gaithersburg, MD.
294. Vázquez-Boland, J. A., M. Kuhn, P. Berche, T. Chakraborty, G. Domínguez-Bernal, W. Goebel, B. González-Zorn, J. Wehland, and J. Kreft. 2001. *Listeria* pathogenesis and molecular virulence determinants. *Clin. Microbiol. Rev.* 14:584-640.
295. Vela, A. I., J. F. Fernandez-Garayzabal, J. A. Vazquez, M. V. Latre, M. M. Blanco, M. A. Moreno, L. de La Fuente, J. Marco, C. Franco, A. Cepeda, A. A. Rodriguez Moure, G. Suarez, and L. Dominguez. 2001. Molecular typing by pulsed-field gel electrophoresis of Spanish animal and human *Listeria monocytogenes* isolates. *Appl. Environ. Microbiol.* 67:5840-5843.
296. Venkitanarayanan, K. S., C. M. Lin, H. Bailey, and M. P. Doyle. 2002. Inactivation of *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* on apples, oranges, and tomatoes by lactic acid with hydrogen peroxide. *J. Food Prot.* 65:100-105.
297. Vitas, A. I., V. Aguado, and V. A. Garcia-Jalon. 2004. Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain). *Int. J. Food Microbiol.* 90:349-356.
298. Vlaemynck, G., V. Lafarge, and S. Scotter. 2000. Improvement of the detection of *Listeria*

- monocytogenes* by the application of ALOA, a diagnostic, chromogenic isolation medium. J. Appl. Microbiol. 88:430-441.
299. Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, and M. Kuiper. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.
300. Waak, E., W. Tham, and M. L. Danielsson-Tham. 2002. Prevalence and fingerprinting of *Listeria monocytogenes* strains isolated from raw whole milk in farm bulk tanks and in dairy plant receiving tanks. Appl. Environ. Microbiol. 68:3366-3370.
301. Wade, W. N., A. J. Scouten, K. H. McWatters, R. L. Wick, A. Demirci, W. F. Fett, and L. R. Beuchat. 2003. Efficacy of ozone in killing *Listeria monocytogenes* on alfalfa seeds and sprouts and effects on sensory quality of sprouts. J. Food Prot. 66:44-51.
302. Wagner, M., B. Auer, C. Trittmel, I. Hein, and D. Schoder. 2007. Survey on the *Listeria* contamination of ready-to-eat food products and household environments in Vienna, Austria. Zoonoses Public. Health. 54:16-22.
303. Walker, S. J., P. Archer, and J. G. Banks. 1990. Growth of *Listeria monocytogenes* at refrigeration temperatures. J. Appl. Bacteriol. 68:157-162.
304. Weis, J., and H. P. Seeliger. 1975. Incidence of *Listeria monocytogenes* in nature. Appl. Microbiol. 30:29-32.
305. Wesley, I. V. 2007. Listeriosis in animals, p. 55-84. In E. T. Ryser and E. H. Marth (eds.), *Listeria, Listeriosis, and Food Safety*. 3rd ed., CRC Press, Boca Raton, FL.
306. Wiedmann, M., J. L. Bruce, C. Keating, A. E. Johnson, P. L. McDonough, and C. A. Batt. 1997. Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. Infect. Immun. 65:2707-2716.
307. Wiedmann, M., and K. Nightingale. 2009. DNA-based subtyping methods facilitate identification of foodborne pathogens. Food Technology. 4:44-49.
308. Winter, C. H., S. O. Brockmann, S. R. Sonnentag, T. Schaupp, R. Prager, H. Hof, B. Becker, T. Stegmans, H. U. Roloff, G. Vollrath, A. E. Kuhm, B. B. Mezger, G. K. Schmolz, G. B. Klittich, G. Pfaff, and I. Piechowski. 2009. Prolonged hospital and community-based listeriosis outbreak caused by ready-to-eat scalded sausages. J. Hosp. Infect. 73:121-128.
309. Wirtanen, G., and S. Salo. 2005. Biofilm risks, p. 46-68. In H. L. M. Lelieveld, M. A. Mostert, and J. Holah (eds.), *Handbook of hygiene control in the food industry*. Woodhead Publishing Limited, Cambridge, England.
310. Yang, H., P. A. Kendall, L. C. Medeiros, and J. N. Sofos. 2009. Efficacy of sanitizing agents against *Listeria monocytogenes* biofilms on high-density polyethylene cutting board surfaces. J. Food Prot. 72:990-998.
311. Yde, M., N. Botteldoorn, S. Bertrand, J. Collard, and K. Dierick. 2010. Microbiological and molecular investigation of an increase of human listeriosis in Belgium, 2006-2007. Euro Surveill. 15:19482.
312. Yoshida, T., T. Sugimoto, M. Sato, and K. Hirai. 2000. Incidence of *Listeria monocytogenes* in wild animals in Japan. J. Vet. Med. Sci. 62:673-675.
313. Zhang, S., and J. M. Farber. 1996. The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. Food Microb. 13:311-321.
314. Åkerstedt, J., and M. Hofshagen. 2004. Bacteriological investigation of infectious keratoconjunctivitis in Norwegian sheep. Acta Vet. Scand. 45:19-26.