PERSISTENT *LISTERIA MONOCYTOGENES* CONTAMINATION IN FOOD PROCESSING PLANTS

JANNE LUNDÉN

Department of Food and Environmental Hygiene
Faculty of Veterinary Medicine
University of Helsinki, Finland

HELSINKI 2004

Department of Food and Environmental Hygiene

Faculty of Veterinary Medicine

University of Helsinki

Finland

PERSISTENT *LISTERIA MONOCYTOGENES* CONTAMINATION IN FOOD PROCESSING PLANTS

JANNE LUNDÉN

ACADEMIC DISSERTATION

To be presented with the permission of the Faculty of Veterinary Medicine, University of Helsinki, for public examination in Auditorium Maximum, Hämeentie 57, Helsinki, on January 16th, 2004 at 12 noon.

Supervising professor

Prof Hannu Korkeala

Department of Food and Environmental Hygiene
Faculty of Veterinary Medicine
University of Helsinki

Supervised by

Prof Hannu Korkeala

Department of Food and Environmental Hygiene
Faculty of Veterinary Medicine
University of Helsinki

CONTENTS

ACKNOWLEDGEMENTS

Finally, I thank my wife Anna and my children Ville and Emma for being incredibly understanding and supportive.

ABBREVIATIONS

AFLP, amplified fragment length polymorphism

BHI, brain heart infusion

CFU, colony forming unit

EPS, exopolysaccharide

MEE, multilocus enzyme electrophoresis

MH, Mueller-Hinton

MIC, minimum inhibitory concentration

MRP, macrorestriction pattern

PCR, polymerase chain reaction

PFGE, pulsed-field gel electrophoresis

QAC, quaternary ammonium compound

RAPD, random amplification of polymorphic DNA

REA, restriction endonuclease analysis

RTE, ready-to-eat

TSB, tryptic soy broth

ABSTRACT

Reasons for persistent *Listeria monocytogenes* food plant contamination were investigated. Properties important in the survival of persistent and non-persistent *L. monocytogenes* strains in food processing plants were examined, and factors in food processing lines that predispose to persistent contamination were identified.

Persistent *L. monocytogenes* strains showed higher adherence levels to stainless steel surfaces than non-persistent strains after short contact times. Because, adherence to stainless steel increases resistance against sanitation procedures, efficient adherence over a short period may have an effect on the initiation of persistent plant contamination.

Differences in initial minimum inhibitory concentrations (MICs) of disinfectants observed between *L. monocytogenes* strains may also have an effect on the survival of these strains in food processing environments. Persistent and non-persistent *L. monocytogenes* strains were observed to adapt to quaternary ammonium compounds (QACs), tertiary alkylamine and sodium hypochlorite at 10ºC and 37ºC. Persistent and non-persistent strains adapted to similar levels. The adaptive response was observed after a 2-h sublethal exposure, indicating rapid response of the cells. The highest increase in resistance was over 15-fold. Although the increased resistance did not exceed the concentrations of disinfectants used at food processing plants, it may influence the survival of cells when the concentration of the disinfectant is sublethal due to inadequate sanitation procedures.

Since, all disinfectants caused cross-adaptation of *L. monocytogenes*, maintaining a high disinfectant efficiency by rotation is difficult. The only disinfectant that *L. monocytogenes* was not observed cross-adapt to was potassium persulphate. However, potassium persulphate caused cross-adaptation of *L. monocytogenes* to the other disinfectants, which reduces the effectiveness of these agents. Cross-adaptation was not seen only to disinfectants with similar mechanisms of action but also to disinfectants with different mechanisms of action, indicating non-specific responses.

Persistent and non-persistent *L. monocytogenes* strains were observed in all meat and poultry processing plants. The persistent strains were often widely spread in the processing

plant, contaminating two or more processing lines. Important factors sustaining contamination were complex processing machines and poor compartmentalization of processing lines. The elimination of *L. monocytogenes* from processing machines was difficult but shown to be possible with regular and thorough disassembly and for example alkali-acid-alkali washes. Compartmentalization, especially the separation of the raw area from the post heat-treatment area, seemed to affect the contamination status of processing lines, with poor compartmentalization increasing contamination.

L. monocytogenes contamination of final food products reflects the contamination status of the manufacturing food plant. Some *L. monocytogenes* pulsed-field gel electrophoresis (PFGE) types were found repeatedly from the product of one producer, indicating a persistent contamination in the food plant. Some PFGE types were also found repeatedly from the products of different producers, indicating persistence of these types in several plants.

In conclusion, persistent *L. monocytogenes* plant contamination appears to be the result of the interaction of several different factors. Properties influencing survival, including enhanced adherence to food contact surfaces and adaptation to disinfectants, in addition to such predisposing factors in the processing line as complex processing machines and poor compartmentalization may lead to persistent *L. monocytogenes* plant contamination.

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original articles referred in the text by the Roman numerals I to V.

I.   Lundén, J. M., M. K. Miettinen, T. J. Autio and H. J. Korkeala. 2000. Persistent *Listeria monocytogenes* strains show enhanced adherence to food contact surface after short contact times. J. Food Prot. 63, 1204-1207.

II   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.

III   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.

IV   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.

V   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.

The original papers have been reprinted with the kind permission from Journal of Food Protection (I, III, IV) and Elsevier (II, V).

1 INTRODUCTION

Listeria monocytogenes was first described in 1924 by Murray et al. (1926). The organism was isolated from rabbits and guinea-pigs and observed to cause monocytosis in the infected animals. This organism originally named *Bacterium monocytogenes*, was reported to be a human pathogen a few years later by Nyfeldt (1929).

Today, the disease listeriosis caused by *L. monocytogenes* is diagnosed regularly. The incidence of listeriosis in developed countries is about 0.2 to 0.8 cases per 100,000 persons annually (Gellin et al. 1991, McLauchlin 1996, Kela and Holmström 2001, Lukinmaa et al. 2003). The incidence is not high, but as the mortality is about 20% (Gellin and Broome 1989), the disease is a public health concern. Listeriosis usually manifests in the elderly, in fetuses or newborns and in individuals with severe underlying diseases. The growing number of people with predisposing factors has increased the size of the population at risk (Schlech III 2000).

The route of transmission of *L. monocytogenes* from foods to humans was finally established at the beginning of the 1980s, when coleslaw was shown by microbiological and epidemiological means to be the vehicle of *L. monocytogenes* in an epidemic in Nova Scotia, Canada (Schlech et al. 1983). After that, several reports have been made of food-borne listeriosis, both epidemics and sporadic cases, due to all kinds of foods (Goulet et al. 1995, Salvat et al. 1995, Loncarevic et al. 1997, Miettinen et al. 1999b, Lyytikäinen et al. 2000).

Although the link between contaminated food and listeriosis in humans was not proved until the 1980s, *L. monocytogenes* was suspected of being transmitted by foods and of causing food-borne epidemics much earlier. The possibility of transmission of *L. monocytogenes* by foods is mentioned by Seeliger in 1961 as follows:

"*Food-borne infection due to Listeria is of considerable epidemiological interest.*"

Observations leading to the above statement were made in Halle, East Germany, where a massive occurrence of listeriosis was identified in the late 1940s to the 1950s.

Consumption of non-pasteurized milk was suspected of being the cause of the outbreak. Seeliger further says:

"The suggested role of infected food, non-pasteurized milk in particular, and probably also meat and game, requires an improvement in and enforcement of control measures."

These words were written at a time when the role of foods as vehicles of *L. monocytogenes* was still obscure. Today, there is no doubt that *L. monocytogenes* is transmitted to humans by a variety of foods. Although the target of preventive measures for decreasing the occurrence of listeriosis is clear, i.e. food processing plants, the eradication of *L. monocytogenes* from foods has been difficult. *L. monocytogenes* contamination has been observed to dominate and persist for extended periods of time in different food processing plants (Rørvik et al. 1995, Unnerstad et al. 1996, Miettinen et al. 1999a). These observations on persistent contaminations set the cornerstones for the present study in which reasons for persistent contamination are sought. The persistent contamination of food processing plants applies a continuous contamination pressure on the products. Increased knowledge is important in successful prevention of *L. monocytogenes*. This study seeks to uncover causes of persistent *L. monocytogenes* contamination.

2 REVIEW OF THE LITERATURE

2.1 *Listeria monocytogenes and listeriosis*

2.1.1 The genus *Listeria*

The genus *Listeria* currently contains six species: *Listeria monocytogenes, Listeria ivanovii, Listeria welshimeri, Listeria innocua, Listeria seeligeri* and *Listeria grayi* (Rocourt 1999, Vazquez-Boland et al. 2001). *L. monocytogenes* and *L. ivanovii* are pathogenic, the former causing disease in humans and animals, and the latter in animals. The other species are non-pathogenic (Vazquez-Boland et al. 2001).

The *Listeria* species are regular Gram-positive non-sporing rods with a diameter of about 0.5 μm and a length of 0.5-2.0 μm. They are facultative anaerobes with no capsule. Moreover, they are catalase-positive, oxidase-negative and motile at 20-25°C due to peritrichous flagella but non-motile at 37°C (Seeliger and Jones 1986).

2.1.2 Growth limits of *Listeria monocytogenes*

L. monocytogenes can grow in a wide temperature range, from -1.5 to 45ºC (Gray and Killinger 1966, Junttila et al. 1988, Hudson et al. 1994). The growth of the organism at -1.5ºC is very slow, with a lag time of 174 h (Hudson et al. 1994). *L. monocytogenes* can grow in laboratory media with a pH ranging from 4.3 (Farber et al. 1989) to 9.6 (Seeliger and Jones 1986). The minimum a_w for growth in a laboratory medium containing glycerol has been reported to be 0.90 (Nolan et al. 1992).

2.1.3 Isolation and identification

The first isolation methods for *L. monocytogenes* included cold enrichment, which was based on *L. monocytogenes* being able to grow at low temperatures (Gray et al. 1947). Cold enrichment was very time-consuming and more rapid methods have subsequently been developed. Modern isolation methods of *Listeria* spp. and *L. monocytogenes* are

based on selective enrichment followed by plating on selective media. Several international method standards, e.g. the standards of the International Standardization Organization (ISO 1996), the International Dairy Federation (IDF 1995) and the Nordic Committee on Food Analysis (NCFA 1999), include a one,- or two-step enrichment followed by plating on two selective plating media.

The identification of *Listeria* spp. is based on Gram-staining and catalase, oxidase and motility tests. The species are distinguished by the haemolysis test, CAMP test and sugar fermentation (Seeliger and Jones 1986). *L. monocytogenes* shows a narrow β-haemolysis on blood media and ferments D-rhamnose but not D-xylose. The CAMP test is positive with β-haemolytic *Staphylococcus aureus*, showing enhanced haemolysis at the intersection of the cultures of *L. monocytogenes* and *S. aureus*. The CAMP-test is negative with *Rhodococcus equi*. Performing the CAMP test is not necessary for the identification of *L. monocytogenes* but can enhance weak haemolysis reactions (Seeliger and Jones 1986). *L. monocytogenes* can also be distinguished from other *Listeria* spp. with commercial biochemical test kits such as API Listeria (Bille et al. 1992).

2.1.4 Subtyping

The subtyping of *L. monocytogenes* isolates offers an approach for investigating the relatedness of isolates and identifying and tracing the sources of epidemics (Miettinen et al. 1999b, Lyytikäinen et al. 2000, de Valk et al. 2001, Sim et al. 2002). Subtyping has also been of great value in identifying sources of contamination in food processing plants (Autio et al. 1999, Berrang et al. 2002, Hoffman et al. 2003, Rørvik et al. 2003). Both phenotyping and molecular typing methods have been applied in the subtyping of *L. monocytogenes* isolates.

Phenotyping methods

Phenotyping of *L. monocytogenes* has been performed by serotyping, phage typing and bacteriocin typing. Serotyping, a classical tool for typing *L. monocytogenes* isolates, is based on differences in the antigenic properties of *L. monocytogenes* isolates (Seeliger and Hohne 1979). *L. monocytogenes* can be divided into 13 serotypes according to somatic (O) and flagellar (H) antigens (Schönberg et al. 1996). Most of the clinical and food isolates

belong to a few serotypes. The method has a poor discriminating power, which limits its use in epidemiological studies that require information on the relatedness of isolates. Serotyping is nevertheless considered useful in dividing *L. monocytogenes* isolates into serogroups in epidemiological studies. Serotyping can be used as a first typing method, but when more discriminatory power is needed, a genotyping method should also be applied (Schönberg et al. 1996, Lukinmaa et al. 2003).

Phage typing is based on the susceptibility of *L. monocytogenes* isolates to certain bacteriophages (Rocourt et al. 1985). Its discriminatory power is high and large numbers of isolates can be typed in a relatively short time. Shortcomings of this method are that some *L. monocytogenes* strains are not typeable (McLauchlin et al. 1996, Ojeniyi et al. 1996) and not all laboratories have access to this method (McLauchlin et al. 1996).

Bacteriocin typing is based on the susceptibility of *L. monocytogenes* isolates to different bacteriocins. Because the method has poor discriminatory power, it is not particularly useful in epidemiological investigations (Curtis and Mitchell 1992).

Molecular typing methods

Several molecular typing methods have been applied to the characterization of *L. monocytogenes* isolates (Bille and Rocourt 1996). Multilocus enzyme electrophoresis (MEE) typing, restriction endonuclease analysis (REA), ribotyping, random amplification of polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) typing were evaluated in a WHO international multicenter subtyping study (Bille and Rocourt 1996). Amplified fragment length polymorphism (AFLP) typing (Fonnesbech Vogel et al. 2001, Autio et al. 2003, Keto-Timonen et al. 2003) and DNA sequence-based typing have also been used to type *L. monocytogenes* (Cai et al. 2002).

DNA macrorestriction analysis by PFGE typing is considered to be the method of choice in the typing of *L. monocytogenes*. This method has a very high discriminatory power and is reproducible. It is also capable of typing *L. monocytogenes* serotype 4b isolates (Brosch et al. 1996). The method is based on the cutting of chromosomal DNA into large fragments with rare cutting restriction endonucleases. The fragments are run in a pulsed-field gel electrophoresis allowing separation of large fragments (Schwartz and Cantor 1984). PFGE

typing has been successfully used in identifying and tracing epidemics (Miettinen et al. 1999b, Lyytikäinen et al. 2000) and contamination routes in food processing plants (Ojeniyi et al. 1996, Unnerstad et al. 1996, Autio et al. 1999, Giovannacci et al. 1999, Miettinen et al. 1999a, 2001b, Senczek et al. 2000, Fonnesbech Vogel et al. 2001, Berrang et al. 2002).

MEE typing has been used in several epidemiological studies (Farber et al. 1991, Rørvik et al. 1995, 2000, Avery et al. 1996, Nesbakken et al. 1996, Buncic et al. 2001). MEE is based on the separation of the soluble metabolic enzymes in a gel electrophoresis, resulting in electrophoretic types. The typeability of the isolates is good, but the discriminatory power is not very high (Caugant et al. 1996). Isolates that have been typed with MEE can further be typed with another method to improve discrimination (Rørvik et al. 2000).

REA is based on cutting chromosomal DNA with frequently cutting restriction endonucleases into fragments. This method has a high discriminatory power, but depending on the enzymes used, may result in numerous fragments, complicating the interpretation of the patterns (Gerner-Smidt et al. 1996).

Ribotyping is based on the restriction of DNA, followed by gel electrophoresis of the fragments obtained. A subset of fragments is visualized by hybridizing with a labelled ribosomal RNA (Stull et al. 1988). Ribotyping shows a good typeability but a limited discriminatory power (Swaminathan et al. 1996), although the discriminatory power can be improved by using different enzymes (de Cesare et al. 2001). Automated ribotyping, which has been used in several epidemiological studies (Tkáčiková et al. 2000, Norton et al. 2001, Berrang et al. 2002, Suihko et al. 2002, Aarnisalo et al. 2003, Hoffman et al. 2003), enables the typing of large amounts of isolates.

RAPD is a polymerase chain reaction (PCR) -based typing method, which randomly amplifies segments of the target DNA by using arbitrarily selected primers (Williams et al. 1990). RAPD shows a high discriminatory power (Boerlin et al. 1995), but obtaining reproducible results is problematic (Wernars et al. 1996). RAPD has been applied in several epidemiological investigations (Lawrence and Gilmour 1995, Giovannacci et al. 1999, Aguado et al. 2001, Fonnesbech Vogel et al. 2001)

AFLP is a PCR-based typing method in which total bacterial DNA is digested by restriction enzymes. An oligonucleotide adapter complementary to the sequence of the restriction site is ligated to the fragment. A subset of fragments are amplified and electrophoresed (Guerra et al. 2002). The discriminatory power of AFLP is similar to that of PFGE (Fonnesbech Vogel et al. 2001, Keto-Timonen et al. 2003).

DNA sequence-based typing methods determine a partial or complete sequence of one or several genes. The data produced by these methods are unambiguous and very sensitive, allowing single nucleotide changes to be observed (Cai et al. 2002).

2.1.5 Listeriosis

Listeriosis in humans is rare, with less than ten cases per one million persons (Gellin et al. 1991, Kela and Holmström 2001). Although listeriosis is uncommon it is of concern because of the high mortality rate of about 20% (Gellin and Broome 1989). Two distinct clinical manifestations of the disease exist, the invasive and the non-invasive form. The invasive form causes life-threatening disease in persons belonging to a specific risk group. This risk group comprises the elderly, pregnant women and people with impaired immune status due to organ transplants or severe underlying disease such as cancer or human immunodeficiency virus (Schlech III 2000, Vazquez-Boland et al. 2001). Clinical signs in adults include sepsis, meningitis and meningoencephalitis. Pregnant women may experience flu-like symptoms, followed by abortion or stillbirth. The infection of neonates can also be of two forms depending on the time of infection and clinical signs (Schuchat et al. 1991). Early onset, which occurs within the first week of birth, is due to infection of the uterus, usually resulting in sepsis. Late onset occurs some weeks after birth, and is probably caused by infection of the birth canal. The clinical presentation in late onset is usually meningitis (Vazquez-Boland et al. 2001). The non-invasive disease causes a self-resolving febrile gastroenteritis, with no predisposing underlying disease detected (Salamina et al. 1996, Miettinen et al. 1999b, Frye et al. 2002).

2.2 *Listeria monocytogenes in foods*

L. monocytogenes is commonly found in different foods. Table 1 presents the prevalence of *L. monocytogenes* in meat, poultry, fish and dairy products. *L. monocytogenes* is frequently found in raw food products. Poultry, in particular, seems to often be contaminated with *L. monocytogenes*, the prevalence being as high as 50% (Lawrence and Gilmour 1994, Miettinen et al. 2001b), but beef and pork can also be highly contaminated (Buncic 1991, MacGowan et al. 1994, Heredia et al. 2001). While *L. monocytogenes* is also found in raw fish and milk, the prevalence is often lower than for meat or poultry (Husu et al. 1990, Rea et al. 1992, Johansson et al. 1999, Jayarao and Henning 2001).

The prevalence of *L. monocytogenes* in processed products varies greatly depending on the product and the study at hand. The ready-to-eat (RTE) foods represent a large variety of foods in which the prevalence of *L. monocytogenes* can range from high to low. Products that are manipulated e.g. sliced are at higher risk for contamination (Uyttendaele et al. 1999a). Cold-smoked and gravad fish have been shown to have a particularly high prevalence (Loncarevic et al. 1996, Keto and Rahkio 1998, Johansson et al. 1999), since *L. monocytogenes* is not destroyed in the processing of these products. The prevalence is higher in vacuum-packed fish products than in products that are not vacuum-packed (Keto and Rahkio 1998). Among processed milk products, soft cheeses are especially susceptible, but *L. monocytogenes* can also be found in other cheeses and processed milk products.

Table 1. Prevalence of *Listeria monocytogenes* in meat, poultry, fish and dairy foods.

Product	Country	Prevalence %	Reference
Meat			
Raw			
Beef	United Kingdom	35	MacGowan et al. 1994
	Mexico	16	Heredia et al. 2001
	Switzerland	6.3	Fantelli and Stephan 2001
Pork	United Kingdom	28	MacGowan et al. 1994
	Switzerland	4.5	Fantelli and Stephan 2001
Minced beef and pork	Yugoslavia	69	Buncic 1991
Processed			
Sausage	Italy	14	Comi et al. 1992
	USA	7.5	Wang and Muriana 1994
Hot-smoked (surface sample)	Yugoslavia	21	Buncic 1991
Hot-smoked (internal sample)	Yugoslavia	ND[a]	Buncic 1991
Full-meat product	Finland	1	Fieandt 1993
Unsliced product	Belgium	1.6	Uyttendaele et al. 1999a
	Belgium	6.7	Uyttendaele et al. 1999a
Pate	Spain	5.4	Dominguez et al. 2001
Fermented sausage	Finland	3.0	Pelttari 1990
	Norway	ND	Rørvik and Yndestad 1991
	Yugoslavia	19	Buncic 1991
Fermented product	USA	3.3	Levine et al. 2001
Different products	USA	3.1	Levine et al. 2001
	Spain	9.2	Aguado et al. 2001
RTE product[b]	Finland	2	Fieandt and Mäkelä 1994
	France	22	Johnson et al. 1990
	Canada	33	Johnson et al. 1990
	USA	ND	Johnson et al. 1990
	New Zealand	1.8	Hudson et al. 1992
Poultry			
Raw			
Broiler	Finland	27	Fieandt 1993
	Finland	62	Miettinen et al. 2001b
	Portugal	41	Antunes et al. 2002
Carcass	Northern Ireland	59	Lawrence and Gilmour 1994
	Belgium	30	Uyttendaele et al. 1999b
	Spain	32	Capita et al. 2001
Ready-to-cook product	Denmark	9.1	Ojeniyi et al. 1996
Processed			
Chicken	New Zealand	13	Hudson et al. 1992
	Northern Ireland	ND	Lawrence and Gilmour 1994
Chicken and turkey	Belgium	25	Uyttendaele et al. 1999b
Turkey	New Zealand	ND	Hudson et al. 1992
Sausages	Denmark	8.8	Ojeniyi et al. 1996
Cooked product	USA	2.1	Levine et al. 2001

Table 1 continued

Product	Country	Prevalence %	Reference
Fish			
Raw	United Kingdom	13	MacGowan et al. 1994
	Finland	ND	Johansson et al. 1999
Processed			
Gravad fish	Switzerland	26	Jemmi 1990[c]
	Iceland	26	Hartemink and Georgsson 1991
	Sweden	21	Loncarevic et al. 1996
	Finland	23	Keto and Rahkio 1998
	Finland	33	Lyhs et al. 1998
	Finland	50	Johansson et al. 1999
	Finland	6	Hatakka et al. 2001
Cold-smoked fish	Switzerland	14	Jemmi 1990[c]
	Sweden	12	Loncarevic et al. 1996
	Finland	14	Keto and Rahkio 1998
	Finland	15	Lyhs et al. 1998
	Finland	17	Johansson et al. 1999
	Spain	22	Dominguez et al. 2001
	Spain	27	Aguado et al. 2001
	Finland	4	Hatakka et al. 2001
	Finland	13	Hatakka et al. 2002
Hot-smoked fish	Switzerland	9	Jemmi 1990[c]
	Sweden	1.5	Loncarevic et al. 1996
	Finland	ND	Keto and Rahkio 1998
	Finland	1	Lyhs et al. 1998
	Finland	2	Johansson et al. 1999
RTE products	Iceland	16	Hartemink and Georgsson 1991
	New Zealand	26	Hudson et al. 1992
	Canada	0.3	Farber 2000[c]
	Canada	ND	Farber 2000
Dairy			
Raw			
Milk	Hungary	3.8	Rodler and Korbler 1989
	United Kingdom	3.6	Greenwood et al. 1991
	Finland	1.7	Husu et al. 1990
	Ireland	4.9	Rea et al. 1992
Bulk tank milk	USA	4.6	Jayarao and Henning 2001
	Finland	2.9	Husu 1990
Dairy silo milk	Sweden	20	Waak et al. 2002
Farm tank milk	Sweden	1	Waak et al. 2002
Pasteurized milk	United Kingdom	1.1	Greenwood et al. 1991
Processed			
Soft cheese	Hungary	ND	Rodler and Korbler 1989
	Italy	1.6	Massa et al. 1990
	United Kingdom	5.9	Greenwood et al. 1991
	Norway	11	Rørvik and Yndestad 1991
	England	0.4	MacGowan et al. 1994

Table 1 continued

Product	Country	Prevalence %	Reference
Dairy			
Processed			
Soft cheese	Australia	3.4	Arnold and Coble 1995
	Finland	ND	Pirhonen 1998
	Europe	6.3	Rudolf and Scherer 2001
Semi-soft cheese	Hungary	ND	Rodler and Korbler 1989
	Europe	7.6	Rudolf and Scherer 2001
Soft and semi-soft	Ireland	ND	Coveney et al. 1994
cheese	Sweden	6	Loncarevic et al. 1995
Fresh cheese	Finland	ND	Saukkonen 1998
	Finland	2.5	Keto and Rantala 1998
	Finland	ND	Pirhonen 1998
Hard cheese	Hungary	ND	Rodler and Korbler 1989
	Europe	4.4	Rudolf and Scherer 2001
Ice cream	United Kingdom	2.0	Greenwood et al. 1991
	Finland	ND	Fieandt and Mäkelä 1994
	Finland	0.5	Miettinen et al. 1999a

[a]ND=Not detected
[b]RTE=ready-to-eat
[c]Imported

2.3 *Listeria monocytogenes* in food processing plants

L. monocytogenes, a common contaminant in food processing plants, has been found in raw materials, in the environment, in the equipment and in final products. *L. monocytogenes* has also been identified in raw areas as well as in post heat-treatment areas. The prevalence of *L. monocytogenes* in the environment and in the equipment of food processing plants is presented in Table 2. The prevalence varies in different food industries, the least contaminated of which appears to be dairy processing plants. However, large differences in prevalence exist between plants within a food industry area. The prevalence also differs considerably in investigations done before and after sanitation, and in those performed in raw and post heat-treatment areas.

Table 2. Prevalence of *Listeria* spp. and *Listeria monocytogenes* in meat, poultry, fish and dairy processing plants.

Food processing plant	Prevalence %		Country	Year	Reference
Sampling site	L [a]	$L.m.$ [b]			
Meat processing plant					
Environ. and equip.	21	NA[c]	USA	1987	Anonymous 1987[d]
Environ. and equip. (raw area)	NA	68	France	NA	Salvat et al. 1995[d]
Environ. and equip.	NA	33	France	NA	Salvat et al. 1995[d]
Environ. and equip. (raw area)	NA	17	France	NA	Salvat et al. 1995[d]
Environ. and equip.	NA	7	France	NA	Salvat et al. 1995[d]
Environ. (raw area)	NA	2.3	France	NA	Chasseignaux et al. 2001
Equip. (raw area)	NA	25	France	NA	Chasseignaux et al. 2001
Environ. (raw area, during activity)	NA	14	France	NA	Chasseignaux et al. 2002[d]
Environ. (raw area, after cleaning)	NA	4	France	NA	Chasseignaux et al. 2002[d]
Equip. (raw area, before cleaning)	NA	53	France	NA	Chasseignaux et al. 2002[d]
Equip. (raw area, after cleaning)	NA	1.4	France	NA	Chasseignaux et al. 2002[d]
Equip. (packing lines)	56	NA	USA	1989	Tompkin et al. 1992[d]
Equip. (packing lines)	41	NA	USA	1990	Tompkin et al. 1992[d]
Equip. (packing lines)	36	NA	USA	1991	Tompkin et al. 1992[d]
Equip. (raw area)	18	13	Greece	NA	Samelis and Metaxopoulos 1999[e]
Equip.	5.4	2.8	Greece	NA	Samelis and Metaxopoulos 1999[e]
Poultry processing plant					
Environ. (raw area)	NA	22	UK	NA	Hudson and Mead 1989
Equip. (raw area)	NA	29	UK	NA	Hudson and Mead 1989
Environ. (raw area)	46	29	Ireland	1992	Lawrence and Gilmour 1994
Environ.	26	15	Ireland	1992	Lawrence and Gilmour 1994
Environ. (raw area)	NA	22	France	NA	Chasseignaux et al. 2001
Equip. (raw area)	NA	16	France	NA	Chasseignaux et al. 2001
Environ. (raw area, during activity)	NA	55	France	NA	Chasseignaux et al. 2002[d]
Environ. (raw area, after cleaning)	NA	27	France	NA	Chasseignaux et al. 2002[d]
Equip. (raw area, before cleaning)	NA	27	France	NA	Chasseignaux et al. 2002[d]
Equip. (raw area, after cleaning)	NA	ND[f]	France	NA	Chasseignaux et al. 2002[d]
Equip.	NA	20	Finland	1996	Miettinen et al. 2001b[d]
Fish processing plant					
Environ. (cold-smoked)	29	29	Norway	1991-92	Rørvik et al. 1995
Environ. (cold-smoked)	NA	11	Finland	NA	Autio et al. 1999
Equip. (cold-smoked)	NA	24	Finland	NA	Autio et al. 1999
Environ. and equip.	7.2	NA	Finland	1996-98	Miettinen et al. 2001a
Environ. and equip.	NA	16	Denmark	1998-99	Fonnesbech Vogel et al. 2001[d]
Environ.	NA	28	USA	1998	Norton et al. 2001[d]
Environ. and equip.	NA	44	USA	2000	Hoffman et al. 2003[d]
Environ. and equip.	NA	1.2	USA	2000	Hoffman et al. 2003[d]

Table 2 continued

Food processing plant	Prevalence %		Country	Year	Reference
Sampling site	L [a]	$L.m.$ [b]			
Dairy processing plant					
Environ. (liquid)	36	ND	Netherlands	NA	Cox et al. 1989[d]
Equip. (liquid)	ND	ND	Netherlands	NA	Cox et al. 1989[d]
Environ. (cheese)	42	3.8	Netherlands	NA	Cox et al. 1989[d]
Equip. (cheese)	12	2.9	Netherlands	NA	Cox et al. 1989[d]
Environ. (ice cream)	57	ND	Netherlands	NA	Cox et al. 1989
Equip. (ice cream)	55	20	Netherlands	NA	Cox et al. 1989
Environ.	19	17	Australia	1988-89	Venables 1989
Environ. and equip. (liquid)	20	11	USA	1987	Charlton et al. 1990[d]
Environ. and equip. (ice cream)	14	5.2	USA	1987	Charlton et al. 1990[d]
Environ. and equip. (cheese)	3.0	1.5	USA	1987	Charlton et al. 1990[d]
Environ. and equip. (cultured milk)	9.3	4.7	USA	1987	Charlton et al. 1990[d]
Environ.	9.3	6.5	USA	NA	Cotton and White 1992[d]
Environ.	18	1.4	USA	NA	Klausner and Donnelly 1991[d]
Environ.	4.7	9.3	France	1988-90	Jacquet et al. 1993
Equip.	ND	11	France	1988-90	Jacquet et al. 1993
Environ.	0-42	NA	USA	NA	Pritchard et al. 1995[d]
Equip.	0-100	NA	USA	NA	Pritchard et al. 1995[d]
Environ. (ice cream)	NA	4.7	Finland	1990-97	Miettinen et al. 1999a
Equip. (ice cream)	NA	5.1	Finland	1990-97	Miettinen et al. 1999a
Environ. (cheese)	ND	ND	Brazil	1999-00	Silva et al. 2003
Equip. (cheese)	25[g]	ND	Brazil	1999-00	Silva et al. 2003
Environ. (cheese)	18	14	Brazil	1999-00	Silva et al. 2003
Equip. (cheese)	ND	ND	Brazil	1999-00	Silva et al. 2003

[a]*Listeria* spp.
[b]*Listeria monocytogenes*
[c]NA=Data not available
[d]Prevalence includes the results of two or more plants
[e]Turkey meat included
[f]ND=Not detected
[g]Milk pipes

2.3.1 Contamination routes and sites of *Listeria monocytogenes*

L. monocytogenes contamination studies have been performed to identify contamination routes and sites in food processing plants and to gain information on how to prevent product contamination (Rørvik et al. 1995, Autio et al. 1999, Miettinen et al. 1999a, 2001b, Norton et al. 2001, Hoffman et al. 2003).

The initial source of contamination in food processing plants appears to be raw materials (Lawrence and Gilmour 1995, Berrang et al. 2002). However, continuous contamination of the plant environment and the final products by raw materials is unlikely as molecular

typing of *L. monocytogenes* isolates has shown that all or some of the types found in raw materials are not seen in the final products (Rørvik et al. 1995, Nesbakken et al. 1996, Autio et al. 1999, Tkáčiková et al. 2000, Norton et al. 2001, Hoffman et al. 2003). Environmental contamination appears to mostly be due to *L. monocytogenes* strains already present in the plant environment (Hoffman et al. 2003), with the processing environment serving as the source of contamination in finished products (Norton et al. 2001).

The contamination sites in meat, poultry, fish and dairy processing plants for the most part are the same, with processing machines standing out. Conveyors are generally contaminated in all plant types, as are processing machines that reduce product size such as slicers (Table 3). Other contaminated machines in different food plants are brining and packing machines and coolers or freezers (Autio et al. 1999, Miettinen et al. 1999a, Hoffman et al. 2003). Identical *L. monocytogenes* types have been found both from the product waste of processing machines or the processing machines themselves and from the final products, indicating that the processing machines have transferred the contamination to the products (Nesbakken et al. 1996, Suihko et al. 2002).

The contamination level has been observed to increase along the processing line (Klausner and Donnelly 1991, Chasseignaux et al. 2001, Rørvik et al. 2003). The contamination level in raw poultry products was higher than that in raw materials, in the processing environment or in the equipment (Chasseignaux et al. 2001), with the level of contamination of poultry carcasses increasing during the processing steps (Rørvik et al. 2003). The increase in contamination might be due to the increase in complex and poorly cleanable processing machines along the line (Klausner and Donnelly 1991).

Table 3. *Listeria monocytogenes* contamination sites in meat, poultry, fish and dairy plants.

Food plant	Contamination site	Reference
Meat	Processing environment	Nesbakken et al. 1996
	Tumbling machine	Samelis et al. 1998, Samelis and Metaxopoulos 1999
	Slicing machine	Suihko et al. 2002
	Conveyor belt	Salvat et al. 1995, Giovannacci et al. 1999, Chasseignaux et al. 2001, Suihko et al. 2002
	Skinning machine	Suihko et al. 2002
	Machines[a]	Salvat et al. 1995
	Mould	Salvat et al. 1995
Poultry	Processing environment	Chasseignaux et al. 2001, Berrang et al. 2002
	Chilling machine	Miettinen et al. 2001b
	Skinning machine	Miettinen et al. 2001b
	Processing machines[a]	Lawrence and Gilmour 1994
	Conveyor belt	Chasseignaux et al. 2001, Miettinen et al. 2001b Berrang et al. 2002
	Cutter	Chasseignaux et al. 2001
Fish	Processing environment	Rørvik et al. 1995, Autio et al. 1999, Rørvik et al. 2000
	Skinning machine	Autio et al. 1999, Johansson et al. 1999, Miettinen et al. 2001a[b], Hoffman et al. 2003
	Cutting table	Norton et al. 2001
	Trimming	Eklund et al. 1995, Johansson et al. 1999
	Brining machine	Autio et al. 1999, Johansson et al. 1999, Miettinen et al. 2001a[b], Norton et al. 2001
	Brining solution	Eklund et al. 1995, Autio et al. 1999, Norton et al. 2001
	Cold smoker	Norton et al. 2001
	Slicing machine	Autio et al. 1999, Johansson et al. 1999, Miettinen et al. 2001a[b], Norton et al. 2001
	Packaging machine	Johansson et al. 1999, Miettinen et al. 2001a[b]
	Conveyor belt	Johansson et al. 1999, Miettinen et al. 2001a[b]
	Cooler	Hoffman et al. 2003
Dairy	Processing environment	Pritchard et al. 1995, Miettinen et al. 1999a
	Milk filler	Pritchard et al. 1995
	Brine pre-filter	Pritchard et al. 1995
	Conveyor	Cotton and White 1992, Pritchard et al. 1995, Miettinen et al. 1999a
	Packing machine	Miettinen et al. 1999a
	Freezer	Pritchard et al. 1995
	Mould	Jacquet et al. 1993

[a]Specific machine not reported
[b]*Listeria* spp.

Drains in the processing areas are often found contaminated with *L. monocytogenes* (Rørvik et al. 1997, Autio et al. 1999, Johansson et al. 1999, Miettinen et al. 2001a, Norton et al. 2001, Hoffman et al. 2003). However, the drains are not considered to be a source of

the contamination, but rather an indicator of *L. monocytogenes* contamination of a processing area (Rørvik et al. 1997, Hoffman et al. 2003).

2.3.2 Persistent plant contamination

Some *L. monocytogenes* strains have been observed to cause food plant contaminations over long periods of time. Prolonged or persistent contaminations have been reported in several food industry areas, with the contamination persisting up to several years (Table 4). Characteristic to the persistent contamination is that not all *L. monocytogenes* strains found cause persistent contamination, some strains are persistent and are found recurrently and others are non-persistent and only recovered sporadically. Another characteristic is that *L. monocytogenes* strains causing persistent contamination are usually not found in raw materials (Rørvik et al. 1995, Nesbakken et al. 1996, Miettinen et al. 1999a). The eradication of persistent contamination has been shown to be difficult but not impossible. Targeted and improved sanitation has led to successful eradication (Miettinen et al. 1999a).

Table 4. Duration of persistent *Listeria monocytogenes* contamination in the food processing industry.

Food industry	Duration of persistent contamination	Reference
Meat industry		
Processing plant	4 years	Nesbakken et al. 1996
Slaughtering and cutting plant	1 year	Giovannacci et al. 1999
Processing plant	2 years	Senczek et al. 2000
Processing plant	3 months	Chasseignaux et al. 2001
Poultry industry		
Processing plant	6 months	Lawrence and Gilmour 1995
Processing plant	9 months	Chasseignaux et al. 2001
Slaughtering plant	16 months	Rørvik et al. 2003
Fish industry		
Cold-smoked fish processing plant	8 months	Rørvik et al. 1995
Cold-smoked fish processing plant	8 months	Fonnesbech Vogel et al. 2001
Cold-smoked fish processing plant	6 months	Norton et al. 2001
Cold-smoked fish processing plant	2 months[a]	Hoffman et al. 2003
Dairy industry		
Cheese plant	7 years	Unnerstad et al. 1996
Ice cream plant	7 years	Miettinen et al. 1999a
Not specified	8 months	Tkáčiková et al. 2000
Fresh sauce industry	17 months	Pourshaban et al. 2000

[a]The plant was investigated earlier (Norton et al. 2001), and the same ribotype was found in both studies, suggesting a persistence of over 2 years.

2.4 Survival of *Listeria monocytogenes* in food processing plants

2.4.1 Adherence and biofilm formation of *Listeria monocytogenes*

The adherence and biofilm formation of bacteria to food contact surfaces have great implications on hygiene because adhered and biofilm cells show increased resistance against stress factors commonly used in the decontamination of food contact surfaces (Frank and Koffi 1990, Ronner and Wong 1993, Oh and Marshall 1995, Aarnisalo et al. 2000). The adherence of cells, which is required for biofilm formation, occurs in a two-stage process (Dunne 2002). The cells first adhere reversibly to the surface mainly due to

20

electrostatic and hydrophobic interactions. This stage is followed by irreversible adherence, which includes the production of exopolysaccharides (EPSs) (Dunne 2002). The EPSs have been described to cement the cells to the surface (Costerton 1999). Biofilms are defined as cells irreversibly adhered to a surface, i.e. cells that are not removed by gentle rinsing, and enclosed in a matrix consisting mainly of EPSs (Donlan 2002).

Adherence to different materials

L. monocytogenes has been shown to adhere to several different food contact materials such as stainless steel, rubber and plastic surfaces. The adhered *L. monocytogenes* cells show increased resistance to cleaning agents, disinfectants and heat (Frank and Koffi 1990, Ronner and Wong 1993, Oh and Marshall 1995, Aarnisalo et al. 2000), all of which are used in the sanitation of the food processing plants.

Differences in adherence of *L. monocytogenes* between food contact materials have been observed, although these differences are small (Beresford et al. 2001). *L. monocytogenes* adheres to stainless steel surfaces in lesser numbers than to rubber or polytetrafluorethylene (Sinde and Carballo 2000) but in higher numbers than to nylon (Blackman and Frank 1996). Buna-N rubber has a bacteriostatic effect on *L. monocytogenes* (Ronner and Wong 1993) and the number of adhered cells on Buna-N rubber has been shown to be lower than on stainless steel (Helke et al. 1993, Ronner and Wong 1993).

Contact time

L. monocytogenes has been demonstrated to adhere to stainless steel, rubber, glass and polypropylene in as little as 20 minutes (Mafu et al. 1990). The organism has also been observed to produce extracellular material (Wirtanen and Mattila-Sandholm 1993) within a one-hour period (Mafu et al. 1990) and a biofilm consisting of cells in two layers on glass surface within 24 hours (Chae and Schraft 2000).

Strain differences in adherence

Differences in the number of adhered cells have been observed between *L. monocytogenes* strains (Ronner and Wong 1993, Norwood and Gilmour 1999, Chae and Schraft 2000,

Kalmokoff et al. 2001). The highest differences in adherence levels between strains are approximately 100-fold (Norwood and Gilmour 1999, Chae and Schraft 2000). Differences in the formation of microcolonies and cell aggregates have also been observed (Kalmokoff et al. 2001).

Temperature

L. monocytogenes has been shown to be capable of adhering to food contact surfaces at a temperature range from 4ºC to 45ºC (Kim and Frank 1994, Smoot and Pierson 1998, Chae and Schraft 2000). Higher adherence to stainless steel was seen at 18ºC than at 4ºC or 30ºC (Norwood and Gilmour 2001). The authors suggested that the optimum adherence at 18ºC could be due to *L. monocytogenes* producing extracellular polymeric substances at 21ºC but not at 10ºC or 35ºC (Herald and Zottola 1988) and having numerous flagella at 20ºC but few at 37ºC (Peel et al. 1988). However, the adherence level has also been demonstrated to be higher at 45ºC than at 10ºC or 30ºC on stainless steel and Buna-N rubber (Smooth and Pierson 1998). This study did not include the test temperature of 18ºC.

Mixed biofilms

L. monocytogenes increases or decreases in biofilms depending on the competing microbes present. The organsim was shown to increase in mixed biofilms with *Pseudomonas fragi* (Sasahara and Zottola 1993), *Pseudomonas fluorescens* (Buchanan and Bagi 1999) and *Flavobacterium* spp. (Bremer et al. 2001). Large amounts of EPSs may improve the conditions for adherence by entrapment (Sasahara and Zottola 1993). The number of *L. monocytogenes* cells has been observed to decrease in a mixed biofilm with *Staphylococcus sciuri* (Leriche and Carpentier 2000) or with *Staphylococcus xylosus* and *Pseudomonas fragi* (Norwood and Gilmour 2001). The difference between the adherence level of *L. monocytogenes* in monoculture and multispecies culture (*S. xylosus* and *P. fragi*) was less than tenfold. The number of *L. monocytogenes* cells in a biofilm with *P. fragi* and *S. sciuri* after 17 days was about 2 % (Norwood and Gilmour 2000) and in a biofilm with *Coryneform*, *Streptococcus* sp., *Pseudomonas* sp. and *Micrococcus* sp. after 25 days around 1 % (Jeong and Frank 1994). In addition to competing microbes, such conditions as temperature influence the proportions of different bacteria on surfaces (Buchanan and Bagi 1999).

2.4.2 Resistance and adaptation of *Listeria monocytogenes* to disinfectants

Resistance

The sanitizing of food processing plants is mainly based on the use of different disinfectants. A wide variety of disinfectants with different mechanisms of action is used (Table 5). The resistance of *L. monocytogenes* to disinfectants has been investigated by determination of minimum inhibitory concentration (MIC). Several studies have shown resistance of *L. monocytogenes* to quaternary ammonium compounds (QACs), with 7% to 26% of strains being resistant (Lemaître et al. 1998, Aase et al. 2000, Mereghetti et al. 2000, Romanova et al. 2002). Resistance has been suggested to be due to the action of an efflux pump (Aase et al. 2000) or to modifications in the cell wall (Mereghetti et al. 2000). Multiple resistance to QAC benzalkonium chloride, hexamidine diisethionate and ethidium bromide has also been observed (Lemaître et al. 1998). This resistance was concluded to be associated with plasmids.

Table 5. Mechanism of action of disinfectants used in the food processing industry.

Disinfectant	Mechanism of action
Quaternary ammonium compounds	Damage of phospholipid bilayers of cytoplasmic membrane
Peroxygens	Disruption of thiol groups in proteins and enzymes
Halogens	Inhibition of DNA synthesis Oxidation of thiol groups
Alcohols	Membrane damage Denaturation of proteins

Adapted from McDonnell and Russell 1999

Adaptation and cross-adaptation

Adaptation to a disinfectant occurs when bacteria are exposed to sublethal concentrations of the disinfectant in question (Gandhi et al. 1993, Aase et al. 2000). *L. monocytogenes* has been observed to adapt to benzalkonium chloride with up to a sixfold increase in resistance

(Aase et al. 2000, Romanova et al. 2002, To et al. 2002). QACs are widely used in the food processing industry and an increase in resistance to these agents is concerning. In one study, all strains reached similar MIC values due to adaptation, and the increased resistance remained during a one-week follow-up period (Aase et al. 2000). Efflux pumps have been suggested to be responsible for the adaptive responses in the initially sensitive strains, whereas changes in fatty acid profile might have been the cause of the increased resistance in the resistant strains.

Cross-adaptation between disinfectants may occur due to specific or non-specific changes and lead to increased resistance to agents with similar or differing modes of action (McDonnell and Russell 1999). Cross-adaptation has been recognized in *Pseudomonas aeruginosa* between QACs (Loughlin et al. 2002), in *Pseudomonas aeruginosa* between a QAC and chlorhexidine (Jones et al. 1989) and in *Serratia marcescens* between chlorhexidine gluconate and benzalkonium chloride (Gandhi et al. 1993).

2.4.3 Persistent *Listeria monocytogenes* strains

L. monocytogenes can contaminate food processing plants for extended periods of time, and the contamination is often caused by a few dominating strains or persistent strains (Lawrence and Gilmour 1995, Rørvik et al. 1995, Nesbakken et al. 1996, Unnerstad et al. 1996, Autio et al. 1999, Giovannacci et al. 1999, Miettinen et al. 1999a, 2001b, Pourshaban et al. 2000, Senczek et al. 2000, Chasseignaux et al. 2001, Fonnesbech Vogel et al. 2001, Norton et al. 2001, Hoffman et al. 2003). Because some *L. monocytogenes* strains cause persistent food plant contaminations and some are non-persistent, other differences likely exist between these strains.

Some phenotypic properties appear to differ between persistent and non-persistent strains. Persistent strains from dairy and non-dairy foods were observed to show significantly higher adherence to stainless steel surfaces than non-persistent strains (Norwood and Gilmour 1999). Differences in susceptibility to disinfectants between persistent and non-persistent *L. monocytogenes* strains have also been suspected to exist (Aase et al. 2000), although not all studies have been able to show that persistence is due to disinfectant resistance (Earnshaw and Lawrence 1998, Holah et al. 2002). Cadmium resistance and

monocin E production have been observed in persistent strains belonging to serogroup 1. Monocin E is active against *L. monocytogenes* serotypes 4a, 4b and 4c, as well as *L. ivanovii*, which may give the producing strains an advantage over other bacteria in the same ecological niche (Harvey and Gilmour 2001).

3 AIMS OF THE STUDY

The objectives of the present studies were to investigate causes of persistent *L. monocytogenes* food plant contamination. Specific aims were as follows:

1. to study the adherence of persistent and non-persistent *L. monocytogenes* strains to stainless steel surface (I).

2. to investigate resistance and adaptation of persistent and non-persistent *L. monocytogenes* strains to disinfecting agents (II, III).

3. to examine cross-adaptation of persistent and non-persistent *L. monocytogenes* strains to disinfectants and to clarify whether rotation of disinfectants is useful (II).

4. to investigate persistent and non-persistent *L. monocytogenes* contamination in food processing plants (III, IV).

5. to identify factors in the processing plant and the processing line that predispose to persistent plant contamination (III, IV).

6. to characterize *L. monocytogenes* food isolates originating from different sources in order to evaluate possible persistence in foods (V).

4 MATERIALS AND METHODS

4.1 *Listeria monocytogenes* strains (I-V)

L. monocytogenes strains used in Studies I (17 strains from the environment, equipment and products) and II (4 strains from the environment and products) were isolated in an ice cream plant and in poultry plants (I, Table 1; II, Table 1). *L. monocytogenes* strains in Study III (39 strains from the equipment and products) were obtained in 1997-2000 as a result of sampling by the meat processing plants. *L. monocytogenes* strains in Study IV (596 strains from the environment, equipment, products and raw materials) were obtained from meat processing plants and a poultry processing plant in 1997-2001 as a result of sampling by the meat and poultry processing plants. The *L. monocytogenes* strains (295) investigated in Study V were isolated in 1988-1999 and originated from products manufactured by different producers in different countries. Cultures were maintained at -70ºC.

4.2 Serotyping of *Listeria monocytogenes* strains (I-V)

Serotyping was done according to the manufacturer's instructions (Denka Seiken, Tokyo, Japan) with some modifications. Determination of the flagellar H-antigens (A, B, C and D) was performed at 26°C instead of 30°C in brain heart infusion (BHI) agar tubes for increased motility. All strains were serotyped in Studies I to III. One to four strains from each PFGE type in Studies IV and V were randomly selected for serotyping.

4.3 DNA isolation and pulsed-field gel electrophoresis (PFGE) typing (I-V)

L. monocytogenes was grown overnight on blood agar for 24 h at 37ºC, after which one colony was inoculated into BHI broth (Difco, Detroit, MI, USA). Cells were harvested from 2 ml of BHI broth after overnight incubation at 37ºC. The cells were resuspended in PIV [10 mM Tris (pH 7.5), 1 M NaCl] and the cell suspension was mixed with an equal volume of 2% low melting point agarose (InCert agarose; FMC Bioproducts, Rockland,

ME, USA). The plugs were formed in GelSyringe dispensers (New England Biolabs, Beverly, MA, USA). Lysis of the cells was performed in lysis solution [6 nM Tris (pH 7.5), 1 M NaCl, 100 mM EDTA (pH 8.0), 0.5% Brij 58 (Sigma, St. Louis, MO, USA), 0.2% deoxycholate, 0.5% sodium laurolyl sarcocine, 20 μg of Rnase/ml (Sigma)] for 3 h at 37ºC with gentle shaking. The lysis was continued in a 1-h wash with ESP [0.5 M EDTA (pH 8.0), 10% sodium laurolyl sarcosine, 100 μg of proteinase K/ml (Finnzymes, Helsinki, Finland)] at 50ºC followed by a 1-h TE wash. The inactivation of proteinase K was accomplished by 1 mM Pefabloc SC (Roche Diagnostics, Mannheim, Germany) at 37ºC overnight. The agarose-embedded DNA was digested with the restriction enzymes *Asc*I (New England Biolabs) and *Apa*I (Boehringer Mannheim, Mannheim, Germany) (I-V) and *Sma*I (New England Biolabs) (I) according to the manufacturer's instructions. The samples were electrophoresed through a 1.0% (wt/vol) agarose gel (Seakem Gold; FMC Bioproducts, Rockland, ME, USA) in 0.5X TBE [45 mM Tris, 4.5 mM boric acid (pH 8.3), and 1 mM sodium EDTA] at 200 V at 10ºC (I, II) or 14ºC (III-V) in a Gene Navigator system with a hexagonal electrode (Pharmacia, Uppsala, Sweden). Pulse times for *Asc*I and *Apa*I ranged from 0.5 to 29.5 s for 20 h (I, II) or from 1 to 35 s for 18 h (III-V) and for *Sma*I from 0.5 to 18 s for 20 h (I). The gels were stained with ethidium bromide, and visualization and digital photographing were performed with an Alpha Imager 2000 documentation system (Alpha Innotech, San Leandro, CA, USA). Mid-range PFGE marker I and Lambda Ladder PFGE marker (New England Biolabs) (I, II) or Low Range PFG marker (New England Biolabs) (III-V) were used for fragment size determination.

4.4   Analysis of PFGE patterns (I-V)

Every restriction enzyme profile was given a number for all restriction enzymes. The PFGE type was obtained by combining two (II-IV) or three (I) restriction enzyme profiles into one unique profile. A PFGE pattern was considered unique when one or more bands differed from other PFGE patterns. In Study V, the macrorestriction patterns (MRP) were analysed with GelComparII software (Applied Maths, Kortrijk, Belgium). Similarity analysis was performed using the Dice coefficient. Optimal position tolerance for *Asc*I and *Apa*I patterns was 1.0% and 1.2%, respectively. Clustering was performed by the unweighted pair group method using arithmetic averages. The faithfulness of cluster analysis was estimated by calculation of the cophnetic correlation.

28

4.5 Persistent and non-persistent strains (I-IV)

L. monocytogenes strains that were found repeatedly (five times or more) over a period of three months or more were considered to be persistent strains. Strains found sporadically (less than five times) or within a limited time period (less than three months) were considered to be non-persistent strains.

4.6 Adherence of cells to stainless steel surface (I, III)

The stainless steel surfaces (type 316, Outokumpu Steel, Tornio, Finland) (20 x 70 x 1 mm in Study I; 5 x 15 x 1 mm in Study III) were cleaned before the adherence test by immersion in 1N NaOH for 24 h. The surfaces were then rinsed in distilled water and immersed in acetone for 1 h to remove possible grease. This was followed by rinsing in distilled water and air-drying. The surfaces were placed vertically in glass jars (I) or test tubes (III) and autoclaved. The strains were cultured to log phase in tryptic soy broth (TSB) at 25ºC. TSB was inoculated with cells in log phase to attain a test suspension with a concentration of 9 x 10^5 cfu/ml (I) or 6 x 10^5 cfu/ml (III). The test suspension was transferred into the autoclaved glass jars (I) or test tubes (III) with the surfaces. The contact times were 1, 2 and 72 h (I) or 2 h (III) at 25ºC with a shaking frequency of 70/min (Promax 2020, Heidolph, Germany) (I). After the contact time the coupons were removed from the test suspension and vortexed at speed one (Cyclo-Mixer, Clay Adams, Parsippany, NJ, USA) for 5 s in a test tube containing 50 ml of sterile water (I) or rinsed in sterile water (III). The surfaces were stained with filter-sterilized (Minisart 0.2 µm, Sartorius, Göttingen, Germany) 0.05% acridine orange (Certistain, Merck, Darmstadt, Germany) for 2 min followed by rinsing and air-drying. Adhered cells were enumerated with an epifluorescence microscope (Optiphot-2, Nikon, Japan). A minimum of 30 fields (I, III) and 10 fields (I) were observed on every short contact surface and long contact surface, respectively. The tests were repeated 3 (I) or 6 (III) times.

4.7 Disinfecting agents (II, III)

The following disinfectants were used in Study II: alkyl-benzyl-dimethyl ammonium chloride (QAC) (Goldschmidt, Pandino, Italy), n-alkyldimethyl ethylbenzyl ammonium chloride (QAC) (Pointing Chemicals, Huddersfield, UK), 1,3-propanediamine-N-(3-aminopropyl)N-dodecyl (tertiary alkylamine) (Lonza, Basle, Switzerland), sodium hypochlorite (active chlorine 10%) (Finnish Chemicals, Äetsä, Finland) and potassium persulphate (Degussa, Hanau, Germany), and in Study III: sodium hypochlorite (active chlorine 10%) (Finnish Chemicals, Äetsä, Finland) and alkyl-benzyl-dimethyl ammonium chloride (Goldschmidt, Pandino, Italy). Filter-sterilized (Minisart 0.45 μm, Sartorius, Göttingen, Germany) stock solutions with a concentration of 10% (w/w) were prepared for all disinfectants and a stock solution of 25% (w/w) for sodium hypochlorite (II, III). The stock solutions were stored at 2ºC and monitored by susceptibility testing (MIC) using *L. monocytogenes* strains as control strains (II).

4.8 Minimum inhibitory concentration (II, III)

Disinfectant resistance of *L. monocytogenes* strains was determined with the microdilution broth method according to the National Committee on Clinical Laboratory Standards (NCCLS 1999). Five colonies grown on blood agar were inoculated into Mueller-Hinton (MH) broth (Difco, Detroit, MI, USA). The suspension was incubated at 37ºC until the concentration was approximately 1-2 x 10^8 cells/ml. The suspension was diluted and inoculated into microwells, resulting in a final concentration of 5 x 10^5 cfu/ml in the wells. The concentration of the suspension was confirmed by plating. Serial twofold dilutions of the disinfectants in four parallel microwells were used for the determination of MIC. The microwell plate was incubated at 37ºC for 20 h, followed by visual analysis of growth. The MIC was determined as the lowest concentration of the disinfecting agent to prevent growth.

4.9 Adaptation and cross-adaptation to disinfectants (II)

4.9.1 Adaptation after 2-h sublethal exposure

The adaptation of four *L. monocytogenes* strains (II, Table 1) were tested by exposure to sublethal concentrations of the disinfectants listed in section 4.7. A suspension of each *L. monocytogenes* strain in MH broth was prepared as described in section 4.8. to give a final concentration of 5 x 10^5 cfu/ml in the microwells. The strains were pre-exposed to sublethal disinfectant concentrations (MIC/8 and MIC/4) at 37ºC for 2 h, followed by exposure to disinfectants at concentrations of 1 x MIC, 2 x MIC or 3 x MIC at 37ºC for 24 h (pre-exposed challenged cells). Each strain was also challenged with disinfectants at concentrations of 1 x MIC, 2 x MIC or 3 x MIC at 37ºC for 24 h without pre-exposure (not pre-exposed challenged cells). Strains not subjected to disinfectants at any time served as controls. Growth was monitored with a Bioscreen C analyser (Labsystems, Helsinki, Finland), which measures turbidity by vertical pathway spectrophotometry. The test was performed twice in four parallel wells.

4.9.2 Adaptation and cross-adaptation after repeated exposure

Strains 41 and 35 (II, Table 1) were exposed to increasing concentrations of the disinfectants listed in section 4.7. The test was performed in microwell plates. A suspension of each *L. monocytogenes* strain in MH broth was prepared as described in section 4.8. The strains were first exposed to a sublethal concentration (MIC/2), after which the concentration was increased. When growth was observed, 20 µl of the suspension was transferred to the next well, which after the transfer contained a concentration of the disinfectant 1.5 times higher than the previous well. This procedure was continued until no growth was observed in the last well after a 3-day incubation. The test was performed twice, both at 10ºC and 37ºC. The suspension of the last well with recorded growth was centrifuged, and the pellet was washed with phosphate-buffered saline to remove any disinfectant. The pellet was resuspended in MH broth and incubated at 37ºC to achieve a concentration of approximately 10^8 cfu/ml. Purity of the culture was controlled by streaking the suspension on a blood agar plate. MICs of all disinfectants were

measured to determine the level of adaptation and possible cross-adaptation of *L. monocytogenes* strains.

The stability of increased resistance was investigated by subculture of adapted strains in MH broth every 48 h for 28 days, both at 37ºC and 10ºC, followed by determination of MICs every seventh day.

4.10 *Statistical analysis (III)*

Student's *t* test was applied in statistical analysis of the adherence of strains.

5 RESULTS

5.1 Adherence of persistent and non-persistent *Listeria monocytogenes* strains to stainless steel surface (I, III)

The adherence of 3 persistent and 14 non-persistent *L. monocytogenes* strains to a stainless steel surface after 1, 2 and 72 h was investigated (I). The poultry plant strains and most of the ice cream plant strains showed higher adherence than non-persistent strains after 1- and 2-h contact times. The adherence of the strains after a 2-h contact time is presented in Figure 1.
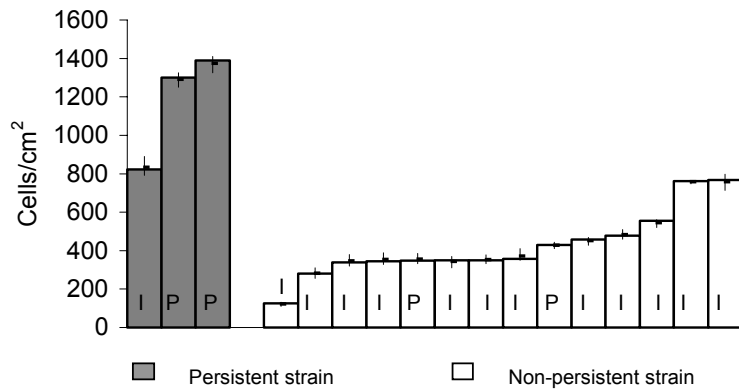
FIGURE 1. Adherence of persistent and non-persistent *Listeria monocytogenes* ice cream plant (I) and poultry plant (P) strains to a stainless steel surface after a 2-h contact time.

The adherence level of the strains following a 72-h contact time was higher than that after short contact times. Several non-persistent strains reached the adherence levels of persistent strains after the 72-h contact time, and three non-persistent strains had a higher adherence level than the persistent strains. The non-motile strain, which had the lowest adherence level at short contact times, showed a higher adherence level than most of the other strains at 72 h.

The persistent strain from the dicing line in the meat processing plant showed a significantly higher adherence to a stainless steel surface after a 2-h contact time that the three non-persistent strains obtained from the same line (III).

5.2 Resistance of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants (II, III)

Initial resistance of persistent and non-persistent *L. monocytogenes* strains to disinfectants was investigated by determining MIC values. Persistent strain 41 had the highest MIC value of the QACs, whereas non-persistent strain 35 had the highest MIC value of the

tertiary alkylamine and sodium hypochlorite (Table 6) (II). No differences were seen in the MICs of potassium persulphate.

Table 6. Minimum inhibitory concentrations (MICs) of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants.

Disinfectant	Strain[a]	MIC (μg/ml)
QAC 1[b]	2919	0.63
	2904, 35	1.25
	41	5
QAC 2[c]	2919, **2904**	1.25
	35	0.63
	41	2.5
Tertiary alkylamine[d]	2919	1.25
	2904	2.5
	35	5
	41	2.5
Potassium persulphate	2919, **2904**, 35, **41**	2500
Sodium hypochlorite[e]	2919, **2904, 41**	2500
	35	5000

[a] Persistent strains are indicated in boldface
[b] Quaternary ammonium compound (alkyl-benzyl-dimethyl ammonium chloride)
[c] Quaternary ammonium compound (n-alkyldimethyl ethylbenzyl ammonium chloride)
[d] 1,3-propanediamine-N-(3-aminopropyl)N-dodecyl
[e] Results represent the MICs of sodium hypochlorite. The active chlorine concentration was 10%.

The persistent and non-persistent strains obtained from the dicing line (III) showed similar MIC values (2500 μg/ml) of sodium hypochlorite and similar MIC values (5 μg/ml) of the QAC (alkyl-benzyl-dimethyl ammonium chloride), with the exception of one non-persistent strain, which was more sensitive than the others (1.25 μg/ml).

5.3 Adaptation of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants (II)

Both persistent (41, 2904) and non-persistent (35, 2919) *L. monocytogenes* strains adapted to QACs and to tertiary alkylamine after a 2-h sublethal exposure. The increase in resistance was two- to threefold. The highest increase was observed in strains with low initial MICs. No increase was observed after the 2-h sublethal exposure in the MICs of potassium persulphate and sodium hypochlorite. Exposure to the sublethal concentrations of MIC/8 and MIC/4 resulted in similar adaptive responses.

Repeated exposure to disinfectants at 10ºC and 37ºC resulted in adaptation of *L. monocytogenes* to all other disinfectants except potassium persulphate (Table 7). The highest increases were observed in strains that showed low initial MICs. The persistent and non-persistent strains reached similar final MIC values as a result of adaptation.

The increased resistance of sodium hypochlorite was lost in one week. However, for QAC 1, it persisted for the 28-day period, and for QAC 2 and the tertiary alkylamine, it decreased from 10 μg/ml to 5 μg/ml in seven days but showed no further decrease. The initial MIC of potassium persulphate persisted throughout the 28-day period.

Table 7. Minimum inhibitory concentrations (MICs) of a persistent (41) and a non-persistent (35) *Listeria monocytogenes* strain before exposure and after repeated exposure to disinfectants.

Disinfectant	Strain	MIC (µg/ml) before exposure	MIC (µg/ml) after repeated exposure
			10ºC / 37ºC
QAC 1[a]	35	1.25	5 / 5
	41	5	5 / 5
QAC 2[b]	35	0.63	5 / 10
	41	2.5	10 / 10
Tertiary alkylamine[c]	35	5	5 / 5
	41	2.5	10 / 5
Potassium persulphate	35	2500	1250 / 2500
	41	2500	1250 / 2500
Sodium hypochlorite[d]	35	5000	5000 / 5000
	41	2500	5000 / 5000

[a] Quaternary ammonium compound (alkyl-benzyl-dimethyl ammonium chloride)
[b] Quaternary ammonium compound (n-alkydimethyl ethylbenzyl ammonium chloride)
[c] 1,3-propanediamine-N-(3-aminopropyl)N-dodecyl
[d] Results represent the MICs of sodium hypochlorite. The active chlorine concentration was 10%.

5.4 Cross-adaptation of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants (II)

All disinfectants, except for potassium persulphate, caused cross-adaptation to other disinfectants. However, potassium persulphate caused cross-adaptation of *L. monocytogenes* to the other disinfectants. The increase in the resistance caused by cross-adaptation was similar to or smaller than the increase in resistance due to adaptive responses. The highest increase in MIC values was eightfold.

5.5 *Listeria monocytogenes* contamination of food processing plants (III, IV)

5.5.1 Distribution of persistent and non-persistent strains (IV)

In total, 596 *L. monocytogenes* isolates from three meat processing plants and one poultry processing plant were characterized by serotyping and PFGE typing to investigate persistent and non-persistent contamination. PFGE typing, which was performed with restriction enzymes *Asc*I and *Apa*I, resulted in 47 types. Nineteen PFGE types were persistent and 28 non-persistent. Nine of the persistent types were found to be non-persistent in another plant. Most of the PFGE types were only found in one plant, but some were common to 2 or 3 plants. Common PFGE types were found in both meat and poultry processing plants. The distributions of the persistent and non-persistent strains in different sampling sites are presented in Table 8, and those in heat-treated and raw products are shown in Table 9.

Table 8. Distribution of persistent and non-persistent *Listeria monocytogenes* PFGE types in different sampling sites in plants A, B, C and D.

Sampling site	Persistent or non-persistent PFGE type (No. of PFGE types)
Processing environment	Persistent (0) Non-persistent (9)
Equipment	Persistent (0) Non-persistent (16)
Product	Persistent (1) Non-persistent (9)
Processing environment and product	Persistent (0) Non-persistent (1)
Equipment and product	Persistent (6) Non-persistent (4)
Processing environment and equipment	Persistent (5) Non-persistent (4)
Processing environment, equipment and product	Persistent (8) Non-persistent (1)

The proportion of persistent PFGE types was higher than that of the non-persistent strains in cooked products. In non-heat-treated products, the reverse was true.

Table 9. Distribution of persistent and non-persistent *Listeria monocytogenes* PFGE types in heat-treated and non-heat-treated products (including raw materials).

Heat treatment of product	Persistent and non-persistent PFGE type (No. of PFGE types)
Yes	Persistent (12)
	Non-persistent (5)
No	Persistent (3)
	Non-persistent (10)

The raw product processing lines were persistently contaminated, while the fermented RTE product processing line was not. The PFGE types found in raw materials or raw products had not established themselves in the processing machines of the post heat-treatment area.

5.5.2 Processing machines (III, IV)

The processing machines in Studies III and IV were contaminated (Table 10). Surfaces both in direct and in indirect contact with foods were contaminated. Several products were contaminated with the same PFGE type found in the processing machine or in machines that manipulated the contaminated products.

Table 10. *Listeria monocytogenes*- contaminated processing machines.

Processing machine	Contamination site
Freezer	Spiral conveyor
	Supporting structures
	Surfaces[a]
Slicing machine	Blades
	Blade cover
	Control panel
	Motor
	Lubricant
	Ball-race screw
	Surfaces[a]
Dicing machine	Cutting blades
	Blade cover
	Surface under blade
	Control panel
	Exterior surface
	Product-remains collector
	Tool kit
Peeling machine	Control panel
	Surface under the peeler
	Surfaces[a]
Weigher (with head system)	Funnel
	Surfaces[a]
Packing machine	Chamber
	Surfaces[a]
Conveyor	Belt
	Supporting structures

[a]Specific site not known.

The dicing machine in Study III was transferred from one plant to another (Table 11). The dicing line in plant A was found to be contaminated with *L. monocytogenes* PFGE type I. The dicing machine was subsequently sold to plant B, which in turn sold the dicing machine after some time to plant C. Both plants B and C were contaminated with PFGE type I soon after the dicing machine had been taken into use. The PFGE type I contamination persisted in the dicing lines in plants B and C. This persistent contamination

was eradicated from the dicing line in plant C after targeted and improved sanitizing procedures.

Table 11. Date of transfer of the dicing machine and first recovery of *Listeria monocytogenes* PFGE type I in plants A, B and C.

Plant	Date of transfer of machine to plant (month/year)	Date of first recovery of *Listeria monocytogenes* PFGE type I (month/year)
A	NA[a]	11/97
B	10/98	10/98
C	3/99	3/99

[a] NA=data not available

5.5.3 Compartmentalization (IV)

The degree of compartmentalization of processing lines was observed to have an effect on the *L. monocytogenes* contamination status. Processing line I in plant B (IV, Table 1), which produced a cooked meat product, was the most compartmentalized line, with the raw area separated from the post heat-treatment area. Processing line II in plant B had intermediate compartmentalization, with inadequate separation of the raw and post heat-treatment areas. This line was contaminated more extensively and over a longer period than processing line I. Processing lines I and II in plant C and line I in plant D were poorly compartmentalized and were continuously contaminated with several different PFGE types in the post heat-treatment area and in processing machines.

5.6 Persistence of *Listeria monocytogenes* in foods (V)

In total, 295 *L. monocytogenes* food isolates, obtained during 1988-1999 were characterized by serotyping and PFGE typing. The combination of *Asc*I and *Apa*I MRPs resulted in 66 PFGE types. Some *L. monocytogenes* strains were found repeatedly from the same producer and the same product over a two-year period, and others were found

repeatedly from different producers for two or more years. Some of the repeatedly found strains were recovered in only one product type, while others were recovered in several different product types, including meat, poultry and fish.

6   DISCUSSION

6.1   Properties of *Listeria monocytogenes* predisposing to persistent contamination (I-III)

6.1.1   Adherence to stainless steel surface (I, III)

Persistent *L. monocytogenes* strains were observed to adhere to stainless steel surfaces more efficiently than non-persistent strains at short contact times. Norwood and Gilmour (1999) have also reported higher adherence levels of persistent *L. monocytogenes* strains than of non-persistent strains. The enhanced adherence of the persistent strains may have an influence on the survival of the strains since adherence increases the resistance of *L. monocytogenes* to disinfectants and heat (Frank and Koffi 1990). Enhanced adherence after short contact times is particularly beneficial because cells can adhere in high numbers before sanitizing procedures take place. This may have an effect on the initiation of persistent plant contamination.

Serotype 1/2c strains showed the highest adherence (I, III), a finding supported by Norwood and Gilmour (1999). Serotype 1/2c differs from the other 1/2 serogroup strains by its flagellar antigens. Flagella have been demonstrated to be important in the early adherence of bacteria (O'Toole and Kolter 1998). The non-motile strain of serotype 1/2 (I) did in fact show the poorest adherence over short contact times. However, the non-motile strain adhered with a high cell count at 72 h. These results suggest that flagella are important in the initiation of adherence. Factors other than flagella seem to be more important in the adherence of cells at longer contact times.

41

Half of the non-persistent strains reached adherence levels similar to those of persistent strains by a contact time of 72 h. Adherence after a long contact time does not offer an explanation to why some strains are persistent and some non-persistent.

6.1.2 Resistance to disinfectants (II, III)

Differences in initial MIC values of disinfectants were observed between *L. monocytogenes* strains in Study II. One of the persistent strains showed higher MIC values of the QACs than the other strains. However, one of the non-persistent strains showed a twofold MIC value of the tertiary alkylamine and sodium hypochlorite compared with the other strains. Differences in MIC values of disinfectants between *L. monocytogenes* strains have been speculated to have an effect on the survival of strains (Lemaître et al. 1998, Aase et al. 2000). Some studies have, however, failed to detect any difference in disinfectant susceptibility between persistent and non-persistent strains (Earnshaw and Lawrence 1998, Holah et al. 2002).

The MIC values between persistent and non-persistent strains in Study III revealed no differences, with the exception of one non-persistent strain that was more susceptible to a QAC than the other strains including the persistent strain. The persistent strain also showed an increased adherence, which has been speculated to have a synergistic effect on disinfectant resistance (Aase et al. 2000).

6.1.3 Adaptation and cross-adaptation to disinfectants (II)

Both the persistent and non-persistent *L. monocytogenes* strains were observed to adapt to QACs, tertiary alkylamine and sodium hypochlorite at 10ºC and 37ºC. The adaptation to the QACs and tertiary alkylamine occurred after a 2-h sublethal exposure, indicating rapid response of the cells. Although the highest increase in resistance was over 15-fold, the resistance did not exceed the concentrations of disinfectants used at food processing plants. Therefore, adaptation appears not to have an important role in the survival of the strains. However, an increased resistance may influence the survival of cells in situations where the concentration of the disinfectant is sublethal. Sublethal concentrations may occur when

disinfectants are applied on dirty or wet surfaces or in hard-to-reach places such as complex processing machines. The increased resistance may also have a synergistic effect with disinfectant resistance in biofilm cells (Aase et al. 2000).

Persistent and non-persistent strains adapted to similar disinfectant levels. Aase et al. (2000) also observed that *L. monocytogenes* strains adapted to benzalkonium chloride at similar levels. These results indicate that the persistence or non-persistence of a strain cannot be explained with differences in the ability to adapt to disinfectants. Nevertheless, the increased resistance to disinfectants of an adapted *L. monocytogenes* strain, persistent or non-persistent, may influence its survival.

The increased resistance of QACs and tertiary alkylamine remained elevated for four weeks. However, the increased resistance of sodium hypochlorite was lost in one week. Potassium persulphate did not show any decrease during the four-week follow-up, which may indicate that the initial MIC values were not due to earlier adaptive responses.

All disinfectants caused cross-adaptation of *L. monocytogenes* strains, resulting in similar or smaller increases in resistance than the increases due to adaptive responses. The only disinfectant that *L. monocytogenes* was observed not to cross-adapt to was potassium persulphate. *L. monocytogenes* cross-adapting not only to disinfectants with similar mechanism of action but also to those with a different mechanism of action indicates that the cross-adaptation was also due to non-specific responses.

Maintaining high disinfectant efficiency by rotation of these agents may thus not be possible due to cross-adaptive responses. Potassium persulphate was the only agent that *L. monocytogenes* did not adapt or cross-adapt to, and it may therefore be suitable for long-term use. However, potassium persulphate did cause cross-adaptation of *L. monocytogenes* to the other disinfectants, which reduces the effectiveness of these agents.

6.2 Persistent and non-persistent food plant contamination (III-V)

6.2.1 Distribution of persistent and non-persistent *Listeria monocytogenes* strains (IV, V)

Persistent and non-persistent *L. monocytogenes* strains were observed in all of the meat processing plants and in the poultry processing plant. The highest proportion of persistent strains compared with the total number of *L. monocytogenes* strains was found in meat processing plant C. This finding suggests that either the sanitation of the plant was inadequate or the contamination was frequently reintroduced, or both.

The majority of the strains were found in only one plant, although some of the strains were common to two or three plants. Some strains were also common to both meat and poultry processing plants. Similar observations were made in Study V, where products that had been processed in different plants were contaminated with identical PFGE types. Plant-specific strains as well as common strains between different types of food plants have also previously been observed (Suihko et al. 2002). However, whether strains are present in one plant only is difficult to determine. Possibly, some of these strains would be found in other plants if the number of samples were increased.

The persistent strains were often widely spread in the processing plant, contaminating two or more processing lines. However, processing lines that produced a fermented uncooked product were not persistently contaminated. This may be due to competing microbes reducing the number of *L. monocytogenes* on surfaces (Jeong and Frank 1994, Leriche and Carpentier 2000). Persistent strains were, in the majority of cases, found in several different sampling sites, illustrating the wide distribution of these strains in the plants, while non-persistent strains were mostly found in only one sampling site.

Some persistent strains were observed to be non-persistent in another plant. The categorization of the same PFGE type as persistent and non-persistent in different plants could have been due to restrictions in the typing method in strain discrimination. Differences may have also been present in the phenotype of strains, which would have influenced their survival. Such phenotypic differences might include variable adherence to

surfaces or susceptibility to disinfectants. Moreover, it cannot be ruled out that an increased number of samples might have resulted in the recategorization of some of the non-persistent strains as persistent strains.

The PFGE types found in the raw materials or raw products were not established in the post heat-treatment lines. This finding is in agreement with several previous studies in which persistent strains were not found in raw materials (Rørvik et al. 1995, Nesbakken et al. 1996, Autio et al. 1999). However, the prevalence of *L. monocytogenes* is high in raw meat and poultry (Johnson et al. 1990, Jay 1996, Samelis and Metaxopoulos 1999), and the contamination was likely originally introduced to the plant by contaminated raw materials. This is supported by Berrang et al. (2002), who found identical *L. monocytogenes* strains from the raw and post heat-treatment areas in a poultry processing line. It is also possible that had the number of samples been increased in Study IV persistent strains would have been found in raw materials at some point.

6.2.2 Factors in the processing line predisposing to persistent contamination (III, IV)

Processing machines (III, IV)

The processing machines in all of the plants were contaminated with persistent strains. The surfaces of the processing machines, which were in direct contact with the product, were contaminated. The persistent PFGE types found in the machines were also found in the products that were manipulated by these machines. This observation indicates that the products were contaminated via the processing machines. It also suggests that the processing machines were poorly sanitized. The only processing machines that were not contaminated on direct surfaces were the peeling machines. This might have been due to the peeling machine applying hot steam during the peeling process.

The dicing machine, which appeared to transfer persistent *L. monocytogenes* contamination from one plant to another, illustrates the difficulties in eliminating contamination (III). This dicing machine and the dicing line were persistently contaminated, and routine sanitation procedures failed to abolish the contamination. Contamination of processing machines has been reported earlier by several authors (Autio et al. 1999, Miettinen et al. 1999a, Suihko

45

et al. 2002), However, structural changes and thorough disassembly of processing machines have resulted in the eradication of *L. monocytogenes* (Autio et al. 1999, Miettinen et al. 1999a). Improved sanitation procedures, including regular disassembly and alkali-acid-alkali washes, also led to the eradication of the contamination in the dicing machine and the dicing line in Study III.

Compartmentalization (IV)

Degree of compartmentalization in the processing lines was observed to have an effect on the *L. monocytogenes* contamination status of plants B, C and D. The effect of compartmentalization on the contamination status of plant A was not evaluated because of the small number of isolates. The most compartmentalized processing line was observed to be the least contaminated. By contrast, the processing line with intermediate compartmentalization showed more extensive contamination and for longer, and the lines with poor compartmentalization were continuously heavily contaminated. The separation of the raw area from the post heat-treatment area in particular appeared to affect the contamination status of processing lines that were otherwise similar.

Contamination in the processing lines of plant C, which had poor compartmentalization, could not be eliminated. The area between the ovens and the slicing and dicing machines was contaminated with several different PFGE types. This area was probably contaminated by raw material from time to time because practically no separation existed between the raw area and the post heat-treatment area. The post-processing machines were also continuously contaminated. Even had the contamination been successfully eliminated from the processing machines, the contamination would likely have been reintroduced at some point due to poor hygiene barriers.

6.3 Persistence of *Listeria monocytogenes* in foods (IV, V)

The observations on the PFGE types found in final food products from different sources reflect the contamination status of the food processing plants where the products were produced. Some of the PFGE types were recurrently found from products of one plant (V), which strongly suggests that the food processing plant was persistently contaminated and that the contamination was spread to the final product. Most of the recurrent strains were

found in different product types, indicating that the strains are not specific to any particular product type. Some of the PFGE types were found recurrently from the products of several producers, indicating their persistence in several plants making the concept of plant specific strains arguable. Similar findings were made in Study IV, where identical persistent PFGE types were found in products and processing environments of different plants.

7 CONCLUSIONS

1. Persistent *L. monocytogenes* strains were observed to adhere to stainless steel surfaces in higher cell numbers than non-persistent strains after short contact times. Such enhanced adherence increases the possibility of survival of the persistent strains due to increased resistance against prevention methods and may have an effect on the initiation of persistent plant contamination.

The adherence level of non-persistent strains was closer to the adherence level of persistent strains after a long contact period. It appears therefore that adherence over long contact times, in contrast to adherence over shorter periods does not explain why some strains are persistent and some non-persistent.

2. The initial resistance of persistent and non-persistent *L. monocytogenes* strains to disinfectants varied. Differences in resistance to disinfectants may influence the survival of strains in food processing plants. Both persistent and non-persistent strains adapted to all disinfectants investigated, with the exception of potassium persulphate. The increase in resistance was similar for persistent and non-persistent strains and did not reach the concentrations of disinfectants used at food processing plants. However, the adaptive response may have an effect on the survival of strains when they encounter suboptimal disinfectant concentrations.

3. The persistent and non-persistent *L. monocytogenes* strains cross-adapted to all disinfectants except potassium persulphate. Potassium persulphate was the only agent that *L. monocytogenes* was observed not to adapt or cross-adapt to, and thus it seems suitable for long-term use. However, potassium persulphate did cause cross-adaptation of *L. monocytogenes* to the other agents. The cross-adaptive responses appeared to be non-specific as cross-adaptation was observed between related and unrelated agents. Maintaining high disinfectant efficiency of these agents by rotation may therefore not be possible, even with agents with different mechanisms of action.

4. The PFGE types found in the raw materials had not established themselves in the post heat-treatment lines. Persistent contamination therefore appears not to be generally due to continuous recontamination by raw materials. However, the contamination may have

originated from raw materials. This hypothesis is supported by several studies that show a high prevalence of *L. monocytogenes* in raw materials. In addition, it is possible that had more raw material samples been investigated in Study IV persistent strains would have been found in raw materials.

Some of the strains were found only in one plant, suggesting that they may have been plant-specific. However, it is difficult to determine whether a strain is plant-specific or not. Increasing the number of samples might have resulted in some of these strains being found in other plants as well. The persistent *L. monocytogenes* strains were widely distributed in the food processing plants and they more often contaminated cooked products than the non-persistent strains illustrating their importance in food safety. The processing lines producing fermented products were not persistently contaminated possibly due to the presence of competing microbes on the processing surfaces.

5. Compartmentalization, and especially the separation of the raw area from the post heat-treatment area, appeared to influence the contamination status of the post heat-treatment line. Poor separation of the raw area from the post heat-treatment line seemed to increase contamination pressure. The processing machines were observed to be contaminated with and to sustain *L. monocytogenes* contamination. Elimination of contamination from complex processing machines was shown to be possible by thorough disassembly of the machines and targeted sanitation. However, in processing lines with inadequate compartmentalization, contamination appeared to be reintroduced at some point due to poor hygiene barriers.

6. The *L. monocytogenes* contamination status of food products reflects the contamination status of the corresponding food plant. Some of the foods of one producer were found to be recurrently contaminated with one PFGE type, indicating that the food processing plant or the processing line was persistently contaminated. Some of the foods produced by several producers were also recurrently contaminated with the same PFGE type, indicating that the PFGE type may have been persistent in several plants.

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. Aarnisalo, K., S. Salo, H. Miettinen, M.-L. Suihko, G. Wirtanen, T. Autio, J. Lundén, H. Korkeala and A.-M. Sjöberg. 2000. Bactericidal efficiencies of commercial disinfectants against *Listeria monocytogenes* on surfaces. J. Food Safety. 20, 237-250.

3. Aase, B., G. Sundheim, S. Langsrud and L. Rørvik. 2000. Occurrence of and possible mechanism for resistance to a quaternary ammonium compound in *Listeria monocytogenes*. Int. J. Food Microbiol. 62, 57-63.

4. Aguado, V., A. I. Vitas and I. García-Jalon. 2001. Random amplified polymorphic DNA typing applied to the study of cross-contamination by *Listeria monocytogenes* in processed food products. J. Food Prot. 64, 716-720.

5. Anonymous 1987. Meat industry research shows *Listeria* widespread, control difficult. Food Chem. News. 29, 27-29.

6. Antunes, P., C. Réu, J. C. Sousa, N. Pestana and L. Peixe. 2002. Incidence and susceptibility to antimicrobial agents of *Listeria* spp. and *Listeria monocytogenes* isolated from poultry carcasses in Porto, Portugal. J. Food Prot. 65, 1888-1893.

7. Arnold, G. J. and J. Coble. 1995. Incidence of *Listeria* species in foods in NSW. Food Australia. 47, 71-75.

8. 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.

9. 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). System. Appl. Microbiol. In Press.

10. Avery, S. M., J. A. Hudson and S. Buncic. 1996. Multilocus enzyme electrophoresis typing of New Zealand *Listeria monocytogenes* isolates. Int. J. Food Microbiol. 28, 351-359.

11. Beresford, M. R., P. W. Andrew and G. Shama. 2001. *Listeria monocytogenes* adheres to many materials found in food processing environments. J. Appl. Microbiol. 90, 1000-1005.

12. Berrang, M. E., R. J. Meinersmann, J. K. Northcutt and D. P. Smith. 2002. Molecular characterization of *Listeria monocytogenes* isolated from a poultry further processing facility and from fully cooked product. J. Food Prot. 65, 1574-1579.

13. 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.

14. Bille, J. and J. Rocourt. 1996. WHO international multicenter *Listeria monocytogenes* subtyping study – rationale and set-up of the study. Int. J. Food Microbiol. 32, 251-262.

15. Blackman, I. C. and J. F. Frank. 1996. Growth of *Listeria monocytogenes* as a biofilm on various food-processing surfaces. J. Food Prot. 59, 827-831.

16. Boerlin, P., F. Bannerman, J. Ischer, J. Rocourt and J. Bille. 1995. Typing *Listeria monocytogenes*: a comparison of random amplification of polymorphic DNA with 5 other methods. Res. Microbiol. 146, 35-49.

17. Bremer, P. J., I. Monk and C. M. Osborne. 2001. Survival of *Listeria monocytogenes* attached to stainless steel surfaces in the presence or absence of *Flavobacterium* spp. J. Food Prot. 64, 1369-1376.

18. Brosch, R., M. Brett, B. Catimel, J. B. Luchansky, B. Ojeniyi and J. Rocourt. 1996. Genomic fingerprinting of 80 strains from the WHO multicenter international typing study of *Listeria monocytogenes* via pulsed-field gel electrophoresis (PFGE). Int. J. Food Microbiol. 32, 343-355.

19. Buchanan, R. L. and L. K. Bagi. 1999. Microbial competition: effect of *Pseudomonas fluorescens* on the growth of *Listeria monocytogenes*. Food Microbiol. 16, 523-529.

20. Buncic, S. 1991. The incidence of *Listeria monocytogenes* in slaughtered animals, in meat, and in meat products in Yugoslavia. Int. J. Food Microbiol. 12, 173-180.

21. Buncic S., S. M. Avery, J. Rocourt and M. Dimitrijevic. 2001. Can food-related environmental factors induce different behaviour in two key serovars, 4b and 1/2a, of *Listeria monocytogenes*? Int. J. Food Microbiol. 65, 201-212.

22. 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.

23. Capita, R., C. Alonso-Calleja, B. Moreno and M. C. García-Fernández. 2001. Occurrence of *Listeria* species in retail poultry meat and comparison of a cultural/immunoassay for their detection. Int. J. Food Microbiol. 65, 75-82.

24. Caugant, D. A., F. E. Ashton, W. F. Bibb, P. Boerlin, W. Donachie, C. Low, A. Gilmour, J. Harvey and B. Nørrung. 1996. Multilocus enzyme electrophoresis for characterization of *Listeria monocytogenes* isolates: results of an international comparative study. Int. J. Food Microbiol. 32, 301-311.

25. de Cesare, A., J. L. Bruce, T. R. Dambaugh, M. E. Guerzoni and M. Wiedmann. 2001. Automated ribotyping using different enzymes to improve discrimination of *Listeria monocytogenes* isolates, with a particular focus on serotype 4b strains. J. Clin. Microbiol. 39, 3002-3005.

26. Chae, M. S. and H. Schraft. 2000. Comparative evaluation of adhesion and biofilm formation of different *Listeria monocytogenes* strains. Int. J. Food Microbiol. 62, 103-111.

27. Charlton, B. R., H. Kinde and L. H. Jensen. 1990. Environmental survey for *Listeria* species in California milk processing plants. J. Food Prot. 53, 198-201.

28. Chasseignaux, E., P. Gérault, M.-T. Toquin, C. Raqimbeau, G. Salvat, P. Colin and G. Ermel. 2002. Ecology of *Listeria monocytogenes* in the environment of raw poultry meat and raw pork meat processing plants. FEMS Microbiol. Lett. 210, 271-275.

29. Chasseignaux, E., M.-T. Toquin, C. Raqimbeau, 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.

30. Comi, G., R. Frigerio and C. Cantoni. 1992. *Listeria monocytogenes* serotypes in Italian meat products. Lett. Appl. Microbiol. 15, 168-171.

31. Costerton, J.W. 1999. Introduction to biofilm. Int. J. Antimicrobial Agents. 11, 217-221.

32. Cotton, L. N. and C. H. White. 1992. *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Salmonella* in dairy plant environments. J. Dairy Sci. 75, 51-57.

33. Coveney, H. M., G. F. Fitzgerald and C. Daly. 1994. A study of the microbiological status of Irish farmhouse cheeses with emphasis on selected pathogenic and spoilage microorganisms. J. Appl. Microbiol. 77, 621-630.

34. Cox, L. J., T. Kleiss, J. L. Cordier, C. Cordellana, P. Konkel, C. Pedrazzini, R. Beumer and A. Siebenga. 1989. *Listeria* spp. in food processing, non-food and domestic environments. Food Microbiol. 6, 49-61.

35. Curtis, G. D. W. and R. G. Mitchell. 1992. Bacteriocin (monocin) interactions among *Listeria monocytogenes* strains. Int. J. Food Microbiol. 16, 283-292.

36. Dominguez, C., I. Gomez and J. Zumalacarregui. 2001. Prevalence and contamination levels of *Listeria monocytogenes* in smoked fish and pâté sold in Spain. J. Food Prot. 64, 2075-2077.

37. Donlan, R. M. 2002 Biofilms: Microbial life on surfaces. Emerg. Infect. Dis. 8, 881-890.

38. Dunne, W. M. 2002. Bacterial adhesion: seen any good biofilms lately? Clin. Microbiol. Rev. 15, 155-166.

39. Earnshaw, A. M. and L. M. Lawrence. 1998. Sensitivity to commercial disinfectants, and the occurrence of plasmids within various *Listeria monocytogenes* genotypes isolated from poultry products and the poultry processing environment. J. Appl. Microbiol. 84, 642-648.

40. Eklund, M. W., F. T. Poysky, R. N. Paranjpye, L. C. Lashbrook, M. E. Peterson and G. A. Pelroy. 1995. Incidence and sources of *Listeria monocytogenes* in cold-smoked fishery products and processing plants. J. Food Prot. 58, 502-508.

41. Fantelli, K. and R. Stephan. 2001. Prevalence and characteristics of shigatoxin-producing *Escherichia coli* and *Listeria monocytogenes* strains isolated from minced meat in Switzerland. Int. J. Food Microbiol. 70, 63-69.

42. Farber, J. M. 2000. Present situation in Canada regarding *Listeria monocytogenes* and ready-to-eat seafood products. Int. J. Food Microbiol. 62, 247-51.

43. Farber, J. M., P. I. Peterkin, A. O. Carter, P. V. Varughese, F. E. Ashton and E. P. Ewan. 1991. Neonatal listeriosis due to cross-infection confirmed by isoenzyme typing and DNA fingerprinting. J. Infect. Dis. 163, 927-928.

44. Farber, J. M., G. W. Sanders, S. Dunfield and R. Prescott. 1989. The effect of various acidulants on the growth of *Listeria monocytogenes*. Lett. Appl. Microbiol. 9, 181-183.

45. Fieandt, E. 1993. Investigation of the microbiological quality of meat and meat products offered for sale during 1992-1993. National Food Administration. Helsinki. Research notes. 14/1993. 41 p.

46. Fieandt, E. and P. Mäkelä. 1994. An investigation of the basic microbiological quality of foods: I ice-creams, II processed foods. National Food Administration. Helsinki. Research notes. 9/1994. 37 p.

47. Fonnesbech Vogel, B., H. Huss, B. Ojeniyi, P. Ahrens and L. Gram. 2001. Elucidation of *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. Appl. Environ. Microbiol. 67, 2586-2595.

48. Frank, J. F. and R. A. Koffi. 1990. Surface-adherent growth of *Listeria monocytogenes* is associated with increased resistance to surfactant sanitizers and heat. J. Food Prot. 53, 550-554.

49. Frye, D. M., R. Zweig, J. Sturgeon, M. Tormey, M. LeCavalier, I. Lee, L. Lawani and L. Mascola. 2002. An outbreak of febrile gastroenteritis associated with delicatessen meat contaminated with *Listeria monocytogenes*. Clin. Infect. Dis. 35, 943-949.

50. Gandhi, P., A. Sawant, L. Wilson and D. Ahearn. 1993. Adaptation and growth of *Serratia marcescens* in contact lens disinfectant solutions containing chlorhexidine gluconate. Appl. Environ. Microbiol. 59, 183-188.

51. Gellin, B. G. and C. V. Broome. 1989. Listeriosis. JAMA. 261, 1313-1320.

52. Gellin, B. G., C. V. Broome, W. F. Broome, W. F. Bibb, R. E. Weaver, S. Gaventa, L. Mascola and the Listeriosis study group. 1991. The epidemiology of listeriosis in the United States – 1986. Am. J. Epidemiol. 133, 392-401.

53. Gerner-Smidt, P., P. Boerlin, F. Ischer and J. Schmidt. 1996. High-frequency endonuclease (REA) typing: results from the WHO collaborative study group on subtyping of *Listeria monocytogenes*. Int. J. Food Microbiol. 32, 313-324.

54. 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.

55. Goulet, V., C. Jacquet, V. Vallant, I. Rebière, E. Mouret, C. Lorente, E. Maillot, F. Stainer and J. Rocourt. 1995. Listeriosis from consumption of raw-milk cheese. Lancet 345, 1501-1502.

56. Gray, M. L. and A. H. Killinger. 1966. *Listeria monocytogenes* and listeric infections. Bacteriol. Rev. 30, 309-382.

57. Gray, M. L., H. J. Stafseth, F. Jr. Thorp, L. B. Sholl and W. F. Jr. Riley. 1947. A new technique for isolating Listerellae from the bovine brain. J. Bacteriol. 55, 471-476.

58. Greenwood, M. H., D. Roberts and P. Burden. 1991. The occurrence of *Listeria* species in milk and dairy products: a national survey in England and Wales. Int. J. Food Microbiol. 12, 197-206.

59. Guerra, M. M., F. Bernardo and J. McLauchlin. 2002. Amplified fragment length polymorphism (AFLP) analysis of *Listeria monocytogenes*. System. Appl. Microbiol. 25, 456-461.

60. Hartemink, R. and F. Georgsson. 1991. Incidence of *Listeria* species in seafood and seafood salads. Int. J. Food Microbiol. 12, 189-196.

61. Harvey, J. and A. Gilmour. 2001. Characterization of recurrent and sporadic *Listeria monocytogenes* isolates from raw milk and nondairy foods by pulsed-field gel electrophoresis, monocin typing, plasmid profiling, and cadmium and antibiotic resistance determination. Appl. Environ. Microbiol. 67, 840-847.

62. Hatakka, M., T. Johansson, L. Rantala, P. Pakkala and T. Honkanen-Buzalski. 2001. Reduced *Listeria monocytogenes* occurrence in Finnish vacuum-packed fish products. International Symposium on Problems of Listeriosis. ISOPOL XIV, Mannheim, Germany, May 13-16.2001.

63. Hatakka, M., L. Rantala, L. Oivanen and T. Johansson. 2002. *Listeria monocytogenes* in vacuum-packed cold-smoked fish products in Finland in 2001. How safe is it to eat Nordic fish? An NMKL seminar. Mariehamn, Åland Islands, 23.8.2002.

64. Helke, D. M., E. B. Somers and A. C. L. Wong. 1993. Attachment of *Listeria monocytogenes* and *Salmonella typhimurium* to stainless steel and Buna-N in the presence of milk and individual milk components. J. Food Prot. 56, 479-484.

65. Herald, P. A. and E. A. Zottola. 1988. Attachment of *Listeria monocytogenes* to stainless steel surfaces at various temperatures and pH values. J. Food Sci. 53, 1549-1562.

66. Heredia, N., S. García, G. Rojas and L. Salazar. 2001. Microbiological condition of ground meat retailed in Monterrey, Mexico. J. Food Prot. 64, 1249-1251.

67. Hoffman, A. D., K. L. Gall, D. M. Norton and M. Wiedmann. 2003. *Listeria monocytogenes* contamination patters for the smoked fish processing environment and for raw fish. Appl. Environ. Microbiol. 66, 52-60.

68. Holah, J. T., J. H. Taylor, D. J. Dawson and K. E. Hall. 2002. Biocide use in the food industry and the disinfectant resistance of persistent strains of *Listeria monocytogenes* and *Escherichia coli*. J. Appl. Microbiol. 92, Suppl, 111-120.

69. Hudson, W. R. and G. C. Mead. 1989. *Listeria* contamination at a poultry processing plant. Lett. Appl. Microbiol. 9, 211-214.

70. Hudson, J. A., S. J. Mott, K. M. Delacy and A. L. Edridge. 1992. Incidence and coincidence of *Listeria* spp., motile aeromonas and *Yersinia enetrocolitica* on ready-to-eat foods. Int. J. Food Microbiol. 16, 99-108.

71. Hudson, J. A., S. J. Mott and N. Penney. 1994. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila*, and *Yersinia enterocolitica* on vacuum and saturated carbon dioxide controlled atmosphere-packaged sliced roast beef. J. Food. Prot. 57, 204-208.

72. Husu, J. R. 1990. Epidemiological studies on the occurrence of *Listeria monocytogenes* in the feces of dairy cattle. J. Vet. Med. 37, 276-282.

73. Husu, J. R., J. T. Seppänen, S. K. Sivelä and A. L. Rauramaa. 1990. Contamination of raw milk by *Listeria monocytogenes* on dairy farms. J. Vet. Med. 37, 268-275.

74. IDF. 1995. Milk and milk products. Detection of *Listeria monocytogenes*. 43A:1995. <http://www.fil-idf.org/StandardsEnglish.htm>

75. ISO. 1996. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 1: Detection method. ISO method 11290-1, 1st ed. International Organization for Standardization. Genève, Switzerland.

76. Jacquet, C., J. Rocourt and A. Reynaud. 1993. Study of *Listeria monocytogenes* contamination in a dairy plant and characterization of the strains isolated. Int. J. Food Microbiol. 10, 13-22.

77. Jay, M. J. 1996. Prevalence of *Listeria* spp. in meat and poultry products. Food Control. 7, 209-214.

78. Jayarao, B. M. and Henning, D. R. 2001. Prevalence of foodborne pathogens in bulk tank milk. J. Dairy Sci. 84, 2157-2161.

79. Jemmi, T. 1990. Occurrence of *Listeria monocytogenes* in imported smoked and fermented fish. Arch. Lebensmittelhyg. 41, 107-109.

80. Jeong, D.K. and J. F. Frank. 1994. Growth of *Listeria monocytogenes* at 10ºC in biofilms with microorganisms isolated from meat and dairy processing environments. J. Food Prot. 57, 576-586.

81. Johansson, T., L. Rantala, L. Palmu and T. Honkanen-Buzalski. 1999. Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. Int. J. Food Microbiol. 47, 111-119.

82. Johnson, J. L., M. P. Doyle and R. G. Cassens. 1990. *Listeria monocytogenes* and other *Listeria* spp. in meat and meat products. J. Food Prot. 53, 81-91.

83. Jones, M. V., T. M. Herd and H. J. Christie. 1989. Resistance of *Pseudomonas aeruginosa* to amphoteric and quaternary ammonium biocides. Microbios. 58, 49-61.

84. 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.

85. Kalmokoff, K. L., J. W. Austin, X.-D. Wan, G. Sanders, S. Banerjee and J. M. Farber. 2001. Adsorption, attachment and biofilm formation among isolate of *Listeria monocytogenes* using model conditions. J. Appl. Microbiol. 91, 725-734.

86. Kela, E. and P. Holmström. 2001. Infectious diseases in Finland 2000. Publications of the National Public Health Institute. KTL B 10/2001. 27 p.

87. Keto, R. and M. Rahkio. 1998. *Listeria* in fish products. National Food Administration. Research notes. Helsinki. 5/1998. 3 p. + app.

88. Keto, R. and M. Rantala. 1998. The microbiological quality and safety of fresh cheeses. National Food Administration. Helsinki. Research notes. 5/1998. 2 p. + app.

89. Keto-Timonen, R., T. Autio and H. Korkeala. 2003. An improved amplified fragmnet length polymorphism (AFLP) protocol for discrimination of *Listeria* isolates. System. Appl. Microbiol. 26, 236-244.

90. Kim, K.Y. and J. F. Frank. 1994. Effect of nutrients on biofilm formation by *Listeria monocytogenes* on stainless steel. J. Food Prot. 58, 24-28.

91. Klausner, R. B. and C. W. Donnelly. 1991. Environmental sources of *Listeria* and *Yersinia* in Vermont dairy plants. J. Food Prot. 54, 607-6011.

92. 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.

93. Lawrence, L. M. and A. Gilmour. 1995. Characterization of *Listeria monocytogenes* isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. Appl. Environ. Microbiol. 61, 2139-2144.

94. Lemaître, J-P., H. Echchannaoui, G. Michaut, C. Divies and A. Rousset. 1998. Plasmid-mediated resistance to antimicrobial agents among Listeriae. J. Food Prot. 61, 1459-1464.

95. Leriche, V. and Carpentier, B. 2000. Limitation of adhesion and growth of *Listeria monocytogenes* on stainless steel surfaces by *Staphylococcus sciuri* biofilms. J. Appl. Microbiol. 88, 594-605.

96. Levine, P., B. Rose, S. Green, G. Ransom and W. Hill. 2001. Pathogen testing of ready-to-eat meat and poultry products collected at federally inspected establishments in the United States, 1990 to 1999. J. Food Prot. 64, 1188-1193.

97. Loncarevic, S., M.-L. Danielsson-Tham, L. Mårtensson, Å. Rigner, A. Runehagen and W. Tham. 1997. A case of foodborne listeriosis in Sweden. Lett. Appl. Microbiol. 24, 65-68.

98. Loncarevic, S., M.-L. Danielsson-Tham and W. Tham. 1995. Occurrence of *Listeria monocytogenes* in soft and semi-soft cheeses in retail outlets in Sweden. Int. J. Food Microbiol. 26, 245-250.

99. Loncarevic, S., W. Tham and M.-L. Danielsson-Tham. 1996. Prevalence of *Listeria monocytogenes* and other *Listeria* spp. in smoked and "gravad" fish. Acta Vet. Scand. 37, 13-18.

100. Loughlin, M. F., M. V. Jones and P. A. Lambert. 2002. *Pseudomonas aeruginosa* cells adapted to benzalkonium chloride show resistance to other membrane-active agents but not to clinically relevant antibiotics. J. Antimicrob. Chem. 49, 631-639.

101. Lukinmaa, S., M. Miettinen, U. M. Nakari, H. Korkeala and A. Siitonen. 2003. *Listeria monocytogenes* isolates from invasive infections: variation of sero- and genotypes during an 11-year period in Finland. J. Clin. Microbiol. 41, 1694-1700.

102. 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, 121-144.

103. 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 from butter in Finland. J. Infect. Dis. 181, 1838-1841.

104. 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.

105. Mafu, A. A., D. Roy, J. Goulet and P. Magny. 1990. Attachment of *Listeria monocytogenes* to stainless steel, glass, polypropylene, and rubber surfaces after short contact times. J. Food Prot. 53, 742-746.

106. Massa, S., D. Cesaroni, G. Poda and L. D. Trovatelli. 1990. The incidence of *Listeria* spp. in soft cheeses, butter and raw milk in the province of Bologna. J. Appl. Bacteriol. 68, 153-156.

107. McDonnell, G. and A. D. Russell. 1999. Antiseptics and disinfectants: activity, action, and resistance. Clin. Microbiol. Rev. 12, 147-179.

108. McLauchlin, J. 1996. The role of Public Health Laboratory Service in England and Wales in the investigation of human listeriosis during the 1980s and 1990s. Food Control. 7, 235-239.

109. McLauchlin, J., A. Audurier, A. Frommelt, P. Gerner-Smidt, C. Jacquet, M. J. Loessner, N. van der Mee-Marquet, J. Rocourt, S. Shah and D. Wilhelms. 1996. WHO study on subtyping *Listeria monocytogenes*: results of phage-typing. Int. J. Food Microbiol. 32, 289-299.

110. Mereghetti, L., R. Quentin, N. van der Mee-Marquet and A. Audurier. 2000. Low sensitivity of *Listeria monocytogenes* to quaternary ammonium compounds. Appl. Environ. Microbiol. 66, 5083-5086.

111. Miettinen, H., K. Aarnisalo, S. Salo and A.-M. Sjöberg. 2001a. Evaluation of surface contamination and the presence of *Listeria monocytogenes* in fish processing factories. J. Food Prot. 64, 635-639.

112. Miettinen, M.K., K. J. Björkroth and H. J. Korkeala. 1999a. Characterization of *Listeria monocytogenes* from an ice-cream plant by serotyping and pulsed-field gel electrophoresis. Int. J. Food Microbiol. 46, 187-192.

113. Miettinen, M.K., L. Palmu, K. J. Björkroth and H. Korkeala. 2001b. Prevalence of *Listeria monocytogenes* in broilers at the abattoir, processing plant, and the retail level. J. Food Prot. 64, 994-999.

114. Miettinen, M. K., A. Siitonen, P. Heiskanen, H. Haajanen, K. J. Björkroth and H. Korkeala. 1999b. Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold-smoked rainbow trout. J. Clin. Microbiol. 37, 2358-2360.

115. Murray, E. G. D., R. A. Webb and M. B. R. Swann. 1926. A disease of rabbit characterised by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus Bacterium monocytogenes (n.sp.). J. Pathol. Bacteriol. 29, 407-439.

116. NCCLS. 1999. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated in animals; approved standard. NCCLS document M31-A. National Committee on Clinical Laboratory Standards. Wayne, PE, USA.

117. NCFA. 1999. *Listeria monocytogenes*. Detection in foods. 136. Nordic Committee on Food Analysis. 2nd ed. 1999. <http://www.nmkl.org/>

118. 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.

119. 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.

120. Norton, D. M., M. A. McCamey, K. Gall, J. M. Scarlett, K. J. Boor and M. Wiedmann. 2001. Molecular studies on the ecology of *Listeria monocytogenes* in the smoked fish processing industry. Appl. Environ. Microbiol. 67, 198-205.

121. Norwood, D.E. and A. Gilmour. 1999. Adherence of *Listeria monocytogenes* strains to stainless steel coupons. J. Appl. Microbiol. 86, 576-582.

122. Norwood, D.E. and A. Gilmour. 2000. The growth and resistance to sodium hypochlorite of *Listeria monocytogenes* in a steady-state multispecies biofilm. J. Appl. Microbiol. 88, 512-520.

123. Norwood, D.E. and A. Gilmour. 2001. The differential adherence capabilities of two *Listeria monocytogenes* strains in monoculture and multispecies biofilms as a function of temperature. J. Appl. Microbiol. 33, 320-324.

124. Nyfeldt, A. 1929. Etiologie de la mononucleose infectieuse. Soc. Biol. 101, 590-592.

125. Oh, D-H. and D. L. Marshall. 1995. Destruction of *Listeria monocytogenes* biofilms on stainless steel using monolaurin and heat. J. Food Prot. 57, 251-255.

126. Ojeniyi, B., H. C. Wegener, N. E. Jensen and M. Bisgaard. 1996. *Listeria monocytogenes* in poultry and poultry products: epidemiological investigations in seven Danish abattoirs. J. Appl. Bacteriol. 80, 395-401.

127. O'Toole, G.A. and R. Kolter. 1998. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol. Microbiol. 30, 295-304.

128. Peel, M., W. Donachie and A. Shaw. 1988. Temperature-dependent expression of flagella of *Listeria monocytogenes* studied by electron microscopy, SDS-PAGE and Western blotting. J. Gen. Microbiol. 134, 2171-2178.

129. Pelttari, E. 1990. A study of *Listeria* bacteria in smoked sausages in 1989, the hygienic quality of prawns in 1989 and a study of *Listeria* bacteria in prepacked fish 1990. National board of trade and consumer affairs. Helsinki. Finland. Research notes 14/1990. 21 p. + app.

130. Pirhonen, T. 1998. The microbiological quality of fresh cheese and soft cheese in retail trade. National Food Administration. Research notes. Helsinki. 5/1998. 7 p.

131. Pourshaban, M., M. Gianfranceschi, A. Gattuso, F. Menconi and P. Aureli. 2000. Identification of *Listeria monocytogenes* contamination sources in two fresh sauce production plants by pulsed-field gel electrophoresis. Food Microbiol. 17, 393-400.

132. Pritchard, T. J., K. J. Flanders and C. W. Donnelly. 1995. Comparison of the incidence of *Listeria* on equipment versus environmental sites within dairy processing plants. Int. J. Food Microbiol. 26, 375-384.

133. Rea, M. C., T. M. Cogan and S. Tobin. 1992. Incidence of pathogenic bacteria in raw milk in Ireland. J. Appl. Bacteriol. 73, 331-336.

134. Rocourt, J. 1999. The Genus *Listeria* and *Listeria monocytogenes*: phylogenetic position, taxonomy, and identification. In: Ryser, E. T. and E. H. Marth. (eds). *Listeria*, listeriosis, and food safety, 2 ed. Marcel Dekker, Inc. New York, USA. p. 1-20.

135. 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*. Zbl. Bakt. Hyg. 259, 489-497.

136. Rodler, M. and W. Korbler. 1989. Examination of *Listeria monocytogenes* in dairy products. Acta Microbiol. Hung. 36, 259-261.

137. Romanova, N., S. Favrin and M. W. Griffiths. 2002. Sensitivity of *Listeria monocytogenes* to sanitizers used in the meat processing industry. Appl. Environ. Microbiol. 68, 6405-6409.

138. Ronner, A.B. and A. C. L. Wong. 1993. Biofilm development and sanitizer inactivation of *Listeria monocytogenes* and *Salmonella typhimurium* on stainless steel and buna-n rubber. J. Food Prot. 56, 750-758.

139. Rudolf, M. and S. Scherer. 2001. High incidence of *Listeria monocytogenes* in European red smear cheese. Int. J. Food Microbiol. 63, 91-98.

140. Rørvik, L. M., B. Aase, T. Alvestad and D. A. Caugant. 2000. Molecular epidemiological survey of *Listeria monocytogenes* in seafoods and seafood-processing plants. Appl. Environ. Microbiol. 66, 4779-4784.

141. Rørvik, L. M., B. Aase, T. Alvestad and D. A. Caugant. 2003. Molecular epidemiological survey of *Listeria monocytogenes* in broilers and poultry products. J. Appl. Microbiol. 94, 633-640.

142. Rørvik, L. M., D. A. Caugant and M. Yndestad. 1995. Contamination pattern of *Listeria monocytogenes* and other *Listeria* spp. in a salmon slaughterhouse and smoked salmon processing plant. Int. J. Food Microbiol. 25, 19-27.

143. 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.

144. Rørvik, L. M. and M. Yndestad. 1991. *Listeria monocytogenes* in foods in Norway. Int. J. Food Microbiol. 13, 97-104.

145. 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.

146. Salvat, G., M. T. Toquin, Y. Michel and P. Colin. 1995. Control of *Listeria monocytogenes* in the delicatessen industries: lessons of a listeriosis outbreak in France. Int. J. Food Microbiol. 25, 75-81.

147. Samelis, J., A. Kakouri, K. G. Geargiadou 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.

148. Samelis, J. and J. Metaxopoulos. 1999. Incidence and principal sources of *Listeria* spp. and *Listeria monocytogenes* contamination in processed meats and a meat processing plant. Food Microbiol. 16, 465-477.

149. Sasahara, K. C. and E. A. Zottola. 1993. Biofilm formation by *Listeria monocytogenes* utilizes a primary colonizing microorganism in flowing systems. J. Food Prot. 56, 1022-1028.

150. Saukkonen, V. 1998. Hygienic quality of vacuum packed fresh cheese and characterization of *Listeria monocytogenes* with pulsed field gel electrophoresis. Graduate studies. Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Helsinki University. 33 p.

151. Schlech III, W. F. 2000. Foodborne listeriosis. Clin. Infect. Dis. 31, 770-775.

152. Schlech, W. F., P. M. Lavigne, A. C. 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.

153. Schuchat, A., B. Swaminathan and C. V. Broome. 1991. Epidemiology of human disease. Clin. Microbiol. Rev. 4, 169-183.

154. Schwartz, D. C. and C. R. Cantor. 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell 37, 67-75.

155. Schönberg, A., E. Bannerman, A. L. Courtieu, R. Kiss, J. McLachlin, S. Shah and W. Wilhelms. 1996. Serotyping of 80 strains from the WHO multicentre international typing study of *Listeria monocytogenes*. Int. J. Food Microbiol. 32, 279-287.

156. Seeliger, H. P. R. 1961. Listeriosis. Hafner, New York, USA.

157. Seeliger, H. P. R. and K. Hohne. 1979. Serotyping of *Listeria monocytogenes* and related species. In. T. Bergan and J. R. Norris (ed.) Methods in microbiology. Vol. 13, Academic press, Inc., New York, USA. p. 31-49.

158. Seeliger, H. P. R. and D. Jones. 1986. Genus *Listeria*. In: Sneath, P. H. A., N. S. Mair, M. E. Sharpe, J. G. Holt (eds.). Bergey's Manual of Systematic Bacteriology, Vol. 2. Williams and Wilkins, Baltimore, USA. p. 1235-1245.

159. Senczek, D., R. Stephan and F. Untermann. 2000. Pulsed-field gel electrophoresis (PFGE) typing of *Listeria* strains isolated from a meat processing plant over a 2-year period. Int J. Food Microbiol. 62, 155-159.

160. Silva, I. M., R. C. Almeida, M. A. Alves and P. F. Almeida. 2003. Occurrence of *Listeria* spp. in critical control points and the environment of Minas Frescal cheese processing. Int. J. Food Microbiol. 81, 241-248.

161. Sim, J., D. Hood, L. Finnie, M. Wilson, C. Graham, M. Brett and J. A. Hudson. 2002. Series of incidents of *Listeria monocytogenes* non-invasive febrile gastroenteritis involving ready-to-eat meats. Lett. Appl. Microbiol. 35, 409-413.

162. Sinde, E. and Carballo, J. 2000. Attachment of *Salmonella* spp. and *Listeria monocytogenes* to stainless steel, rubber and polytetrafluorethylene: the influence of free energy and the effect of commercial sanitizers. Food Microbiol. 17, 439-447.

163. Smoot, L.M. and M. D. Pierson. 1998. Effect of environmental stress on the ability of *Listeria monocytogenes* scott A to attach to food contact surfaces. J. Food Prot. 61, 1293-1298.

164. Stull, T. L., J. J. LiPuma and T. D. Edlind. 1988. A broad-spectrum probe for molecular epidemiology of bacteria: ribosomal RNA. J. Infect. Dis. 157, 280-286.

165. Suihko, M-L., S. Salo, O. Niclasen, B. Gudbjörnsdóttir, G. Torkelsson, S. Bredholt, A.-M. Sjöberg and P. Gustavsson. 2002. Characterization of *Listeria monocytogenes* isolates from the meat, poultry and seafood industries by automated ribotyping. Int. J. Food Microbiol. 72, 137-146.

166. Swaminathan, B., S. B. Hunter, P. M. Desmarchelier, P. Gerner-Smidt, L. M. Graves, S. Harlander, R. Hubner, C. Jacquet, B. Pedersen, K. Reineccius, A. Ridley, N. A. Saunders and J. A. Webster. 1996. WHO-sponsored international collaborative study to evaluate methods for subtyping *Listeria monocytogenes*: restriction fragment length polymorphism (RFLP) analysis using ribotyping and Southern hybridization with two probes derived from *L. monocytogenes* chromosome. Int. J. Food. 32, 263-278.

167. Tkáčiková, L., M. Kantíková, A. Dimitriev and I. Mikula. 2000. Use of the molecular typing methods to evaluate the control of *Listeria monocytogenes* contamination in raw milk and dairy products. Folia Microbiol. 45, 157-160.

168. To, M., S. Favrin, N. Romanova and M. Griffiths. 2002. Postadaptational resistance to benzalkonium chloride and subsequent physicochemical modifications of *Listeria monocytogenes*. Appl. Environ. Microbiol. 68, 5258-5264.

169. Tompkin, R. B., L. N. Christiansen, A. B. Shaparis, R. L. Baker and J. M. Schroeder. 1992. Control of *Listeria monocytogenes* in processed meats. Food Australia. 44, 370-376.

170. Unnerstad, H., E. Bannerman, J. Bille, M-L. Danielsson-Tham, E. Waak and W. Tham. 1996. Prolonged contamination of a dairy with *Listeria monocytogenes*. Neth. Milk Dairy J. 50, 493-499.

171. Uyttendaele, M., P. De Troy and J. Debevere. 1999a. Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market. Int. J. Food Microbiol. 53, 75-80.

172. Uyttendaele, M., P. De Troy and J. Debevere. 1999b. Incidence of *Salmonella*, *Campylobacter jejuni*, *Campylobacter coli*, and *Listeria monocytogenes* in poultry carcasses and different types of poultry products for sale on the Belgian retail market. 62, 735-740.

173. de Valk, H., V. Vaillant, C. Jacquet, J. Rocourt, F. le Querrec, F. Stainer, N. Quelquejeu, O. Pierre, V. Pierre, J.-C. Descenclos and V. Goulet. 2001. Two consecutive nationwide outbreaks of listeriosis in France, October 1999-February 2000. Am. J. Epidemiol. 154, 944-950.

174. Vazquez-Boland, J. A., M. Kuhn, P. Berche, T. Chakraborty, G. Dominguez-Bernal, W. Goebel, B. Gonzalez-Zorn, J. Wehland and J. Kreft. 2001. *Listeria* pathogenesis and molecular virulence determinants. Clin. Microbiol. Rev. 14, 584-640

175. Venables, L. J. 1989. *Listeria monoctogenes* in dairy products - the Victorian experience. Food Australia. 41, 942-943.

176. 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.

177. Wang, C. and P. Muriana. 1994. Incidence of *Listeria monocytogenes* in packages of retail franks. J. Food Prot. 57, 382-386.

178. Wernars, K., P. Boerlin, A. Audurier, E. G. Russell, G. D. W. Curtis, L. Herman and N. van der Mee-Marquet. 1996. The WHO multicenter study on *Listeria monocytogenes* subtyping: random amplification of polymorphic DNA (RAPD). Int. J. Food Microbiol. 32, 325-341.

179. Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey. 1990. DNA polymorphism amplified by arbitrary primers useful as genetic markers. Nucl. Acids Res. 18, 6531-6535.

180. Wirtanen, G. and T. Mattila-Sandholm. 1993. Epifluorescence image analysis and cultivation of foodborne biofilm bacteria grown on stainless steel surface. J. Food Prot. 56, 678-683.