



Kaarina Aarnisalo

Equipment hygiene and risk
assessment measures as tools in the
prevention of *Listeria monocytogenes*
-contamination in food processes

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**Equipment hygiene and risk
assessment measures as tools
in the prevention of *Listeria
monocytogenes* -contamination
in food processes**

Kaarina Aarnisalo

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Keywords food hygiene, *Listeria monocytogenes*, risk assessment, hygienic design, disinfectant, lubricant, maintenance, microbial modelling, transfer of bacteria, automated ribotyping

Abstract

Several factors affect on the hygiene level of food processing equipment. A problematic pathogen occurring in food processing is *Listeria monocytogenes*, causing listeriosis with high mortality (20–30%) especially for individuals with reduced immunity. This bacterium is very tolerant to different stress factors and as it can be present in most of the raw materials of food processes, its total elimination is almost impossible. Efficient control of *L. monocytogenes* at the processing plant level requires good equipment hygiene, including functioning good manufacturing and hygiene practices used by all employees, effective means of decontamination and rapid detection of contamination sources, as well as hazard analysis systems supported by risk assessment procedures. The present thesis focuses on deficiencies and improvements in these equipment hygiene and risk assessment practices with the aim of elucidating and developing the most efficient practices against *L. monocytogenes*.

The hygienically most problematic types of equipment in the Finnish food industry were investigated by using a mail-survey. These were identified as the packaging machines, conveyers, dispensers, slicing machines and cooling machines. The main reason for the equipment being considered as problematic was poor hygienic design. The results show clearly that equipment designers must focus their performance on more suitable equipment design. Additionally, an investigation based on a mail-survey and microbiological sampling was made concerning hygiene performance of the maintenance personnel in food processing plants. Clear deficiencies were found e.g. in use of protective clothing, washing of hands and tools as well as avoiding foreign bodies left on the production lines. The results of these studies also indicate that *L. monocytogenes* may be transferred through maintenance work. Training of maintenance personnel with reference to hygienic practices must be increased.

Topics connected to the maintenance operations which have received only minor attention in previous studies include the growth and survival of *L. monocytogenes*

in lubricants used in the equipment as well as control of the bacterium with disinfectants at cold temperatures. In the current thesis the survival, growth and transfer of the bacterium in lubricants used in food processing equipment was studied. The results showed that lubricants used in maintaining the equipment may act as contamination vehicles of *L. monocytogenes*. As the temperatures in food processing premises are usually low, an investigation of the efficiency of eight commonly used commercial disinfectants against *L. monocytogenes* strains at +5 °C was performed. The tested agents were generally efficient at the recommended concentrations and effect times. Thus they appear to be suitable for control of *L. monocytogenes* at the plant level, with only a few exceptions.

Rapid, reliable and easy-to-use methods are needed at the processing plant level. Consequently the suitability of automated ribotyping was compared with the traditionally accepted and successfully used pulsed-field gel electrophoresis (PFGE) to discriminate *L. monocytogenes* isolates and thus trace contamination sources in food plants. PFGE had a higher discriminatory power for *L. monocytogenes* isolates than automated ribotyping. However, based on its automation and rapidity automated ribotyping can be considered a good method for control purposes, although in epidemiological studies identical results must be confirmed with PFGE.

Additionally, in this thesis risk assessment practices were developed by investigating and modelling recontamination of a product and by a plant-level quantitative risk assessment. Transfer of *L. monocytogenes* from slicing blade to slices of cold-salted salmon was investigated and modelled. Transfer with a progressive exponential reduction in the quantity of bacteria (log CFU/g) in slices was detected. The results provide an example to food processors of how limited data from microbiological analysis can be used to assess the level of recontamination for risk assessment purposes. The principles of microbiological risk assessment can be used at the processing plant level to assist in developing Hazard Analysis Critical Control Point (HACCP)- systems in order to provide a more scientific and comprehensive approach to the control of *L. monocytogenes* and other microbiological hazards. As a concluding example, a practical approach to quantitative risk assessment of *L. monocytogenes* for one product at the plant level is presented. This approach helps food processors in illustrating the risks caused by the products for consumers and thus rationalizing risk management actions against *L. monocytogenes*.

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Avainsanat food hygiene, *Listeria monocytogenes*, risk assessment, hygienic design, disinfectant, lubricant, maintenance, microbial modelling, transfer of bacteria, automated ribotyping

Tiivistelmä

Elintarviketuotantolaitteiden hygieniatasoon vaikuttavat useat tekijät. *Listeria monocytogenes* -bakteeri on elintarvikeprosesseissa esiintyvä ongelmallinen patogeeni, joka aiheuttaa listerioosia erityisesti riskiryhmiin kuuluville henkilöille. Tautiin sairastuneiden kuolleisuus on 20–30 %. Bakteeri kestää hyvin erilaisia stressitekijöitä ja koska bakteeria on voitu todeta myös useimmista elintarvikeprosesseissa käytetyistä raaka-aineista, sen täydellinen hävittäminen prosesseista on lähes mahdotonta. *L. monocytogenes* -bakteerin tehokas hallinta tuotantolaitostasolla koostuu hyvästä laitehygieniasta ja sisältää toimivat, hyvät tuotanto- ja hygieniakäytännöt kaikkien työntekijöiden osalta, tehokkaat bakteerin tuhoamiseen tarkoitetut menetelmät ja nopeat kontaminaatiolähteiden toteamisen menetelmät yhdistettynä riskinarviointikäytännöillä tuettuun prosessin vaara-analyysiin. Tässä työssä selvitettiin puutteita ja parannuksia nykyisin käytössä olevien laitehygieniakäytäntöjen osalta ja kehitettiin riskinarviointikäytäntöjä mahdollisimman tehokkaiden *L. monocytogenes* -bakteerin torjumiskeinojen varmistamiseksi.

Tuotantohygienialtaan ongelmallisimpia laitteita Suomen elintarviketeollisuudessa selvitettiin kyselytutkimuksena. Ongelmallisimmiksi laitteiksi todettiin pakkaus-koneet, kuljettimet ja annostelu-, siivutus- ja jäähdytyskoneet. Pääasialliseksi syyksi kaikkien näiden laitteiden osalta todettiin huono hygieeninen laitesuunnittelu. Tulokset osoittavat selvästi, että laitevalmistajien on paneuduttava asiaan paremmin ja kehitettävä elintarviketeollisuuteen tarkoitettujen laitteiden hygieenisyyttä. Selvitys tehtiin myös laitteita huoltavan kunnossapitohenkilöstön hygieenisistä toimintatavoista kyselytutkimuksena ja mikrobiologisella näytteenotolla. Selkeitä puutteita toimintatavoissa voitiin todeta mm. suojavaatetuksessa, käsien ja työkalujen puhdistamisessa sekä vierasesineiden tuotantolinjoille jäämisen osalta. Tutkimuksessa löydettiin myös viite siitä, että *L. monocytogenes* voisi siirtyä huoltotyön yhteydessä tehtaan sisällä. Kunnossapitohenkilöstön hygieniakoulutuksen määrää on lisättävä.

Kunnossapitotyöhön liittyviä, aiemmin vain vähän tutkittuja aihealueita ovat *L. monocytogenes* -bakteerin kasvu ja selviytyminen laitteissa käytetyissä voiteluaineissa sekä bakteerin tuhoaminen desinfiointiaineilla kylmissä lämpötiloissa. Työssä selvitettiin *L. monocytogenes* -bakteerin selviytymistä, kasvua ja siirtymistä laitteistoissa käytetyissä voiteluaineissa. Tulokset osoittivat, että voiteluaineet voivat toimia *L. monocytogenes* -kontaminaation lähteinä ja siirtymisvälineenä. Koska tuotantolämpötilat elintarvikeprosesseissa ovat yleensä alhaiset, kahdeksan nykyisin käytössä olevan kaupallisen desinfiointiaineen tehoa *L. monocytogenes* -bakteeriin selvitettiin +5 asteessa. Desinfiointiaineet tehosivat yleensä valmistajan antamalla alhaisimmilla käyttökonsentraatioilla ja käyttöajoilla, vain joitakin poikkeuksia lukuun ottamatta.

Koska tehdastasolla tarvitaan nopeita, luotettavia ja helppokäyttöisiä menetelmiä, automaattisen ribotyypityksen soveltuvuutta *L. monocytogenes* -isolaattien eroteluun ja siten kontaminaatiolähteiden jäljittämiseen elintarvikeprosesseissa verrattiin hyväksi todettuun pulssikenttägeelielektroforeesimenetelmään. Tulokset osoittivat, että pulssikenttägeelielektroforeesi oli erottelukyvyltään parempi. Nopeutensa ja automaattisuutensa takia automaattinen ribotyypitys soveltuu kuitenkin myös hyvin hygieniavalvontaan, mutta epidemiologisissa selvityksissä ribotyypityksellä saadut identtiset tulokset on varmistettava pulssikenttägeelielektroforeesilla.

Työssä kehitettiin lisäksi riskinarviointikäytäntöjä selvittämällä ja mallintamalla tuotteen jälkikontaminaatiota sekä kuvaamalla tehdastason riskinarviointi. *L. monocytogenes* -bakteerin siirtymistä siivutettaessa terästä graavilohisiivuihin tutkittiin ja sitä mallinnettiin. Selkeää siirtymistä todettiin bakteerimäärien eksponentiaalisesti pienentyessä siivuissa. Tulokset toimivat esimerkkinä elintarvikeyrityksille rajatun mikrobiologisen tutkimusaineiston käytöstä jälkikontaminaation vaikutuksen arvioimiseen. Kvantitatiivisen mikrobiologisen riskinarvioinnin periaatteita voidaan soveltaa tuotantolaitostasolla Hazard Analysis Critical Control Point (HACCP) -järjestelmien kehittämisessä tieteellisemmän ja kokonaisvaltaisemman listeriavalvonnan aikaansaamiseksi. Tässä työssä viimeisenä esimerkkinä kuvataan käytännönläheinen yhden tuotteen tuotantolinja -tason *L. monocytogenes* -riskinarviointi. Tämä lähestymistapa auttaa elintarviketuottajia hahmottamaan kuluttajille tuotteista aiheutuvaa riskiä ja siten vertailemaan ja rationalisoimaan eri riskinhallintakeinoja *L. monocytogenes* -bakteerin torjumisessa.

Preface

This study was carried out at VTT Technical Research Centre of Finland during the years 2000–2007. The financial support of VTT, Tekes – the Finnish Funding Agency for Technology and Innovation as well as the Jenny and Antti Wihuri Foundation is gratefully acknowledged. All studies were undertaken in cooperative projects with industrial partners, who are also gratefully acknowledged.

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Kaarina Aarnisalo

List of publications

- I Aarnisalo, K., Tallavaara, K., Wirtanen, G., Maijala, R. and Raaska, L. 2006. The hygienic working practices of maintenance personnel and equipment hygiene in the Finnish food industry. *Food Control*, Vol. 17, pp. 1001–1011.
- II Aarnisalo, K., Autio, T., Sjöberg, A.-M., Lundén, J., Korkeala, H. and Suihko, M.-L. 2003. Typing of *Listeria monocytogenes* isolates originating from the food processing industry with automated ribotyping and pulsed-field gel electrophoresis. *Journal of Food Protection*, Vol. 66, No. 2, pp. 249–255.
- III Aarnisalo, K., Raaska, L. and Wirtanen, G. 2007. Survival and growth of *Listeria monocytogenes* in lubricants used in the food industry. *Food Control*, Vol. 18, pp. 1019–1025.
- IV Aarnisalo, K., Lundén, J., Korkeala, H. and Wirtanen, G. 2007. Susceptibility of *Listeria monocytogenes* strains to disinfectants and chlorinated alkaline cleaners at cold temperatures. *LWT – Food Science and Technology*, Vol. 40, pp. 1041–1048.
- V Aarnisalo, K., Sheen, S., Raaska, L. and Tamplin, M. 2007. Modelling transfer of *Listeria monocytogenes* during slicing of ‘gravad’ salmon. *International Journal of Food Microbiology*, Vol. 118, pp. 69–78.
- VI Aarnisalo, K., Vihavainen, E., Rantala, L., Maijala, R., Suihko, M.-L., Hielm, S., Tuominen, P., Ranta, J. and Raaska, L. Use of results of microbiological analyses for risk-based control of *Listeria monocytogenes* in marinated broiler legs. *International Journal of Food Microbiology*, *in press*.

The author's contribution to the appended publications

- I The author had the main responsibility in planning the work on hygienic working practices of maintenance personnel, together with Dr. Laura Raaska; in collecting and analyzing the data and in writing the paper. The author supervised and participated in analyzing the microbiological samples. The author had the main responsibility in planning the questionnaire on hygienic equipment design and in collecting and analyzing the data.
- II The work was planned together with other authors. Kaarina Aarnisalo had the main responsibility in choosing the *L. monocytogenes* isolates for the study and had participated in collecting most of them. She carried out the serotyping, collected the result tables to the paper and compared the results by calculating the *DI* values. She wrote the paper together with Dr. Maija-Liisa Suihko and other authors. Dr. Maija-Liisa Suihko was responsible for the results of automated ribotyping and Dr. Tiina Autio for pulsed-field gel electrophoresis.
- III The author had the main responsibility in designing the experiments, together with Dr. Gun Wirtanen; in interpreting the results and writing the paper. She supervised and partially participated in the laboratory work. Statistical analysis was performed together with Anne Arvola.
- IV The author had the main responsibility in designing the experiments, together with other authors, and in writing the paper. She carried out the transfer experiments. She participated in the modelling work, the main responsibility for modelling being with Dr. Shiowshu Sheen.
- V The author had the main responsibility in planning the work and interpreting the results concerning suspension and surface experiments and in writing the paper. She supervised and partially participated in the laboratory work concerning suspension and surface experiments. Dr. Janne Lundén had main responsibility in the MIC and adaptation experiments.

VI The author had the main responsibility in planning the work and in writing the paper, together with Dr. Laura Raaska and other authors. The author acted as supervisor of and participated in collecting and analyzing the broiler legs and in performing the laboratory heating experiments. The risk assessment was performed together with Dr. Riitta Maijala and Dr. Jukka Ranta (responsible for modelling). Dr. Maija-Liisa Suihko was responsible for the results of automated ribotyping and Leila Rantala for those of pulsed-field gel electrophoresis and serotyping.

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List of symbols

| | |
|-------|--|
| AISI | American Iron and Steel Institute |
| ALOA | Agar <i>Listeria</i> Ottavani and Agosti |
| ANSI | American National Standards Institute |
| a_w | water activity |
| CCD | charge-coupled device camera |
| CCP | Critical Control Point |
| cDNA | complementary deoxyribonucleic acid |
| CEN | European Committee for Standardization |
| CFU | colony forming units |
| DI | discrimination index |
| DIN | Deutsches Institut für Normung |
| DNA | deoxyribonucleic acid |
| EHEDG | European Hygienic Engineering and Design Group |
| ELGI | European Lubrication Grease Institute |
| EPDM | ethylene propylene diene monomer rubber |
| FDA | American Food and Drug Administration |
| GHP | Good Hygiene Practice |
| GMP | Good Manufacturing Practice |
| HACCP | Hazard Analysis Critical Control Point |
| HDPE | high density polyethylene |
| ISO | International Standardization Organisation |
| LMBA | <i>Listeria monocytogenes</i> Blood Agar |

| | |
|--------|--|
| ME | microbicidal effect |
| MPN | most probable number |
| NBR | nitrile butyl rubber |
| NLGI | National Lubricating Grease Institute |
| OCP | own-checking plan |
| PALCAM | Polymyxin Acriflavine Lithium Chloride Ceftazidime Aesculin Mannitol agar |
| PC | polycarbonate |
| PCR | polymerase chain reaction |
| PFGE | pulsed-field gel electrophoresis |
| PMP | Pathogen Modeling Program |
| PP | polypropylene |
| PU | polyurethane |
| PVC | polyvinyl chloride |
| QAC | quaternary ammonium compound |
| rRNA | ribosomal ribonucleic acid |
| RT | ribotype |
| RTE | ready-to-eat food |
| SD | standard deviation |
| TSB | Tryptone Soya Broth |
| USDA | United States Department of Agriculture |
| UVM | University of Vermont broth |
| WTO | World Trade Organization |

1. Introduction

1.1 Equipment hygiene in food processing

Several factors have been reported to affect on the hygiene level of food processing equipment, including hygienic design of the equipment, hygienic practices of personnel, cleaning and disinfection of the equipment, lubricants used in the equipment as well as lay-out of the processing, air-currents, type of food product and cleanliness of the processing environment (Lelieveld et al., 2003). In this thesis, the first four factors were studied more specifically, with focus on occurrence of the pathogenic bacterium *Listeria monocytogenes* – a problematic contaminant in food processing.

1.1.1 Hygienic design of food processing equipment

Hygiene problems in equipment are caused when micro-organisms attach to the surfaces, survive on them and later become detached thus contaminating and reducing the quality of the product (Wirtanen, 1995). This can be due to a poor hygienic design in cases where the machines cannot be cleaned properly. Constructions that cause problems include sites where soil, product debris and micro-organisms can accumulate, e.g. dead ends, sharp corners and low-quality seals and joints (Anon., 1993; Anon., 1995). Equipment has been identified as the source of contamination in the food industry in many studies (see e.g. Table 4). In Finland hygiene in dairy plants was investigated by the Finnish Food Safety Authority in 2001–2005. Deficiencies were reported e.g. in topics concerning equipment hygiene as presented in Table 1. Additionally, *L. monocytogenes* was found in 313 (40–83 per year)/13876 (2342–4242 per year), i.e. 2.3% (1.7–2.9%) of the samples, which were taken from the processing equipment and the working environment (NFA, 1998, 2000–2005). Good hygienic design of food processing equipment protects the product from contamination with substances harmful to consumer health and provides access for cleaning, maintenance and inspection (Lelieveld et al., 2003). Criteria for good hygienic design are presented in Table 2.

Table 1. Deficiencies affecting on equipment hygiene observed in Finnish dairy plants during 2001–2005, inspected by the Finnish Food Safety Authority (397–450 inspections/year) (NFA, 1998, 2000–2005).

| Topic | No. of lacks in inspections^a (%) |
|--|--|
| Constructions and surfaces in the plant; type or wear of materials | Range: 21–29 (4.7–6.7) Total: 126 (5.8) |
| Layout of production rooms and arrangements of production | Range: 12–17 (3.0–3.8) Total: 74 (3.4) |
| Processing equipment and methods, temperature control | Range: 1–10 (0.2–2.3) Total: 30 (1.4) |
| Personnel: hair, clothing, jewellery, make-up, practices | Range: 2–7 (0.5–1.6) Total: 23 (1.1) |
| Cleaning and disinfection | Range: 6–8 (1.4–1.8) Total: 14 (1.6) |

^a No. of plants inspected were 132 (2001), 132 (2002), 131 (2003), 126 (2004), 124 (2005).

1.1.2 Hygiene of personnel working with equipment

An important factor affecting the hygiene level of food processing equipment is the hygienic practices of people working with the equipment. Many papers have been published on unsatisfactory hygienic practices in food handling (Upmann and Reuter, 1998; Haupt et al., 1999; Henroid and Sneed, 2004) and outbreaks caused by these (Guzewich and Ross, 1999; Duncanson et al., 2003; LaPorte et al., 2003). Contamination of foods by food handlers has been identified as one of the most important causes of foodborne outbreaks (FDA, 2000; Michaels et al., 2004). One group of employees who frequently work with the equipment are the maintenance personnel, who dismantle machinery for cleaning procedures and reassemble it after cleaning in addition to maintaining the operation of machinery during production. No earlier reports are available on the effect of this specific group of personnel on production hygiene.

Personnel are both reservoirs and vectors of micro-organisms (Marriott, 1999; Holah and Taylor, 2003). The level and risk of contamination from personnel is difficult to measure as it depends on various factors such as the different activities and the range of personnel movement patterns during the working day as well as the perceptions and attitudes of the personnel (Troller, 1993; Holah and Taylor, 2003).

Table 2. Criteria for good hygienic design of food processing equipment (Anon., 1993; Anon., 1995; CEN, 1997; Lelieveld et al., 2003).

| Design parameters | Generally recommended criteria for the food area^a in equipment |
|-------------------------------------|--|
| Construction materials | durable, cleanable, disinfectable; resistant to cracking, abrasion and corrosion; non-toxic, non-absorbent; do not transfer undesirable odours etc.; do not contribute to contamination of food. Suitable materials are e.g. stainless steels EN 1.4301 (AISI 304), EN 1.4404 (AISI 316L), EN 1.4435 (AISI 316I), EN 1.4571 (AISI 316Ti) and plastics (see Conveyer belts) |
| Surface finish | cleanable, disinfectable, smooth, continuous, prevents trapping of microbes, $R_a \geq 0.8\mu\text{m}$ |
| Drainability | self draining |
| Corners | rounded, no dead spaces, cleanable, disinfectable |
| Joints | sealed, hygienic, no gaps or crevices, protruding ledges and seals should be avoided |
| Welds | smooth, continuous; no misalignments, cracking or porosity; sloped edges |
| Fasteners (screws, bolts) | avoid if possible; cleanable, disinfectable |
| Seals/Gaskets | tolerate processing conditions without changes, cleanable, disinfectable, suitable materials include e.g. EPDM ^b , NBR ^c , nitrile rubber, silicone rubber, Viton rubber |
| Rims | no ledges where product can lodge; cleanable, top rims rounded and sloped |
| Bearings, shafts | located outside the food area, cleanable and disinfectable, food grade lubricant used |
| Panels, covers, doors | prevent entry of soil and contaminants, cleanable, disinfectable |
| Instrumentation and control devices | prevent ingress of contamination, sanitary couplings |
| Conveyer belts | non-absorbent, covered edges, rounded rims, cleanable, disinfectable, tolerant; suitable materials PP ^d , PVC ^e , acetal copolymer, PC ^f , HDPE ^g |
| Placing and installation | electronic devices in the non-food area, sealed to floor, rounded pedestal, clear space everywhere around the equipment to enable cleaning |

^a Area composed of surfaces in contact with food; the food area also includes the surfaces with which the product may come into contact under intended conditions of use, after which it returns into the product (CEN, 1997).

^b ethylene propylene diene monomer

^d polypropylene

^f polycarbonate

^c nitrile butyl rubber

^e polyvinyl chloride

^g high density polyethylene

Micro-organisms in the human body can be divided into resident and transient microbes. The face, neck, hands and hair contain both a higher proportion of transient micro-organisms and a higher general bacterial density than other parts of the body (Troller, 1993; Holah and Taylor, 2003). Hands are the major source of infection from transient and resident micro-organisms. *L. monocytogenes* has been found on hands or gloves used in food-handling (Destro et al., 1996; Autio et al., 1999). Hand hygiene has been considered to be the most important single operation affecting reduction of hygiene risk (Paulson, 2000; Holah and Taylor, 2003). It has also been estimated that approximately 5% of healthy people are asymptomatic carriers of *L. monocytogenes* in their intestines (Farber and Peterkin, 1991; Farber and Harwig, 1996; Skinner, 1996). Of the employees of poultry houses and slaughterhouses as many as 10–30% have been shown to be carriers (Bennett, 1986).

Contamination from personnel can be direct or indirect. Direct contamination can occur by contact between the body and the food product. In indirect contamination, people act as vectors and transfer contamination from one area or surface to another by e.g. using the same equipment in raw and cooked product areas (Holah and Taylor, 2003). Clothing or footwear can contaminate work surfaces when personnel move around the plant (Holah and Taylor, 2003). Several investigations on food-handling practices of personnel in hospitals (Angelillo et al., 2000; Askarian et al., 2004; Danchaivijitr et al., 2005) and food service environments (Henroid and Sneed, 2004; Sneed et al., 2004; Tang and Wong, 2004) have been published. Less information is available on hygienic practices of food employees in food plants or abattoirs, but several deficiencies in hygienic practices have been identified in the studies performed (Table 3).

Important means of avoiding contamination of products and equipment include Good Hygiene Practices (GHPs) and regular medical screening of the personnel, sufficient training of hygiene aspects and control of indirect contamination (Marriott, 1999; Holah and Taylor, 2003). The regulation on food hygiene (EU) No. 852/2004, Annex II (Anon., 2004a) state that food -handlers must be supervised and instructed in food hygiene matters commensurate with their work activity. Even if the personnel maintaining the equipment do not actually touch the raw materials or food products, they probably touch a multitude of surfaces in contact with unpacked products while maintaining the machinery.

Table 3. Studies on hygienic working practices and attitudes on food hygiene of food industry personnel.

| Food sector and country | Type of survey | Main conclusions of hygienic practices | | Reference |
|--|---|--|--|---------------------------|
| | | Positive | Negative | |
| 10 Meat plants, Austria | Survey of self-assessment | | <ul style="list-style-type: none"> - relative frequency of non-conformities regarding own-checking was 25% at best, for premises/equipment/tools 10%, personal hygiene 21%, specific process hygiene 30% and for cutting plant hygiene 9% at best. Simple hygiene fundamentals were not used. | Haupt et al., 1999 |
| 54 Abattoirs, the Netherlands | Inspection with checklist, microbiological sampling, two visits | + during the follow-up study improved consciousness of Good Manufacturing Practices (GMPs) among employees was observed | <ul style="list-style-type: none"> - better consciousness of importance of GMPs required, e.g. more frequent hand washing | Heuvelink et al., 2001 |
| 5 Abattoirs, Finland | Visual observation and microbiological sampling | + frequency of slaughtering errors and touching of the carcasses was low | <ul style="list-style-type: none"> - designing slaughtering lines so as to make hygienic working possible and the enforcement of hygienic practices, such as the regular disinfection of working tools, were needed. Hygienic practice was associated with the carcass contamination level. | Rahkio and Korkeala, 1996 |
| Meat abattoir, Austria | Visual observation | | <ul style="list-style-type: none"> - defects in personnel clothing and behaviour, personal belongings stored in slaughter room | Upmann and Reuter, 1998 |
| Abattoir, South Africa | A structured questionnaire | + basic hygiene practices were used and the employees adhered to majority of them | <ul style="list-style-type: none"> - improved communication between management and workers and more training needed | Nel et al., 2004 |
| Small food manufacturing plants, UK | Structured interviews with managers on hygiene training | + all the firms had taken some hygiene training, managers had positive attitudes towards training | <ul style="list-style-type: none"> - hygiene programmes unstructured and unrecorded, regular refresher training given in less than half of the plants, lack of time and money were perceived to be a major restricting factors to training | Worsfold, 2005 |
| Bakery, USA | Microbiological sampling | | <ul style="list-style-type: none"> - food handler was a source of viral contamination in baked goods | Weltman et al., 1996 |
| Flamenguin plant, Spain | Microbiological sampling | | <ul style="list-style-type: none"> - raw materials and food handlers were the principal sources of microbiological contamination | Cordoba et al., 1998 |
| Food industry (meat, dairy, bakery, fish), Finland | Mail survey on attitudes towards own-checking and HACCP plans was distributed to 87 plants, with response rate of 34.9% | + attitudes exclusively positive; all 30 companies had a functioning own-checking plan (OCP), while other quality management programs were less prevalent | <ul style="list-style-type: none"> - most difficulties in devising the OCP/HACCP plan caused choosing the critical control points, committing the firm's entire workforce and organizing the documentation | Helm et al., 2006 |
| Food handlers in general, Italy | Interview with a structured questionnaire within a random sample from 411 food handlers | + positive attitude toward foodborne disease control by the great majority, more likely achieved when more training received | <ul style="list-style-type: none"> - the attitude was not supported by hygienic practices, e.g. only 20.8% used gloves when touching unwrapped raw food, strong need for educational programs for improving knowledge | Angelillo et al., 2000 |
| 20 Food premises, UK | An audit proforma, questionnaires on effectiveness of food hygiene training | | <ul style="list-style-type: none"> - formally trained staff work more hygienically than non-trained | Kirby and Gardiner, 1997 |
| 52 Small and medium size (SME) food businesses, UK | Questionnaires on food handler (including food handlers working at retail, catering or, manufacturing) beliefs and self-reported practices on food safety | + food handlers were aware of the food safety actions they should be carrying out, 95% had received food hygiene training (100% in the manufacturing sector). Food handlers in the manufacturing sector were more likely to report carrying out food safety behaviours than food handlers in the catering and retail sector. | <ul style="list-style-type: none"> - 63% admitted to sometimes not carrying out food safety procedures, lack of time, personnel, resources | Clayton et al., 2002 |

1.1.3 Lubricants used in equipment

The purpose of the use of lubricants in food-processing equipment is to reduce friction and wear, inhibit the access of outside particles to equipment surfaces, protect surfaces against corrosion and remove wear particles, increase the efficiency of systems and the transfer of heat, power and electricity (Netuschil, 1995; Lewan, 2003). The lubricants usually used in the food industry are composed of base oil (e.g. mineral oil, white oil, silicone), thickeners and additives. The lubricants can be completely oil-based but many are emulsions containing water. To achieve high technical performance, the lubricants are often made of different synthetic components (Netuschil, 1995).

Lubricants can be contaminated with water, organic material, residues of other lubricants, physical or chemical substances causing oxidation and other chemical reactions, particles from corrosion (Anon., 2003), or with micro-organisms. Contamination in lubricants can lead to contamination of food products e.g. through leakage from bearings, dripping from open lubrication points e.g. chains, leakage from oil circulation systems or from corroded joints of oil-filled heat exchange systems or contact between oil-coated machine surfaces (Anon., 2003). The microbes must often tolerate anaerobic conditions and low water activity in lubricants. *L. monocytogenes* is capable of withstanding the above mentioned conditions (Buchanan et al., 1989; Lou and Yousef, 1999). It has been shown to survive in butter, which was the vehicle in a Finnish *L. monocytogenes* epidemic in 1998–1999 (Lyytikäinen et al., 2000). Rossmore (1988) reported findings of *L. monocytogenes* in dairy conveyer lubricants. Use of lubricants in conveyers has also caused hygiene problems in breweries (Heinzel, 1988). *Acinetobacter* sp., *Algalgigenes* sp., *Pseudomonas* sp. and sulphate reducing bacteria have been isolated from lubricants (Ortiz et al., 1990; Hamilton, 1991). Petitdemange et al. (1995) observed clear differences between strains of *Clostridium butyricum* in their ability to survive and grow in industrial glycerol.

During processing and cleaning the quality of lubricants is impaired. The survival of microbes in lubricants has been reported to be enhanced when the lubricants are contaminated with organic material and water (Netuschil, 1995; Lou and Yousef, 1999). The addition of antimicrobial substances such as glutaraldehyde (25 ppm) or isothiazoline (10 ppm) has been reported to inhibit the growth of microbes, e.g. *L. monocytogenes* (Rossmore, 1988; Hsu, 1991).

1.1.4 *Listeria monocytogenes* in equipment hygiene

L. monocytogenes is a bacterium of special concern in food processing as it has been connected to several food-borne outbreaks and it causes listeriosis, a severe illness especially for immunocompromized individuals, with high mortality of approximately 20–30% (Rocourt, 1996; Rocourt et al., 2003; Lyytikäinen et al., 2006). The bacterium causes problems in food processes, as it tolerates well various stress factors that it encounters in food processing plants and also has a very good ability to attach to different surfaces and thus to persist in the food plants for years. *L. monocytogenes* has been detected from food processing equipment which has been implicated in the contamination of final products (Table 4). It has been isolated from the equipment and processing environments of various food sectors in several studies (Gravani, 1999; Tompkin, 2002). The characteristics of this bacterium are presented in detail in Section 1.3.

1.2 Legislation and standards

1.2.1 Legislation on hygienic design of food processing equipment

In Europe the most important legislation giving criteria for hygienic design of equipment is the Council Directive on the approximation of the laws of Member States relating to machinery (89/392/CEE, revised 98/37/EC, Anon., 1998). It contains safety requirements and a few very basic principles of hygienic design on constructions and surfaces, which must be cleanable and safe for production. This directive also obliges the equipment manufacturers to provide guidelines on sanitizing the equipment. EC Regulation No. 1935/2004 on materials and articles intended to come into contact with food applies to materials and articles which already are or are intended to come into contact with food (Anon., 2004b). According to this regulation the materials shall not transfer their constituents to food in quantities which could endanger human health or cause changes in composition or organoleptic characteristics of food. There is also a EC Regulation No. 2023/2006 on good manufacturing practice for materials and articles intended to come into contact with food (Anon., 2006).

Table 4. L. monocytogenes findings in food processing equipment which have been associated with contamination of final product.

| Equipment | Food sector and product | Country | Reference |
|--|--|----------------|--------------------------------|
| Air chiller | Poultry, raw | Finland | Miettinen et al., 2001 |
| Cold-smoker | Fish, cold-smoked salmon | USA | Norton et al., 2001 |
| Conveyer belts | Meat | Nordic country | Suihko et al., 2002 |
| | Seafood | Nordic country | Suihko et al., 2002 |
| Dicing machine | Poultry, raw | Finland | Miettinen et al., 2001 |
| | Meat, cooked | Finland | Lundén et al., 2002 |
| Freezer | Poultry, cooked | USA | Berrang et al., 2002 |
| Packaging machine | Dairy, butter | Finland | Maijala et al., 2001 |
| | Dairy, ice cream | Finland | Miettinen et al., 1999a |
| Salting machine | Fish, cold-smoked salmon | Finland | Autio et al., 1999 |
| | Fish, cold-salted, cold-smoked and smoked salmon | Finland | Johansson et al., 1999 |
| Skinning machine | Meat | Nordic country | Suihko et al., 2002 |
| | Poultry, raw | Finland | Miettinen et al., 2001 |
| Slicing machine | Fish, cold-salted, cold-smoked and smoked salmon | Finland | Johansson et al., 1999 |
| | Fish, cold-smoked salmon | Finland | Autio et al., 1999 |
| | Fish, cold-salted, cold-smoked and smoked salmon | Finland | Johansson et al., 1999 |
| – slicer switches | Meat | Nordic country | Suihko et al., 2002 |
| | Meat, rillets | France | Goulet et al., 1998 |
| Tumbling machine | Meat | - ^a | Lelieveld et al., 2003 |
| | Meat, cooked | Greece | Samelis and Metaxopoulos, 1999 |
| Evisceration machine, pluck sorter, spin chiller | Poultry, raw | Denmark | Ojeniyi et al., 1996 |

^a not reported

A basic standard concerning hygiene requirements for the design of machinery is the International Standardization Organisation (ISO) standard 14159:2002 (ISO, 2002). It specifies hygiene requirements of machines and user information to be provided by the manufacturer. It applies to all types of machines and associated equipment used in applications where hygiene risks to the consumer of the product can occur. The European Committee for Standardization (CEN) issues standards for equipment manufacturers to enable fulfilling the requirements of the EU directive. One important basic standard is the standard EN 1672-2 “Food processing machinery – Safety and hygiene requirements – Basic concepts – Part 2; Hygiene requirements” (CEN, 1997). There are also a number of standards for different specific food processing equipment, e.g. for slicing, cutting and filling machines (<http://www.cen.eu/>; <http://www.3-a.org>). Guidelines and methods in

accordance with the standards are published by different organizations, e.g. by the European Hygienic Engineering and Design Group (EHEDG) (<http://www.ehedg.org>), 3-A Sanitary Standards Inc. (3-A, <http://www.3-a.org>) and NSF International, The Public Health and Safety Company™ (<http://www.nsf.org>).

1.2.2 Legislation on lubricants used in food processing

Lubricants used in food processing equipment at points where incidental contact with food products may occur (i.e. food-grade lubricants), have special requirements. They must fulfil the requirements of legislation and be internationally accepted, physiologically safe and neutral in taste and odour (Netuschil, 1995; Köhler, 2001). Use of food-grade lubricants is recommended in food-processing plants especially at critical control points (Anon., 2003). Definitions of food-grade lubricants can be found in the document FGL1/2001/issue 2 of the European Lubrication Grease Institute (ELGI), the National Lubricating Grease Institute (NLGI) and the EHEDG. The standard of Deutsches Institut für Normung (DIN) V 0010517 and the NSF International draft for an American National Standards Institute (ANSI) standard are also sources for official information on lubricants (Anon., 2003). These definitions include the registration of food-grade lubricants (H-1) by a competent third party organisation, as also required in the new ISO standard “Safety of machinery – Lubricants with incidental product contact – Hygiene requirements” (ISO, 2006). This standard specifies definitions and hygiene requirements for the formulation, manufacture, use and handling of lubricants which may come into incidental contact with products during manufacture and processing. The United States Department of Agriculture (USDA) has classified the lubricants into food-grade and non-food-grade. USDA H1 lubricants contain only components approved by The American Food and Drug Administration (FDA) and they can be used in places in which there is incidental contact with food. These classifications are also generally accepted in Europe, where legal requirements are less stringent (Köhler, 2001). The USDA stopped registering lubricants in 1998 and these records are nowadays maintained by NSF International (Yano, 2005). Additionally, a guideline on “Production and use of food-grade lubricants” has been published by EHEDG (Anon., 2003).

1.2.3 Legislation on *L. monocytogenes*

According to EC Regulation No. 2073/2005 on Microbiological criteria for foodstuffs (Anon., 2005), the limit of *L. monocytogenes* is 100 CFU/g at the end of the shelf-life of ready-to-eat (RTE) food products. In the same regulation it is also stated that food businesses manufacturing RTE foods, which may pose a *L. monocytogenes* risk for public health, shall sample the processing areas and equipment for *L. monocytogenes* as part of their sampling scheme (Anon., 2005). The presence of *L. monocytogenes* in meat and fish products is not regulated by Finnish food legislation, but in recent years several guidelines on control of *Listeria* in the food chain, targeted at meat and fish processing facilities and retailers, have been published by the authorities (<http://www.evira.fi>, <http://www.ktl.fi>). The National Food Agency of Finland has given press releases on vacuum-packed fish products, advising consumers to pay attention to the time for which the products are stored; to the home refrigerator temperature, which should be below 3 °C; and to consuming products before the best-by-date (Lyytikäinen et al., 2006). In the United States there is currently a zero tolerance policy for the levels of *L. monocytogenes* allowed in food (McLauchlin et al., 2004). In Canada, the limit is 100 CFU/g in RTE foods (Health Canada, 2004).

1.3 Characteristics of *L. monocytogenes* – a problematic food processing contaminant

1.3.1 The genus *Listeria* and *L. monocytogenes*

The genus *Listeria* consists of six species and two subspecies: *L. monocytogenes*, *L. ivanovii* subs. *ivanovii*, *L. ivanovii* subsp. *londoniensis*, *L. seeligeri*, *L. innocua*, *L. welshimeri* and *L. grayi*. Two of the species are pathogenic: *L. monocytogenes*, the foodborne human pathogen and *L. ivanovii*, an animal pathogen. *L. ivanovii* and *L. seeligeri* have also occasionally been associated with human listeriosis (Cummings et al., 1994; Lessing et al., 1994; Khelef et al., 2006).

Bacteria belonging to the genus *Listeria* are Gram-positive and non-sporeforming. They are catalase-positive, oxidase-negative and methyl red-positive bacteria. Voges-Proskauer reaction is positive. They are able to move with the aid of flagella at 20–25 °C. The bacteria are regular, short rods; some of the cells may

be curved. Their diameter is 0.4–0.5 µm and length 0.5–2 µm (Seeliger and Jones, 1986).

L. monocytogenes was first described by Murray et al. (1926) and was then named as *Bacterium monocytogenes* because it caused severe monocytosis in infected laboratory rabbits and guinea pigs. Later, in 1927 Pirie renamed the bacterium as *Listerella hepatolytica* and further in 1940 to its current name (Gray and Killinger, 1966).

1.3.2 Occurrence

Listeria, including *L. monocytogenes*, are ubiquitous in nature and can be found in water, mud, sewage and vegetation as well as in cattle milk, faeces of animals and humans, animal feed as well as in food and almost all food raw materials (Seeliger and Jones, 1986; Bell and Kyriakides, 2004; Khelef et al., 2006).

L. monocytogenes has been isolated from a large variety of both raw and cooked food products including meat products e.g. raw beef, lamb, pork, ground meats, ham, fermented and dried sausages, processed meats and pâté; poultry products e.g. raw and cooked poultry and eggs; sea food products such as raw fish, smoked salmon, uncooked and cooked shellfish; dairy foods e.g. raw and pasteurized milk, creams, soft, semisoft and hard cheeses and ice cream; vegetables including cabbage, cucumber, potatoes, tomatoes and frozen vegetables; and RTE foods (Farber and Peterkin, 1991; Bell and Kyriakides, 2004). The bacterium was recognized as a foodborne pathogen after several outbreaks in the early 1980s (Schlech et al., 1983; Bell and Kyriakides, 2004). Food products which have caused outbreaks of listeriosis include a variety from different areas of the food industry: cole slaw in Canada 1981 (Schlech et al., 1983), pasteurized milk in USA 1983 (Fleming et al., 1985), Mexican style cheese in USA 1985 (Linnan et al., 1988), soft cheese in Switzerland 1983–1987 (Bille, 1990), pâté in UK 1987–1989 (McLauchlin et al., 1991), jellied pork tongue in France 1992–1993 (Goulet et al., 1993), chocolate milk in USA 1994 (Dalton et al., 1997), corn and tuna salad in Italy 1997 (Aureli et al., 2000), rainbow trout in Sweden 1994–1995 (Ericsson et al., 1997) and in Finland 1997 (Miettinen et al., 1999b), delicatessen turkey meat in USA 2000 (Olsen et al., 2005), 2001 (Frye et al., 2002) and 2002 (Gottlieb et al., 2006), as well as butter

in Finland 1998–1999 (Lyytikäinen et al., 2000). Cases have also been caused by e.g. uncooked chicken meat (Schwartz et al., 1988; Anon., 1989; Kaczmarski and Jones, 1989). Summaries on the occurrence of this pathogen worldwide in different food products have been published (Farber and Peterkin, 1991; Autio, 2003) and therefore in this work a summary of the occurrence and amounts of *L. monocytogenes* in various food products in Finland, where the studies of the current thesis were also performed, is presented in Table 5.

1.3.3 Tolerance of pH, temperature and a_w

L. monocytogenes is able to survive in various environmental conditions. It is a facultatively anaerobic bacterium, which grows well in aerobic conditions (Khelef et al., 2006). It grows over a wide pH-range of 4.3–9.6 (Lou and Yousef, 1999), optimal growth being at neutral to slightly alkaline pH (Seeliger and Jones, 1986). Survival in as low pH as 1.4 has been reported (Reimer et al., 1988).

The temperature growth range of *L. monocytogenes* is 1–45 °C (Seeliger and Jones, 1986), but slow growth at as low as –0.4 °C (Walker et al., 1990) and even at –1.5 °C (Hudson et al., 1994) have been reported as well as good survival in much lower freezing temperatures (Lou and Yousef, 1999), e.g. many weeks in foods at –18 °C (Golden et al., 1988; Olsen et al., 1988). The optimum growth temperature is 30–37 °C (Seeliger and Jones, 1986). As the bacterium grows in refrigeration temperatures, it is a potential risk in cold rooms of food processing plants (Gravani, 1999). Cold stress adaptation of *L. monocytogenes* is a function of many molecular adaptation mechanisms and is an important characteristic of *L. monocytogenes*, enabling it to survive and proliferate in refrigerated foods and cold environments (Tasara and Stephan, 2006).

Table 5. Occurrence and amount of *L. monocytogenes* (L.m.) in various food products in Finland during 1996–2005.

| Food product | No. of <i>L.m.</i> positive samples / analyzed samples (%) | Quantity of <i>L.m.</i> (CFU/g): no. of samples / no. of all samples (%) | No. of producers with <i>L.m.</i> (%) | Year of study | Reference |
|--|--|--|---------------------------------------|--------------------|-----------------------------|
| Fish and other seafood | | | | | |
| cold-salted fish; – vacuum-packed | 44/172 (25.6) | ≤ 100: 10/16 (62.5) >100: 6/16 (37.5) | 16/46 (34.8) | 1996–2000 | Johansson et al., 2003 |
| | 22/110 (20.0) | | | 1996 | Johansson et al., 1999 |
| – rainbow trout, non-sliced | 4/12 (33.3) | | | | Lyhs et al., 1998 |
| – rainbow trout, sliced | 10/31 (32.3) | | | | Lyhs et al., 1998 |
| | 28/204 (13.7) | | | 2003–04 | Aalto et al., 2006 |
| – not vacuum-packed | 0/24 (0) | | | | Johansson et al., 2003 |
| | 13/81 (16.0) | | | 2003–04 | Aalto et al., 2006 |
| cold-smoked fish; – vacuum-packed | 44/223 (19.7) | <100: 32/43 (74.4) ≥ 100: 11/43 (25.6) | 7/37 (18.9) | 2003–04 | Aalto et al., 2006 |
| | 46/356 (13) | | | 2001 | Hatakka and Johansson, 2002 |
| | 76/720 (10.6) | | | 1996–2000 | Johansson et al., 2003 |
| | 5/30 (17) | ≤ 100: 1/5 (20.0) >100: 4/5 (80.0) | 3/12 (25) | 1996 | Johansson et al., 1999 |
| – salmon | 22/22 (100) | | 1/1 (100) | 1997 | Autio et al., 1999 |
| – rainbow trout, non-sliced | 4/42 (9.5) | | | | Lyhs et al., 1998 |
| – rainbow trout, sliced | 5/20 (25.0) | | | | Lyhs et al., 1998 |
| cold-smoked fish; not vacuum-packed | 4/54 (7.4) | | | 2003–04 | Aalto et al., 2006 |
| | 1/10 (10.0) | | | 1996–2000 | Johansson et al., 2003 |
| cold-salted and cold-smoked fish products; – vacuum-packed | 61/369 (16.5) | | | 1996–1999 | Anon., 2000b |
| | 6/42 (14.3) | | | 1996–1999 | Anon., 2000b |
| hot smoked fish; – vacuum-packed | 2/147 (1.4) | ≤ 100: 1/1 (100.0) | 1/12 (8.3) | 1996–2000 | Johansson et al., 2003 |
| | 1/48 (2) | | | 1996 | Johansson et al., 1999 |
| – rainbow trout | 1/42 (2.4) | | | | Lyhs et al., 1998 |
| – salmon | 0/6 (0) | | | | Lyhs et al., 1998 |
| hot smoked fish – not vacuum-packed | 0/8 (0) | | | 1996–2000 | Johansson et al., 2003 |
| roe | 0/29 (0) | | 0/11 (0) | 2003–04 | Aalto et al., 2006 |
| | 7/147 (4.8) | | 7/26 (26.9) | 1999 | Miettinen et al., 2003 |
| crustaceans and molluscs | 3/18 (17) | | | 1996–2000 | Johansson et al., 2003 |
| Dairy | | | | | |
| soft and semisoft cheeses from pasteurized milk | 0/132 (0) | | | 2003–04 | Aalto et al., 2006 |
| soft and semi-soft cheeses from raw milk | 5/90 (5.5) | 1.1 – 6×10 ³ | | 2003–04 | Aalto et al., 2006 |
| soft cheese | 0/49 (0) | | | 1996–2000 | Johansson et al., 2003 |
| unripened cheese | 2/144 (1.4) | | | 1996–2000 | Johansson et al., 2003 |
| milk and milk products | 6/842 (0.7) | | | 2001 | Hatakka and Majjala, 2003 |
| ice cream | 6/1129 (0.5) | | 1/1 (100) | 1990–1997 | Miettinen et al., 1999a |
| ice creams and desserts | 0/193 (0) | | | 2001 | Hatakka and Majjala, 2003 |
| milk products | 43 (1–19/year)/ 15476 (1531– 2961/year), i.e. 0.3 (0.04–0.7)% | | | 1998, 2000–2005 | (NFA, 1998, 2000–2005) |
| butter | 34/59 (57.6) | ≤ 100: 16/18 (88.9) 2/18 (11.1) | 1/1 (100) | 1998–1999 | Majjala et al., 2001 |
| edible fats and oils | | | 0/27 (0) | 2001 | Hatakka and Majjala, 2003 |

Table 5. Cont.

| Food product | No. of <i>L.m.</i> positive samples / analyzed samples (%) | Quantity of <i>L.m.</i> (CFU/g): no. of samples / no. of all samples (%) | No. of producers with <i>L.m.</i> (%) | Year of study | Reference |
|--------------------------------------|--|--|---------------------------------------|---------------|----------------------------|
| <u>Meat and meat products</u> | | | | | |
| meat and meat products | 128/857 (14.9) | | | 2001 | Hatakka and Maijala, 2003 |
| cold cuts | 0/236 (0) | | 0/4 (0) | 2002 | Johansson et al., 2002 |
| – liver sausages | 0/30 (0) | | | 1996–2000 | Johansson et al., 2003 |
| pieces of raw broiler | 35/100 (35) | | | 1996–2000 | Johansson et al., 2003 |
| | 38/61 (62.0) | | 3/3 (100) | 1997–1998 | Miettinen et al., 2001 |
| | 47/85 (55.3) | 5.01×10^3 MPN/kg ^a | 4/4 (100) | | Husu et al., 1993 |
| – frozen carcasses | 27/80 (33.8) | ≤ 2250 CFU/carcass | | | Johansson and Hirn, 1992 |
| eggs and egg products | 0/6 (0) | | | 2001 | Hatakka and Maijala, 2003 |
| <u>Vegetables and fruits</u> | | | | | |
| fresh vegetables and products | 2/158 (1.3) | | | 1996–2000 | Johansson et al., 2003 |
| frozen vegetables | 61/313 (19.5) | | | 1996–2000 | Johansson et al., 2003 |
| fruits and vegetables and products | 9/452 (2.0) | | | 2001 | Hatakka and Maijala, 2003 |
| berries | 4/344 (1.2) | | | 1996–2000 | Johansson et al., 2003 |
| <u>Others</u> | | | | | |
| RTE foods | 2/78 (2.6) | | | 1996–2000 | Johansson et al., 2003 |
| | 6/119 (5.0) | | | 2001 | Hatakka and Maijala, 2003 |
| soups and sauces | 0/88 (0) | | | 2002 | Hatakka and Maijala, 2003 |
| confectioneries | 1/23 (4.3) | | | 1996–2000 | Johansson et al., 2003 |
| salted mushrooms | 1/1(100) | 3.8×10^6 | | | Junttila and Brander, 1989 |
| grain and grain products | 0/11 (0) | | | 2001 | Hatakka and Maijala, 2003 |
| nuts, nut products and snacks | 0/2 (0) | | | 2001 | Hatakka and Maijala, 2003 |
| herbs and spices | 0/1 (0) | | | 2001 | Hatakka and Maijala, 2003 |

^a MPN = most probable number

L. monocytogenes does not survive heating at 60 °C, 30 min and thus is destroyed in pasteurization treatment (Seeliger and Jones, 1986). However, if the heating process does not reach all the material to be treated, or if the amount of bacterial cells is very high, e.g. 10^5 – 10^6 CFU/ml, the bacterium may survive (ICMSF, 1996). Tolerance against heating may also vary depending on several factors (grease content, NaCl-content, atmosphere, heating profile, strain) (Embarek and Huss, 1993). Unusual tolerance of *L. monocytogenes* to thermal processing in chicken/broiler meat has been observed in some studies, e.g. as high as 82.2 °C internal temperature was not sufficient to kill *L. monocytogenes* when inoculated on raw chicken meat surface at a level of 10^5 – 10^6 CFU/g

bacteria (Carpenter and Harrison, 1989). Thorough and sufficient heat treatment (e.g. 85 °C, 15 min or 80 °C, 20 min) is generally an effective way to destroy *L. monocytogenes* from cleaned production surfaces (Marriott, 1999). However, grease has been suggested to protect *L. monocytogenes* from heat, at least in some food products (Embarek and Huss, 1993; Murphy et al., 2004).

L. monocytogenes is able to grow at water activity (a_w) values of ≥ 0.90 (Farber et al., 1992; Lou and Yousef, 1999). It tolerates well high salt concentrations ($>20\%$) and thus it can cause problems in brine solutions and brining machines in food processing (Gravani, 1999). The nutritional requirements of *L. monocytogenes* are similar to those of many other gram-positive bacteria (ICMSF, 1996; Jay, 1996).

1.3.4 Listeriosis

Listeriosis is an illness which is severe especially to individuals belonging to risk groups, i.e. very young or old people, pregnant women, cancer and AIDS patients and patients receiving immunosuppressive therapy (Rocourt, 1996). Approximately 20% of the population typically belong to risk groups (Miller et al., 1997; Maijala et al., 2001). The fatality rate of the disease is approximately 20–30% (Rocourt, 1996; Rocourt et al., 2003; Lyytikäinen et al., 2006). The main symptoms include miscarriages in pregnant women and meningitis and bacteraemia in neonates and adults (Rocourt, 1996). However, milder food poisoning symptoms including vomiting and diarrhoea are possible, or in case of zoonotic infection localised skin lesions. The disease can also be asymptomatic or very mild, but may later develop into infections such as meningitis. The incubation time of the disease varies from <24 h to several months (Bell and Kyriakides, 2004). The factors predisposing infection are not fully understood, but include host immunity, level of inoculum and virulence of the strain (Bell and Kyriakides, 2004).

L. monocytogenes has 13 serovars based on the expression of somatic (O) and flagellar (H) antigens. Approximately 95% of human isolates belong to serovars 4b, 1/2a and 1/2b (Graves et al., 1999). Invasive outbreaks have mainly been caused by serovar 4b (Slutsker and Schuchat, 1999). *L. monocytogenes* is transmitted to humans via three main routes; contact with animals, cross-infection

of new-born babies in hospital, and foodborne infection, of which the last two routes apply to the majority of human listeriosis cases (Bell and Kyriakides, 2004).

In Finland 18–53 cases of listeriosis have been reported annually during 1995–2004 (Lyytikäinen et al., 2006) and have been connected e.g. to consumption of salted mushrooms (Junttila and Brander, 1989), rainbow trout (Miettinen et al., 1999b) and butter (Lyytikäinen et al., 2000). Almost 25% (78/315) of listeriosis cases in Finland have been caused by a certain sero-genotype or closely related genotypes, which have also been found from vacuum-packed cold-smoked or cold-salted (i.e. ‘gravad’) fish products, which accordingly are considered risk products especially to people belonging to risk groups (Lyytikäinen et al., 2006).

For healthy adults, the doses of *L. monocytogenes* causing listeriosis have been reported to vary from 10^5 to 10^9 CFU/g or /ml (Junttila and Brander, 1989; Misrachi et al., 1991; Dalton et al., 1997; Miettinen et al., 1999a, b; Aureli et al., 2000). However, for risk groups the doses have been reported to be lower, varying from <10 to 10^4 CFU/g (Berrang et al., 1988; Ericsson et al., 1997; Anon., 2000a). Despite the recent emphasis on studying this bacterium, the sources and routes of contamination, as well as the infective dose have still in many cases remained unknown (Rocourt et al., 2003). Currently the opinion is that high levels of *L. monocytogenes* are needed for causing the illness in the normal population (Chen et al., 2003; FAO/WHO, 2004).

1.3.5 Detection, identification and typing

Direct plating, selective enrichment, cold enrichment and several rapid methods based on e.g. polymerase chain reaction (PCR) can be used in various combinations to detect *L. monocytogenes* in food, clinical and environmental samples. Several methods for typing of *L. monocytogenes* isolates are also available (Graves et al., 1999).

A typical method for isolation of *L. monocytogenes*, especially from food products, includes one- or two-step enrichment followed by plating on selective agar. Direct plating is also used in detection from contaminated or infected material (Khelef et al., 2006). Standards of different organizations are available and one of the most commonly used are the methods of the International

Standardization Organization (ISO), ISO 11290-1 for detection (ISO, 1996) and ISO 11290-2 (ISO, 1998) for enumeration. Typical enrichment broths used in enrichment of *L. monocytogenes* are e.g. Fraser-broth (Fraser and Sperber, 1988) and University of Vermont broth (UVM) (Donnelly and Baigent, 1986; ICMSF, 1996). The bacterium grows well in many common media, e.g. brain heart infusion and trypticase or tryptose broth (Jay, 1996). Suspected samples are cultivated on selective agars e.g. Oxford- (Curtis et al., 1989), Modified Oxford- (MOX) (Lee and McClain, 1986), or Polymyxin Acriflavine Lithium Chloride Ceftazidime Aesculin Mannitol -agar (PALCAM) after an enrichment step, (van Netten et al., 1989). Special agars have also been developed for identification of *L. monocytogenes*, such as a *Listeria monocytogenes* Blood Agar (LMBA) based on the β -hemolytic reaction on sheep blood and *Listeria* selective agents (Johansson, 1998).

Confirmation tests of *L. monocytogenes* are carried out after incubation on selective agar plates and strain purification on non-selective agar plates. The genus confirmation is typically based on gram-staining, determination of motility at 20–25 °C, biochemical tests e.g. catalase test and especially for *L. monocytogenes* also the β -hemolysis test. *L. monocytogenes* produces β -hemolysis on blood agar plates, with a narrow zone of hemolysis around colonies. However, *L. ivanovii* and *L. seeligeri* also produce hemolysis. Additional tests are based on utilization of different sugars (ICMSF, 1996). These tests can be carried out with commercial kits, e.g. API Listeria[®] test (Bio-Mérieux SA, Marcy l’Etoile, France).

In identifying *L. monocytogenes* -genotypes more than ten different molecular typing methods have been applied (Graves et al., 1999). Hitherto, the only fully automated typing method available is ribotyping (Bruce, 1996). Ribotyping refers to the use of nucleic acid probes to recognize ribosomal genes (Farber, 1996). In ribotyping DNA is first extracted from cells, digested with an endonuclease (restriction enzyme), e.g. *EcoRI*, followed by separation of fragments by agarose gel electrophoresis. Separated fragments are transferred to a nylon membrane and hybridized with a labelled cDNA-probe derived from rRNA by reverse transcriptase. A chemiluminescent pattern is created and recorded (Jay, 1996). In automated ribotyping, the system performs all the process steps required to characterize the isolated bacterial colony to the strain level, from cell lysis to image analysis (Bruce, 1996). The process contains the following steps, of which only the first and last are performed manually: sample

preparation from bacterial cultures, DNA preparation, DNA separation and transfer, membrane processing using a labelled 16s rRNA probe from *E. coli*, detection of the chemiluminescent fragments using a charge-coupled device (CCD) camera, analysis by comparison of the patterns and optionally data manipulation and sorting (Farber, 1996). The results for 32 strains processed in batches of 8 can be obtained in 24 h. Each strain produces a unique DNA fragment pattern, which the system uses in a series of proprietary algorithms to generate a RiboPrint pattern. The pattern is characterized, archived and compared to a supplied database as well as against all the other patterns which have been run on the system to determine similarity (Bruce, 1996). Manual (Baloga and Harlander, 1991; Graves et al., 1994; Jensen et al., 1996; Louie et al., 1996; Ojeniyi et al., 1996; Kerouanton et al., 1998) and automated (Ryser et al., 1996; Arimi et al., 1997; Wiedmann et al., 1997; Allerberger and Frischel, 1999; Gendel and Ulaszek, 2000; Norton et al., 2001; Suihko et al., 2002; Lukinmaa et al., 2004; Grif et al., 2006; Saunders et al., 2006) ribotyping have been used for typing of *L. monocytogenes* in the studies cited.

In PFGE the genomic DNA is digested by one or more restriction enzymes, the fragments are separated by field inversion electrophoresis and fragments are resolved in agarose gels. The alternating electrical fields force molecules to change directions, resulting in electrophoretic profiles, i.e. designated pulsovars (Jay, 1996). Many factors, such as electric field strength, field angle and shape, agarose type and concentration, pulse time, ionic strength and temperature, are known to affect the resolution of this highly discriminating and reproducible method (Farber, 1996). PFGE has proved to be a very accurate and reproducible method for molecular typing of *L. monocytogenes* (Brosch et al., 1996) and has been used in numerous studies on contaminants and in epidemiology (Destro et al., 1996; Autio et al., 1999; Graves et al., 1999; Lukinmaa et al., 2004; Miettinen and Wirtanen, 2006). PFGE is the standard method used in PulseNet, the molecular subtyping network for foodborne disease surveillance. Via PulseNet public health officials can share molecular epidemiological information in real-time (Swaminathan et al., 2006).

Serotyping is the traditional and still routinely used phenotyping method in cases of outbreaks. However, it has a relatively poor discrimination power and some industrial isolates may be untypeable with standard typing antisera (Graves et al., 1999). It is based on differing antigenic determinants expressed on the cell

surface. Antigenic variations are produced by many different surface structures, which can be identified by serological typing. In the case of *Listeria*, the strains are divided into serotypes based on somatic (O) and flagellar (H) antigens. *L. monocytogenes* has 13 different serovars (Seeliger and Jones, 1986).

1.3.6 Attachment and transfer

L. monocytogenes attaches to and grows on different kinds of surfaces even at low temperatures (Mafu et al., 1990; Wirtanen and Mattila-Sandholm, 1993; Smoot and Pierson, 1998) and forms biofilms (Jeong and Frank, 1994; Blackman and Frank, 1996). According to Mafu et al. (1990) *L. monocytogenes* attaches to stainless steel, glass and plastic surfaces both at 20 °C and 4 °C in 20 min or 1 h, respectively. Several factors affect attachment and biofilm formation of *L. monocytogenes*, such as the type of surface and the level of nutrients available (Ronner and Wong, 1993), the bacterial strain (Lundén et al., 2000; Norwood and Gilmour, 1999) as well as the presence of other micro-organisms (Sasahara and Zottola, 1993; Jeong and Frank, 1994). According to a study of Mai et al. (2006), the overall wettability of the surfaces appeared to be a primary determinant of attachment of *L. monocytogenes*. Also corrosion enhances the attachment (Mai et al., 2006).

Recontamination, i.e. transfer of pathogens to the product from the processing environment after an inactivation step (den Aantrekker et al., 2003), has been identified as a significant cause of contamination of foods and thereby foodborne illnesses (Reij and den Aantrekker, 2004). Recontamination is effected by transfer of contaminants from surface to surface. Transfer of persistent *L. monocytogenes* contamination between food-processing plants was associated with a dicing machine in the study of Lundén et al. (2002). Only a few studies have been made of the transfer of *L. monocytogenes* from production surfaces to products or vice versa. Studies reported, and predictive models available, are summarized in Table 6. These studies show that *L. monocytogenes* is readily transferred to different products from various processing surfaces.

Table 6. Studies on transfer of *L. monocytogenes* to food products and predictive models constructed.

| Transfer/cross-contamination | | Model | Reference |
|---|--|----------|------------------------------------|
| from | to | (yes/no) | |
| Stainless steel slicer blade | Turkey breast, bologna and salami; slicer surfaces | No | Vorst et al., 2006a |
| Stainless steel slicer blade | Uncured, oven roasted turkey, bologna, salami; slicer housing, conveyer belt | No | Lin et al., 2006 |
| Stainless steel kitchen knives | Turkey breast, bologna and salami; slicer surfaces | No | Vorst et al., 2006b |
| Stainless steel, PVC, polyurethane (PU) | Beef | Yes | Midelet and Carpentier, 2002 |
| Stainless steel, high density polyethylene (HDPE) | Bologna and American cheese | No | Rodríguez and McLandsborough, 2007 |
| Stainless steel, PVC and PU (pure and two-species biofilms) | Tryptone soya agar (TSA) | Yes | Midelet et al., 2006 |
| Food contact surfaces, gloves, environment | Fish products | Yes | Ivanek et al., 2004 |
| Food processing environment | Different foods | Yes | Schaffner, 2004 |
| From inoculated oranges to work surfaces | From work surfaces to orange juice | No | Martinez-Gonzales et al., 2003 |
| From contaminated utensils to uninoculated oranges | To orange juice | | |
| Bare or gloved hands | Cooked ham | Yes | Pérez-Rodríguez et al., 2006 |

In these studies factors enhancing the amount of *L. monocytogenes* being transferred were a high amount of bacteria, or biofilm; stainless steel surface compared to polyethylene surface; hydration of biofilm and higher force of the cutting blade. Additionally food type and the presence of non-*Listeria* species on the surface had a clear impact on transfer (Midelet and Carpentier, 2002; Lin et al., 2006; Vorst et al. 2006a; Rodríguez and McLandsborough, 2007). Ivanek et al. (2004) and Schaffner (2004) presented mathematical models describing *L. monocytogenes* cross-contamination in food-processing plants.

1.4 Prevention of *L. monocytogenes* in food processes

In the following, aspects on prevention of *L. monocytogenes* in food processes are presented with special focus on disinfection.

1.4.1 General aspects in prevention

As *L. monocytogenes* is widely distributed in nature and survives in various environmental conditions, total elimination of this bacterium from most food processes is impractical or even impossible. However, it is possible to reduce and control the level of this bacterium and minimize the risk to public health. By using a variety of physico-chemical factors, singly or in combinations, effective control of growth and survival of *L. monocytogenes* can be achieved. It has been suggested that the cause of *L. monocytogenes* contamination in products is due more to contamination from the processing environment and equipment than from the raw materials (Bell and Kyriakides, 2004). Strict temperature control and storage time limitations in products supporting the growth, and absence or lowest possible initial level of the bacterium, are needed to minimize the occurrence and amount of this bacterium in food products (FAO/WHO, 2004). This pinpoints the importance of a high level of equipment hygiene.

Tompkin (2002) presented six strategies for controlling *L. monocytogenes* in food plants. These included (I) prevention of the establishment and growth of *Listeria* in niches or other sites that can lead to the contamination of RTE foods; (II) implementation of a sampling program that can assess whether the environment is under control; (III) as rapid and effective a response as possible to each positive product contact sample; (IV) verification by follow-up sampling that the source has been detected and corrected; (V) a short-term assessment of last samplings to detect problems and trends and (VI) a longer-term assessment.

Examples of successful eradication means of *L. monocytogenes* have been published. In a study by Autio et al. (1999) of *L. monocytogenes* contamination in a cold-smoked rainbow processing plant, an *L. monocytogenes* eradication program consisting of the use of hot steam, hot air and hot water was successfully implemented. None of the control samples taken five months after eradication contained *L. monocytogenes*. In a study by Samelis et al. (1998),

cooked ham products were contaminated with *L. monocytogenes* during tumbling step, but the product was absent from vacuum packed product provided that heating to a core temperature of 70 °C occurred and recontamination during slicing and packing was prevented. *L. monocytogenes* has been found to contaminate products from the body of the meat slicer switches. In this case a simple solution, rubber switch covers, solved the problem (Lelieveld et al., 2003).

1.4.2 Efficiency of disinfectants on *L. monocytogenes*

L. monocytogenes, as in general for gram-positive bacteria, is more sensitive to disinfectants than most gram-negative bacteria. Gram-positive bacteria lack the outer surface layer of gram-negative bacteria, which restricts the entry of various antimicrobial substances (McDonnell and Russell, 1999). A summary of the effects of a variety of disinfecting compounds on *L. monocytogenes* in different test conditions is presented in Table 7. Due to many varying factors in the disinfect tests the efficacy against *L. monocytogenes* is difficult to compare. Furthermore, commercial products containing a variety of compounds are also difficult to compare. As can be concluded from the studies presented in Table 7 and from studies made with commercial products (Sallam and Donnelly, 1992; Aarnisalo et al., 2000), many disinfecting or sanitizing agents commonly used in the food industry, such as quaternary ammonium compounds (QACs), chlorine and iodofors, are effective against *L. monocytogenes* cells in suspension.

The microbicidal effect of quaternary ammonium compounds is based on membrane damage in the phospholipid bilayers. Halogens e.g. chlorine inhibit DNA-synthesis and cause oxidation of thiol groups in proteins and enzymes. Oxidation is also caused by peroxygens. The mode of action of alcohols is not well known, but most probably they cause membrane damage and denaturation of proteins (McDonnell and Russell, 1999).

The surface attachment and biofilm formation of *L. monocytogenes* (Mustapha and Liewen, 1989; Frank and Koffi, 1990; Dhaliwal et al., 1992; Wirtanen and Mattila-Sandholm, 1992b; Mosteller and Bishop, 1993) as well as the presence and type of organic material (El-Kest and Marth, 1988a; Best et al., 1990; Wirtanen and Mattila-Sandholm, 1992a; van de Weyer et al., 1993; Aarnisalo et al., 2000) affects the disinfectant efficacy and a thorough cleaning of the

surfaces should be performed before disinfection (Krysinski et al., 1992). Other factors affecting the disinfectant efficacy against *L. monocytogenes* include the concentration, duration, pH and temperature of the use solution (El-Kest and Marth, 1988b; Tuncan, 1993). Furthermore, variation between *L. monocytogenes* strains in resistance against disinfectants has been reported (Earnshaw and Lawrence, 1998; Teodorovic et al., 2000; Lundén et al., 2003).

Only a few publications are available on disinfectant efficacy against *L. monocytogenes* at low temperatures (Orth and Mrozek, 1990; Tuncan, 1993), although *L. monocytogenes* is problematic especially in cold areas of food processing, e.g. in the dairy and meat industry (Lundén, 2004; Wirtanen and Salo, 2004). Coolers and freezers are potential habitats for this bacterium (Gravani, 1999), and efficient disinfection of these premises must be performed. In the study of Tuncan (1993) the efficacy of disinfectants containing quaternary ammonium compounds and iodofors in low concentrations (50 ppm) was clearly reduced when the temperature was reduced from 25 °C to 2 °C. Cold temperature did not affect the efficacy of chlorine (25–200 ppm). However, increased effectiveness of chlorine due to an increase in temperature has also been reported (El-Kest and Marth, 1988b; Orth and Mrozek, 1990). Additional factors reported to affect the efficacy include the quality of used water (Marriott, 1999), the age of the bacterial cells (El-Kest and Marth, 1988a; Lee and Frank, 1991b), the time that biofilm has grown on a surface (Shin-Ho-Lee and Frank, 1991), the surface material (Krysinski et al., 1992; Mosteller and Bishop, 1993; Ronner and Wong, 1993) as well as the nutrient level and growth temperature of the *L. monocytogenes* cells (Lee and Frank, 1991a).

In places which are difficult to reach *L. monocytogenes* may encounter suboptimal concentrations of disinfectants. Such places are common in unhygienic food processing equipment. It is possible that the resistance of the organism towards the agent increases through adaptive responses (Aase et al., 2000). This can also occur through cross-adaptive response to different disinfectants (McDonnell and Russell, 1999; Lundén et al., 2003). Adaptive response is a characteristic of *L. monocytogenes*, which partly explains its ability to persist in food processing equipment (Lundén, 2004; Møretro and Langsrud, 2004).

Table 7. Crude estimates of efficacy of different disinfecting compounds on L. monocytogenes under various experimental conditions.

| Disinfecting agent | Use-concentration | | Test conditions | | | Efficacy ^a | | Reference | |
|----------------------------|-------------------------------|---------------------------------|-----------------|----------------|---|---|---|--|----------------------------|
| | (ppm) | | t (min) | T (°C) | Soiled (soil type and load) | Suspension | Surface ^b | | |
| <u>Ethanol</u> | 70% vol/vol | | 1 | 20 | TSB whole human serum pasteurized milk (2% fat) | ++ ++ ++ | + - + | Best et al., 1990 | |
| | 10, 60 (AvCl ^c) | | 1 | 20 | TSB whole human serum pasteurized milk (2% fat) | ++/ND ^d +/++ ++/++ | -/++ -/- +/+ | Best et al., 1990 | |
| | 200 (AvCl) | | 10 | 20 | TSB pasteurized milk (2% fat) | ND | -/- ^e / ^e - ^f | Krynski et al., 1992 | |
| <u>Sodium hypochlorite</u> | 200 (AvCl) | | 0.5 | 20 | TSB | ND | +(4h) ^g / ^g -(8d) ^g | Shin-Ho-Lee and Frank, 1991 | |
| | 200 (AvCl) | | 0.5 | 25 | TSB | ++ | ND | Mosteller and Bishop, 1993 | |
| | 200 (AvCl) | | 0.5 | 25 | steamed milk (2% fat) | ND | + ^{h,i} | Mustapha and Liewen, 1989 | |
| | 50, 100, 200, 400, 800 (AvCl) | | 1, 2, 5 | 20 | TSB | +/++(≥200 ppm) | + / ++ (≥800, ≥400, ≥800ppm) ^j + / ++ (≥800 ppm) ^k | (1h) ^g (1h) ^g | |
| | 50, 100, 200, 400, 800 (AvCl) | | 1, 2, 5 | 20 | TSB | +/++ (≥200 ppm) | + / ++ (≥400, ≥400, ≥400 ppm) ^j + / ++ (≥800, ≥400, ≥200, ppm) ^k | (24h) ^g (24h) ^g | |
| | 25, 50, 100, 200 (AvCl) | | 0.5 | 2; 7; 15.5; 25 | saline | +/++/++/++ | ND | Tuncan, 1993 | |
| | <u>Iodofoor</u> | 80 (titratable I ₂) | | 1 | 20 | TSB whole human serum pasteurized milk (2% fat) | + - ++ | - - - | Best et al., 1990 |
| | | 25 | | 10 | 20 | TSB | ND | - / - ^e / - ^f | Krynski et al., 1992 |
| | | 25 | | 0.5 | 25 | steamed milk (2% fat) | ++ | ND | Mosteller and Bishop, 1993 |
| | | 25 | | 0.5 | 25 | steamed milk (2% fat) | ND | + ^{h,i} | Tuncan, 1993 |
| 25, 50 | | | 0.5 | 2 | saline | +/+ | ND | | |
| 25, 50 | | | 0.5 | 7 | saline | +/++ | ND | | |
| 25, 50 | | | 0.5 | 15.5; 25 | saline | +/++ | ND | | |

Table 7. Cont.

| Disinfecting agent | Use-concentration (ppm) | Test conditions | | | | Efficacy ^a | | Reference | |
|-------------------------------------|----------------------------|-----------------|---------|--------|--------------------------------|-----------------------|-------------------------------------|--|--|
| | | t (min) | T (°C) | Clean | Soiled (soil type and load) | Suspension | Surface ^b | | |
| <u>Quaternary ammonium compound</u> | 400, 500 | 1 | 20 | TSB | whole human serum | ++ / ND + / + | + / ++ - / - | Best et al., 1990 | |
| | 200 (pH 4.4 or 6.4) | 10 | 20 | TSB | | ++ | + | Krysinski et al., 1992 Mosteller and Bishop, 1993 | |
| | 200 | 0.5 | 25 | | | ND | + / - ^e / - ^f | | |
| | 200 | 0.5 | 25 | | steamed milk (2% fat) | ND | - ^h / + ⁱ | Mustapha and Liewen, 1989 Tuncan, 1993 | |
| | 50, 100, 200, 400, 800 | 1, 2, 5 | 20 | TSB | | ++ | ++ | | |
| | 25, 50, 100, 200 | 0.5 | 2 | saline | | + / + / ++ / ++ | ND | Krysinski et al., 1992 Mosteller and Bishop, 1993 | |
| | 25, 50, 100, 200 | 0.5 | 7; 15.5 | | | + / ++ / ++ / ++ | ND | | |
| | 25, 50, 100, 200 | 0.5 | 25 | | | ++ / ++ / ++ / ++ | ND | | |
| | <u>Peracetic acid</u> | 105 | 10 | 20 | TSB | | ND | + / + ^e / - ^f | Krysinski et al., 1992 Mosteller and Bishop, 1993 |
| | | 200 | 0.5 | 25 | | | ++ | ND | |
| 200 | | 0.5 | 25 | | steamed milk (2% fat) | ND | + ^h / - ⁱ | | |
| <u>Acid anionic</u> | 200 | 0.5 | 25 | | | ++ | ND | Mosteller and Bishop, 1993 | |
| | 200 | 0.5 | 25 | | steamed milk (2% fat) | ND | + ^{h,i} | | |

^a ++ = good efficacy (≥5 log reduction in suspension, ≥4 log reduction on surface), + = poor efficacy (<5 log reduction in suspension, <4 log reduction on surface), - = no efficacy (<1 log reduction)

^b stainless steel, ^c available chlorine, ^d not detected, ^e polyester, ^f polyester/polyurethane, ^g attachment time, ^h buna-N-rubber, ⁱ Teflon[®], ^j smooth, ^k pitted

1.5 Microbiological risk assessment (MRA) in the food industry

1.5.1 Background and components of MRA

Microbiological risk assessment was launched after the implementation of the Sanitary and Phytosanitary Agreement by the World Trade Organization (WTO) (WTO, 1994) in 1995 in order to assist in fulfilling and clarifying global trade regulations, and has been used by national and international authorities in risk management decision making. Codex Alimentarius (1999) defines microbiological risk analysis to consist of three components: risk assessment, risk management and risk communication. The microbiological risk assessment process should have a sound scientific basis, be transparent, and be conducted using a structured approach including hazard identification, exposure assessment, hazard characterization and risk characterization. It should explicitly consider the dynamics of microbiological growth, survival and death in foods and the interaction between human and agent following consumption.

The purpose of the hazard identification is to identify the micro-organisms or the microbiological toxins of concern with food. Exposure assessment includes an assessment of the extent of human exposure by considering the frequency and levels of contamination of foods over time and at the time of consumption. In the hazard characterization step, a qualitative or quantitative description of the severity and duration of possible adverse effects from consumption of a micro-organism or its toxin is provided. Ideally, a dose-response relationship should be established but in the absence of a known dose-response relationship, expert opinions can be used. Risk characterization combines information obtained from hazard identification, hazard characterization and exposure assessment to obtain a risk estimate, which is a qualitative or quantitative estimate of the likelihood and severity of the adverse effects which could occur in a given population (Codex Alimentarius, 1999).

1.5.2 Microbiological risk assessment of *L. monocytogenes*

FAO/WHO (2004) has conducted a risk assessment of *L. monocytogenes* in four categories of RTE foods, namely pasteurized milk, ice cream, fermented meat and cold-smoked fish. It was concluded that the food matrix, virulence of the strain and susceptibility of the consumer were all important factors for the probability of listeriosis. The models developed predicted that a high number of *L. monocytogenes* is needed for illness and that the greatest impact on reducing rates of listeriosis would be obtained by reducing high levels of contamination. In foods where growth of *L. monocytogenes* is supported, better temperature control and limitation of storage times would be beneficial. Other risk assessments of *L. monocytogenes* have been performed with the following products: soft cheese from raw milk (Farber et al., 1996; Bemrah et al., 1998; Sanaa et al., 2004), Hispanic-style cheese (FAO/WHO, 2004), Swiss Emmental cheese (Aebi et al., 2003), smoked fish (Buchanan et al., 1997; Lindqvist and Westöö, 2000), Deli meats (FSIS, 2003), RTE products (FDA/USDA, 2003) and butter (Maijala et al., 2001).

For *L. monocytogenes*, dose-response relationship models are available from some studies and have been summarized in the FAO/WHO document on risk assessment of *L. monocytogenes* in RTE foods (FAO/WHO, 2004). A variety of dose-response models based on epidemiological data (Buchanan et al., 1997), expert elicitations (Farber et al., 1996) and data derived from surrogate pathogens or animals (Haas et al., 1999), are available for risk assessment of *L. monocytogenes* (FAO/WHO, 2004). These generally include the use of the exponential model (Rose et al., 1991), but e.g. the Beta-Poisson (Haas, 1983) and Weibull-Gamma models (Todd and Harwig, 1996) are also used.

1.5.3 Use of predictive models in MRA and risk management

Predictive microbiology can be used to estimate changes in bacterial numbers, allowing the exposure of an individual to a pathogen to be assessed. Without predictive models this has been difficult to carry out in microbiological risk assessment (Walls and Scott, 1997). Predictive microbiological growth models can be divided into primary, secondary and tertiary models. Models that describe the population growth curve are primary models (Whiting and Buchanan, 1993).

This description requires definition of the initial number of cells, the lag time, the rate of growth and the maximum population density. Models describing inactivation of micro-organisms have also been developed (van Gerwen and Zwietering, 1998). Secondary models describe the influence of environmental conditions on the parameters of the primary model, or the primary characteristics of the population and environment interactions, e.g. lag time of the microbe and exponential growth rate. Tertiary models are computer software interfaces that enable rapid predictions of microbiological growth from values selected by the user (Ross and McMeekin, 2003).

Predictive models can be used as tools for process development and optimization in predicting the bacterial levels in the final products and the effect of cross-contamination, as described e.g. for *L. monocytogenes* by Rasmussen et al. (2002) and Ivanek et al. (2004). Many models are also available in computer programs such as Pathogen Modeling Program (PMP, <http://www.arserrc.gov/mfs/pathogen.html>), and Seafood Spoilage and Safety Predictor (<http://www.difres.dk/micro/sssp/>). Data underlying many predictive models can be found in ComBase (<http://www.ifr.ac.uk/combase/>). The predictive models have limitations, which should be recognized in order to avoid unrealistic scenarios. Such limitations include uncertainty, which is the expression of knowledge gaps, and variability which describes the heterogeneity of the quantities and characteristics modelled (Ross and McMeekin, 2003).

1.5.4 Use of MRA and predictive models at the food plant level

Although risk assessment has been used mainly on a national and international level, it is a beneficial approach for food companies in product and process development and optimization, as an extension of or in validation of an HACCP-plan (Notermans and Mead, 1996; van Schothorst, 1997; Serra et al., 1999; Hoornstra et al., 2001; Hoornstra and Notermans, 2001). In risk assessments for governments use is mainly made of epidemiological data, whereas for food companies product and process information is generally used. In most HACCP-systems a qualitative approach is used. Quantitative risk assessment is still challenging for producers, but it could be used to quantify the effect of control measures, to estimate the occurrence of contaminants in the end products and in deriving and validating control measures and critical limits at Critical Control

Points (CCPs). Either worst-case or what-if scenarios, or a statistical approach using probability distributions can be applied (Hoornstra et al., 2001). At the plant level, a compact and simple way of performing the risk assessment is needed. Van Gerwen et al. (2000) presented a general method for stepwise quantitative risk assessment of food products and their production processes. Computer programs for performing a risk assessment have recently been launched (Ross and Sumner, 2002; Sumner and Ross, 2002; Tuominen et al., 2003; McMeekin et al., 2006), e.g. a practical semiquantitative hygiene risk assessment model HYGRAM[®] by Tuominen et al. (2003). This model includes a hazard module for assessing the risk of *L. monocytogenes*. The principles of risk assessment underlying the programs should also be familiar to their users. Predictive microbiology models provide a scientific basis to support key aspects of HACCP and quantitative microbiological risk assessment (Kleer and Hildebrandt, 2001; McMeekin et al., 2006).

2. Aims of the study

The aim of this thesis was to identify deficiencies in and to improve means of equipment and process hygiene in the food industry and to develop risk assessment procedures for prevention of *L. monocytogenes* in food plants. Based on the results, suggestions for improved manufacturing and risk management practices for assuring end product safety are given. Specific aims were:

1. To identify and evaluate food processing equipment and the hygienic practices of maintenance personnel in the food industry in order to identify aspects that can negatively affect equipment hygiene and enhance the occurrence of *L. monocytogenes* in equipment (Paper I).
2. To compare the discriminatory power of automated ribotyping to that of PFGE in distinguishing *L. monocytogenes* isolates and to determine the suitability of the methods for tracing contamination sources in food processes (Paper II).
3. To enhance means of *L. monocytogenes* decontamination in equipment by evaluating commercial lubricants and disinfectants used in the food processing equipment; by determining the survival, growth and transfer of *L. monocytogenes* in lubricants (Paper III); and by investigating the susceptibility of the bacterium to disinfectants in cold conditions (Paper IV).
4. To develop risk assessment procedures by using predictive modelling to investigate transfer of *L. monocytogenes* from equipment to product during slicing to assess recontamination (Paper V); and by performing a production plant level risk assessment of *L. monocytogenes* for one food product (Paper VI).

3. Materials and methods

The mail-surveys performed in equipment and maintenance hygiene studies are described (Paper I). The methods used for sampling and analyses of *L. monocytogenes* (Papers I–VI) are summarized and methods used in susceptibility (Papers III and IV), transfer (Paper V) and risk assessment (Paper VI) studies, as well as the mathematical methods used in the studies, are also described. Cold-salted (i.e. ‘gravad’) salmon (Paper V) and raw marinated broiler legs (Paper VI) were chosen as example foods in laboratory experiments because *L. monocytogenes* is often detected in these products (see Table 5).

3.1 Questionnaires on equipment hygiene and hygienic working practices of maintenance personnel (Paper I)

Two mail surveys to Finnish food companies were sent in spring 2002 to 1) identify and evaluate equipment causing hygiene problems in food processing and 2) to study the hygienic practices of maintenance personnel in the food industry in order to identify aspects that can have a negative effect on equipment hygiene. The respondents were able to answer anonymously. The companies were chosen from the company registers of the Finnish Food and Drink Industries’ Federation and VTT.

The questionnaire on equipment hygiene was sent to 184 food companies. An employee responsible for equipment hygiene was asked to answer the survey. The questionnaire on hygienic working practices of maintenance personnel was sent to the quality managers of 106 food companies, who were asked to distribute it in addition to themselves to maintenance personnel (330), food-handlers (118), and cleaning personnel (224), i.e. a total of 778 questionnaires. More detailed descriptions of the surveys can be found in Paper I.

3.2 Sampling, detection and identification of *L. monocytogenes*

3.2.1 *L. monocytogenes* strains used in laboratory experiments

The *L. monocytogenes* strains used in laboratory experiments (Papers III and IV) were obtained from the VTT culture collection (except strain F2365 in Paper V, which was obtained from the United States Department of Agriculture) and maintained in 5% glycerol at -70°C . For each study the strains were chosen mainly on the basis of their site of isolation and the food sector they represented. The strains used are presented in Table 8. For comparing the discriminating ability of automated ribotyping to that of pulsed-field gel electrophoresis (PFGE) in distinguishing *L. monocytogenes* isolates (Paper II), a total of 486 *L. monocytogenes* isolates originating from 17 Finnish food processing plants were collected from 1997 to 1999. For further typing with *Pvu*II enzyme as well as with PFGE, a set of 121 isolates was selected from these isolates, representing all the *Eco*RI ribotypes generated and 16 food plants.

Table 8. Strains of *Listeria monocytogenes* used, their origin, and serotypes.

| Strain | Origin | Serotype | Papers |
|--------------------|---|----------|---------|
| I (VTT E-981041) | Meat plant, product | 1/2 | IV |
| II (VTT E-991508) | Dairy, cheese machine | 1/2 | IV |
| III | Meat plant, freezer | 4b | III, IV |
| IV (VTT E-021893) | Meat plant, freezer | 1/2 | IV |
| V | Meat plant, product | 4b | IV |
| VI (VTT E-991599) | Meat plant, product | 1/2 | IV |
| VII (VTT E-991513) | Fish plant, fish net | 1/2 | IV |
| VIII | Meat plant, conveyer | 1/2 | IV |
| IX (VTT E-981012) | Dairy, raw milk | 1/2 | III, IV |
| X (VTT E-981045) | Meat plant, product | 1/2 | IV |
| XI (VTT E-991205) | Dairy, butter | 3a | III |
| F2365 | Isolate from the 1985 Mexican-style soft cheese outbreak (Linnan et al., 1988), rifampicin-resistant mutant | 4b | V |

3.2.2 Analyses of tools, work environment and protective clothing of maintenance personnel (Paper I)

In addition to the questionnaires (see 3.1), the working practices of maintenance personnel were studied at four food companies (a meat company, a poultry company, a dairy and a bakery) during two normal work shifts by taking microbiological samples. For analysis of *L. monocytogenes* a total of 71 samples from three food plants were taken with moisturised gauze pads kept in 10 ml of a peptone saline solution (Maximal Recovery Diluent, Lab M, Amersham, Bury, UK). The isolation and detection were carried out according to the ISO 11290-1 method (ISO, 1996) with the following modifications: only Oxford agar (Oxoid, Hampshire, UK) was used as the selective agar and the preliminary identification was carried out using API Listeria strips (bioMérieux, Marcy-l'Etoile, France) in accordance with the manufacturer's instructions.

Additionally, samples for analysing total aerobic bacteria were taken with commercial Petrifilm™ Count Plates (3M Health Care, St. Paul, MN, USA) and for *Enterobacteriaceae* with 3M Petrifilm™ Enterobacteriaceae Count Plates from four food plants. Altogether, 95 and 96 samples were taken and incubated at 30 °C for 3 d and at 37 °C for 2 d, respectively. After this the colonies were counted.

3.2.3 Analyses of cold-salted salmon slices in a transfer study (Paper V)

For analyzing the amount of *L. monocytogenes* transferred during slicing of cold-salted salmon from inoculated blade to product (see 3.5), the slices (i.e. samples) were transferred directly to sterile filter (280 µm mesh) stomacher bags (Spiral Biotech, Norwood MA, USA) and weighed for more accurate enumeration. Peptone water (0.1% wt/wt) was added to the sample at a 5:1 ratio (wt/wt) and the mixture was processed in a stomacher (Model Bag Mixer 400, Interscience Inc., Weymouth, MA, USA) at room temperature for 30 s. The swabs used for surface sampling were placed individually into 10 ml of peptone water, mixed in a vortex mixer for 30 s, and then 10-fold serial dilutions (100 µl from each tube) were plated on two plates of Modified Oxford (MOX) -agar

containing 0.1% rifampicin (Sigma Aldrich, St Louis, MO, US). The plates were incubated at 37 °C for 2 d and the colonies were counted.

3.2.4 Analyses of broiler legs in a risk assessment study (Paper VI)

For analysing the prevalence and numbers of *L. monocytogenes* in raw marinated broiler legs for the risk assessment study (see 3.6.5), a total of 186 packages of raw marinated broiler legs were purchased from 41 retail stores in the Helsinki metropolitan area during one year in 2002–2003. The packages were transferred to the laboratory in an insulated box, and were stored at 6 °C or 10 °C, and analysed at the end of their shelf life. To quantify *L. monocytogenes*, one leg from each package was transferred to a sterile bag and weighed, and half of the sample weight of buffered peptone-water was added. The broiler leg was hand massaged for 3 min, followed by a pre-incubation for 1 h at room temperature. The rinse diluent was used for enumeration and isolation conducted according to the ISO 11290-2 method (ISO, 1998) and the detection of *L. monocytogenes* after heating experiments according to the ISO 11290-1 method (ISO, 1996), with some modifications as described in Paper VI.

Information on levels of *L. monocytogenes* at the point of consumption is needed when performing risk assessment and therefore a small scale heating experiment was performed. For investigating the temperatures normally used by consumers cooking broiler legs, cooking practices of 20 consumers, mainly students and young adults, were investigated using a temperature logger (DataSquirrel, Eltek Limited, UK). Consumers were given a package of broiler legs, corresponding to those investigated in the microbiological survey, and asked to cook them in their home kitchen oven as they normally would. No instructions were given except on the correct use of the temperature logger, which measured both the oven's air temperature and the meat temperature at the thickest portion of the leg. They were also asked to fill in a small questionnaire concerning their cooking practices.

The thermal inactivation of *L. monocytogenes* from naturally contaminated broiler legs was investigated in the laboratory from 21 samples kept at 10 °C. The packages (samples) purchased from retail shops contained 3–4 broiler legs in a modified atmosphere package. The numbers of *L. monocytogenes* was investigated from one leg and two legs were cooked one at 132 °C and the other

at 175 °C, for 50 min. The former temperature was chosen as it was the lowest average temperature detected in consumer ovens during cooking. The other temperature was the temperature recommended by the producers. The cooking time was the shortest recommended by the producers. After heating, the samples were cooled and stored at 10 °C until the next day when numbers of *L. monocytogenes* were determined. A more detailed description of the experiments is presented in Paper VI.

3.2.5 Typing of *L. monocytogenes* (Paper II)

The discriminatory power and usability of automated ribotyping was compared with traditionally used PFGE for distinguishing *L. monocytogenes* strains isolated from food processing plants. Additionally the strains were serotyped. The isolates were ribotyped using the RiboPrinter[®] System (DuPont Qualicon[™], Inc., Wilmington, DE, USA) as described by Bruce (1996). The restriction enzymes used were *EcoRI* (Qualicon) and *PvuII* (Qualicon). Similarity values were calculated using the software provided by Qualicon (version 11.2 (c) 1999).

In situ DNA isolation and PFGE were performed as described by Autio et al. (1999). The restriction enzymes used were *AscI* and *SmaI* (New England Biolabs, Beverly, Mass., USA). The serotyping was carried out by the agglutination method using Denka Seiken's *L. monocytogenes* serotyping antisera (Denka Seiken, Tokyo, Japan) according to the manufacturer's instructions.

3.3 Analysing survival and transfer of *L. monocytogenes* in lubricants (Paper III)

3.3.1 Survival of *L. monocytogenes* in lubricants

The survival of *L. monocytogenes* in lubricants used in food processing equipment was investigated in 11 different types of lubricants used in food processing plants (Table 9). A mixture of 0.1% bovine albumin and 0.1% potato starch was added as soil. Culturing of the *L. monocytogenes* strains (Table 8) is presented in detail in Paper III. The inoculated tubes were incubated both at room (20 °C) and refrigerated (5 °C) temperatures. The samples (0.5 g) were

taken in triplicate after 0.5 h (control sample), 4 h, 24 h, 3 d and 14 d and pipetted to 4.5 ml of neutralization solution (Aarnisalo et al., 2000) and the solution was allowed to stand for 5 min before culturing. To enhance the emulsification of the lubricants, a dilution series was prepared in pre-warmed (35 °C) 0.85% Tween-saline solution, plated on duplicate plates of Oxford agar (Oxoid Ltd., UK) and incubated at 30 °C for 3 d. The microbicidal effect (*ME*) of the lubricants was calculated as described in 3.6.1.

3.3.2 Transfer from lubricants to stainless steel surfaces and vice versa

For investigating transfer from lubricants to stainless steel surfaces the first seven lubricants (A–G) given in Table 9 were soiled and inoculated with strains III and XI (VTT E-991205). The lubricant (2 g) was transferred to a sterile Petri dish containing a filter paper (Whatman, qualitative no. 2, Maidstone, UK) and spread to cover the whole paper. Sterile stainless steel discs were placed on top of the filter paper and after 0.5 h, 1 h, 4 h and 24 h three discs were analyzed as replicate samples. They were transferred to test tubes containing 5 ml of neutralization solution. The lubricant-contaminated stainless steel disc was mixed with the inactivation solution in a test tube mixer and the disc was further rubbed with a cotton swab for 30 s. The neutralization solution was allowed to stand for 5 min before culturing. After the same time periods (0.5 h, 1 h, 4 h and 24 h), samples were taken from the lubricant in order to detect changes in the *L. monocytogenes* concentration in the lubricant during the 24 h incubation period.

Table 9. Lubricants, composition and usage as given by the manufacturers.

| Lubricant | Composition | Usage |
|----------------|---|---|
| A ¹ | Synthetic hydrocarbon (70–80%), hydro treated polymer (10–20%), antioxidant (0.5–2%), fumed silica (7–10%), thermal stabilizer (3–5%), polyglycol (1–2%), polytetrafluorethylene (Teflon) (0.5–2%), additives (0.25–1%) | Food industry lubricant (grease, USDA H1) |
| B ² | Silicone, non-ionic surface active compounds (<5%), preservatives | Conveyer belt lubricant (3 ml/L aqueous solution of lubricant in use) |
| C ² | Crude oil (30–60%), butane (20–50%), propane (5–30%) | Chain lubricant |
| D ¹ | Rapeseed oil (100%) | Cooking oil |
| E ² | Mineral oil containing a mixture of mainly saturated hydrocarbons with C15–C50 (>90%), additives | Hydraulic oil |
| F ² | “Dry” (not diluted with water), polyhydric alcohols and a small amount of silicone emulsion | Conveyer belt lubricant |
| G ¹ | White oil (>97%), dialkyl-dimethyl-aluminum silicate, additives (against corrosion, wear, pressure and oxidation) | Food industry lubricant (grease, USDA H1) |
| H ² | Mineral oil containing a mixture of mainly saturated hydrocarbons with C15–C50 (>90%), additives | Gear oil |
| I ² | Mineral oil containing a mixture of mainly hydrocarbons with C12–C50 (>90%), lithium thickener, additives | Multi-purpose grease for vehicles |
| J ¹ | White oil, Al-salt of stearic acid (5–15%), Al-salt of benzoic acid (1–5%) | Aluminium complex grease (USDA H1) |
| K ² | Hydrodesulfurized light dearomatized naphtha (petroleum) (30–60%), hydrocarbon propellant (10–30%), additives | Multi-purpose grease |

¹ food grade ² non food grade

For investigating transfer of bacteria from surfaces into lubricants, growth broth (0.1 ml) containing 10⁸ CFU/ml *L. monocytogenes* was pipetted onto the surface of sterile stainless steel discs (#AISI 304, 2B, 10.5 mm in diameter, Happoteräs Oy, Helsinki, Finland). The discs were left to dry for 1.5 h at 30 °C, after which they were further dried for 10 min with compressed air. The discs were transferred to 10 g of soiled lubricant. After 1 h, 4 h and 24 h, three discs were removed from the test tubes and the tubes were incubated at room (20 °C) and refrigerated (5 °C) temperatures. The soiled lubricants were mixed and three replicate samples from each tube were plated as described in 3.3.1.

3.4 Susceptibility of *L. monocytogenes* to disinfectants (Paper IV)

The efficacy of commercially available disinfectants commonly used in the food industry against *L. monocytogenes* at cold temperature (+5 °C) was investigated using both suspension and surface methods.

3.4.1 Suspension method

The efficacy of eight disinfectants (Table 10) was studied in both clean and soiled solutions with ten strains I–X (Table 8) using a method slightly modified from Aarnisalo et al. (2000). A more detailed description of the test is given in Paper IV. Microbicidal effect (*ME*) was calculated as described in 3.6.1. The disinfectant was considered to be effective if it reduced the amount of vegetative bacterial cells by 5 log CFU-units.

3.4.2 Surface method

The surface method used was modified from Charaf et al. (1999) using 5 strains (Table 8) and disinfectants presented in Table 10. The surfaces used were stainless steel discs (AISI 304, 2B, 10.5 mm in diameter, Happoteräs Oy, Helsinki, Finland) and glass bead blasted PE-discs (10.5 mm in diameter, Vulganus Oy, Nastola, Finland). The culturing, inoculation and disinfection efficacy methods are described in detail in Paper IV. Microbicidal effect (*ME*) was calculated as described in 3.6.1. The disinfectant was considered to be effective if it reduced the amount of vegetative bacterial cells by 4 log CFU-units.

Table 10. The composition, purpose of use and recommended in-use concentrations of disinfectants.

| Agent | Composition | Purpose of use | Recommended in-use concentrations (%) |
|-------|--|-----------------------------------|---------------------------------------|
| A | Peracetic acid (<5%), hydrogen peroxide (15–30%), acetic acid (5–15%), phosphonic acid (<1%) | Disinfectant for closed processes | 0.05–3 |
| B | Peracetic acid (<5%), hydrogen peroxide (5–15%), acetic acid (5–15%), phosphonic acid (<5%), non-ionic tensides (<5%) | Disinfectant for open processes | 1–3 |
| C | Peracetic acid (<5%), hydrogen peroxide (5–15%), acetic acid (15–30%), phosphonic acid (<5%), anionic tensides (5–15%) | Disinfectant for closed processes | 0.05–1 |
| D | Ethanol (<70%) | Disinfectant for open processes | 100 |
| E | Sodium hypochlorite (>60%, active chlorine 13%), sodium hydroxide (<5%) | Disinfectant for open processes | 0.05–2 |
| F | Alkyl dimethyl benzyl ammonium chloride (>30%), synthetic tensides | Disinfectant for closed processes | 0.1–0.5 |
| G | Isopropanol (15–30%), 1-propanol (>30%) | | 100 |
| H | Peracetic acid (5–15%), hydrogen peroxide (15–30%), acetic acid (5–15%) | Disinfectant for closed processes | 0.1–4 |

3.5 Analysing transfer of *L. monocytogenes* during slicing of cold-salted salmon (Paper V)

Fresh Atlantic salmon (*Salmo salar*) fillets were stored at 0 °C for up to experimentation time (no longer than one week). The fillets were salted as described in detail in Paper V. The thickness of the slices was set at 3.5 mm and slicing was performed manually. After each trial, the slicer and blade were cleaned and disinfected. All trials were repeated three times on different days.

The slicer used in this study was a delicatessen slicer (Globe 3975 Variable Speed Automatic Slicer, Globe Food Equipment Co., Dayton, OH, USA) made of #304 stainless steel, as also was the blade. The roughness (A^2/A ratio, which is the scanned rough surface area/unit surface area) and sharpness of the blade

were measured at the beginning and the end of the tests by reflection confocal microscopy as described by Flores et al. (2006). The blade was contaminated with *L. monocytogenes* resulting in approximately 8, 5 or 3 log CFU/blade. The effect of slicing temperature was studied at room temperature, 10 ± 0.5 °C, and 0 ± 0.2 °C. The slicer and blade were pre-cooled to the experimental temperature before inoculation. For experiments, the first 11 slices and then every second slice to the 39th slice were collected. Surface samples (from approximately 10 cm² area each) were collected from the slicer blade, holding plate and blade safety guard using sterile cotton-tip swabs during slicing.

Transfer from inoculated salmon fillet to slicing machine and to slices of uninoculated fillets was studied by surface-inoculating the salmon fillet to contain approximately 8 log CFU of *L. monocytogenes*. The trials were made at room temperature. After slicing the inoculated fillet, an uninoculated fillet was sliced into 39 slices and the slices were analyzed. Surface samples as described above were collected.

3.6 Mathematical methods

3.6.1 Microbicidal effect (Papers III, IV)

The microbicidal effect (*ME*) of lubricants and disinfectants was calculated according to the following Equation (1):

$$ME = \log N_c - \log N_d, \quad (\text{Eq. 1})$$

where N_c is CFU/ml or CFU/cm² for the control and N_d is CFU/ml or CFU/cm² for the treatment.

3.6.2 Discrimination index (Paper II)

The discrimination power of the automated ribotyping and PFGE was determined by calculating the discrimination index (*DI*) using the following Equation (2):

$$DI = 1 - \frac{1}{N(N-1)} \sum_{j=1}^s n_j(n_j - 1) \quad (\text{Eq. 2})$$

where N is the total number of strains in the sample population, s is the total number of types described and n_j is the number of strains belonging to the j th type (Hunter and Gaston, 1988).

3.6.3 Statistical analyses (Papers I, III, V)

In the study on survival and growth of *L. monocytogenes* in lubricants (Paper III), repeated measures analysis of variance was used to analyze whether there were significant differences in reduction in the amount of *L. monocytogenes* due to change in time, lubricant, temperature, purity of lubricant and bacterial strain. Differences within the lubricants and different strains were further analyzed by the multiple comparisons Tukey-test.

The significance of different experiment conditions on transfer of *L. monocytogenes* to slices of cold-salted salmon (Paper V) were analyzed with a general linear model univariate analysis. Differences in numbers of aerobic bacteria on maintenance personnel tools, protective clothing and in the work environment (see 3.2.2) were calculated using the non-parametric Mann-Whitney test.

All statistical analyses were conducted using SPSS for Windows v. 12.0.1. (Chicago, USA). The level of significance used was $p < 0.05$.

3.6.4 Predictive modelling (Paper V)

TableCurve 2D Version 5.01 (SYSTAT Software Inc., Richmond, CA, USA) was used in the study on transfer of *L. monocytogenes* in order to select an empirical model to best fit the experimental data, based on the simplicity, applications (predictions vs. time in slicing – convergence and no singularity in long time prediction), fitted coefficients with standard error, t -test results, $P > |t|$, r^2 -value and F -value.

3.6.5 Producer level quantitative risk assessment (Paper VI)

An example of a robust risk assessment for the single plant level using worst-case and average point estimates was produced. The steps of microbiological risk assessment are hazard identification, exposure assessment, hazard characterization and risk characterization (see 1.5). For exposure assessment purpose, the prevalence and numbers of *L. monocytogenes* in marinated broiler legs were investigated and a laboratory scale heating study was performed to estimate the numbers of *L. monocytogenes* at the point of consumption (see 3.2.4). A scheme/flowchart of the factors and probabilities needed for producing the risk estimate was drawn (Fig. 1, Paper VI).

Output estimates of *L. monocytogenes* -positive samples were then compared to the data obtained in the cooking studies (see 3.2.4). Since the cooking data were very limited, the assessed probabilities of cooking time (min) and oven temperature (°C) combinations (t, T -values) were sensitive to possible outliers in the data. Therefore, for assessing the probability of the highest risk cooking combinations, a simple simulation which could be performed using e.g. Excel was performed. Natural logarithms of t (time, min) and T (temperature, °C) were used to avoid negative values in simulations. Functions for calculating separately for both data variables, $\ln(t)$ and $\ln(T)$, the means and standard deviations as well as correlations between these variables were needed. For simulation, a function generating random numbers from normal distribution was used and the simulated values for t and T could be calculated (see Paper VI for more details). To assess the probability of product being cooked within any t, T -segment the percentage of simulated data points (t, T) belonging to the specified t, T -segment was calculated.

An exponential dose-response model, which was also chosen for the recent FAO/WHO (2004) risk assessment study on RTE foods, was used in risk assessment:

$$P = 1 - e^{-rN}, \quad (\text{Eq. 3})$$

where P is the probability of an adverse effect, N is the number of biological agents consumed and r is a constant specific to each pathogen. The three r -values used in this study (presented in Buchanan et al., 1997; Linqvist and Westö, 1997)

2000 and FAO/WHO, 2004) are presented in Table 1 of Paper VI along with the main characteristics of the models. The biological end point in all of these models was invasive listeriosis. The step-by-step presentation of calculating the risk with the exponential model using the r -value from the study of Buchanan et al. (1997) and worst-case point-estimates is presented in 4.7.3.

4. Results and discussion

4.1 Hygienically most problematic food processing equipment (Paper I)

The response rate for the questionnaire on equipment hygiene was 23.9% (44/184): 14 plants representing the meat, poultry and RTE food industry, 11 bakeries, 8 fish companies, 6 dairies and 5 plants from other branches of the food industry answered. The response rates obtained from both questionnaires (see also 4.2.1) are comparable with the rates of other recent postal surveys sent to the food industry (Hielm et al., 2006). There were most often 10–90 employees in the plants (69.2%) ($n=39$). The respondents ($n=43$) were most often working as quality managers or hygiene-responsible operatives (44.2%) or production managers (25.6%). As the equipment used by Finnish food producers is purchased mainly from other countries (mainly Europe), but also from Finland, the problems in hygienic design and the conclusions of this study are also applicable to other countries.

The respondents were asked to name the five hygienically most problematic pieces of equipment in their plant. There were 39 equipment choices given in the questionnaire and an option to mention other equipment as well. Altogether 61 types of equipment were mentioned at least once, which showed clearly that the hygiene problems in each company were specific. Packaging machines, conveyers, dispensers, slicing machines and cooling machines were considered the most problematic equipment (Table 11). In previous studies, packaging machines, conveyers, slicing machines and cooling machines have also been found to be a source of *Listeria* contamination (Humphrey and Worthington, 1990; Gravani, 1999; Miettinen et al., 1999a; Tompkin, 2002). The main reason was poor hygienic design. Self-made and domestic equipment were considered more hygienic than equipment purchased from outside Finland. This may be due to easier communication of problems and their solutions between manufacturers and food processors.

Packaging machines were cited most often as unhygienic equipment (Table 11). The shelf-life of a product depends to a great extent on proper packaging, the last step before the product is transported to retailers and to the consumers. A common problem with packaging machines is that they often do not tolerate water because of electronic circuits, which makes the cleaning and disinfection procedures very difficult.

Table 11. The hygienically most problematic equipment in different food industry sectors (n = number of respondents).

| Most problematic equipment | No. of responses | | | | |
|----------------------------|------------------|--------------------------|----------------|-------------|----------------|
| | All n=44 | Meat and poultry n=14 | Bakery n=11 | Fish n=8 | Dairy n=6 |
| Packaging machines | 20 | 7 | 4 | 5 | 3 |
| Conveyors | 17 | 7 | 6 | 1 | 3 |
| Dispensing equipment | 15 | 4 | 8 | 2 | 1 |
| Slicing machines | 15 | 9 | 3 | 3 | - ^a |
| Cooling equipment | 9 | 6 | 2 | - | 1 |
| Proving drawers | 6 | - | 6 | - | - |
| Pasteurising equipment | 5 | - | 1 | - | 4 |
| Cold stores | 4 | 1 | - | 1 | 2 |
| Heat exchangers | 4 | 1 | - | - | 3 |
| Pumps | 4 | 1 | - | - | 2 |
| Filleting machines | 3 | - | - | 3 | - |

^a “-“ = not mentioned by the respondents

The respondents (n=30) stated that food processing equipment should be easy to dismantle and clean, and more simple constructions should be used to improve equipment hygiene. The materials should tolerate strong cleaning and disinfecting agents as well as heat. Furthermore, it should also be possible to use water in cleaning the equipment and process surfaces. Coverings should be easy to open for cleaning. These results stress the importance of including hygienic aspects in the equipment design at the beginning of the design process. However, the technical and occupational safety aspects must also be taken into account. If cleaning of the equipment is difficult due to these factors, the best possible solution for assuring cleanability, e.g. targeted cleaning programs to problematic sites, should be established.

Operational reliability (34%) was clearly the most important factor affecting the acquisition of equipment, followed by costs (20%) and cleanability (11%) ($n=38$). According to 43.2% of respondents, the manufacturers have provided instructions on how to clean the equipment and according to 40.9% some of the manufacturers have ($n=44$). When instructions were available, most respondents (76.7%) followed them ($n=43$), although 58.1% of respondents found the instructions inadequate. Many (38.1%) of the respondents ($n=21$) answered e.g. that equipment manufacturers are not interested in or do not understand the hygienic aspects of their equipment. However, manufacturers are required by the EU Machine Directive (89/392/EEC, revised 98/37/EC) to give instructions for cleaning the equipment. This Directive does not specify what kind of instructions should be included in the material. Many of the equipment manufacturers do not have sufficient knowledge of cleaning to give sufficient information to the client and because of this, instructions should be made in cooperation with cleaning specialists. There should also be cooperation on hygiene aspects between equipment and sanitizing agent manufacturers and food processors already at the design phase of the equipment.

4.2 Hygienic working practices of maintenance personnel (Paper I)

4.2.1 Significant aspects according to the questionnaire

In the questionnaire on hygienic working practices of maintenance personnel answers were obtained from 23.6% of the plants ($n=106$). Out of 778 employees 127 (16.3%) answered the mail survey. Of these, 59 were maintenance personnel and the rest (68) were food-handlers, quality managers and cleaning personnel. One third (33.9%) of the respondents worked in the meat and poultry industry, 26% in the bakery industry, 20.5% in the dairy industry, 1.6% in the fish industry and 18% in other sectors of the food industry.

According to this study, consumer complaints could rarely/seldom be linked directly to the work of maintenance personnel (62.5%, $n=16$). However, as pathogens such as *L. monocytogenes* can be transferred between processing surfaces and food products (Table 6), the hygienic practices of employees are of utmost importance in preventing contamination of equipment by these bacteria.

Most of the maintenance personnel worked in the production area continuously (61%) or at least visited the production area 5 times (23.7%) during a work shift and 42.4% reported having touched surfaces in contact with food often or always. Whereas they were conscious of this and they knew (91.5%, $n=59$) which surfaces came into contact with food, only 55.9% of them usually wore gloves when working in the food production area and even fewer washed their hands in situations where they should in order to work hygienically (13.6% after smoking and 23.7% before starting to work without gloves). One reason for not wearing gloves is probably that they hinder the performance of some work tasks. In situations like this the importance of washing of hands should be highlighted.

Almost all (89.8%) of the maintenance personnel had personal tools which they themselves cleaned ($n=59$). One third (32.2%) of the respondents answered that they washed their tools once a day or always after work. The others answered that they washed their tools more seldom and 32.2% only once a year or never. The majority of maintenance personnel and quality managers (69%, $n=87$) considered that there were enough washing points and adequate cleaning agents and disinfectants available (74.7%, $n=83$). For tools that are in common use, no specified persons were in charge of the cleaning in 71.9% of all the cases reported ($n=57$).

According to the majority (88.2%, $n=59$) of maintenance personnel, foreign bodies were never or seldom left on surfaces after the maintenance work but, according to the majority of food and cleaning personnel (68.2%, $n=66$), they were sometimes or even often left. Clear differences between opinions were identified. The responsibility for cleaning the equipment after work by the maintenance personnel was not always defined. All responsibilities in hygiene matters in food companies should be clear.

According to the maintenance personnel and quality managers, most of the maintenance personnel had written hygiene rules given by the food company (64.2%) or by the maintenance company (32.3%) ($n=81$). Most (63.6%) of the maintenance personnel knew where to find the hygiene rules ($n=55$). It is very important that the rules are available for all. These rules must be clearly written and should also include the maintenance personnel and their work.

Meetings between maintenance, food-processing and cleaning personnel in which hygiene issues were discussed, were not arranged in 52.9% of the plants ($n=119$). One third (33.9%) of maintenance personnel reported that they had not received sufficient information about hygiene aspects commensurate with their work. Studies on food hygiene were not included in their basic education (89.9%, $n=59$). The requirement for sufficient hygiene knowledge of especially this personnel group should also be stated in legislation and studies on food hygiene should be obligatory in their basic education. As publications on hygienic working practices of maintenance personnel have not previously been available, the results can only be compared with results of studies made on hygienic practices of food employees. Several deficiencies were also detected in these studies (Table 5).

4.2.2 Microbiological sampling in food processing

The occurrence of *L. monocytogenes* and the total number of aerobic bacteria in samples taken from the tools, environment and personnel (protective clothing and hands of maintenance personnel) are presented in Table 12. *Listeria* spp. was found in six samples (8.5%), of which *L. monocytogenes* was found in only one sample (1.4%) taken from a screwdriver. Tools contained clearly ($p<0.05$) less aerobic bacteria than samples from personnel and environment. Samples from the personnel contained on average more bacteria than samples from the environment, but the difference was not significant ($p>0.05$). *Enterobacteriaceae* were only found on one of the gloves (100 CFU/cm²).

Although *L. monocytogenes* was not found from the samples taken from clothes, the amount of aerobic bacteria on maintenance personnel clothes and tools was on average high (Table 12). This result pinpoints the need for regular and sufficiently frequent changing of the protective clothing and the need to change them whenever they get dirty. As *L. monocytogenes* was sampled from one screwdriver and another *Listeria* spp. from a pneumatic machine in common use (see Paper I) tools are potential vectors of *L. monocytogenes* transfer within food processing. This emphasizes the need to clean and disinfect them regularly, preferably daily and always whenever they get soiled.

Table 12. Results of microbiological samples taken from clothes, tools and work environment of the maintenance personnel. The samples were taken with gauze pads for *Listeria* spp. and with Petrifilm™ plates for aerobic bacteria sampling.

| Samples | <i>Listeria</i> spp. | Aerobic total bacteria | | | |
|-------------|--|------------------------|------------------------------|--------------------------------|---------------------------------|
| | No. of positive samples / No. of samples | No. of samples | Range (CFU/cm ²) | Average (CFU/cm ²) | St. dev. (CFU/cm ²) |
| Tools | 2/40 ^a | 50 | 1–40 | 5 | 8 |
| Personnel | 4/24 | 36 | 1–100 | 14 | 23 |
| Environment | 0/7 | 9 | 3–20 | 8 | 5 |

^a *L. monocytogenes* was detected from one sample taken from a screwdriver.

4.3 Discriminatory power of automated ribotyping compared with PFGE in distinguishing *L. monocytogenes* isolates (Paper II)

Identification of *L. monocytogenes* isolates to strain level by typing is needed in food plants for tracing contamination routes and identifying possible plant-specific strains causing problems. Rapid tracing of *L. monocytogenes* contamination sources is of utmost importance in the prevention of product contamination. Hitherto the only fully automated typing method is automated ribotyping and thus the discriminatory power of this method was compared to the generally recognized method PFGE and the traditionally used serotyping. Digestion with *EcoRI* generated 16 different ribogroups or ribotypes (RTs) from the 486 *L. monocytogenes* isolates and when 121 isolates representing all the 16 RTs were further typed with *PvuII*, 19 RTs were generated (Table 2, Paper II). When PFGE was used, digestion with *AscI* resulted in 42 and with *SmaI* in 24 macrorestriction patterns from the 121 isolates analyzed. When the patterns were combined, 46 final PFGE types were generated. The *DI* was 0.878 for ribotyping with *EcoRI* and 0.867 with *PvuII*. The overall *DI* for ribotyping with both enzymes was 0.906. In the case of PFGE, the *DI* of typing with *AscI* was 0.960 and with *SmaI* 0.920, the overall *DI* being 0.966. Most of the isolates (109/121) belonged to the serotype 1/2 (Table 2, Paper II). Only four of the isolates were of serotype 4b (3.3% of the isolates) and two of serotype 3 (1.7%). Some isolates (6/121) were not typeable with the commercial kit used.

From the isolates of four plants (E[29 isolates], I[7 isolates], J[25 isolates], and P[25 isolates]), PFGE generated more PFGE types (8, 5, 11 and 14, respectively) than ribotyping RTs (5, 4, 7 and 9, respectively). PFGE could further divide 10 RTs into a total of 33 PFGE types, indicating more genetic types from different plants than ribotyping. By contrast, ribotyping could also divide 3 PFGE types into a total of 6 RTs, indicating higher discrimination between these isolates than that obtained by PFGE.

Thus the discrimination power is not only dependent on the method, but also on the isolates and enzymes used. With automated ribotyping it is possible to ribotype the first pure culture colonies without any conventional time-consuming preliminary tests in 8 h. More expertise and careful manual work is needed for running PFGE. With PFGE, identification of an isolate from pure culture can be performed in 30 h (Graves and Swaminathan, 2001).

According to the results presented above (see Paper II), as well as in Paper VI, PFGE had higher discriminatory ability than automated ribotyping. However e.g. in the Paper VI, the strain occurring frequently during one year in products of one producer, and surviving milder heat treatment, could be distinguished from the other isolates with both methods. Automated ribotyping has successfully been used for investigating contamination sources in food plants (Suihko et al., 2002), and mainly based on its automation and rapidity, it is a good tool for screening large numbers of isolates in contamination studies. However, in cases of outbreaks, the identical patterns must be confirmed by PFGE.

4.4 Survival and transfer of *L. monocytogenes* in lubricants

4.4.1 Hygiene of lubricants according to the questionnaire (Paper I)

Lubricants are needed for various purposes in almost all equipment to maintain proper functioning (see 1.1.3). The questionnaire on equipment hygiene (3.1) also contained questions on hygiene of lubricants used in food plants. 21.4% of the respondents ($n=42$) had noticed hygiene problems in the use of lubricants. The respondents ($n=11$) gave the following reasons for hygiene problems in lubricants: lubricants collect a lot of soil (72.7%); traces of lubricants are left on

production surfaces after maintenance work (63.6%); inability to clean surfaces from lubricant residues (54.5%); and high levels of micro-organisms being found in the samples taken from sites containing lubricants (36.4%). A few earlier studies also showed the ability of lubricants to support the growth of micro-organisms (van der Waa, 1995; Rossmoore, 1988; Ortiz et al., 1990; Hamilton, 1991).

4.4.2 Survival of *L. monocytogenes* in lubricants (Paper III)

Only a few investigations have been available on the occurrence and survival of any micro-organisms, not to mention *L. monocytogenes*, in lubricants used in the food industry. The ability of *L. monocytogenes* to survive in lubricants used in food processing equipment was investigated by inoculating bacteria to lubricants and taking samples during a 14 d test period. The amount of *L. monocytogenes* in lubricants decreased significantly ($p < 0.05$) during the 14 d test period except in the case of synthetic conveyer-belt lubricant B and rapeseed oil D, in which the amount even marginally ($p > 0.05$) increased at 20 °C. Because of the clear survival of *L. monocytogenes* in rapeseed oil, it would not be recommendable for lubricating purposes in the food industry unless it were to be cleaned off the surfaces and replaced with new oil daily. *L. monocytogenes* also survived well in the synthetic conveyer-belt lubricant B diluted in water, which clearly indicates that water enhanced the survival of *L. monocytogenes*. The dry synthetic lubricant proved to be a better choice for lubricating conveyer belts.

Clear differences in survival of *L. monocytogenes* in different lubricants were observed. The following pure lubricants reduced the amount of *L. monocytogenes* $> 3 \log$ CFU/g during the 14 d incubation period at room temperature: mineral-oil based hydraulic oil E, dry synthetic conveyer-belt lubricant F, mineral-oil based multipurpose grease I and chain lubricant C (Fig. 1a, Paper III). In these lubricants the reduction was statistically significant ($p < 0.05$) already after 24 h. Two lubricants (the white-oil based aluminium complex grease J [USDA H1] and synthetic multipurpose grease K) killed *L. monocytogenes* in both pure and soiled conditions both at room (20 °C) and refrigerated (5 °C) temperatures already 0.5 h after inoculation. Thus, the use of these lubricants can be recommended.

Cold temperature and soiling had different effects on each lubricant. The listericidal effect of the dry synthetic conveyer-belt lubricant F and mineral-oil based hydraulic oil E (both pure and soiled) was reduced ($p < 0.05$) when the temperature was decreased from 20 °C to 5 °C. Conversely, low temperature clearly ($p < 0.05$) increased the listericidal effect of chain lubricant C (Fig. 1b, Paper III). Soiling reduced the listericidal effect of chain lubricant C used as chain lubricant and mineral-oil based hydraulic oil E especially at 5 °C, but it did not have a clear effect ($p > 0.05$) on the lubricants in general (not shown in Figs.). Organic material has been reported to reduce the listericidal effects of sanitizers and lubricants containing bactericidal agents (Lou and Yousef, 1999). Non-food-grade lubricants reduced the amount of *L. monocytogenes* better ($p < 0.05$) than food-grade lubricants, but use of food-grade lubricants is required in food contact areas.

Although the amount of *L. monocytogenes* in most lubricants, both pure and soiled, decreased significantly ($p < 0.05$) during the 14 d test period, lubricants may act as a source of contamination on the basis of the results obtained on the survival of *L. monocytogenes*. Water in lubricants should be avoided. In food manufacturing processes the change period for lubricants is often longer than two weeks, which may result in higher quantities of soil and better survival and growth of *L. monocytogenes* than detected in this study. Suitability for use in different temperatures and in clean or soiled conditions should also be considered from the microbicidal point of view.

4.4.3 Transfer of *L. monocytogenes* from lubricants to stainless steel surfaces and vice versa (Paper III)

The ability of lubricants to act as vectors for transferring *L. monocytogenes* was investigated by studying the transfer of *L. monocytogenes* from lubricants to stainless steel surfaces and vice versa. Significant ($p < 0.05$) transfer of *L. monocytogenes* from lubricants to the surfaces of stainless steel discs was detected (Table 3, Paper III). The largest amount of *L. monocytogenes* (CFU/g) was detected on surfaces of discs which were incubated in soiled grease lubricants A and G. *L. monocytogenes* bacteria were also well transferred to the stainless steel surfaces from synthetic conveyer-belt lubricant B and the rapeseed oil D.

Significant ($p < 0.05$) transfer of *L. monocytogenes* from stainless steel surfaces to lubricants at room and refrigerated temperature was seen in the case of both conveyer-belt lubricants, B and F (Table 4, Paper III). No transfer into synthetic grease lubricant A containing Teflon (USDA H1) was detected during the 24 h follow-up study. *L. monocytogenes* was transferred >4 log CFU/g to mineral-oil based hydraulic oil E at 5 °C (strain III) (Table 4, Paper III) and sporadic transfer to other lubricants (C, D and G) was detected. No previous studies on the transfer of bacteria from surfaces to lubricants or vice versa have been reported. The results indicate that lubricants, especially conveyer belt lubricants, may act as contamination vectors between processing surfaces. The lubricants should be regularly changed and the surfaces cleaned and disinfected (see 4.5) before adding new lubricant. The requisite changing frequency should be determined by monitoring the microbiological contamination level by sampling.

4.5 Susceptibility of *L. monocytogenes* to disinfectants (Paper IV)

4.5.1 Efficacy of disinfectants in suspension

Successful daily cleaning and disinfection are needed as means of decontamination of food processing equipment and premises for producing safe foods. As sanitizing is usually performed in cold premises, the efficiencies of commonly used disinfectants were investigated at +5 °C. All the disinfectants (Table 10), except alkaline hypochlorite-containing disinfectant E, were effective ($ME > 5$) in both clean and soiled conditions in suspensions. However, the peracetic acid-based disinfectant A in both clean and soiled suspensions, as well as the peracetic acid-based disinfectants C and H in soiled conditions failed to inactivate some strains at the lowest recommended concentrations given by the manufacturer (Table 3, Paper IV). When the concentrations were increased and the duration was extended to 10 min, the three latter disinfectants were also effective.

Tuncan (1993) stated that cold temperature did not affect the efficacy of chlorine. However, increased effectiveness due to an increase in temperature has also been reported (El-Kest and Marth, 1988b; Orth and Mrozek, 1990). The alkaline hypochlorite-containing disinfectant E was also tested as recommended

by the manufacturer at 20 °C. However, the increase in temperature and an increase of the concentration from the lowest recommended (0.05%) in-use concentration to 0.2% using a duration time of 10 min was not sufficient to inactivate all the strains. The pH of in-use concentrations of agent E was between 8 and 10. According to McDonnell and Russell (1999) in aqueous solution between pH 4 and 6, chlorine exists predominantly as hypochlorous acid (HClO), the active moiety, whereas above pH 9, the hypochlorite ion (OCl⁻) predominates. The pH of this disinfectant should be lower to increase its microbicidal efficiency.

4.5.2 Efficacy of disinfectants on surfaces

As attachment of the cells to surfaces has previously been shown to reduce the efficacy of disinfectants (see 1.4.2), the efficacy of disinfectants was tested also on surfaces. In this thesis, all the disinfectants (Table 10) were effective ($ME > 4$) when applied to stainless steel and PE surfaces, except the QAC-based disinfectant F and the peracetic acid-based disinfectant H against one strain (Table 5, Paper IV). The QAC-based disinfectant F was ineffective ($ME < 4$) against two of the strains on both surfaces. Previously cold temperature has also been shown to reduce the efficacy of QACs and of iodofor on *Listeria* spp., especially at low concentrations (Orth and Mrozek, 1990; Tuncan, 1993). However, the recommended duration for this QAC-based product to act was 0.5–1 h. The alkaline hypochlorite-containing disinfectant E was effective on the surface, which may be explained by the fact that *L. monocytogenes* was growing on the surfaces on the agar plate covered with a filter paper and the amount of protein on the surface was low.

According to Taormina and Beuchat (2001), heat resistance of *L. monocytogenes* was increased after exposure to alkali. The safety of foods requiring heat treatment may be endangered if they are contaminated by *L. monocytogenes* cells which have survived exposure to processing environments ineffectively cleaned or sanitized with alkaline detergents or disinfectants. Therefore the efficiency of cleaning and sanitizing treatments should be assured at the plant level, e.g. in cooperation with research laboratories, in the conditions prevailing in the process, and residues of the agents should be rinsed off the surfaces completely.

4.6 Transfer of *L. monocytogenes* during slicing of cold-salted salmon (Paper V)

As recontamination has been identified as a significant cause of food contamination, information on the level of recontamination for risk assessment purposes is needed. Slicing machines are one of the hygienically most problematic pieces of equipment in the food industry (Table 11). As cold-salted salmon is a RTE product, which has been associated with contamination by *L. monocytogenes* in several studies (Table 5), potential cross-contamination from a contaminated blade to uncontaminated cold-salted salmon slices was simulated.

4.6.1 Transfer of *L. monocytogenes* from slicing blade to slices

Transfer of *L. monocytogenes* was calculated over a total of 39 slices. There was a progressive exponential reduction in the quantity of *L. monocytogenes* transferred (Fig. 1). When compared to the inoculum level of the blade, clearly ($p < 0.05$) lower total numbers of *L. monocytogenes* were transferred when the inoculum level was lower, the temperature was colder or the attachment time was longer compared to the experiment made at room temperature with a high (8.4 ± 0.4 log CFU/g) inoculum level and a short attachment time (10 min). For example 5.3 ± 0.3 log CFU/g was transferred to the second slice when the inoculum level was 8.4 ± 0.4 log CFU/blade and the amount was reduced ca. 1.6 log CFU/g during slicing of 39 slices (Fig. 2a, Paper V). Based on the result data of the samples (2–39), when compared to the inoculum level of the blade, there were no statistically significant differences in the logarithmic reduction of *L. monocytogenes* numbers in slices between the different experiments ($p > 0.05$), although a marginally lower reduction was detected at 0 °C compared to room temperature. The transfer percentage (the amount of *L. monocytogenes* in all 39 slices compared to the inoculum) varied between 0.00011 and 0.17%, being lowest at 0 °C. However, when calculated with the predicted values, instead of using the results of 39 slices directly, a significantly ($p < 0.05$) lower logarithmic reduction in the number of *L. monocytogenes* between slices was found when slicing was at 0 °C compared to the experiment made at room temperature with a high inoculum level and a short attachment time, indicating that cold temperature prolonged the transfer. However, in all experimental conditions, the number of bacteria decreased quite rapidly (i.e. after slicing the fourth fillet) to < 1 log CFU/g.

The reduction in quantity of *L. monocytogenes* transferred was lower than that reported by Vorst et al. (2006a) for turkey breast, bologna and salami (2 log CFU/20 slices). Soft salmon material (salmon fillets consisting mainly of protein, fat and moisture) on slicer surfaces as well as solidification of fat most probably slowed the transfer at colder temperatures. According to Midelet and Carpentier (2002), who studied transfer of bacteria including *L. monocytogenes* from various materials to pieces of beef, in most cases the inoculation concentration had the strongest influence on the total number of CFU detached. The attachment strength of bacteria on different materials also had a significant ($p<0.05$) effect on transfer. In the current study, based on the predicted values higher numbers ($p<0.05$) of *L. monocytogenes* were also transferred to slices from the slicing blade when the inoculum was higher or when the attachment time was shorter. When the blade inoculum was low, ca. 3 log CFU/blade, only a few colonies were found on the first 10 slices and no colonies were detected on the slicer surfaces, including the blade. Levels below the agar detection limit were most probably present but were not examined by enrichment.

When a salmon fillet was inoculated with *L. monocytogenes* (surface inoculum of fillet was 7.6 ± 0.1 log CFU *L. monocytogenes* per fillet) and sliced, the blade contained 3.9 ± 0.8 log CFU/10 cm² (i.e. 5.5 ± 2.4 log CFU/blade). 3.2 ± 0.4 log CFU/g was initially transferred to the subsequent sliced uninoculated fillet and the overall reduction in transfer was 1.5 log CFU after 39 slices. The reduction was only marginally ($p>0.05$) lower compared to reduction when the blade was initially contaminated. When the predicted values were used with higher (156) slice numbers (Fig. 1), significantly smaller logarithmic reduction was observed compared to the experiment, in which the blade was directly inoculated (8.4 ± 0.4 log CFU/blade, 10 min) at room temperature.

The contamination level on the holding plate and cover depended on the level of *L. monocytogenes* on slices, especially in experiments made at room temperature, i.e. the higher the level was on slices, the higher the level was on equipment surfaces (Table 1, Paper V).

The roughness (A'/A) of the slicing blade was 2.3 ± 0.1 on the sharp side and 1.7 ± 0.1 on the flat side in the beginning of the experiments, and 2.0 ± 0.2 and 2.7 ± 0.6 , respectively, at the end of the experiments. The results and pictures taken of the new blade and the blade at the end of the experiments (pictures not

shown) by reflection confocal microscopy clearly demonstrated blade wear over the course of the experiments, emphasizing the importance of regular maintenance of the blade in food processing operations. The blade, the blade guard and holding plate should be periodically dismantled, cleaned and sanitized to avoid attachment of and prolonged product contamination with *L. monocytogenes*. Additionally, the blade should be sharpened regularly.

4.6.2 Model of transfer of *L. monocytogenes*

Based on the results of the transfer experiments performed in the laboratory, transfer in different conditions was modelled. An exponential model of transfer as a function of slice number provided a reasonable fit across all treatments, e.g. average $r^2 > 0.7$, except that in the 0 °C study the r^2 -value was 0.63:

$$y = a * e^{(-x/b)}; \quad (\text{Eq. 4})$$

The values for constants a and b are different for each experiment. The model describes a microbiological decay curve as a function of slice number. The results of the predictions are most reliable when the data is obtained from experiments made in conditions prevailing in the process where the model will be applied (e.g. temperature, attachment time of inoculum, type of slicer etc.).

In this thesis, limited data from microbiological analysis was used to assess transfer of *L. monocytogenes* among processing surfaces and product. According to the results, *L. monocytogenes* was readily transferred from contaminated slicing blade to cold-salted salmon slices. The highest numbers of *L. monocytogenes* on slices were detected in the beginning of the slicing (except in the very first slice). Based on the results, the expected numbers of *L. monocytogenes* on slices due to recontamination from the blade are low, especially after slicing the first slices. The results obtained in this study can be used to assess the level of recontamination in the salmon process and they also provide an example how to estimate the impact of recontamination on other products.

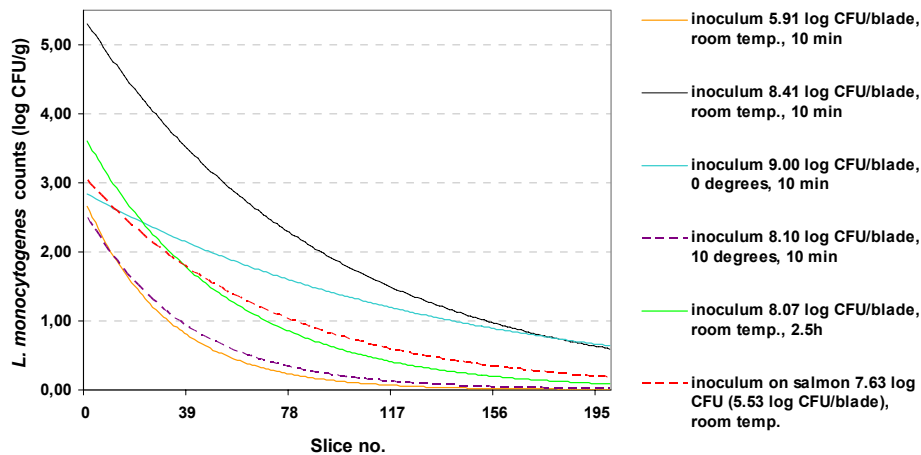


Figure 1. Predicted transfer of *L. monocytogenes* from inoculated blade to uninoculated salmon fillets during slicing of 200 slices in different conditions. After slice 39 the predictions are extrapolations.

4.7 Producer level quantitative risk assessment of *L. monocytogenes* (Paper VI)

4.7.1 Prevalence and numbers of *L. monocytogenes* in broiler legs

Information of prevalence and levels of *L. monocytogenes* in raw marinated broiler legs was collected for performing a producer level risk assessment (4.7.3). Overall, 39% (72/186) of the samples were contaminated with *L. monocytogenes* (Table 13) (36% of the samples stored at 6 °C and 57% of the samples stored at 10 °C). The prevalence of *L. monocytogenes* varied between the producers, being 18% (11/62) for producer A, 69% (43/62) for B and 29% (18/62) for C (see Paper VI). The numbers of *L. monocytogenes* were low, between 0.3 and 147 CFU/g in all the samples stored at 6 °C and between 0.5 and 300 CFU/g in all the samples stored at 10 °C.

In Finland prevalences between 35% and 62% have been reported for retail raw broiler meat (Miettinen et al., 2001; Johansson et al., 2003). In other countries levels of *L. monocytogenes* positive samples in raw broiler meat have been similar (Cox and Bailey, 1999). Only a few investigations are available on the

levels of *L. monocytogenes* in raw broiler or chicken meat. According to Mead et al. (1990) and Rørvik and Yndestad (1991), raw chicken normally contains *L. monocytogenes* 10^3 CFU/g. Our results are thus in agreement with these previous findings. Franco et al. (1995) reported levels of >math>10^3</math> CFU/g for raw chicken leg skin and leg muscle meat in many samples taken directly from a poultry processing plant, and found them most contaminated from the poultry pieces.

Table 13. Prevalence and levels of L. monocytogenes (L.m.) in marinated broiler legs obtained from retail shops and stored at 6 °C or at 10 °C until the end of their shelf life.

| Producer | Production period | No. of <i>Listeria</i> spp. positive samples (%) | No. of <i>L. m.</i> positive samples (%) | No. (CFU/g) of <i>L. m.</i> Range (Average) |
|----------|--------------------------|--|--|---|
| A | Feb/Mar – Nov/Dec -02 | 26/55 (47) | 9/55 (16) | 0.3–29.7 (5.1) |
| | Jan/Feb ^a -03 | 2/7 (29) | 2/7 (29) | 0.5–5.0 (2.8) |
| B | Feb/Mar – Nov/Dec -02 | 40/55 (73) | 37/55 (67) | 0.3–147 (7.4) |
| | Jan/Feb ^a -03 | 6/7 (86) | 6/7 (86) | 2.0–180 (39.2) |
| C | Feb/Mar – Nov/Dec -02 | 14/55 (25) | 14/55 (25) | 0.3–2.7 (0.9) |
| | Jan ^a -03 | 4/7 (57) | 4/7 (57) | 0.5–300 (84.6) |
| Total | | 92/186 (49) | 72/186 (39) | 0.3–300 (12.7) |

^a stored at 10 °C

4.7.2 Thermal inactivation of *L. monocytogenes* by heating

The temperatures used in the laboratory experiments were based on the consumer tests. For cooking the broiler legs, the consumers used 43–105 min (average 64 min) and the oven was set to 175 °C, 200 °C, 205 °C or 225 °C. The lowest average temperature detected in consumer ovens during cooking was 132 °C. 74% (14/19) of the consumers kept the broiler legs longer than 50 min in the oven, which was the producer's recommendation for cooking at 175 °C.

The consumer cooking data was divided into four (t,T) segments (A–D) with (A) ≥ 50 min, ≥ 175 °C; (B) ≥ 50 min, between ≥ 132 °C and < 175 °C; (C) ≥ 50 min, < 132 °C; (D) < 50 min, any temperature (Fig. 2, Paper VI). When calculated using a simulation as presented in 3.6.5, the corresponding simulated probabilities were: $P(A) = 0.25$, $P(B) = 0.51$, $P(C) = 0.03$ and $P(D) = 0.21$. However, we limited our example to calculating conditional risk when consumers heat the broiler legs according to producer’s instructions, for ≥ 50 min. Accordingly a simulation was performed for this data to obtain the conditional probabilities $P(t, T | t > 50 \text{ min})$. Thus,

$$P(T > 175 | t > 50 \text{ min}) = 0.25 / (1 - 0.21) = 0.32$$

$$P(132 < T \leq 175 | t > 50 \text{ min}) = 0.51 / (1 - 0.21) = 0.64$$

$$P(T \leq 132 | t > 50 \text{ min}) = 0.03 / (1 - 0.21) = 0.04.$$

In laboratory experiments, of the 21 samples in each of the two experiments (132 °C 50 min and 175 °C 50 min), 12 (57%) were contaminated with *L. monocytogenes* from < 1 to 300 CFU/g. The heating eliminated *L. monocytogenes* from all the samples, except for one sample at 132 °C. The level of *L. monocytogenes* in this sample before heating was 22 CFU/g. The worst-case cooking scenario was 132 °C ≥ 50 min, as none of the consumers in this study actually used the combination of ≤ 132 °C ≤ 50 min (see Fig. 2, Paper VI).

4.7.3 Quantitative risk assessment

Quantitative risk assessment was performed on the basis of the contamination (4.7.1) and heating study (4.7.2) data (see Fig. 3, Paper VI). The worst-case probability of a sample being *L. monocytogenes* -positive after heating would be calculated using the probabilities calculated in 4.7.2 as follows:

$$P(L. m. \text{-positive after heating} | L. m. \text{-positive before heating and } t > 50) = 0.32 \times 0 + 0.64 \times (1/12) + 0.04 \times 1 = 0.09.$$

As the worst-case probability, the producer could choose the highest % of positive samples obtained during the time period of interest. In our study we used 39%, the value obtained from the samples, as the worst-case value. Then,

$$P(L. m.-positive after heating; worst) = P(L. m.-positive samples before heating; worst) \times P(L. m.-positive after heating | L. m. - positive before heating and t > 50) = 0.39 \times 0.09 = 0.04. \quad (\text{Eq. 5})$$

L. monocytogenes was only detected after cooking but, because the number of *L. monocytogenes* in broiler leg in which the bacteria survived was 22 CFU/g before cooking, this number was used as the worst-case number of *L. monocytogenes* after cooking. When this number was multiplied by the greatest weight of a broiler leg (456 g), we obtained a worst-case approximation of the level of exposure/portion/individual, $N(\text{max})$, of 1.032×10^4 CFU, with the assumption that one portion is one broiler leg. According to Eq. (3), the worst-case dose-response from one broiler leg would then be:

$$P(\text{listeriosis from } L. m.-\text{positive broiler leg; worst}) = 1 - e^{(-1.18 \times 10^{10} \times 1.032 \times 10^4)} = 1.18 \times 10^{-6}.$$

As 6.3 million kg of marinated broiler legs were consumed in Finland, the number of consumed legs was 6.3 million kg / 0.301 kg / 5.3 million consumers (population of Finland) = 3.95 marinated broiler legs/person/year. Of the population of Finland, 16–22% belong to risk groups (Maijala et al., 2001). As a worst-case estimate, 22% of the population was estimated to belong to the risk group. Thus the worst-case listeriosis risk from heated marinated broiler legs for people belonging to a risk groups would be:

$$P(\text{listeriosis; risk group, worst}) = P(L. m.-positive after heating; worst) \times P(\text{listeriosis from } L. m.-\text{positive broiler leg; worst}) \times P(\text{risk group; worst}) = 0.04 \times 1.18 \times 10^{-6} \times 0.22 = 9.14 \times 10^{-9}. \quad (\text{Eq. 6})$$

When the number of portions eaten/person/year is included, this gives the expected cases of listeriosis as $9.14 \times 10^{-9} \times 3.95$ portions/person/year $\times 5.3$ million = 0.19 cases/year.

The risk for the general population was also calculated (see Table 14) as well as values obtained by using the following average point estimates: level of *L. monocytogenes* -positive broiler legs 34% (estimated mean level, see Paper VI); $P(L. m.-positive after heating | L. m. -positive before heating and t > 50) = (0.32 \times 0 + 0.64 \times (1/12) + 0.04 \times 1)/2 = 0.05$, which assumption was made

based on the use of a time × temperature combination of 50 min × 175 °C or more by 15/19 of the consumers; number of *L. monocytogenes* after heat treatment, 2 CFU/g (estimated average concentration in raw marinated broiler legs see Paper VI); average weight of a broiler leg portion, 301 g; and the portion of the total population belonging to the risk group (16 + 22)/2%=19%. The results obtained with different *r*-values of dose-response models are presented in Table 14.

Table 14. Expected cases of listeriosis/year from heated marinated broiler legs when heated according to the producer's instructions, based on different dose-response models.

| Dose-response model used | No. of listeriosis cases/year | | | |
|---------------------------|-------------------------------|------------|-----------------------|-----------------------|
| | High-risk population | | General population | |
| | average | worst-case | average | worst-case |
| Buchanan et al., 1997 | 4.32×10^{-3} | 0.19 | - ^a | - |
| Linqvist and Westöö, 2000 | 2.05×10^{-2} | 0.91 | - | - |
| FAO/WHO, 2004 | - | - | 8.34×10^{-6} | 3.07×10^{-4} |

^a“-“ = No dose-response model available.

According to the results of the point estimate-based risk assessment 4.32×10^{-3} – 2.05×10^{-2} (on average) people belonging to the risk group in Finland would annually be at risk of suffering severe listeriosis from marinated broiler legs after cooking according to the instructions given by producers. As the risk was negligible for the general population, the results of the point estimate-based risk assessment indicated that the risk is very low. At the plant level it should be considered which products require a thorough risk assessment. In addition, factors causing uncertainty in risk assessment must be described in order to be able to evaluate the level of confidence and to avoid unrealistic scenarios (see description in Paper VI).

The producer can affect the prevalence and numbers of *L. monocytogenes* on a product before it leaves the factory, and adjust the sell-by date and the guidelines for cooking it. Using this kind of risk-based approach, the effects of different management actions on the number of cases of listeriosis can be estimated and thus a more comprehensive understanding of the risk to consumers obtained. A similar approach can be used to assess the risk of *L. monocytogenes* and other microbiological hazards for different products.

Additionally, the results indicate that Finnish oven-cooked marinated broiler legs do not present a significant risk from *L. monocytogenes* if recontamination after heating is avoided. Recontamination can be a major issue in contamination of the processed products (see 1.3.6) and should be prevented. This emphasizes once again the importance of good equipment hygiene.

5. Conclusions

Occurrence of *L. monocytogenes* in different food products is a universal problem in the food industry as the bacterium is widely distributed and tolerant against various environmental conditions. *L. monocytogenes* is known for its ability to persist in food processing equipment and therefore hygiene should be optimized against this pathogen. In this thesis, deficiencies in current process hygiene measures and improvements for minimizing the occurrence of *L. monocytogenes* were investigated and the following conclusions were drawn:

1. Equipment design and maintenance practices in food processing

Packaging machines, conveyers, dispensers, slicing machines and cooling machines were found to be the hygienically most problematic equipment in food processing plants. The main reason was generally poor hygienic design. In previous studies, these machines have also been found to be sources of *L. monocytogenes* -contamination. Thus equipment designers should focus their performance on more suitable equipment design. Additionally, their training in this topic must be increased.

The effect of hygienic practices of maintenance personnel on equipment hygiene and on occurrence of *L. monocytogenes* in processing equipment has often been a topic in food safety discussions but has not been studied to date. In the current thesis it was found that the maintenance personnel in food processing plants is a potential source of contamination of food processing equipment, and thus food products. Clear deficiencies in hygiene performance of this employee group were found. An indication was also found that *L. monocytogenes* could be transferred through maintenance work. Training of maintenance personnel in hygiene matters must therefore be increased and included in the legislation.

2. Automated ribotyping and PFGE in identification and tracing of *L. monocytogenes* in food processing

Typing of *L. monocytogenes* -isolates is needed in tracing contamination sources in food plants. The results of this thesis showed that PFGE had a higher discriminatory power for *L. monocytogenes* isolates than ribotyping. However, due to its automation and rapidity, automated ribotyping can be considered a good method for *L. monocytogenes* control purposes and for detecting contamination sources in food processing. In epidemiological studies, the results obtained with the automated ribotyping system must be confirmed by PFGE.

3. Lubricants and cold disinfectant treatments as vehicles in *L. monocytogenes* contamination routes in the food industry

Recently an ISO-standard and guidelines on Good Manufacturing Practices for manufacturing and use of food-grade lubricants have been published. However, the microbiological quality of lubricants has received little attention. The studies made in this thesis showed that *L. monocytogenes* survived during a 14 d experiment period in 9 out of 11 lubricants used in food-processing equipment. Clear differences between lubricants were found. It was shown, for the first time, that *L. monocytogenes* was transferred to stainless steel surfaces from lubricants and in many cases from surfaces to lubricants, indicating that lubricants may act as vectors in *L. monocytogenes* contamination from one surface to another. In addition to the requirements of toxicological safety and good technical performance, based on this study, prevention of survival and growth of bacteria should be considered when choosing lubricants for maintenance of food-processing equipment. Moreover, samples for detection of *L. monocytogenes* should be regularly collected from lubrication points of food processing equipment.

The eight commercial disinfectants were in general efficient against *L. monocytogenes* strains at +5 °C at the concentrations and effect times recommended by the manufacturer. Thus they were suitable for control of *L. monocytogenes* at the plant level, with only a few exceptions. The results pinpoint the need to use appropriate concentrations and duration, especially

in the case of QAC and hypochlorite-based agents. In some cases the lowest recommendations given by the manufacturer may not be sufficient. The efficiency of cleaning and sanitizing treatments at production plant level should be confirmed, e.g. in cooperation with research laboratories, under conditions prevailing in the processes.

4. Risk assessment at the food processing plant level

Information on the level of recontamination of products is needed for risk assessment purposes. An example of how predictive modelling and limited data from microbiological analysis can be used to assess the level of recontamination was given in the thesis. To assess recontamination, transfer of *L. monocytogenes* from slicing blade to slices of cold-salted salmon was investigated and clearly observed. An exponential model was suitable for predicting the expected number of *L. monocytogenes* (log CFU/g) on the salmon slices. In all test conditions, the numbers of *L. monocytogenes* were predicted to be reduced to <1 log CFU/g after the fourth uninoculated fillet was sliced. Salmon processors can use the results as a guide in risk management decisions.

A robust quantitative risk assessment of *L. monocytogenes* for one product was presented. Marinated broiler legs were used as an example product. This approach helps food processors in illustrating the risks caused by the products for consumers by estimating the effects of different risk management actions on the number of cases of listeriosis. This information can be utilized when planning risk management actions for own-checking systems. The results indicate that Finnish oven-cooked marinated broiler legs are not a significant *L. monocytogenes* risk, if recontamination after cooking is avoided. The results of the studies presented in this thesis also emphasize that risk assessment may be needed especially in case of products where recontamination after processing may typically occur.

Efficient control of *L. monocytogenes* at plant level is a combination of good equipment hygiene including functioning Good Hygiene Principles (GHP's) used by all employee groups, and rapid methodology in detecting contamination sources, as well as efficient hazard analysis systems utilizing

a risk assessment approach. Optimally hygienic solutions of the food plants for control of *L. monocytogenes* and other pathogens should be included in the design phase of the food processes and processing equipment as early as possible. They should be optimized for the conditions prevailing at each processing plant, which emphasizes cooperation between food processors, research institutes and suppliers of hygiene solutions. The measures should be environment- and energy-saving, which requires a high standard of equipment design. New design solutions, and materials as easy to clean and disinfect as possible, should be developed for reduced need of sanitizing and maintenance, e.g. changing parts of machinery. As rapid tracing of contaminants is a key issue in prevention of food-borne illnesses caused by contaminated products, development of rapid, reliable and easy-to-use automated methods for detecting contamination sources in food processes is needed.

The impact of different maintenance procedures on transfer of *L. monocytogenes* inside food plants should be studied further. Studies on the importance and mechanisms of recontamination have been launched during recent years. Several factors regarding transfer of *L. monocytogenes* need further investigations, including the effect of strain variability and product composition. Additionally, transfer from production scale slicers should be studied. Investigations on bacterial levels in lubricants used in maintenance of food processing equipment and their role in contamination of the equipment should be performed. Moreover, it should be investigated whether lubricants lead to the development of resistance and cross-resistance of *L. monocytogenes*.

Future risk assessment at the plant level should have a more comprehensive, quantitative approach with the assistance of computer programs. For this purpose, there is a clear need for additional quantitative data on *L. monocytogenes* contamination levels in different foods and production equipment.

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| Author(s) Aarnisalo, Kaarina | | |
| Title Equipment hygiene and risk assessment measures as tools in the prevention of <i>Listeria monocytogenes</i> -contamination in food processes | | |
| Abstract <p>A problematic pathogen occurring in food processing is <i>Listeria monocytogenes</i>. Its efficient control at the processing plant level requires good equipment hygiene, including functioning good manufacturing and hygiene practices used by all employees, effective means of decontamination and rapid detection of contamination sources, as well as hazard analysis systems supported by risk assessment procedures. The present thesis focuses on deficiencies and improvements in these equipment hygiene and risk assessment practices with the aim of elucidating and developing the most efficient practices against <i>L. monocytogenes</i>.</p> <p>The hygienically most problematic types of equipment in the Finnish food industry were identified as the packaging machines, conveyers, dispensers, slicing machines and cooling machines. The main reason for the equipment being considered as problematic was poor hygienic design. Equipment designers must focus their performance on more suitable equipment design. Clear deficiencies were also found in hygiene performance of the maintenance personnel in food processing plants. The results of these studies also indicate that <i>L. monocytogenes</i> may be transferred through maintenance work. Training of maintenance personnel with reference to hygienic practices must be increased. The results of the thesis showed that lubricants used in food processing equipment may act as contamination vehicles of <i>L. monocytogenes</i>. An investigation of the efficiency of eight commonly used commercial disinfectants against <i>L. monocytogenes</i> strains at +5 °C was performed. The tested agents were generally efficient at the recommended concentrations and effect times, with only a few exceptions. The suitability of automated ribotyping was also compared with the traditionally accepted and successfully used pulsed-field gel electrophoresis (PFGE) to discriminate <i>L. monocytogenes</i> isolates and thus trace contamination sources in food plants. PFGE had a higher discriminatory power for <i>L. monocytogenes</i> isolates than automated ribotyping. However, based on its automation and rapidity automated ribotyping can be considered a good method for control purposes. Additionally, risk assessment practices were developed by investigating and modelling recontamination of a product and by a plant-level quantitative risk assessment. Recontamination was modelled by investigating transfer of <i>L. monocytogenes</i> from slicing blade to slices of cold-salted salmon. Transfer with a progressive exponential reduction in the quantity of bacteria in slices was detected. As a concluding example, a practical approach to quantitative risk assessment of <i>L. monocytogenes</i> for one product at the plant level is presented. This approach helps food processors in illustrating the risks caused by the products for consumers.</p> | | |
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| Tekijä(t) Aarnisalo, Kaarina | | |
| Nimeke Laitehygienia- ja riskinarviointitoimenpiteet työvälineinä <i>Listeria monocytogenes</i> -kontaminaation torjumisessa elintarvikeprosesseissa | | |
| Tiivistelmä <i>Listeria monocytogenes</i> -bakteeri on elintarvikeprosesseissa esiintyvä ongelmallinen patogeeni. Sen tehokas hallinta tuotantolaitostasolla koostuu hyvästä laitehygieniasta ja sisältää toimivat, hyvät tuotanto- ja hygieniakäytännöt kaikkien työntekijöiden osalta, tehokkaat bakteerin tuhoamiseen tarkoitettut menetelmät ja nopeat kontaminaatiolähteiden toteamismenettelmät yhdistettynä riskinarviointikäytännöillä tuettuun prosessin vaara-analysiin. Työssä selvitettiin puutteita ja parannuksia nykyisin käytössä olevien laitehygieniakäytäntöjen osalta ja kehitettiin riskinarviointikäytäntöjä mahdollisimman tehokkaiden <i>L. monocytogenes</i> -bakteerin torjumiskeinojen varmistamiseksi. Tuotantohygienialtaan ongelmallisimmiksi laitteiksi Suomen elintarviketeollisuudessa todettiin pakkauskooneet, kuljettimet ja annostelu-, siivutus- ja jäähdytyskooneet. Pääasialliseksi syyksi todettiin huono hygieeninen laitesuunnittelu. Laitevalmistajien on kehitettävä elintarviketeollisuuden tarkoitettujen laitteiden hygieenisyyttä. Työssä tehdyssä selvityksessä laitteita huoltavan kunnossapitohenkilöstön hygieenisistä toimintatavoista voitiin näissä tavoissa todeta selkeitä puutteita. Löydettiin myös viite siitä, että <i>L. monocytogenes</i> voisi siirtyä huoltotyön yhteydessä tehtaan sisällä. Kunnossapitohenkilöstön hygieniakoulutuksen määrää on lisättävä. Työn tulokset osoittivat myös, että tuotantolaitteistoissa käytettävät voiteluaineet voivat toimia <i>L. monocytogenes</i> -kontaminaation lähteinä ja siirtymisvälineinä. Työssä testatut käytössä olevat kaupalliset desinfiointiaineet (8 kpl) tehosivat <i>L. monocytogenes</i> -bakteeriin yleensä valmistajan antamalla alhaisimmilla käyttökonsentraatioilla ja käyttöajoilla +5 asteessa, vain joitakin poikkeuksia lukuun ottamatta. Automaattisen ribotyyppityksen soveltuvuutta <i>L. monocytogenes</i> -isolaattien erotteluun ja siten kontaminaatiolähteiden jäljittämiseen elintarvikeprosesseissa verrattiin hyväksi todettuun pulssikenttägelelektroforeesi (PFGE) -menetelmään, ja tulokset osoittivat, että PFGE oli erottelukyvyltään parempi. Nopeutensa ja automaattisuutensa takia automaattinen ribotyyppitys soveltuu kuitenkin myös hyvin hygieniavalvontaan. Työssä kehitettiin lisäksi <i>L. monocytogenes</i> -bakteerin riskinarviointikäytäntöjä selvittämällä ja mallintamalla tuotteen jälkikontaminaatiota sekä kuvaamalla tehdastason riskinarviointi. Jälkikontaminaatiota mallinnettiin tutkimalla <i>L. monocytogenes</i> -bakteerin siirtymistä siivutettaessa terästä graavilohisiiivuihin. Selkeää siirtymistä todettiin bakteerimäärien eksponentiaalisesti pienentyessä siivuisissa. Viimeisenä esimerkkinä kuvataan käytännönläheinen yhden tuotteen <i>L. monocytogenes</i> -riskinarviointi. Tämä lähestymistapa auttaa elintarviketuottajia hahmottamaan kuluttajille tuotteista aiheutuvaa riskiä. | | |
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