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REVIEW ARTICLE

An updated review of *Listeria monocytogenes* in the pork meat industry and its products

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Abstract

Pork meat and processed pork products have been the sources of outbreaks of listeriosis in France and in other European countries during the last decade. The aim of this review is to understand how contamination, survival and growth of *Listeria monocytogenes* can occur in pork meat products. This study discusses the presence of *L. monocytogenes* in raw pork meat, in the processing environment and in finished products. The prevalence of *L. monocytogenes* generally increases from the farm to the manufacturing plants and this mainly due to cross-contamination. In many cases, this pathogen is present in raw pork meat at low or moderate levels, but foods involved in listeriosis outbreaks are those in which the organism has multiplied to reach levels significantly higher than 1000 CFU g^{-1}. In such cases, *L. monocytogenes* has been able to survive and/or to grow despite the hurdles encountered during the manufacturing and conservation processes. Accordingly, attention must be paid to the design of food-processing equipment and to the effectiveness of the cleaning and disinfecting procedures in factories. Finally, the production of safe pork meat products is based on the implementation of general preventive measures such as Good Hygiene Practices, Good Manufacturing and the Hazard Analysis Critical Control Point.

Introduction

Listeria monocytogenes is a Gram-positive ubiquitous bacterium which is widely distributed in the environment. It has been isolated from a variety of sources including soil, vegetation, silage, faecal material, sewage and water (AFSSA 2000). This facultative intracellular bacterium can cause listeriosis, a severe invasive illness in humans, which may result in death. The risk of contracting listeriosis is high in immuno-compromised persons, the elderly, pregnant women and neonates. Thirteen serotypes of *L. monocytogenes* have been identified, but only three serotypes (1/2a, 1/2b and 4b) are associated with the majority of sporadic cases of listeriosis; serotype 4b is linked to almost all recent outbreaks (Rocourt and Bille 1997).

The transmission of this pathogen by contaminated food was first conclusively demonstrated by epidemiological and laboratory investigations in 1983 (Schlech *et al.* 1983). *Listeria monocytogenes* has been recovered from many different foods, and conversely, a variety of different food items such as raw and processed meats, soft cheese, raw milk, hot dogs, seafood and fresh vegetables have been linked to both sporadic cases and outbreaks of listeriosis (AFSSA 2000; FICT 2002). Pork meat and processed pork products, such as deli meats, have been implicated in listeria outbreaks in France (Jacquet *et al.* 1995; Goulet *et al.* 1998) and in other European countries (Jay 1996; Loncarevic *et al.* 1997) during the past decade. *Listeria monocytogenes* is of particular concern in raw, undercooked or ready-to-eat foodstuffs (AFSSA 2000). Because the organism is ubiquitous, food-processing industries are easily contaminated through raw food stuffs (AFSSA 2000). *Listeria monocytogenes* may then subsist in these industries because it grows at low temperatures,

adheres to various food contact surfaces and certain strains have adapted to disinfectants (Salvat *et al.* 1995). In fact, Nesbakken *et al.* (1996) found *L. monocytogenes* at every stage of the fresh pork meat industry, with increasing prevalence from the slaughterhouse to the cutting room.

The human population responses to exposures to a foodborne pathogen are highly variable. Disease incidence is dependent on a variety of factors, including virulence of the pathogen, dose (the number of pathogens ingested), the general health and immune status of the host and the attributes of the food matrix that alter microbial or host status (Risk Assessment Drafting Group 2004). However, the incidence of *L. monocytogenes* in meat products is generally low, even if the pathogen is present at low or moderate levels (Encinas *et al.* 1999; AFSSA 2000; FICT 2002). Even if a single bacterial cell has the potential to cause disease, epidemiological data indicate that foods involved in listeriosis outbreaks are those in which the organism has multiplied and in general have reached levels significantly >1000 CFU g^{-1} (Ross *et al.* 2002; Risk Assessment Drafting Group 2004).

Criteria or recommendations for tolerable levels of *L. monocytogenes* in processed foods have been established in some countries. For example, the USA practices 'zero tolerance' (no organisms found in 25 g of a food product, Shank *et al.* 1996) while Canada and France apply different norms according to the foodstuff. The probability of contracting listeriosis is thought to be very low when food contamination was below 100 CFU g^{-1}. Therefore, a level of not more than 100 CFU g^{-1} is tolerated in certain foodstuffs, while zero tolerance is applied to foods which support *L. monocytogenes* growth and have extended shelf-lives (AFSSA 2000). There is a great deal of uncertainty concerning *L. monocytogenes* counts because the actual level in serving of food may vary considerably from one portion to another (Risk Assessment Drafting Group 2004). Several countries have therefore concluded that the complete absence of *L. monocytogenes* in certain foods is an unrealistic and unattainable requirement which does not have a significant positive impact on public health and may even divert resources and attention from other preventive measures. Despite these recommendations and drastic economic losses to industries due to recall products contaminated with *L. monocytogenes*, several processed food-related outbreaks of listeriosis have occurred during the last decade (Goulet *et al.* 1998).

The aim of this review is first, to update knowledge about the origin of *L. monocytogenes* contamination in pork meat, products and processing environment; and secondly, to understand how the organism is able to develop or survive the manufacturing processes, and then cause sporadic cases or outbreaks of listeriosis. This review details and discusses our current understanding of these issues. Special attention is drawn to the different serotypes and clonal lineages that are found in foods and are implicated in foodborne listeriosis.

Contamination of raw pork meat, meat processing environments and pork meat products

Raw pork meat

Listeria monocytogenes occurs frequently in raw pork meat (Norrung *et al.* 1999), although the origin of the contamination is unclear. *Listeria monocytogenes* has been occasionally isolated on farms from the faeces and skin of presumably healthy pigs (Skovgaard and Norrung 1989). The organism is thought to be harboured in the intestinal tract: the prevalence of *L. monocytogenes* in pig faecal samples ranges from 0% to 47%; the highest prevalences are reported in Eastern Europe (Felon *et al.* 1996). Husbandry practices that involve feeding pigs dry feed or silage, rearing pigs in closed houses, and maintaining specific pathogen-free herds may account for some of the reported variation in the incidence of *L. monocytogenes* in healthy pigs (Felon *et al.* 1996).

Carcasses are thought to be contaminated when the large intestine is ruptured during evisceration (Skovgaard and Norrung 1989). However, Kanuganti *et al.* (2002), detected *L. monocytogenes* in only 4% of pork carcasses sampled but not in the rectal contents of animals prior to slaughter. This may be due to major slaughter plants practice of evacuating the rectum prior to evisceration, thus minimizing rectal contents and thus subsequent carcass contamination. Authors have suggested not all *L. monocytogenes* detected in carcasses have a faecal origin. Bunčić *et al.* (1991) showed that pigs were more likely to harbour *L. monocytogenes* in their tonsils than to excrete the bacteria in their faeces.

Autio *et al.* (2000) also found that 14% of pig tongues and 12% of tonsils sampled in slaughterhouses contained *L. monocytogenes*. Interestingly, Kanuganti *et al.* (2002) showed that *L. monocytogenes* was detected more often in pig tonsil homogenates (7·1% of 252 samples) than in tonsil scrapings collected on the farm (3% of samples). Reported prevalence of *L. monocytogenes* in tonsils range from 0% to 61%; this range is probably due to differences in sampling techniques and/or farm management methods (Felon *et al.* 1996).

Autio *et al.* (2000) found that the occurrence of *L. monocytogenes* differed among slaughterhouses. Furthermore, the level of contamination of viscera (tongue, oesophagus, trachea, lungs, heart, diaphragm, kidneys and liver); was particularly high (64%). They hypothesized that *L. monocytogenes* spread through contact between the

tonsils and tongue and the other viscera and carcass during the evisceration process. Moreover, Kanuganti *et al.* (2002) detected *L. monocytogenes* less often in the tissues of freshly slaughtered pigs (0·8–2·4% of samples) or of small intestines (8·3–9·3% of samples) than in ground pork (45–50·2% of samples). Furthermore, an extensive study of meat contamination levels in the meat processing industry indicated that chilling and cutting significantly increased the contamination of pork meat (Nesbakken *et al.* 1996) while Van der Elzen and SNIJders (1993) found that the environmental prevalence of the pathogen in chilling–cutting areas to be as high as 71–100%. These findings strongly suggest that postslaughter processing is a significant cause of meat contamination, and that contamination is amplified in the chilling and cutting room environment (Nesbakken *et al.* 1996). Pulsed field gel electrophoresis (PFGE) studies of *Listeria* strains collected both from raw meat and work surfaces during meat processing have demonstrated conclusively that raw meat can contaminate meat processing environments. Reciprocally, contaminated equipment can, in turn, contaminate meat products during processing (Giovannacci *et al.* 1999, Thevenot *et al.* 2004).

Meat is mainly contaminated with *L. monocytogenes* serotypes 1/2a, 1/2b and 1/2c (Hof and Rocourt 1992). We also found that this is true for raw pork meat (Thevenot *et al.* 2005b). Most listeriose cases are sporadic and frequently involve serotypes 1/2a and 1/2b (Schuchat *et al.* 1991). The presence and predominance of serotype 1/2a and to a lesser extent 1/2b in meat processing plants might be a source of sporadic cases. We found serotype 4b strains in raw pork meat entering French deli meat factories (Thevenot *et al.* 2005b), although Giovannacci *et al.* (1999) did not found this strain in pork slaughtering and cutting plants. Nevertheless, this is of major public health importance as serotype 4b is responsible of the majority of listeria outbreaks in France (Goulet *et al.* 1998).

Pork meat processing environments

In general, the primary source of food contamination by *L. monocytogenes* before release to consumers appears to be the processing environment (Kathariou 2002).

Several studies have shown that *L. monocytogenes* strains isolated from meat processing environments are frequently of serotypes 1/2a, 1/2b and 1/2c (Jay 1996; Chasseignaux *et al.* 2002; Thevenot *et al.* 2005b). We also isolated serotypes 4b and 4e in French meat curing factories (Thevenot *et al.* 2005b).

Different molecular techniques (amplified fragment length polymorphism, PFGE, PCR and ribotyping) have shown that the genotypes of *L. monocytogenes* strains collected from pork meat processing environments are extremely diverse (Chasseignaux *et al.* 2001; Lundén *et al.* 2003a,b; Martinez *et al.* 2003; Thevenot *et al.* 2006). High genotype diversity among strains collected both from surfaces during processing and processed products may indicate either a continuous source of inoculation by raw material or the persistence of several strains in spite of the cleaning and disinfection operations. Indeed, both Lundén *et al.* (2003a, 2003b) and Thevenot *et al.* (2006) found both transient (sporadic) and resident (persistent) *L. monocytogenes* strains in meat processing environments.

Listeria monocytogenes may become established in the processing environment and survive for long period of time. Giovannacci *et al.* (1999) and Lundén *et al.* (2002) pointed out the persistence of *L. monocytogenes* strains over a year for two pork processing plants and for 3 years in a grinder respectively.

Listeria monocytogenes adheres to the inert surfaces encountered in the food-processing environments, although there are differences in both the extent and adsorption rate according to the type of surface, pretreatment, environmental conditions and bacterial serotypes (AFSSA 2000). Biofilms develop as a result of both the adsorption and adherence of free-floating cells, and the continued growth of cells in the biofilm matrix. *Listeria monocytogenes* can incorporate into a biofilm, although Chae and Schraft (2000) showed that not all *L. monocytogenes* strains had the same ability to evolve into a mature biofilm. These authors noted that the multiplication rate of free-floating *L. monocytogenes* cells *vs* biofilm cell forms was different and suggested that growth behaviour differed according to the support media. Kalmokoff *et al.* (2001) found little differences in the adsorption rates of different strains but significant differences in their adherence and ability to form a biofilm. The highly adherent strains produced fibrils which were generally absent in low-adherence strains (Vatanyoopaisatn *et al.* 2000). Norwood and Gilmour (2000) examined 111 *L. monocytogenes* strains and found that serotype 1/2c adhered significantly more to stainless steel than other serotypes over a 24-h period. Lundén *et al.* (2000) also noted differences in the short-term adsorption of resident and nonresident strains onto a stainless steel model surface, with strains within the 1/2c serotype demonstrating the highest degree of adsorption.

A study led by Autio *et al.* (2003) on *L. monocytogenes* strains recovered in 11 food-processing plants showed that certain genotypes were better able to cause resident (persistent) contamination of food facilities than other genotypes. These authors hypothesized that resident strains adapt to food-processing facilities via natural selection. However, Lundén *et al.* (2003a, 2003b) observed

several *L. monocytogenes* strains with similar PFGE types which were classified as persistent in one factory but not another, emphasizing the complex nature of persistent and nonpersistent contamination. Lundén *et al.* (2003a, 2003b) did suggest that with additional samples been collected, some of the nonpersistent *L. monocytogenes* strains might have been recovered more frequently, and thus have been categorized as persistent.

Processed meat products

Processed meat products may be contaminated by *L. monocytogenes* at several stages: either the raw ingredients are contaminated and the manufacturing process is insufficient to sterilize the product or by contact with contaminated unprocessed raw materials, unclean surfaces or people (Samelis *et al.* 1998; Chasseignaux *et al.* 2001). The latter routes are well recognized (Reij and De Aantrekker 2004). Poor personnel hygiene, including simple procedures such as hand washing, has been identified as a causative mode of transmission of the pathogens (AFSSA 2000). Cross-contamination may occur at any stage between the meat processing plant to the final consumer in the home (Reij and De Aantrekker 2004). Contamination in the home often occurs when the same cooking utensils such as cutting boards are used on raw contaminated foods and then sterile food (either cooked or ready-to-eat processed) (AFSSA 2000).

Till now, there has been sporadic information on the occurrence of *L. monocytogenes* in processed pork meat products. First, because contamination may occur after manufacturing, in the home kitchen (AFSSA 2000). Secondly, many meat products are speciality items which are region or country specific.

Nevertheless, studies have documented the prevalence of *L. monocytogenes* processed raw pork meat products. In Japan, Inoue *et al.* (2000) observed that 20·6% of ready-to-cook minced pork samples were contaminated with essentially 1/2a and 1/2b serotypes. In the USA, 22·9% of the ground pork meat products and sausages sampled by Duffy *et al.* (2001) were contaminated with the pathogen. Additionally, *L. monocytogenes* was found in 10·6% and 10% of dried raw sausages in Chile (Cordano and Rocourt 2001) and France (Thevenot *et al.* 2005b) respectively.

A survey of cooked pork meat products by the French hygiene control services (DGCCRF 1996) between 1993 and 1996 showed that 16% of tongue, 9% of 'pâté', 11·7% of 'rillettes', 6·02% of salami and 13·1% of hams sampled were contaminated by *L. monocytogenes*; 1%, 1% 1·7%, 0·2% and 1·1% of the contaminated products, respectively, had more than 10 CFU g^{-1} of *L. monocytogenes*.

As with raw pork meat, serotypes 1/2a, 1/2c and 1/2b strains are most common in processed pork products (Jay 1996), while serotype 4b is rare (Greenwood *et al.* 1991; Hayes *et al.* 1991). However, in our recent study (Thevenot *et al.* 2006), we isolated several 4b strains in two different French pork meat curing factories that had the same PFGE types as the serotype 4b reference strain (CIP 78·38). An additional 45 strains (collected mostly from one factory) shared nearly identical PFGE profiles with a strain of *L. monocytogenes* isolated from a human clinical case of listeriosis (CIP 105550). The similitude between profiles suggests that these strains are closely related. Martinez *et al.* (2003) also found the same PFGE types between strains isolated from a human listeriosis case and a chicken processing company. The presence of 4b strains in the meat processing industry may result in sporadic cases or an outbreak of listeriosis, particularly if foodstuffs are consumed by persons in the at-risk population.

Meat processing environment and meat adapted clones

The presence of strains sharing identical pulsotypes in raw meat, meat processing environment and products is evidence that certain clones have adapted to meat processing plants and processed products (Thevenot *et al.* 2006). Chasseignaux *et al.* (2001) observed the same profiles in several plants which were from different geographical location. Autio *et al.* (2002) also observed that certain strains were recovered repeatedly in the finished pork-meat products of several unrelated factories, indicating that strains are not always plant specific. Certain strains may be more widely distributed in nature, and thus be more easily (re)introduced into processing plants via raw material. What is more, some clones may be better adapted both to raw meat and to meat processing environments and finished products.

The war between *L. monocytogenes* and the pork meat processing industry

Listeria monocytogenes has been isolated from an extensive range of pork meat products (AFSSA 2000; FICT 2002). The growth and survival of a micro-organism is dependent on its ability to overcome environmental hurdles encountered during the manufacturing and conservation process, such as temperature, pH, water activity, metal ions and nutrient availability. Most bacteria, including *L. monocytogenes*, are generally resistant to small changes in a specific environmental parameter, but severe or multiple changes stimulate complex stress responses that are generally directed to survival instead of growth (Booth 1998). The various environmental obstacles that are utilized by the food-processing industry are discussed below.

Food-mediated hurdles to *L. monocytogenes* growth

Physical and chemical hurdles

Temperature. Temperature is the most important hurdle encountered by *L. monocytogenes*. It grows in a temperature range of +0·4 to 45°C, while the optimum growth temperature lies between 30 and 37°C (AFSSA 2000). *Listeria monocytogenes* grows in refrigerated foods (Augustin 1999), and this is of major importance for risk assessment of foodstuffs: even when initial contamination is low, the organism can multiply during refrigeration and reach levels up to 100 CFU g^{-1} (AFSSA 2000). Besides, the temperature home refrigerators is often closer to 9°C than 4°C (Sergelidis *et al.* 1997), which also favours *L. monocytogenes* growth.

Fleming *et al.* (1985) showed that the thermotolerance of *L. monocytogenes* is higher than many other nonspore-forming foodborne pathogens. Its thermotolerance may increase after exposure to a variety of environmental stress conditions including heating at sublethal temperatures or osmotic and acidic shocks. Linton *et al.* (1992) observed that the effect of a heat shock on *L. monocytogenes* was highly dependent on temperature and treatment duration: higher temperatures and longer treatments increased resistance. Factors that are intrinsic to the underlying foodstuff also affect influence thermotolerance (AFSSA 2000). Pork meat products which are cooked at very high temperatures are quite likely free of live *L. monocytogenes* cells. However, products such as 'rillettes', which are cooked at fairly low temperatures (50–60°C) may not be *L. monocytogenes* free even after if cooking times are long (FICT 2002).

At present, there is little evidence that certain strains of *L. monocytogenes* are more thermotolerant than others (De Jesus and Whiting 2003).

pH. Listeria monocytogenes is reported to exhibit viability at pH levels between 4·6 and 9·6 with an optimum at 7·1 (AFSSA 2000).

Listeria monocytogenes may be subject to low pH during processing of fermented specialties such as cured dried sausages (saucisson and chorizo) (Foegeding *et al.* 1992; Thevenot *et al.* 2005a). It also survives highly acidic pH characteristic of human stomachs and macrophage phagosomes (Cotter and Hill 2003). This property can be considered as a virulence factor as many other bacteria are not able to survive such conditions. The glutamate decarboxylases (GAD) system, which modulates intracellular pH in certain Gram-positive cells (Cotter and Hill 2003), is thought to be the key mechanism by which *L. monocytogenes* maintains pH homeostasis in acid environments. Strains which display reduced GAD activity are more sensitive to gastric fluid, and furthermore, GAD activity is significantly correlated with acid tolerance in this organism (Cotter and Hill 2003). The GAD system plays a role in the acid tolerance during both logarithmic and stationary growth phases and is required for the induction of an optimal acid tolerance response (Cotter and Hill 2003). However, Vialette *et al.* (2003) tested the pH sensitivity of *L. monocytogenes* strains which were isolated from meat products and food-processing environments, and found that bacteria were usually more sensitive in the exponential growth phase than in the stationary phase.

The usual pH of meats (5·0–5·5) can induce an acid tolerance response in *L. monocytogenes*, which in turn decreases the organisms sensitivity to even lower pH (Faleiro *et al.* 2003). Several authors have noted that acid adaptation can induce cross-protection against several other damaging factors including osmotic stress (O'Driscoll *et al.* 1996; Vasseur *et al.* 2001), and Faleiro *et al.* (2003) have indicated that such cross-stress protection is strain dependent. The molecular processes involved have not yet been identified. Finally, sublethal stresses other than acid, i.e. osmotic, heat and low temperatures, do not appear to affect the acid resistance of this bacterial species (Koutsoumanis *et al.* 2003).

NaCl. Listeria monocytogenes may also encounter high salt concentrations in dried sausages, raw dried ham or cooked meats (Foegeding *et al.* 1992; Dabin and Jussiaux 1994). This organism is able to grow at high salt concentrations (up to 10% NaCl), although salt tolerance varies with pH and temperature (AFSSA 2000). For example, *L. monocytogenes* survives in cheese brining systems (Faleiro *et al.* 2003). Strains of *L. monocytogenes* that have adapted to acid environment may become cross-protected against several damaging factors including osmotic stress (O'Driscoll *et al.* 1996; Vasseur *et al.* 1999). However, osmo-adaptation does not induce strong cross-protection against other stress factors (Lou and Yousef 1997).

A_w. Water activity is strongly linked with the pH and salt content of the meat products. For example, during the drying stage of cured raw sausage products (e.g. saucisson and chorizo), the water holding capacity decreases, as does the pH, which approaches the isoelectric point (Tyopponen *et al.* 2003). The addition of salt to the sausage mix limits water activity, thereby inhibiting the growth of many spoilage and pathogenic bacteria including *L. monocytogenes* (Lücke 1985). After drying, the water activity is <0·90, which also inhibits bacterial growth (Tyopponen *et al.* 2003). Unfortunately, some manufacturers tend to reduce drying times in order to increase profitability. Insufficiently dried products may have water activity levels which permit the growth of

L. monocytogenes ($A_w = 0{\cdot}92$) (AFSSA 2000), and could be associated with a higher risk of listeriosis.

Other factors. Listeria monocytogenes can be subjected to other potential stress in meat products. Microbial growth in certain meat products may consume all the available oxygen in the matrix. The growth of aerobic spoilage bacteria will be affected, but not that of *L. monocytogenes* because it is an aerobic/anaerobic organism. Encinas *et al.* (1999) found significant differences in numbers of *Listeria* between chorizos formulated with hot and mild paprika. Other spices, such as pepper, cardamom and garlic may also have antioxidative and antimicrobial properties (Tyopponen *et al.* 2003). Finally, smoke, which contains phenols, carbonyls and different organic acids, may inhibit different bacteria on the surfaces of cured pork products. According to Encinas *et al.* (1999), the manufacturing process and smoking significantly reduced *Listeria* counts in sausages and ham.

Microflora

Natural microflora. Three groups of different bacteria are present in raw pork meat. The first regroups 'useful' organisms such as *Micrococcus*, *Staphylococcus* (levels ranging from 10^4 to 10^5 CFU g^{-1}), lactic acid bacteria (LAB) (from 10^3 to 10^4 CFU g^{-1}); and yeast (from 10^3 to 10^4 CFU g^{-1}) (Samelis *et al.* 1998). LAB produce lactic acid, resulting in low pH values (Leistner 1995). This can be beneficial in raw meat products. For example, certain raw sausage specialties are obtained with natural LAB contamination (Dabin and Jussiaux 1994). Manufacturers may add LAB starters and glucose (which is an energy source for LAB) to the meat matrix enhance and control the drop in pH (Ross *et al.* 2002). *Micrococcus*, *Staphylococcus* and yeast produce aromatic compounds which contribute to the flavour of cured products (Montel *et al.* 1996).

The second group is composed of spoilage bacteria, such as hetero-fermentative LAB, pseudomonads and enterobacteria (Samelis *et al.* 1998). Their presence in raw meats can lead to spoilage during the manufacturing process (Dabin and Jussiaux 1994). The last group is represented by pathogenic bacteria such as *Campylobacter*, *Clostridium botulinum* and *Clostridium perfringens*, *Salmonella* spp. and *L. monocytogenes* (Dabin and Jussiaux 1994).

Microbial stability of cooked, fermented or raw meat products occurs when spoilage and pathogenic bacteria are reduced/removed and when useful micro-organisms are utilized properly (Leistner 2000; Ross *et al.* 2002).

Starters. Modern food processing employs a range of technologies to ensure that the bacteriological quality of food is maintained from the time of manufacture to the time of consumption (Ross *et al.* 2002). Food fermentation involves the oxidation of carbohydrates into various metabolites (principally organic acids, alcohols and carbon dioxide) which limit the growth of spoilage and/or pathogenic flora. A number of desirable compounds are also synthesized, which affect the flavour of foods (diacetyl and acetaldehyde) or their nutritive value (vitamins, antioxidants and bioactive peptides) (Ross *et al.* 2002; Tyopponen *et al.* 2003). Food fermentation has been practiced for millennia, and as a result there is a tremendous variety of fermented meat specialties worldwide (Ross *et al.* 2002).

LAB are primarily responsible for many of the microbial transformations found in the more common fermented meat products (Ross *et al.* 2002). LAB produce high amounts of lactic acid that in turn lower the pH value (5·0) of the meat matrix, effectively eliminating or inhibiting spoilage and pathogen contaminants (Tyopponen *et al.* 2003).

LAB may also inhibit the growth of *L. monocytogenes*: Encinas *et al.* (1999) isolated *L. monocytogenes* from chorizo manufactured without lactic starters, but not in chorizos with starters. Kang and Fung (2000) confirmed the inhibitory effect of LAB starters. They counted *L. monocytogenes* in salami manufactured with and without LAB starters; the level was 2·2 $\log_{10}$ higher in the latter. Furthermore, meat processing lines are continuously contaminated through incoming raw products, yet lines which utilize the fermentation process do not remain persistently contaminating (Lundén *et al.* 2003a,b). This suggests that competing flora may prevent *L. monocytogenes* from becoming established on processing surfaces. Finally, Mahoney and Henriksson (2003) showed that the ability of *L. monocytogenes* to cause foodborne listeriosis is dependent on the nature of the food matrix; a traditional meat starter culture inhibits growth of the pathogen during passage through the gastrointestinal tract.

Many bacteria of the LAB family produce protein inhibitors referred to collectively as bacteriocins (Ross *et al.* 2002). In general, bacteriocins either depolarize the target cell membrane or inhibit cell wall synthesis. They can be divided into three main groups: the lantibiotic family [small bacteriocins composed of one or two peptides of *c.* 3 kDa (Nisin; Sigma-Aldrich, St Quentin Fallavier, France)], small nonmodified peptides (<30 kDa) and large heat-labile proteins (>30 kDa). De Martinis and Franco (1998) found that *Lactobacillus sake* produces a bacteriocin-like substance that can be used to inhibit the growth of *L. monocytogenes* in a Brazilian sausage speciality. Foegeding *et al.* (1992) further showed that pediocin increased food safety during both the fermentation and drying portions of sausage manufacturing.

Cleaning and disinfecting products and procedures

Typical cleaning and disinfecting processes in food-processing environments involve a series of steps, each with a specific purpose (Tamplin 1980). A pre-rinse is performed using water to remove gross, loose material, followed by cleaning with suitable detergents (often alkaline) in order to remove residual substances. The detergent is then rinsed off, antimicrobial chemicals are applied in order to sanitize surfaces, and a final post-rinse with water removes these chemicals. The cleaning step is important because it removes organic matter from processing surfaces (Chasseignaux *et al.* 2001; Thevenot *et al.* 2005b). Besides harbouring bacterium, organic matter can reduce the efficiency of disinfectants [such as quaternary ammonium compounds (QACs)] or prevent disinfectants from reaching bacterium at the recommended concentrations (Gibson *et al.* 1999). Furthermore, Taormina and Beuchat (2002) showed that *L. monocytogenes* strains that were exposed to cleaning solutions that which did not affect cell viability were more sensitive to subsequent treatment with sanitizing chemicals.

Several studies have indicated that adherent micro-organisms may be much more resistant to sanitizing compounds than free-floating cells (Somers *et al.* 1994; Fatemi and Frank 1999). Furthermore, antimicrobial agents that are highly effective in liquid media may be less active in more complex foodstuffs (Norwood and Gilmour 2000). Multispecies biofilms, which combine the effects of bacterial shielding and the production of extracellular polymeric substances, also increase bacterial resistance to antimicrobial agents (Costerton *et al.* 1995). Finally, food and food-processing equipment, together with the highly buffered system typical of meats, offer micro-organisms a complex environment that suppresses the efficacy of antimicrobial agents (Korber *et al.* 2002).

During the last decade, numerous studies have focussed on sanitizing (disinfecting) products and their effects on *L. monocytogenes*. In a survey by Holah *et al.* (2002), QACs were markedly the most commonly used disinfectant in the food industry. This is primarily due to their biocide property (QACs cause generalized membrane damage and inactive cellular enzymes) combined with their excellent nontainting, nontoxic and noncorrosive nature (on human skin and hard surfaces), which makes them the ideal choice for manual application in the food industry. Still, a minimum inhibition concentration has increased the resistance of *L. monocytogenes* to QACs in strains isolated from a range of environmental, food, animal and clinical sources (Mereghetti *et al.* 2000). Mechanisms of such a resistance remain unclear. Lemaître *et al.* (1998) found extrachromosomal DNA in all resistant strains tested and concluded that this resistance was plasmid mediated. On the other hand, Mereghetti *et al.* (2000) observed resistant *L. monocytogenes* strains with and without plasmids. Romanova *et al.* (2002) confirmed the latter hypothesis: indeed all isolates tested in their study contained the *mdrL* gene, which encodes an efflux pump that confers resistance to QACs and is both chromosome- and plasmid borne. Finally, Holah *et al.* (2002) suggested that persistence was not due to resistance towards disinfectants but to physical adaptation mechanisms (surface attachment, biofilm formation, reduced growth rate and quiescence).

Several studies underlined the importance of utilizing proper procedures and concentrations during the sanitizing process. For example, the concentrations of sodium hypochlorite recommended by suppliers are more than sufficient to eliminate free-floating *L. monocytogenes* but are ineffective on bacteria in multispecies biofilms cultured on a stainless steel surface (Norwood and Gilmour 2000). There is also a significant difference in the effectiveness of sanitizing solutions on *L. monocytogenes* when applied to stainless steel or conveyor belt surfaces (Bremer *et al.* 2002). This study suggested that the decreased effectiveness of chlorine on the conveyor belt surface was due to limited penetration of the disinfectant: bacteria were relatively protected by the fabric weave. The authors concluded that during the sanitizing process, little consideration is given to the physical properties of the material being sanitized.

Authors have also tested different procedures to improve sanitizing processes. For example, Vasseur *et al.* (2001) tested combinations of chemicals that could decrease or eliminate *L. monocytogenes* surviving in food-processing plants. Chemicals that induced pH shocks were more efficient when used in combination with other parameters. An acid treatment (pH = 5·4) followed by an alkaline treatment (pH = 10·5) was not very effective against *L. monocytogenes*, whereas the opposite combination led to a three-log reduction of the bacterial population. Whatever the bacterial species, the most efficient treatments were combinations of alkaline, osmotic and biocide shocks (Vasseur *et al.* 2001).

Mereghetti *et al.* (2000) suggests that the rotation of disinfectants will prevent the development of resistant strains. However, Lundén *et al.* (2003a, 2003b) demonstrated that rotation of disinfecting agent with differing mechanisms of action in food-processing plants may not have the desired effects because of disinfectant cross-adaptation. Rotation of disinfectants may nevertheless lengthen the adaptation periods.

Finally, Holah *et al.* (2002) observed an increasing tendency for disinfectants to be left on surfaces and not rinsed off prior to recommencing production. Manufacturers claim that residual disinfectants will prevent subsequent surface microbial development, although low, sublethal concentrations may in fact enhance resistant.

In most food-processing environments and more precisely in pork meat industries, the daily use of sanitizers at correct concentrations preceded by detergent-aided cleaning, will remove all adhering organisms or biofilms (Norwood and Gilmour 2000).

Food processing lines

Food processing equipment plays an important part in sustaining contamination and in contaminating processed foodstuffs (Lundén *et al.* 2002; Reij and De Aantrekker 2004). Factories with complex processing lines are more susceptible to persistent *L. monocytogenes* contamination (Thevenot *et al.* 2005b), and furthermore, it is difficult to eradicate *L. monocytogenes* from contaminated processing lines and equipment (Miettinen *et al.* 1999; Autio *et al.* 2003).

Mechanical cleaning is the most effective way to detach adhered cells and biofilms (Gibson *et al.* 1999). However, poorly designed equipment will have small spaces and narrow openings that are difficult or impossible to reach, resulting in poor mechanical cleaning. It is from these locations that the main danger of cross-contamination of pathogens from mature biofilm exists (Miettinen *et al.* 1999; Autio *et al.* 2003). Even sanitizers may not reach the site of contamination at effective concentrations when applied according to the manufacturer's recommendations. It may well be necessary to use sanitizer at much higher concentrations in these particular areas of the food-processing environment.

Attention must be paid to a correct design of equipment and helpful recommendations and guidelines for the validation of equipment cleanability have been published (European Hygienic Equipment Design Group (EHEDG) 1997; Reij and De Aantrekker 2004). Finally, compartmentalization of the processing line and disassembly processing machines should help control *L. monocytogenes* contamination in meat and poultry processing plants (Lundén *et al.* 2003a,b).

In summary, cleaning and disinfecting procedures for food-processing machines combine mechanical energy and chemical products. When these procedures are adequately applied, both persistent and nonpersistent *L. monocytogenes* strains can be eliminated (Reij and De Aantrekker 2004).

Conclusion

Listeria monocytogenes poses a microbiological risk in processed foods containing pork meats. The bacterium is able to adapt to hurdles encountered during the manufacturing and conservation process. In many European countries, a concentration of up to 100 CFU g^{-1} of food is tolerated in processed foodstuffs. The production of safe foods containing pork meat is contingent on proper hygiene at all levels of the food chain.

Suppliers of raw pork products must abide by strict microbiological standards, thus limiting initial contamination of processing lines and food products with *L. monocytogenes*. Even so the contamination of processing lines is unavoidable at present. Therefore, special care must be taken to ensure that cleaning and disinfecting procedures are applied correctly. General preventive measures such as Good Hygiene Practices and Good Manufacturing Practices will help limit the risk of spread food-borne pathogens, including *L. monocytogenes*. Hazard Analysis Critical Control Point techniques should be applied to each food-processing plant in order to identify and control sources of *L. monocytogenes* contamination and dissemination. End product control, challenge testing and *L. monocytogenes* predictive growth models can all be used to ensure that the end product is safe. An alternative approach could be to group food products according to intrinsic and extrinsic parameters, which limit microbial growth. In the case of *L. monocytogenes*, the main factors are pH, A_w, composition of the food product, natural antimicrobial components, biological structures, temperature, atmosphere, and competitive flora. It is a scientific sound and interesting option to group foods based on the set of hurdles encountered during manufacturing process in order to establish their stability towards *L. monocytogenes* and determine their status: safe or with potential danger for persons at risk. Finally, the consumers must scrupulously respect the product's shelf life and the cold chain.

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References

AFSSA (2000) *Rapport de la Commission D'étude des Risques liès à Listeria monocytogenes*. Available at: http://www.afssa.fr (accessed 9 May 2006).

Augustin, J.C. (1999) Modélisation de la dynamique de croissance des populations de *Listeria monocytogenes* dans les aliments. Thèse de Doctorat, Lyon: Université Lyon I.

Autio, T., Sateri, T., Fredriksson-Ahoma, M., Rahko, M., Lunden, J. and Korkeala, H. (2000) *Listeria monocytogenes* contamination pattern in pig slaughterhouses. *J Food Prot* **63**, 1438–1442.

Autio, T., Lunden, J., Fredriksson-Ahomaa, M., Bjorkroth, J., Sjoberg, A.M. and Korkeala, H. (2002) Similar *Listeria monocytogenes* pulsotypes detected in several foods originating from different sources. *Int J Food Microbiol* **77**, 83–90.

Autio, T., Keto-Timonen, R., Bjorkroth, J. and Korkeala, H. (2003) Characterization of persistent and sporadic *Listeria monocytogenes* strains by pulsed-field gel electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP). *Syst Appl Microbiol* **26**, 539–545.

Booth, I. (1998) The bacteria strike back. *Biochemistria* **5**, 8–11.

Bremer, P.J., Monk, I. and Butler, R. (2002) Inactivation of *Listeria monocytogenes/Flaviobacterium* spp. biofilms using chlorine: impact of substrate, pH, time and concentration. *Lett Appl Microbiol* **35**, 321–325.

Bunčić, S., Paunovic, L. and Radisic, D. (1991) The fate of *Listeria monocytogenes* in fermented sausages and in vacuum-packaged frankfurthers. *J Food Prot* **54**, 413–417.

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

Chasseignaux, E., Toquin, M.T., Ragimbeau, C., Salvat, G., Colin, P. and Ermel, G. (2001) Molecular epidemiology of *Listeria monocytogenes* isolates collected from the environment, raw meat and raw products in two poultry- and pork processing plants. *J Appl Microbiol* **91**, 888–899.

Chasseignaux, E., Gerault, P., Toquin, M.T., Salvat, G., Colin, P. and Ermel, G. (2002) Ecology of *Listeria monocytogenes* in the environment of raw poultry meat and raw meat processing plants. *FEMS Microbiol Lett* **210**, 271–275.

Cordano, A.M. and Rocourt, J. (2001) Occurrence of *Listeria monocytogenes* in food in Chile. *Int J Food Microbiol* **70**, 175–178.

Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R. and Lappin-Scott, H.M. (1995) Microbial biofilms. *Annu Rev Microbiol* **49**, 711–745.

Cotter, P.D. and Hill, C. (2003) Surviving the acid test: responses of gram positive bacteria to low pH. *Microbiol Mol Biol Rev* **67**, 429–453.

Dabin, E. and Jussiaux, R. (1994) Modifications des saucissons au cours de l'étuvage et du sèchage. In *Le Saucisson Sec* ed. Frentz, J.C. pp. 136–138. Paris: Erti.

De Jesus, A.J. and Whiting, R.C. (2003) Thermal inactivation, growth, and survival studies of *Listeria monocytogenes* strains belonging to three distinct genotypic lineages. *J Food Prot* **9**, 1611–1617.

De Martinis, E.C.P. and Franco, B.D.G.M. (1998) Inhibition of *Listeria monocytogenes* in a pork product by *Lactobacillus sake* strain. *Int J Food Microbiol* **42**, 119–126.

DGCCRF (1996) *Surveillance des Produits de Charcuterie de 1993 à 1996*. Available at: http://www.finances.gouv.fr/DGCCRF (accessed 9 May 2006).

Duffy, E.A., Belk, K.E., Sofos, J.N., Bellinger, G.R., Pape, A. and Smith, G.C. (2001) Extent of microbial contamination in United States pork retail products. *J Food Prot* **64**, 172–178.

European Hygienic Equipment Design Group (EHEDG) (1997) EHEDG guildness and test methods. *Trends Food Sci Technol* **8**, 1–90.

Encinas, J.P., Sanz, J.J., García-López, M.L. and Otero, A. (1999) Behaviour of *Listeria* spp. in naturally contaminated chorizo (Spanish fermented sausage). *Int J Food Microbiol* **46**, 167–171.

Faleiro, M.L., Andrew, P.W. and Power, D. (2003) Stress response of *Listeria monocytogenes* isolated from cheese and other foods. *Int J Food Microbiol* **84**, 207–216.

Fatemi, P. and Frank, J.P. (1999) Inactivation of *Listeria monocytogenes/Pseudomonas* biofilms by peracid sanitizers. *J Food Prot* **62**, 761–765.

Felon, D.R., Wilson, J. and Donachie, W. (1996) The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. *J Appl Microbiol* **81**, 641–650.

FICT (2002) *Production Industrielle*. Available at: http://www.fict.fr (accessed 9 May 2006).

Fleming, D.W., Cochi, S.L., McDonald, K.L., Brondum, J., Hayes, P.S., Plikaytis, B.D., Holmes, M.B., Audurier, A. *et al.* (1985) Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *New English J Med* **3120**, 404–407.

Foegeding, P.M., Thomas, A.B., Pilkington, D.H. and Klaenhammer, T.R. (1992) Enhanced control of *Listeria monocytogenes* by *in situ*-produced pediocin during dry fermented sausage production. *Appl Environ Microbiol* **58**, 884–890.

Gibson, H., Taylor, J.H., Hall, K.E. and Holah, J.T. (1999) Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. *J Appl Microbiol* **87**, 41–48.

Giovannacci, I., Ragimbeau, C., Queguiner, S., Salvat, G., Vendeuvre, J.L., Carlier, V. and Ermel, G. (1999) *Listeria monocytogenes* in pork slaughtering and cutting plants. Use of RAPD, PFGE, and PCR-REA for tracing and molecular epidemiology. *Int J Food Microbiol* **53**, 127–140.

Goulet, C., Rocourt, J., Rebiere, I., Jacquet, C., Moyse, C., Dehaumont, P., Salvat, G. and Veit, P. (1998) Listeriosis outbreak associated with the consumption of rillettes in France in 1983. *J Infect Dis* **177**, 155–160.

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

Hayes, P.S., Graves, L.M., Ajello, G.W., Swaminathan, B., Weaver, R.E., Wenger, J.D., Schuchat, A. and Broome, C.V. (1991) Comparison of cold-enrichment and U.S. Department of Agriculture methods for isolating *Listeria* spp. from naturally contaminated foods. *Appl Environ Microbiol* **57**, 2109–2113.

Hof, H. and Rocourt, J. (1992) Is any strain isolated detected in food a health risk? *Int J Food Microbiol* **16**, 683–692.

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

Inoue, S., Nakama, A., Yoshiteru, A., Kokubo, Y., Maruyama, T., Saito, A., Yoshida, T., Terao, M. *et al.* (2000) Prevalence and contamination levels of *Listeria monocytogenes* in retal foods in Japan. *Int J Food Microbiol* **59**, 73–77.

Jacquet, C.B., Catimel, R., Brosch, R., Buchreiser, C., Dehaumont, P., Goulet, V., Lepoutre, A., Veit, P. *et al.* (1995) Investigations related to the epidemic strain involved in French listeriosis outbreak in 1992. *Appl Environ Microbiol* **61**, 2242–2246.

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

Kalmokoff, M.L., Austin, J.W., Wan, X.D., Sabders, G., Banerjee, S. and Farber, J.M. (2001) Adsorption, attachment and biofilm formation among isolates of *Listeria monocytogenes* using model conditions. *J Appl Microbiol* **91**, 725–734.

Kang, D.H. and Fung, Y.C., (2000) Stimulation of starter culture for further reduction of foodborne pathogens during salami fermentation. *J Food Prot* **63**, 1492–1495.

Kanuganti, S.R., Wesley, I.V., Reddy, P.G., McKean, J. and Hurd, H.S. (2002) Detection of *Listeria monocytogenes* in pigs and pork. *J Food Prot* **65**, 1470–1474.

Kathariou, S. (2002) *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. *J Food Prot* **65**, 1811–1829.

Korber, D.R., Greer, G.G., Wolfaardt, G.M. and Kohlman, S. (2002) Efficacy enhancement of trisodium phosphate against spoilage and pathogenic bacteria in model biofilms and on adipose tissue. *J Food Prot* **65**, 627–635.

Koutsoumanis, K.P., Kendall, P.A. and Sofos, J.N. (2003) Effect of food processing-related stresses on acid tolerance of *Listeria monocytogenes*. *Appl Environ Microbiol* **69**, 7514–7516.

Leistner, L. (1995) Stable and safe fermented sausages world-wide. In *Fermented Meats* ed. Campbell-Platt, G., Cook, P.E. pp. 160–175. UK: Blackie Academic and Professional.

Leistner, L. (2000) Basic aspects of food preservation by hurdle technology. *Int J Food Microbiol* **55**, 181–186.

Lemaître, J.P., Echannaoui, H., Michaut, G., Davies, C. and Rousser, A. (1998) Plasmid-mediated resistance to antimicrobial agents among Listeriae. *J Food Prot* **61**, 1459–1464.

Linton, R.H., Wesber, J.B., Pierson, M.D., Bishop, J.R. and Hackney, C.R. (1992) The effect of sublethal heat shock and growth atmosphere on the heat resistance of *Listeria monocytogenes* Scott A. *J Food Prot* **55**, 84–87.

Loncarevic, S., Danielsson-Tham, M.L., Martensson, L., Ringner, A., Runehagen, A. and Tham, W. (1997) A case of listeriosis in Sweden. *Lett Appl Microbiol* **24**, 65–68.

Lou, Y. and Yousef, A.E. (1997) Adaptation to sublethal environmental stresses protects *Listeria monocytogenes* against lethal preservation factors. *Appl Environ Microbiol* **63**, 1252–1255.

Lücke, F. (1985) Fermented sausages. In *Microbiology of Fermented Foods*, Vol. 2 ed. Wood, B. pp. 41–84. London: Elsevier.

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

Lundén, J.M., Autio, T.J. and Korkeala, H.J. (2002) Transfer of persistent *Listeria monocytogenes* contamination between food-processing plants associated with a dicing machine. *J Food Prot* **65**, 1129–1133.

Lundén, J., Autio, T., Annukka, A., Hellstrom, S. and Korkeala, H. (2003a) Adaptative and cross-adaptative responses of persistent and no-persistent *Listeria monocytogenes* strains to disinfectants. *Int J Food Microbiol* **82**, 265–272.

Lundén, J., Autio, T.J., Sjoberg, A.M. and Kokeala, H.J. (2003b) Persistent and nonpersistent *Listeria monocytogenes* contamination in meat and poultry processing plants. *J Food Prot* **66**, 2062–2069.

Mahoney, M. and Henriksson, A. (2003) The effect of processed and meat starter cultures on gastrointestinal colonization and virulence of *Listeria monocytogenes* in mice. *Int J Food Microbiol* **84**, 255–261.

Martinez, I., Rorvik, L.M., Brox, V., Lassen, J., Seppola, M., Gram, L. and Fonnesbech-Vogel, B. (2003) Genetic variability among isolates of *Listeria monocytogenes* from food products, clinical samples and processing environments, estimated by RAPD typing. *Int J Food Microbiol* **84**, 285–297.

Mereghetti, L., Quentin, R., Marquet-Van Der Mee, N. and Audurier, A. (2000) Low sensitivity of *Listeria monocytogenes* to quaternary ammonium compounds. *Appl Environ Microbiol* **66**, 5083–5086.

Miettinen, L., Björkroth, K.J. and Korkeala, H. (1999) Characterisation of *Listeria monocytogenes* from an ice-cream plant by serotyping and pulsed-field gel electrophoresis. *Int J Food Microbiol* **46**, 187–192.

Montel, M., Reitz, J., Talon, R., Berguade, J. and Rousset-Akrim, S. (1996) Biochemical activities of *Micrococcaceace* and their effect on the aromatic profiles and odours of a dry sausage model. *Food Microbiol* **13**, 229–240.

Nesbakken, T., Kapperud, G. and Caugant, D.A. (1996) Pathways of *Listeria monocytogenes* contamination in the meat processing industry. *Int J Food Microbiol* **31**, 161–171.

Norrung, B., Andersen, J.K. and Schlundt, J. (1999) Incidence and control of *Listeria monocytogenes* in foods in Denmark. *Int J Food Microbiol* **53**, 195–203.

Norwood, D.E. and Gilmour, A. (2000) The growth and resistance to sodium hypochlorite of *Listeria monocytogenes* in a steady-state multispecies biofilm. *J Appl Microbiol* **88**, 512–515.

O'Driscoll, B., Gahan, C.G.M. and Hill, C. (1996) Adaptative acid tolerance response in *Listeria monocytogenes*: isolation of an acid-tolerant mutant which demonstrates increased virulence. *Appl Environ Microbiol* **62**, 1693–1698.

Reij, M. and De Aantrekker, E.D. (2004) Recontamination as a source of pathogens in processed foods. *Int J Food Microbiol* **91**, 1–11.

Risk Assessment Drafting Group (2004) *Risk Assessment in Ready to Eat Foods*. Available at: http://www.fao.org/es/esn (accessed 9 May 2006).

Rocourt, J. and Bille, J. (1997) Foodborne listeriosis. *World Health Stat Q* **50**, 67–73.

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

Ross, R.P., Morgan, S. and Hill, C. (2002) Preservation and fermentation: past, present and future. *Int J Food Microbiol* **79**, 3–16.

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

Samelis, J., Metaxopoulos, J., Vlassi, M. and Pappa, A. (1998) Stability and safety of traditional Greek salami – a microbiological ecology study. *Int J Food Microbiol* **44**, 69–82.

Schlech, W.F., Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haladane, V., Wort, A.J., Hightower, A.W., Johnson, S.E. *et al.* (1983) Epidemic listeriosis evidence for transmission by food. *New English J Med* **308**, 203–206.

Schuchat, A., Swaminathan, B. and Broome, C.V. (1991) Epidemiology of human listeriosis. *Clin Microbiol Rev* **4**, 169–183.

Sergelidis, D., Abrahim, A., Sarimvei, A., Panoulis, C., Karaioannoglou, P. and Genigeorgis, C. (1997) Temperature distribution and prevalence of *Listeria* spp. in domestic, retail and industrial refrigerators in Greece. *Int J Food Microbiol* **34**, 171–177.

Shank, F.R., Elliot, E.L., Wachsmuth, I.K. and Losikoff, M.E. (1996) US position on *Listeria monocytogenes* in foods. *Food Control* **7**, 229–234.

Skovgaard, N. and B. Norrung (1989) The incidence of *Listeria* spp in faeces of Danish pigs and in minced pork meat. *Int J Food Microbiol* **8**, 59–63.

Somers, E.B., Schoemi, J.L. and Wong, A.C.L. (1994) Effect of trisodium phosphate on biofilm and planktonic *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium*. *Int J Food Microbiol* **22**, 269–276.

Tamplin, T.C. (1980) CIP technology, detergents, and sanitizers. In *Hygienic Design and Operation of Food Plant* ed. Jowitt, R. pp. 183–225. Westport, CT, USA: AVI Publishing.

Taormina, P.J. and Beuchat, L.R. (2002) Survival of *Listeria monocytogenes* in commercial food processing equipment cleaning solutions and subsequent sensitivity to sanitizers and heat. *J Appl Microbiol* **92**, 71–80.

Thevenot, D., Delignette-Muller, M.L., Christieans, S. and Vernozy-Rozand, C. (2005a) Fate of *Listeria monocytogenes* in experimentally contaminated French sausages. *Int J Food Microbiol* **101**, 189–200.

Thevenot, D., Delignette-Muller, M.L., Christieans, S. and Vernozy-Rozand, C. (2005b) Prevalence of *Listeria monocytogenes* in 13 dried sausage processing plants and their products. *Int J Food Microbiol* **102**, 85–94.

Thevenot, D., Delignette-Muller, M.L., Denburg, A., Christieans, S. and Vernozy-Rozand, C. (2006) Serological and molecular epidemiology of *Listeria monocytogenes* strains collected in 13 French salting plants and their products. *Int J Food Microbiol* (in press).

Tyopponen, S., Petaja, E. and Mattila-Sandholm, T. (2003) Bioprotectives and probiotics for dry sausages. *Int Food Microbiol* **83**, 233–244.

Van der Elzen, A.M.G. and Snijders, J.M.A. (1993) Critical points in meat production lines regarding the introduction of *Listeria monocytogenes*. *Vet Q* **15**, 143–145.

Vasseur, C., Rigaud, N., Hebraud, M. and Labadie, J. (2001) Combined effects of NaCl, NaOH, and biocides (Monolairin or Lauric acid) on inactivation of *Listeria monocytogenes* and *Pseudomonads* spp. *J Food Prot* **64**, 1442–1445.

Vatanyoopaisatn, S., Nazli, A., Dodd, C.E.R., Rees, C.E.D. and Xaites, W.M. (2000) Effect of flagella on initial attachment of *Listeria monocytogenes* to stainless steel. *Appl Environ Microbiol* **66**, 860–863.

Vialette, M., Pinon, A., Casseignaux, E. and Lange, M., (2003) Growth kinetics comparison of clinical and seafood *Listeria monocytogenes* isolates in acid and osmotic environment. *Int J Food Microbiol* **82**, 121–131.