Food Standards Agency
Project FS101057

Development of an initial report - Reducing the risk of vulnerable groups contracting listeriosis

Literature review - report 1
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Literature review

1. Executive summary

The aim of project FS101057 undertaken on behalf of the Food Standards Agency (FSA) was to draft guidance to reduce the risk of vulnerable groups contracting listeriosis in healthcare settings.

To ensure this guidance was based on sound, reliable evidence one branch of the research examined the body of literature available, taking the findings into consideration when drafting the guidance. This comprehensive literature review (report 1) gathered and summarised the existing, and at the time current body of evidence on the nature and extent of the problem of listeriosis in vulnerable groups.

This research was undertaken in parallel with two other reports upon which the guidance was drafted:

- Investigation into current practices used to control listeriosis in healthcare settings (report 2), via:
    - Site visits
    - A survey
- Examination of previous outbreaks of listeriosis and lessons learned from UK hospitals (report 3).

This literature review included both white and grey literature.

2. Limitations

The limited time available for the review was the main constraint, as the literature on the topic is enormous. We also understand axiological values do influence our interpretation of literature within the review. The professional backgrounds of the authors include research, teaching, local authority enforcement and catering. The majority of information was sourced via electronic databases and search engines, so a considerable amount of the information available is not easily accessed, particularly guidance within food safety management systems. This imbalance is addressed by the two reports produced by STS mentioned above in relation to the *Listeria* guidance project for healthcare organisations.

The researchers were, however, confident these limitations did not adversely affect key issues discussed within the review and believe they have comprehensively covered *Listeria*, *Listeria monocytogenes* and listeriosis in a variety of contextual settings.

3. Methodology

The research methodology considered both quantitative and qualitative data within the critical review. “Quantitative methods are ideal for measuring pervasiveness of

known phenomena and central patterns of association, including inferences of causality. Qualitative (mainly inductive) methods allow for identification of previously unknown processes, explanations of why and how phenomena occur, and the range of their effects (Pasick et al., 2009)". This triangulation (Denzin 1978) of different data sources, researchers, theories and multiple methods provides answers to questions from different perspectives.

Most literature used has been identified through the use of a comprehensive database, Zetoc, and follow-up of relevant articles from reference lists. Zetoc covers academic literature from all disciplines, thus allowing retrieval of literature from different perspectives, and with different areas of focus. Keyword searches were used, which resulted in sometimes very large numbers of papers (Table 1).

Keywords	Additional keywords	Papers identified by Zetoc
Listeria		12,270
Listeria monocytogenes		10,094
	Food	4082
	Food hospital	10
	Hospital	48
	Immunosuppressed	9
	Vulnerable	11
	Transmission	97
	Infective dose	5
	Incidence	201
	Care	31
	Nursing	1
Listeriosis		1216
	Hospital	21
	Food	345
	Care	8
	Nursing	1

Table 1 Numbers of papers identified in the review using different keyword searches of ZETOC.

Using the narrower searches, the titles of papers so identified were read, and those that indicated the paper was useful recorded. Abstracts were then read to further filter the articles to retrieve and read in full. Only one or two papers could not be retrieved. These papers were read in detail to extract useful insights for inclusion in the review.

The critical review on scientific literature was restricted largely to papers published from 2003, as McLauchlin et al (2004) conducted a review as part of FSA project B01020: A microbiological risk assessment of *Listeria monocytogenes* in cooked meat and poultry. Some pre-2003 papers were included, where their content was deemed important to include, for instance where the focus of the 2004 review meant they were not relevant. All papers were published in English, and where incidence of *L. monocytogenes* in foods was the focus of the paper, these were restricted to surveys of foods in the developed world. If a paper involved experimental studies,

e.g. antimicrobial effects of certain ingredients, it could come from any part of the world.

Every source of information should be considered in the exploration of an issue or subject. Therefore, the critical review also examined grey literature because such materials may not be accessible through indexed academic databases and other conventional channels. The authors of this review understand the use of grey literature may raise questions about authenticity and reliability, but these sources of information tend to be recent and original. Peripheral materials reviewed include conference proceedings, non-indexed journals, guidance documents, reports, surveys, news articles and student dissertations. Grey literature is particularly useful in gauging micro and macro perspectives on outbreaks on listeriosis, results from microbiological surveys and recommendations to reduce risk and categorising at-risk groups.

The internet is a major source of grey literature that is accessible to a wide international audience and there are several websites that provide a refined gateway to this type of literature. Examples include:

- MAGiC (Managing Access to Grey Literature Collections)
- GrayLIT Network
- Google Scholar

Most internet searches use Yahoo, Bing or Google. All three search engines were tested and Google UK was selected as the preferred choice. Google (UK) search parameters included all results on the web.

Keywords	Additional keywords	Google results
		1,630,000
Listeria	Guidance	470,000
	*Vulnerable	5,880,000
	Outbreaks	349,000
	Reports	2,360,000
		502,000
Listeria monocytogenes	Guidance	157,000
	*Vulnerable	1,930,000
	Outbreaks	180,000
	Reports	1,850,000
		1,020,000
Listeriosis	Guidance	681,000
	*Vulnerable	20,300,000
	Outbreaks	118,000
	Reports	296,000

Table 2 Google results identified using different keywords. * Indicates FSA as the first result in the Google UK search.

All sources of grey literature reviewed were in English. The main countries for the geographic sources of the literature include EU countries (mainly UK and Ireland),

Scandinavian countries, USA, Canada, Australia and New Zealand. The timeline for the review of grey literature mainly included information from the period 2000 - 2013. As the review progressed the manner in which the information was presented to the reader was also examined. The NHS webpages on *Listeria* are particularly good and include an informative video and allows readers to leave feedback and rate the guidance.

4. Listeria monocytogenes

4.1. Background

L. monocytogenes, as a microorganism, and its associated disease, listeriosis, were first recognised in animals in 1924 (McLauchlin et al, 2004). In the 1980s it became recognised as an opportunistic pathogen (Goh et al, 2014; Aguilar et al, 2013; Lungu et al, 2011; Todd & Nottermans, 2011), due to a rise in human cases across several countries, and evidence for foodborne transmission raised interest (McLauchlin et al, 2004). It continues to be a public health problem now, owing to the organism's unusual growth and survival characteristics and its ability to adhere to food contact surfaces, which contribute to the complexity of eliminating it from the environment (Earnshaw & Lawrence, 1998).

Although the incidence of infection is low, listeriosis has a high mortality rate of 20-25%, and if accompanied by other severe symptoms up to 50% (Aguilar et al, 2013). Hospital acquired infection is not commonly reported, but medical outcomes are often poor, especially amongst particular at-risk groups (Coetzee et al, 2011). This review forms part of the evidence that informs the development of FSA guidance to minimise the risk of at-risk groups contracting listeriosis in hospital and nursing/care home settings.

4.2 Nature and natural habitat of organism

L. monocytogenes is a Gram positive, non-spore forming rod (Angelidis et al, 2013; Munoz et al, 2012; da Silva & Martinis, 2013). It is a facultative anaerobe and is motile due to peritrichous flagella (da Silva & Martinis, 2013). It is catalase positive, beta haemolytic (Elsner et al, 1997), and, being intracellular, grows within host cells (Munoz et al, 2012). *Listeria* spp. are widely distributed (ubiquitous) in nature (McLauchlin et al, 2004; Ding et al, 2013; Angelidis et al, 2013) and extremely adaptable (Grassi et al, 2013), with genetic mechanisms for survival and adaptation, including biofilm formation, quorum sensing and antibiotic resistance (da Silva & Martinis, 2013).

Listeria includes six species, one of which is *L. monocytogenes*. Listeriosis is the disease caused by the genus *Listeria*, with *L. monocytogenes* being the major pathogenic species. Other species are almost non-pathogenic in humans (Hof, 2003). Of more than 3000 human cases in the UK between 1965 and 2002, there were only single figure incidences arising from other species (McLauchlin et al, 2004). *L. monocytogenes* can be differentiated into four serogroups and 13 distinct

serovars. The majority of human cases result from three serovars – 1/2a, 1/2b and 4b (Renier et al, 2011) , with 1/2a more often found in foods, and 4b most frequently associated with human listeriosis (Doorduyn et al, 2006).

The organism has evolved the ability to invade and mobilise within eukaryotic cells. Thus it is an opportunistic pathogen with multiple routes of infection and disease presentations. Owing to this, there is unlikely to be a single infective dose. In addition, responses vary with food compositions which can influence, for instance, survival of the organism in the stomach and upper intestine (McLauchlin et al, 2004). It has been estimated that incidence of exposure to *L. monocytogenes* is five – nine events per person per year (Grif et al, 2003). Asymptomatic carriage is common in healthy individuals, with estimates of 3% (Grif et al, 2003), 1-15% (Allerberger & Wagner, 2009), 2-20% (Elsner et al, 1997), 5–10% in USA, and up to 26% in Germany (Painter & Slutsker, 2007). Those with higher occupational exposure to *L. monocytogenes* tend to have higher carriage rates (Painter & Slutsker, 2007) and diets high in foods prone to contamination with *L. monocytogenes* may also lead to higher rates of asymptomatic carriage (Grif et al, 2003). Prolonged gastrointestinal carriage has been reported (Kruszyna et al, 2008), although other studies has suggested a maximum shedding time of four days in healthy adults, suggesting the organism can be eliminated effectively from the intestine (Grif et al, 2003).

L. monocytogenes can become endemic in food processing environments (McLauchlin et al, 2004), with examples of persistence for up to seven years (Parisi et al, 2013), partly owing to the ability to form biofilms, which are particularly resistant to elimination. As such it is not uncommon for food to be contaminated at low levels (Angelidis et al, 2013). The organism has been isolated from a wide variety foods (e.g., Ding et al, 2013; Goh et al, 2014), although not always implicated in outbreaks. There has been no evidence of seasonality in occurrence of contamination or infection (Grif et al, 2003).

4.3. Distinguishing features

L. monocytogenes is characterised by a number of unusual properties, which contribute to the difficulty in controlling the organism in foods and food production or preparation environments, and allow organisms to grow to levels that will cause illness (Luber et al, 2011). It can survive temperatures of 0°C, pH close to 3.0, a_w near to 0.91, salt content of 20–30%, and undissociated acid concentrations of 0.25mM (Aguilar et al, 2013). It can grow at pH levels as low as 4.4 (or 4.8; Grassi et al, 2013), and salt concentrations up to 14%. It can grow at temperatures between 1 and 45°C, and some strains can grow at even lower temperatures (Kramarenko et al, 2013). The ability to grow to significant numbers at refrigeration temperatures, with sufficient time (Ding et al, 2013) can lead to proliferation of the organism to levels potentially threatening to human health (Spanu et al, 2013). The organism is also able to persist in production environments, including under adverse conditions, in the form of biofilms (Todd & Nottermans, 2011).

Low temperature growth has been a contributing factor in a number of outbreaks. The organism is, however, psychrotolerant rather than psychrophilic, as its optimum temperature for growth is in the range 30–37°C (Chan & Wiedmann, 2009). There are also characteristically prolonged incubation periods (Parisi et al, 2013), which may mean that long-shelf life foods are eaten by many people prior to recall as a result of being implicated in disease.

4.3.1. Low-temperature tolerance

Understanding of the strategies employed by *L. monocytogenes* to survive low temperature is limited. Although various *L. monocytogenes* genes have been identified that are activated in response to cold growth, their precise role in cold adaptation is not well understood (Arguados-Villa & Tasara, 2010). Arguados-Villa & Tasara (2010) found significant cold growth phenotypic variability amongst strains grown at 4°C, but not those grown at 37°C. Most of the phenotypes found to be able to overcome cold stress were those associated with human listeriosis. Five strains were tested. The three isolated from food had lag phases of seven to 30 hours, whereas others had lag phases of 54 to 140 hours. Although ultimate counts were similar, shorter lag times meant reaching the stationary phase more quickly, which may explain the disproportionate distribution of certain strains in cold production environments and cold preserved foods. Certain food components (short peptides, glycine betauine and carnitine) are also known to promote cold growth of *L. monocytogenes* (Arguados-Villa & Tasara, 2010).

Reduced temperature leads to a decrease in membrane fluidity (converting the normal liquid crystalline state of a lipid membrane to a gel-like phase), which leads to reduced ability of the organism to take up nutrients; increased superhelical coiling of DNA, which can affect ability to replicate; changes to the secondary structures in RNA affecting translation; reduced enzyme activities; slow protein folding and the need to adapt ribosomes to function at low temperatures. Cells respond by changing the membrane composition and by altering gene expression to overcome these problems. Thus, synthesis of cold-shock proteins in a state of cold shock increases, but decreases as the organism becomes adapted, and the fatty acid composition of the membrane is adjusted to adapt membrane fluidity (Chan & Wiedmann, 2009).

Cold-stress resistance and low temperature growth capacity are biological properties to maintain membrane structural integrity, to ensure nutrient uptake, to maintain ribosome functionality and to face slow protein folding, reduced enzyme activities, decreased ability to replicate DNA and altered RNA translation (Cacace et al, 2010). As such, results (using proteomic approaches) have shown cells grown at 4°C to have increased levels of chaperones, folding catalysts and transport for osmolyte and oligopeptide uptake. In addition, proteins involved in metabolic processes for energy production are present at higher levels, suggesting more energy is required to sustain cold growth (Cacace et al, 2010). These mechanisms are not very well understood, and improved understanding could lead to the identification of inhibitors of these adaptive mechanisms (Chan & Wiedmann, 2009).

4.3.2: Thermotolerance

Perversely, the organism is also relatively thermotolerant, and under some circumstances may be able to survive pasteurisation. Thermotolerance is dependent on strain and food properties/formulations. For instance, in meat products thermotolerance is affected by meat spices, muscle type, pH and fat content (Aguilar et al, 2013), with fat, for instance, possibly offering protection.

4.3.3: Acid tolerance

L. monocytogenes also appears to have enhanced ability to survive the high acid levels in the stomach, although this is strain dependent. Virulent strains can survive passage through the stomach, with some foods acting as buffers and so protecting the organism. Fat in food may also protect organisms through the gastrointestinal tract. In simulated digestion, acid followed by exposure to pepsin and bile salts lead to greater cell death, than the individual compounds alone. It is thought that acid damage to the lipopolysaccharide of the outer membrane and denaturation of the cytoplasm may lead to greater susceptibility to pepsin and bile, as cells were not affected by pepsin or bile without acid exposure first (Ramalheira et al, 2010). This might suggest that if stomach acid is reduced, then the effectiveness of pepsin and bile will be reduced.

4.3.4: Antibiotic resistance

Another important characteristic of *L. monocytogenes,* contributing to its importance as a foodborne pathogen, is that the organism has developed resistance to some therapeutic antibiotics. Resistance may have derived from food sources, possibly as a result of exposure to antibiotics and other antimicrobials used in agriculture and in the food sector. This could reflect pre-exposure adaptation of *L. monocytogenes*, but could also be linked to starvation of the organism in areas of processing plants where there are fewer nutrients, which may convey more general resistance to control methods (Lungu et al, 2011).

4.3.5: Biofilms

Biofilm formation is a particular feature of *L. monocytogenes*, allowing it to survive and persist for long periods, including on food-contact surfaces (Koo et al, 2013). A biofilm is cell aggregates adhered to each other and/or to surfaces or interfaces by self-produced extracellular polymers. *L. monocytogenes* is capable of attaching to inert surfaces and subsequently forming biofilms on food production equipment and environments. Cells can be released during production, colonising new substrates or becoming direct sources of contamination (Cruz & Fletcher, 2012; da Silva & Martinis, 2013). Biofilm-coated surfaces are particularly difficult to decontaminate, as the biofilm protects organisms from a variety of stresses (Renier et al, 2011). Thus, cells in biofilms show a greater level of resistance to several physicochemical stresses, including antibacterial agents, where resistance is related to the age of the biofilm (Cruz & Fletcher, 2012), ultraviolet rays, toxic metals, acids, desiccation, salinity, antimicrobials and high concentrations of disinfectants and sanitisers.

Therefore the organism is very difficult to remove from its niches, and thus persists (da Silva & Martinis, 2013).

The formation of biofilms comprises five steps: suspended bacterial cells adhere to surface through van der Waals forces, electrostatic forces and hydrophobic interaction; cells proliferate and produce extracellular polymeric substances (EPS); more complex structures are constructed; a mature biofilm is formed, with channels to allow flow of nutrients and excreta; cell dispersion leading to colonisation of other surfaces (da Silva & Martinis, 2013). The structure of the biofilm depends on the cultivation system. If formed in a static environment, the biofilm comprises a homogeneous layer of rod-shaped cells or micro colonies; under continuous flow it manifests as ball-shaped colonies surrounded by a network of knitted chains composed of elongated cells (da Silva & Martinis, 2013; Renier et al, 2011). EPS mediates bacterial adhesion to surfaces, provides mechanical stability to biofilms, protects cells from external stresses, provides a network that interconnects and immobilises cells, and functions as an external digestive system keeping extracellular enzymes close to cells for the metabolism of biopolymers. EPS has also been shown to contain extracellular DNA, possibly as a result of quorum sensing molecules inducing autolysis of some of the cells, leading to release of DNA. Released DNA may be involved in horizontal gene transfer, promoting beneficial mutations in remaining cells and biofilm formation (da Silva & Martinis, 2013).

There is some indication that efficacy of biofilm production is strain dependent. Serotype 1/2c shows the highest levels of attachment, but correlation between strain and ability to form biofilms has only been shown in relation to strains 1/2a and 4b. 4b is a low density biofilm producer, but can form a higher density biofilm in the presence of another high biofilm producer (e.g. 1/2a; Renier et al, 2011).

L. monocytogenes is able to adhere to a variety of food-contact surfaces found in food-processing/preparation environments, including both hydrophobic (e.g. PTFE) and hydrophilic (e.g. Stainless steel) surfaces (Renier et al, 2011). It has been found to occur on surfaces in populations reaching 10^4-10^7cfu/cm^2 (da Silva & Martinis, 2013). Factors affecting ability to adhere and form biofilms include electrostatic charge and hydrophobicity of the contact surface, diversity of serotypes, expression of flagella, EPS composition, environmental conditions (e.g. pH and temperature) and the culture medium (da Silva & Martinis, 2013). In addition, biotic factors can influence biofilm development. For instance, resident biofilms can affect biofilm formation positively or negatively. Biofilm formation may be inhibited as a result of competition for nutrients or the secretion of antimicrobials (e.g. bacteriocins produced by lactic acid bacteria), whilst biofilm development may be favoured if the structure of the resident biofilm is porous, thus facilitating adhesion (Renier et al, 2011). The use of probiotics (genetically engineered Lactic acid bacteria) to prevent adhesion and colonisation of *L. monocytogenes* in the gut has been suggested (Koo et al, 2012).

5. Common food vehicles

Once *L. monocytogenes* emerged as an important food pathogen, many studies were published reporting the presence of the organism in a wide range of food products (Crerar et al, 2011). This was, perhaps, unsurprising given the ubiquitous nature of the organism. Whilst the organism was found in many food types, not all of the foods found to have the potential to harbour the organism have been implicated in outbreaks of listeriosis. In part at least, this is because of the long incubation period, meaning that identification of the source of infection is rare (Parisi et al, 2013). The predominant serotype identified in a variety of food products is serotype 1/2a (Kramarenko et al, 2013) or 1/2a, 1/2b and 4b, which are also the strains most frequently associated with foodborne outbreaks and sporadic cases of listeriosis (O'Connor et al, 2010).

In general, higher counts have been found in raw foods than ready to eat (RTE) products. Whilst this is as may be expected, the presence of the organism in RTE products, especially those with long refrigerated shelf lives allowing the organism to multiply (Kramarenko et al, 2013), is of more concern, as there is no subsequent listericidal treatment to remove organisms prior to consumption (Grassi et al, 2013). Investigations of RTE foods are usually associated with a high prevalence of *L. monocytogenes*, but low total counts. Thus, Kramarenko et al (2013) reported that 98.4% of positive samples, where enumerated, had counts below 10cfu/g. There have, however, been incidences of contamination levels over the legal (EU) limit of 100cfu/g during the shelf life of RTE fishery and meat products and cheeses (Kramarenko et al, 2013). Little et al (2009) tested 6984 RTE products in the UK, between May 2006 and April 2007. *L. monocytogenes* was isolated from sandwiches (7%), and sliced meats (3.7% during shelf life; 4.2% at end of shelf life) and of these, 0.4% and 0.7/0.9% respectively had counts above 100cfu/g. This illustrates the importance of correct storage conditions in maintaining low levels of the organism through to consumption. Contamination of RTE products has more often been the result of post-heat treatment contamination, than inadequate heat treatments leading to survival of organisms (Kramarenko et al, 2013).

L. monocytogenes has been isolated from salad products (including those packed in modified atmospheres), meat and fish products – especially cold smoked fish products, such as smoked salmon (Kramarenko et al, 2013), dairy products including soft cheeses, pate and sandwiches. A high prevalence and high counts have been associated with particular foods types – RTE meat products, soft cheeses, blue mould cheeses, smoked fish, pate, deli-meats, unpasteurised milk, fermented raw-meat sausages, non-reheated frankfurters, hot dogs and deli-salads (Kramarenko et al, 2013). It has also been suggested that reducing salt levels in RTE foods, as a result of dietary advice and consumer preferences, could be a contributor to the growth of the organism where present as a contaminant (Allerberger & Wagner, 2009). Given the global nature of the food supply and the wide distribution of

products across national borders, contamination studies conducted on foods produced in different countries become relevant.

5.1. Seafood

The identified prevalence of *L. monocytogenes* in cold-smoked fish and in gravad fish in Sweden was reported as 14%, compared to 2% for hot smoked fish. 96% of isolates were serotype 1/2a. The organism was more often isolated in imported products, highlighting the impact of the global market and the importance of being mindful of the implementation of food safety controls in other places, including other EU countries (Thisted Lambertz et al, 2012). Mahmoud (2012) also found 26% of frozen seafood samples to be contaminated with *L. monocytogenes*, which was, in the case of cold smoked salmon, linked to the raw salmon and contaminated equipment and environment.

5.2. Meat products

L. monocytogenes has been isolated from a wide range of meat products. Globally incidence of *L. monocytogenes* contamination of RTE meat products ranges from 2.7–20% (Awaisheh, 2013). A 6.7% incidence has been reported in sliced vacuum-packed RTE meat (Aguilar et al, 2013). Prevalence has been shown to be greater where meats were prepacked, or in larger (300g+) pack sizes, and in sandwiches containing vegetable ingredients (Little et al, 2009). Prevalence in European industries manufacturing fermented sausages has been extensively documented both in product and equipment, with a 15% incidence of contamination in samples from Italy and Spain (Marco et al, 2013). Organisms may not grow in the fermented product, as a result of a low a_w and also the presence of nitrite and lactate in the formulation, but can survive (Marco et al, 2013). This raises the importance of the presence of preservative in controlling the growth of the organism. Presence of *L. monocytogenes* on cooked meat is most often the result of cross-contamination, including reuse of the same cutting boards for raw and RTE foods, irrespective of the material of the cutting board (Goh et al, 2014). Another issue with meat and meat products is that *L. monocytogenes* concentrates in organs, so undercooked organ meat is potentially a greater hazard than undercooked muscle tissue (Mor-Mur & Yuste, 2010). Fresh and frozen chicken and cooked and RTE poultry products have been found to be contaminated, with the latter revealing counts up to 700cfu/g at retail level (Earnshaw & Lawrence, 1998). This highlights the importance of adequate controls at all points along the food chain.

5.3. Fresh fruit and vegetables

L. monocytogenes has been isolated from many different varieties of fresh fruit and vegetables (Domenach et al, 2013). For instance, lettuce has, in various studies, been shown to be contaminated at a rate of 0–2%. Vegetables are likely to contain pathogens owing to growing methods, for instance from the soil, or through the application of manure (Ding et al, 2013). For vegetables that are not cooked prior to

consumption, this highlights the importance of thorough decontamination measures, such as washing and sanitising using chlorine-based disinfectants.

5.4. Sandwiches

Ready-made sandwiches have frequently been linked to outbreaks of listeriosis (Dufour, 2011). The contracted annual value of sandwich sales into the NHS is £13million, equating to c 16 million sandwiches, not including those bought from non-contracted suppliers or made in-house (HPA, 2008), which gives an indication of the potential of contaminated sandwiches to cause major problems. HPA (2008) reported two recent studies of sandwiches served to vulnerable groups in the UK. Of 3000 sandwiches served in hospitals/care homes, 2.7% contained *L. monocytogenes* at levels of less than 10cfu/g and 1% at 20cfu/g. A similar rate of contamination was found in a study of 950 sandwiches sampled in Wales (3.1%). Contamination was more evident in bought-in sandwiches than those made on site, although poor temperature control during storage has also been noted as a feature of the outbreaks (HPA, 2008).

5.5. Contamination during food production

As *L. monocytogenes* is usually killed by commercial food processing heat treatments, foods positive for the organism at point of consumption can usually be traced back to post-process contamination from environmental sources, including in processing plants and retail environments, as well as, most likely domestic kitchens and institutional kitchens (Chan & Wiedmann, 2009). Post-processing contamination is often exacerbated by poor temperature control, allowing the organism to grow, as initial contamination levels are likely to be low (Chan & Wiedmann, 2009). RTE foods are easily re-contaminated post-processing (Goh et al, 2014), and this is an almost inevitable consequence of processing practices such as cutting, slicing and packaging, as well as contamination from environmental sources (water, dust, processing surfaces; Awaisheh, 2013). Growth of the organism on food can occur as a result of poor temperature control along the food chain, including during transport and distribution of the product (Ding et al, 2013). It is also of note that the ability of *L. monocytogenes* to grow at temperatures that inhibit competitive organisms (Chan & Wiedmann, 2009), means the organism is effectively selectively cultured. Todd & Notermans (2011) suggested that contamination from raw materials was generally associated with short term problems with the organism, whereas errors in facility maintenance, allowing the persistence of the organism, were associated with longer term problems.

A number of outbreaks have been linked to dairy products. *L. monocytogenes* has been found in different environmental sites within dairy plants (Parisi et al, 2013). Parisi et al (2013) found *L. monocytogenes* in seven of 34 cheese factories, with a higher proportion of isolates deriving from floor drains than other areas or in the product or raw ingredients. Contaminated drains, cleaned using high power hoses, will lead to the production of aerosols that can transfer the organisms to other surfaces, including foods. Overall, 6.4% of environmental samples yielded *L.*

monocytogenes, compared with 2.4% of food samples. Predominant serotypes were 1/2b, 1/2a and 4b. This confirmed that dairy plants constitute a good ecological niche for colonisation of these organisms, and pointed to cross-contamination post processing as being more important than contamination of raw ingredients (Parisi et al, 2013).

It is of note that the serotypes frequently isolated from processing equipment and environments (as above) are most often those that have been implicated in human pathogenesis. For instance, in Sweden, the organism was found in environmental samples in 64% of the meat processing plants studied, and 87% of the isolates were serotype 1/2a (Thisted Lambertz et al, 2012); in Ireland the serotypes isolated from food processing environments were – 1/2a, 1/2b, 1/2c, 4b and 4c (O'Connor et al, 2010).

Refrigerators have also been investigated as a source of contamination, and their colonisation with *L. monocytogenes* has been demonstrated (Azevedo et al, 2005). Azevedo et al (2005) found 3/86 domestic fridges positive for *L. monocytogenes*. The authors noted that fridges often do not operate at 5°C or below, with temperatures in excess of 10°C reported, and often are not cleaned often enough, thereby posing a risk of cross-contamination of foods, especially if the food is not covered. Jackson et al (2007) also investigated domestic fridges, finding 1.2% of fridges *L. monocytogenes* positive. The authors noted raw, unwrapped foods, open and leaking packs and hands amongst possible sources of contamination.

6. Listeriosis

Listeriosis is the disease caused by consumption of *L. monocytogenes* in sufficient numbers. It is a rare foodborne disease compared with Salmonella, for instance, but its importance lies in a high fatality rate and the severity of the disease (Goh et al, 2014).

Listeriosis can take different forms, invasive or non-invasive, with the former being associated with high mortality rates, particularly amongst at-risk groups. As 90% of adults have immune lymphocytes against *L. monocytogenes*, exposure is thought to be common (Hof, 2003). In general, however, the attack rate is very low. For those contracting invasive forms of the illness, the prognosis is poor, with survivors sometimes developing serious long-term health problems (McLauchlin et al, 2004). Most cases are likely to be sporadic (see below), but a key factor is immunosuppression (McLauchlin et al, 2004) for both epidemic and sporadic cases.

6.1. Nature/severity of disease

When ingested, *L. monocytogenes* causes a generally, self-limiting, flu-like disease in healthy people, although more recently, mild fever and gastroenteritis have been reported (da Silva & Martinis, 2013). Healthy individuals can, however, develop more serious forms by ingesting large numbers of cells, or when taking certain medications (Aguilar et al, 2013).

Listeriosis takes invasive and non-invasive forms. Invasive listeriosis leads to meningoencephalitis, encephalitis, sepsis and abortion and has a high mortality rate (20–30%, Awaisheh, 2013); (20–50%, Smith et al, 2010). Amongst Spanish cases the number of deaths has reduced in the period 1997 – 2007 (26.6%), compared to the previous ten years (53.1%, Munoz et al, 2012). Fatality rates and severity of disease depend on other factors (Goh et al, 2014), and in high risk groups case fatality can reach 75% (Awaisheh, 2013). EFSA reported a fatality rate of 16.6% in 2011, with the elderly especially affected (Parisi et al, 2013). Listeriosis mainly affects newborn infants, the elderly, pregnant women and immunocompromised persons. Non-invasive listeriosis causes febrile gastroenteritis: fever, diarrhoea, muscle pain, headache, nausea, vomiting and abdominal pain (Parisi et al, 2013), and mainly affects healthy adults (Parisi et al, 2013; Painter & Slutsker (2007)), and does not lead to serious illness (Kramarenko et al, 2013). These patients have raised levels of anti-Listeriolysin O (Painter & Slutsker, 2007).

The organism has mainly been reported as a cause of intra-uterine infection, meningitis and septicaemia. In pregnancy (10–20% of all cases; McLauchlin et al, 2004) it manifests as a severe systemic infection of the unborn or newly delivered infant, but as little more than a flu-like bacteraemic illness in the mother. 20% of listeriosis cases in pregnancy lead to abortion or still birth, and 63% of remaining pregnancies result in neonatal infection, either in first week of life (early onset) or one to several weeks after birth (late onset, Painter & Slutsker, 2007).

For adults/juveniles the illness mainly presents as central nervous system (CNS) infection or septicaemia, the latter mainly in those who are immunosuppressed. Many also present with bacteraemia without evidence of meningitis (Painter & Slutske, 2007) or with multi-organ dysfunction (Kruszyna et al, 2008). In a small number of CNS infections, macroscopic brain abscesses have been observed (Cone et al, 2003; Lecuit, 2007), which have been associated with higher mortality levels (Roed et al, 2012) and neurological sequelae in adult survivors (Lecuit, 2007; Roed et al, 2012). Other rarer presentations include infections of specific parts of body (McLauchlin et al, 2004; Cokes et al, 2011).

L. monocytogenes accounts for 3.8% of foodborne disease hospitalisations (Painter & Slutske, 2007) and of those who contract invasive forms of the disease there is a 94% hospitalisation rate (Koo et al, 2013). It is responsible for 27.6% of foodborne disease deaths (Painter & Slutsker, 2007), and is the main cause of death associated with foodborne pathogens in the UK (Little et al, 2010). After Salmonellosis, it is the second most frequent cause of foodborne infection-related deaths in Europe (Allerberger & Wagner, 2009). Listeriosis is the third most common cause of acute bacterial meningitis in the Western World, responsible for 4–12% of all cases (Roed et al, 2012) and among all bacterial meningitides, *L. monocytogenes* meningitis has the highest case mortality rate (22%, Lecuit, 2007). Amaya-Viller et al (2010) reported it to be the third most frequent cause of bacterial meningitis in adults in nine Spanish hospitals over a 39 month period. There is concern about health-

care associated listeriosis, as *L. monocytogenes* is naturally resistant to many antibiotics prescribed for treatment of healthcare associated infections (Martins et al, 2010).

6.2. Incidence

One issue when estimating incidence, is being able to associate apparently disparate cases. Marcus et al (2009) notes the importance of nationwide databases in linking sporadic cases and implicated foods. Other factors that must be taken into consideration when interpreting data across European countries, and perhaps globally, include different case definitions, diagnostic practices and surveillance systems (Kvistholm et al, 2010).

The incidence of illness from *L. monocytogenes* is lower than for other pathogens, but the resultant invasive illness has a high fatality rate (20–30%, Ding et al, 2013), and the highest mortality rate of all food pathogens (Kramarenko et al, 2013). It is therefore of public health significance (Ding et al, 2013). It is believed that incidence is under-reported as it is rarely diagnosed as a cause of gastroenteritis (non-invasive form of illness), and some miscarriages/stillbirths (one of the manifestations of the invasive form of the illness) are not correctly attributed to *L. monocytogenes* (da Silva & Martinis, 2013), as the appropriate tests are not routinely carried out (McLauchlin et al, 2004). In addition, sub-clinical listeriosis can occur, which may present as a mild flu-like illness that is unlikely to be either investigated or reported (McLauchlin et al, 2004). Being under-diagnosed, particularly at early stages, has the unfortunate consequence of delaying administration of appropriate treatments, which is critical to a favourable outcome (Lecuit, 2007). Accounting for underreporting (a very conservative half of actual cases reported) would equate to an actual burden of 3473 hospital bed days and 68 deaths in the UK, making it third only to Salmonella and Campylobacter in relation to bed days, and the fourth most common cause of death (McLauchlin et al, 2004).

In England and Wales, and in other EU states, the number of cases is increasing (Table 3), after a decline in the 1990s (Shetty et al, 2009). The decline was thought to be the result of advice to pregnant women to avoid particular foods. Thus, there has been a change from pregnancy-related illness to illnesses affecting the elderly (Munoz et al, 2012). For instance, the mean age of patients increased from 53 years to 60 years over an eleven year period (1997 – 2007) in hospital cases in Spain, and the proportion of people over 65 years increased from 21.9% to 45.6% (Munoz et al, 2012). Incidence in the UK has doubled since 1990; 109 cases were reported per annum between 1990 – 2000; 189 cases between 2001 – 2008, with more infections in those over 60 years old, with underlying conditions, such as cancer, or with treatments leading to stomach acid suppression. Patients also more often presented with bacteraemia in the absence of CNS involvement (Gillespie et al, 2010). Across the EU, cases of listeriosis increased by 19% (to 1645 cases; 270 deaths) in 2009 compared with 2008 (c 0.4 cases per 100,000 population (Parisi et al, 2013). There were a similar number of cases reported in 2010 (1601 cases; 181 deaths,

Kramarenko et al, 2013; Angelidis et al, 2013). The rise mainly affected people over 65 years (58% of cases, Thisted Lambertz et al, 2012; Kramarenko et al, 2013). Similarly, Crerar et al (2011) found 69.2% of cases were amongst those over 60 years in New Zealand. The reason for the increase in the mean age of adult infections (above 55 years; McLauchlin et al, 2004) is not clear, but is likely to include poor food storage habits (the elderly are more likely to eat foods beyond their shelf life; Milne, 2011) and increased susceptibility as a result of age, underlying conditions and their treatment (McLauchlin et al, 2004).

The Advisory Committee on the Microbiological Safety of Food (ACMSF) report on the rise of listeriosis in England and Wales since 2001 state two main reasons for the increase in infection in those aged 60 years and over: improved case recognition and those within this age group surviving longer with chronic conditions. A news article published on the FSA website in 2009 (FSA 2009) suggests the over 60s are more likely to take risks with 'use by' dates than younger people because they are reluctant to throw away food. Other research commissioned by the FSA (FSA 2009) suggest the over 60s were confused by fridge temperatures.

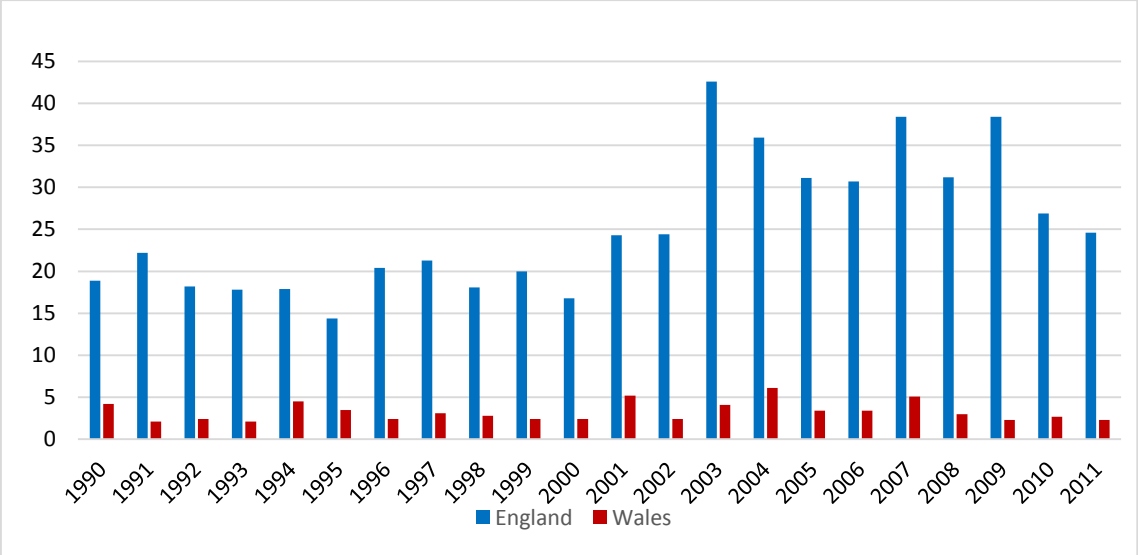

Table 3 Rates of human infections due to *Listeria monocytogenes* in England and Wales by region, reported to the HPA 1990 - 2011 (rates per 1,000,000 population). Source: HPA (2013)

Attack rate is low in outbreaks, and assumed to be low in sporadic cases. For instance, only one individual contracted listeriosis from cheese (some found to be contaminated with above 10^7cfu/g), despite 57% of products being shown to be contaminated, over eleven months, with a three to six month shelf life (McLauchlin et al, 2004). Retail cheeses contained 10^1-10^5 cfu/g. Doubling times for the organism were estimated at one – two days. Another seven cases were infected with a similar strain, but it was not possible to confirm they had eaten the cheese. Nonetheless, many people had eaten the highly contaminated cheese, and did not develop serious infection. Similarly, in as outbreak involving butter in a Finnish hospital, there was an estimated 7% attack rate amongst immunocompromised patients, and an estimated daily dose of 10^1–10^5cfu/g. Prolonged exposure to low daily doses increased

susceptibility (McLauchlin et al, 2004). However, attack rate for febrile gastroenteritis can be high. 72% of individuals consuming contaminated corn salad (10^6cfu/g) developed gastroenteritis and fever; 19% were hospitalised with more severe symptoms (McLauchlin et al, 2004).

Incidence varies around the world, possibly as a result of dietary preferences and food safety policies (Munoz et al, 2012). Denmark has the highest number cases in Europe (Smith et al, 2010). Taking the same reporting period (1995 – 1999) there was an incidence of 1.7 – 2.4 cases per million in the UK; 5.4 cases per million in France and 9.4 per million in the USA (McLauchlin et al, 2004). Possible reasons for these differences are the French preferences for eating meat that is less well cooked and high consumption levels of soft cheeses, and a practice of home-food manufacture in the USA, with potentially less stringent food safety controls. In addition, a number of cases in the USA have been associated with a particular fresh cheese, popular with the Hispanic community, who were disproportionately represented in the statistics. A recent paper, reported 1591 cases annually in the USA, 1455 hospitalisations, and 255 deaths – equating to c 2.9 cases per million of the population (Ding et al, 2013), representing a significant drop in incidence. The number of listeriosis cases per 1,000,000 of the population in Canada increased sharply between 2001 and 2007, rising from 2.7 to 4.2 (Farber et al, 2011).

6.3. Mode of infection

The discussion below pertains to invasive forms of listeriosis, where the organism is spread from food, through the blood to other organs, leading to serious disease and possibly death. Non-invasive forms, i.e., febrile gastroenteritis, are hypothesised to be due to limited invasion of the gut mucosa (Painter & Slutsker, 2007), and are generally short-lived and self-limiting.

The principle route of infection is through food (McLauchlin et al, 2004), with 99% of listeriosis cases thought to be foodborne (Norton & Braden, 2007). A small number of cases derive from contact with a contaminated environment, infected animals or via cross-contamination at birth. Invasion of the body is through sites in the nasopharyngeal tract or the upper parts of the alimentary tract (McLauchlin et al, 2004). It is also thought that invasion can be through the oral mucosa (leading to lesions in base of brain), and that contaminated aerosols (including from foods) may also lead to infection via a respiratory route (McLauchlin et al, 2004).

L. monocytogenes has evolved sophisticated strategies to infect various tissues of the host (Seveau et al, 2007). The extent of intraluminal multiplication and precise locations at which it crosses the intestinal barrier is still debated (Lecuit, 2007), and there is still much to learn about the roles of specific proteins in infection (Seveau et al, 2007), however, the account below summarises the various theories.

The organism invades the gastrointestinal epithelium (Ramalheira et al, 2010), animal studies showing that the small intestine is the primary site of invasion

(McLauchlin et al, 2004). Specifically, it invades the epithelium at multicellular junction sites at the tip of intestinal villus, where enterocytes undergo programmed cell death (Seveau et al, 2007). Exposure to bile may enhance biofilm formation and consequently contribute to survival and colonisation of the gastrointestinal tract, and it is suggested that extracellular DNA is important in the early stages of biofilm formation (Renier et al, 2011). It enters phagocytic host cells where it survives and multiplies and then is disseminated to other organs through the blood (Ramalheira et al, 2010), where it transgresses either the blood-brain barrier or the placenta (Goulet et al, 2013). Its intracellular nature may also lead to successive invasion of organs, which may explain the long incubation times (up to three months) in some patients after consumption of contaminated foods (McLauchlin et al, 2004). In pregnancy-related cases, neonates are infected in-utero through the placenta or amniotic fluid, or possibly via contamination from the birth canal (Painter & Slutsker, 2007), then spreading to the foetus' internal organs (McLauchlin et al, 2004).

In vitro tests, using mammalian cells, show *L. monocytogenes* to have properties of invasion and spreading (McLauchlin et al, 2004). The organism is able to penetrate into, survive and multiply within virtually every nucleated cell of the body (Hof, 2003). The major steps in intracellular parasitism involve the cell wall proteins Internalin A and B for adhesion to the surface of host cells and entry into the cells via phagocytosis. Extracellular proteins are also important in initial attachment to the surface (Renier et al, 2011). Listeriolysin O and phospholipase C then enable escape from the phagocytic vacuole, and actin assembly is responsible for cell-to-cell spread (Renier et al, 2011).

Internalin A is a listerial surface protein involved in the initial stages of cell invasion (all cell types). Other surface proteins are required for invasions of other cell types (Internalin B – hepatocyte-like cells; p60 - invasion of fibroblasts). Internalin A and B are known as invasins (Seveau et al, 2007). Some mammalian cells naturally encapsulate external materials into a membrane-bound compartment, but *Listeria* has the ability to induce the process in cells that would not normally do this, e.g., the enterocytes comprising the epithelial cells lining the intestine (McLauchlin et al, 2004). Thus the action of Internalin A and/or B triggers host cells to internalise the attached bacteria (Hof, 2003). The bacteria then mediate the dissolution of the vacuole membrane through the action of listeriolysin, haemolysin and, possibly, phospholipase C. This allows the organism to leave the phagocytic vacuole and enter the host cytoplasm, where it grows (McLauchlin et al, 2004). It then facilitates the polymerisation of actin from the host cell cytoskeleton, mainly at the apical tip of the bacterium, so new actin filaments act like a driving force allowing the organism to move around the host cell. If it gets under the surface of the host cell, the cell membrane is induced to produce extrusions that penetrate the neighbouring cell, which then engulfs it. The bacterium lyses the double cell membrane (using a second phospholipase enzyme – McLauchlin et al, 2004) and invades the cytoplasm of the neighbouring cell. The organism thus moves between cells, while avoiding the

host cells' defence mechanisms (Hof, 2003). Having crossed the intestinal epithelium, it invades mesenteric lymph nodes and the blood. Most bacteria are then trapped in the liver, and are cleared from the circulatory system. Surviving bacteria reproduce in hepatocytes. At some point hepatocytes undergo lysis, releasing bacteria. If infection is not controlled at this stage (e.g. owing to the use of immunosuppressive therapies), a secondary bacteraemia develops (Goulet et al, 2013).

6.4. Vulnerable groups

A number of factors have been identified that predispose individuals to contracting invasive forms of listeriosis, with a recognised risk factor identified in 80-90% cases (Buchholz & Mascola, 2001). These include age, pregnancy, underlying conditions and consumption of high-risk foods. In non-pregnant individuals, listeriosis is usually secondary to impaired cell-mediated immunity, which is congenital, acquired, iatrogenically induced or associated with metabolic disorders (Cone et al, 2003). The increased elderly population and the increase in the number of people with immunosuppression have been proposed as a possible reason for the increase in the number of cases of listeriosis.

Increased age has clearly been linked to increased incidence of listeriosis. In the USA, up to 40 years of age, less than one case per million of the population is reported per year, for those over 70 years, 21 cases are reported per million per year (Cone et al, 2003). The FAO/WHO Listeria Assessment Risk Group indicated (in 2003) that the elderly (over 60 years) were 2.6 times more susceptible (Swaminathan & Garner-Smidt, 2007), and a Danish study of hospital patients with listeriosis between 1977 and 2006, reported a median age at diagnosis of 61.7 years (Roed et al, 2012). In England and Wales the highest infection rates (Table 4) are for those between the ages of 50 to 80+ and the lowest is between the ages of 10-19 (HPA 2012). It is thought that the elderly may be more prone to infection as a result of declining T-cell function with age (Swaminathan & Garner-Smidt, 2007), and possibly use of over the counter medications that reduce stomach acid concentrations (see below). T-cells are required for final clearance of bacteria from the body (Zenewicz & Shen, 2009). Reduced T-cell function is also the proposed mechanism for vulnerability in pregnancy-related cases, as T-cell immunity is most impaired in the third trimester, such that the body does not reject the 'alien' baby (Allerberger & Wagner, 2009). T-cell immunosuppression, neutropenia and possibly hepatic disease also predispose to brain abscesses (Cone et al, 2003). It is, however, notable that most cases in the elderly also involve a least one other underlying condition.

Year	Age group in years								
	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80+
1990	0	2	11	14	6	13	22	22	16
1991	4	4	11	14	9	15	14	18	20
1992	0	1	9	10	10	16	11	14	16
1993	1	1	8	11	5	10	17	27	14
1994	0	2	12	10	6	7	18	27	19
1995	5	1	0	9	5	14	11	24	18
1996	0	4	6	12	8	15	16	33	18
1997	2	2	5	12	5	15	19	30	25
1998	3	2	7	9	10	12	12	21	24
1999	1	4	6	9	7	10	25	23	18
2000	2	1	7	5	2	14	18	27	22
2001	4	3	8	10	10	15	26	35	32
2002	2	3	6	9	9	13	22	43	30
2003	7	7	14	20	16	26	44	58	38
2004	8	3	13	12	11	14	53	56	34
2005	10	4	6	17	11	15	40	48	36
2006	13	1	6	14	6	17	45	43	39
2007	15	3	7	12	13	26	51	53	47
2008	13	1	7	4	6	26	46	45	34
2009	15	1	9	19	13	26	41	42	48
2010	15	2	4	13	5	16	24	45	32

Table 4 *L. monocytogenes* cases in England and Wales by age, reported to the HPA 1990 – 2011.

A number of underlying conditions have been linked to increased risk of contracting listeriosis, and Hof (2003) provided a useful summary of incidence amongst people with different predisposing factors (Table 5).

Predisposing conditions, some of which result in a compromised or weakened immune system include AIDS, diabetes, organ transplants, where immunity is suppressed through medication to prevent rejection of the new organ (Angelidis et al, 2013; Kramarenko et al, 2013) and malignancy (Painter & Slutsker, 2007). T-cell inhibitors are used to prevent rejection of organs (Swaminathan & Garner-Smidt, 2007). The most common predisposing factors amongst patients with listeriosis have been reported to be malignancy and use of immunosuppressive therapy (Doorduyn et al, 2006). Haematologic cancers have been reported to be the main predisposing cancer (Roed et al, 2012), although the most common underlying condition in Spain over 22 years from 1986 was non-haemotologic malignancy (Munoz et al, 2012). It has also been suggested that listeriosis risk is linked to inflammatory bowel disease, and that ingestion of *L. monocytogenes* may result in an abnormal immunologic response in susceptible people (Painter & Slutsker, 2007). In addition, conditions or events that disrupt the gastrointestinal micro-environment/flora, e.g. colonoscopy as well as colon cancer may increase the risk of the organism invading the body (Buchholz & Mascola, 2001).

	Incidence listeriosis in certain populations at risk (per 100,000 individual per year)
Normal population	0.7
>70 years	2
Alcoholics	5
Diabetics	5
Iron overload	5
Pregnant women	12
Cancer patients	15
Steroid therapy	20
Lupus erythematosus	50
Kidney transplant patients	100
Chronic lymphatic leukaemia	200
AIDS	600
Leukenia (acute monocytic + acute lymphoblastic)	1000

Table 5 Incidence of listeriosis in certain populations at risk (Hof, 2003)

A frequent feature of these conditions, or the therapies used to control them, is a decreased level of cell-mediated immunity (Painter & Slutsker, 2007). Patients with compromised T-cell function have a lifetime risk of contracting listeriosis 100-300 times higher than the general population (Kruszyna et al, 2008). Similarly, AIDS patients have been reported to be 145 times more likely to contract listeriosis compared to the general population (Chan & Wiedmann, 2009). The increase in the number of immunocompromised individuals as a result of the emergence of diseases such as AIDS, and use of intensive cancer therapies, immunosuppressive drug therapies, organ transplants, as well as the rise in the number of elderly people means *L. monocytogenes* has become a pathogen of serious concern (Lungu et al, 2011).

Taking antacids and/or cimetidine-containing compounds used to treat heart burn or stomach ulcers (Aguilar et al, 2013), reduces the acidity of the stomach (Norton & Braden, 2007). As acid in the stomach kills many of the organisms, it is possible that use of antacids may increase the susceptibility of some patients (McLauchlin et al, 2004). Increased use of proton pump inhibitors (PPI) in the elderly (Munoz et al, 2012) has also been mooted as a risk factor, as PPI are more effective at gastric acid suppression than, for instance, antacids (Winter et al, 2009). Painter & Slutsker (2007) and Allerberger & Wagner (2009) also noted the use of laxatives as a possible factor, and Grif et al (2003) reported increased prevalence of *L. monocytogenes* in the faeces of patients receiving long-term H_2-antagonists, compared with those with normal gastric secretions.

Level of exposure to the organism may also be important, affected by the amount and frequency of consumption of contaminated food, frequency and levels of contamination in food, likelihood of growth on food during refrigerated storage, storage temperature and duration of storage prior to consumption (Swaminathan & Garner-Smidt, 2007). Consuming RTE meat products has been highlighted and

possible links made to changes in diet and food preparation practices, e.g. increased consumption of refrigerated foods in Europe (Munoz et al, 2012).

6.5. Infective dose and incubation times

The infectious dose remains unclear (Kramarenko et al, 2013; Swaminathan & Garner-Smidt, 2007), but is suspected to be high (counts of above 10^4 cfu/g are often found in implicated foods) or caused by prolonged consumption of foods containing lower levels of contamination (Kramarenko et al, 2013; Chan & Wiedmann, 2009). Infectious dose may, however, be low in susceptible individuals (Swaminathan & Garner-Smidt, 2007). Long incubation time also means that the affected person may have consumed infected foods multiple times, so determining a dose for a single exposure is difficult and data are scarce (Goulet et al, 2013).

If the infective dose is high, then post-process multiplication of *L. monocytogenes* in food products initially contaminated at low levels is required for onset of disease (Cacace et al, 2010), which would suggest that incidence could be reduced by adequate controls. Determining a single infective dose has been difficult, not least because of the wide range of factors that interact to determine if an individual becomes ill. In addition, the long incubation time, and subsequent lack of availability of implicated foods, adds to the difficulty. Also extrapolation from models to populations of interest is problematic, especially in relation to situations, as in this case, where particular subgroups show greater susceptibility to infection. Then, to have a single dose-response outcome is of questionable validity (McLauchlin et al, 2004).

Infectious dose is dependent on the type of food product, strain virulence, and host susceptibility (immunological status), which is why relatively low doses may cause illness in high-risk populations (Little et al, 2010). Mor-Mur & Yuste (2010) estimated that the dose could be as low as several hundred organisms or less in some cases. Attempts to estimate doses are biased towards cases with shorter incubation times, where suspect foods are available (McLauchlin et al, 2004). In addition, the effects of food vehicle characteristics have not been successfully integrated into models. Thus, typically, efforts to define a minimum infective dose of infectious agents through mathematical modelling have been unsuccessful (McLauchlin et al, 2004). It is known that, generally, an increased dose leads to increased frequency of cases.

Some studies have attempted to estimate infective doses, including using animal models. Cynomologous monkeys required 10^9 organisms orally to present with listeriosis symptoms; when fed 10^5 or 10^7 bacteria the monkeys shed the organism in their faeces for two and 21 days respectively (McLauchlin et al, 2004). Questions have, however, been raised about the adequacy of animal models. Other studies have used probabilities, based on risk assessments. Risk assessment modelling has suggested a 10^{-9} to 10^{-13} probability of infection with doses of 100 organisms; the probability increasing to 10^{-6} to 10^{-9} if the dose is 1,000,000 organisms (Allerberger & Wagner, 2009). Other estimates of infective dose include: Estimated ingested dose

of $1x10^8$ in a Norwegian hospital outbreak linked to camembert (Johnsen et al, 2010); 10^9 organisms estimated to cause infection (Buchholz & Mascola, 2001); prolonged exposure (related to butter in a Finnish hospital) to an estimated daily dose of 10 – 1000 cfu/day, but if a higher contamination level was used, then 10^4-10^5 cfu/day leading to a single dose prediction to cause illness of $7.7x10^4$cfu (Norton & Braden, 2007). USDA-FSIS estimated 1 x 10^6 cfu/serving to lead to a median death rate of 1 per 100,000 servings, but with very wide uncertainty bounds (McLauchlin et al, 2004).

The food matrix can also affect the survival of organisms in gastric acid, and thus susceptibility to infection. One issue is neutralising stomach acid by the food, but also other aspects of the food matrix e.g. reduced transit times, reducing acid secretion, protection of organism in high fat foods or emulsion, may affect the dose required to present as illness. Similarly if the organism is exposed to stressors prior to consumption, it can affect its survival within the host. For instance, prior exposure to acid stress can improve survival and also ability to attach to and invade the host (McLauchlin et al, 2004).

Chan & Wiedmann (2009) put forward an interesting notion. The average healthy person in the USA is estimated to consume 10^6 to 10^9cfu *L. monocytogenes* in a single serving once every two years, with no ill effect. The authors estimated that if levels of *L. monocytogenes* in RTE foods in the USA were reduced so no food had more than 1000cfu/g at the time of consumption, the number of cases of listeriosis would reduce from 2500 to six.

In the case of febrile gastroenteritis, it appears that in a normal host a dose of several million bacteria is required (Grif et al, 2003), but the density of *L. monocytogenes* in implicated foods has ranged from 30cfu/g to $1.6x10^9$cfu/g (Painter & Slutsker, 2007).

There have been various attempts to define the incubation time between consumption of a contaminated food and onset of symptoms of invasive listeriosis. Estimates have varied widely, but broadly for febrile gastroenteritis are short, and for the invasive form of listeriosis are long. Incubation time for febrile enteritis has been estimated to be a median of 24 hours, ranging from six to 240 hours (Farber et al, 2011), for instance, in an outbreak (mainly of febrile gastroenteritis) in a Norwegian hospital, a mean of three to four days was reported (range one to 24 days, Johnsen et al, 2010).

The ranges of times may reflect the level of contamination of the source food, quantity of food consumed, virulence of the specific strain of *L. monocytogenes* and the immunological status of the patient (Farber et al, 2011). Estimates of incubation times, for a variety of outbreaks with different food sources, vary from one day to 67 days, with no clear link to food type as a determinant of incubation time (Goulet et al, 2013). Farber et al (2011) estimated incubation time based on historical data for

listeriosis cases. The authors determined the median incubation period for invasive forms to be eight days (range one to 67 days); a longer incubation was suggested for pregnancy-related cases (median 27.5 days, range 17 – 67 days); than for CNS cases (median nine days, range one to 14 days) and bacteraemia cases (median, two days, range one to 12 days). The longer incubation time for pregnancy-related cases may be due the time required to colonise the placenta (Goulet et al, 2013).

Other estimates of incubation times include a mean of 31 days, range 11 – 70 days (Painter & Slutsker, 2007; Buchholz & Mascola, 2001); one to above 90 days (Shetty et al, 2009); two - 88 days (Yde et al, 2009).

6.6. Virulence

Virulence differences between serovars are not consistent, with sometimes known virulent strains behaving as a virulent in animal or in vitro models (McLauchlin et al, 2004). There are 13 serovariants of *L. monocytogenes*, with 4b, 1/2a and 1/2b most often causing disease. There are a wide range of strains within serovars, with strains within 4b predominating as causes of outbreaks and sporadic cases (McLauchlin et al, 2004). These strains of serovar 4b may, therefore, be more virulent, and possibly result in higher mortality rates (Swaminathan & Garner-Smidt, 2007), although McLauchlin et al (2004) suggested there was no evidence of differences in mortality or severity of disease in people contracting listeriosis from different serovars. Those that have underlying diseases are affected by a wider range of serovars than healthy or pregnant individuals (McLauchlin et al, 2004).

7. Epidemiology – including routes of transmission

Examining a range of implicated foods, reveals the high mortality rate of the disease, the predominance of cases where listeriosis is secondary to another condition, and the narrow range of serovars isolated from individuals and/or implicated foods (McLauchlin et al, 2004), 95% of infections are caused by three serotypes – 1/2a, 1/2b and 4b (Swaminathan & Garner-Smidt, 2007). Norton & Braden (2007) discussed various outbreaks and implicated foods to 2002, and it is notable that in 25% of cases it was not possible to identify the vehicle of infection. Munoz et al (2012) also noted that it has not always been possible to demonstrate a clear relationship to a concrete food. Within hospital outbreaks, the inadequacy of records of patients' food consumption was noted as an issue in following up and identifying common foods, especially where the patient was unable to communicate (e.g. had died, Martins et al, 2010). Hence, sometimes, links to hospital catering were based on the most likely scenario, given the fact that a number of those affected had eaten in hospital in the time preceding symptoms, including eating in outpatient units (Martins et al, 2010). In addition, sometimes there were no further cases following corrective actions in suspect areas, suggesting the root cause of the infection had been successfully tackled (Martins et al, 2010).

Factors affecting human exposure to *L. monocytogenes* include amount and frequency of consumption of a food, frequency and levels of *L. monocytogenes* in RTE foods, potential of the food to support growth of the organism in food in refrigerated storage, refrigerated storage temperature and duration refrigerated storage prior to consumption (Farber et al, 2011). A preponderance of RTE foods as vehicles is notable (Awaisheh, 2013). Also of note is that outbreaks are often widely geographically spread, where products are centrally produced and then widely distributed (Norton & Braden, 2007), either within a country of internationally. Frequent underlying breakdowns in food safety management include cross-contamination after production, followed by growth of the pathogen in (sometimes prolonged) storage (Ding et al, 2013).

8. Food vehicles and recorded outbreaks

It is worth noting that foods contaminated with *L. monocytogenes* look, smell and taste normal (Kruszyna et al, 2008), so it can be difficult to identify when the food should not be consumed. This is especially pertinent for *Listeria* spp., as most spoilage organisms will not grow at refrigeration temperatures, but *Listeria* spp. will.

Foodborne listeriosis has been associated with a wide range of foods – vegetables, meat, dairy and seafood (McLauchlin et al, 2004). RTE foods (Grassi et al, 2013; Thisted Lambertz et al, 2012) with a long shelf life (one+ weeks) have been shown to be an important source of infection, particularly associated with recontamination after listericidal treatments (Thisted Lambertz et al, 2012). RTE foods are reported to be responsible for 90% of cases in the USA (Koo et al, 2013).

Examining cases in the UK (2005-8) showed that those infected were more likely than the general population to have eaten cooked meats (beef, ham/pork), cooked fish (particularly smoked salmon), prawns, dairy products (milk, butter, hard cheese (not Cheddar), blue cheese, camembert and mixed salads). They were less likely to report consumption of other seafood, dairy spreads, sandwiches and fresh vegetables (Gillespie et al, 2010). Table 6 shows a number of listeriosis cases.

Milk and dairy products have been implicated in around half of all listeriosis outbreaks and several sporadic outbreaks in Europe (Parisi et al, 2013). They have been linked to both invasive and non-invasive forms (Parisi et al, 2013). Various types of cheese have been implicated, and although counts of the organism in cheese are rarely above 100 cfu/g, there have been sporadic cases and outbreaks associated with dairy products in various countries (Spanu et al, 2013). Those most often implicated are fresh, soft, blue veined and mould ripened. The main route of contamination has been contact with infected surfaces in production plants and infected equipment (Spanu et al, 2013). A number of outbreaks, and their causes (where known) are outlined below.

Year	Location	Persons affected	Deaths	Vehicle
2008	Canada	57	23	RTE deli meats
2008	Quebec	38	3	Pasteurised milk cheese
2008	New York	5	3	Tuna salad
2008	Connecticut	2	1	Chicken salad
2008	Multistate	20	0	Sprout
2008/9	Multistate	13	0	Mexican-style cheese
2008/9	Chile	119	5	Brie and camembert
2009	Illinois	6	0	Undetermined
2009	Washington	2	0	Mexican-style cheese
2009	Multistate	8	0	Mexican-style cheese
2009/10	Multistate	33	8	'Quargel'
2010	Oregon	4	0	Fresh cheese
2010	Louisiana	8	2	Meats
2010	New York	5	1	Mexican-style cheese
2010	Washington	2	0	Sushi
2010	Washington	4	0	Undetermined
2010	Texas	10	5	Undetermined
2010	Multistate	6	1	Mexican-style cheese
2011	Multistate	147	33	Cantaloupe melons
2011	Belgium	12	4	Hard cheese
2011	England	3	0	Prepacked sandwiches and salads
2012	Multistate	22	4	Ricotta salata cheese
2012	Spain	10	0	Latin-style fresh cheese

Table 6 Selected listeriosis foodborne outbreaks (2008-12) – adapted from da Silva & Martinis (2013)

8.1 Outbreaks linked to dairy products

- All but one of 34 cases of invasive listeriosis in Austria, Germany and the Czech Republic could be explained by consumption of 'Quargel', a sour milk curd cheese, before it was withdrawn from the market. Eleven of 20 samples tested yielded less than 100cfu/g, and nine samples above100 cfu/g. One sample taken from a patient's fridge yielded 2,100,000cfu/g (Fretz et al, 2010).
- Spain, 2012, two cases who had both consumed Latin-style fresh cheese in the two-months prior to symptoms. *L. monocytogenes* was found in samples of cheese, one having counts well above 100cfu/g ($3.2x10^4$cfu/g). The strains found in the cheese matched those isolated from patients. The population was advised not to consume this batch of cheese, even though it was supposedly within shelf life, which is long (de Castro et al, 2012).
- An outbreak (n=12) in Belgium, where a hard cheese made from pasteurised milk (Pave du Nord) was implicated. The cheese was manufactured in Belgium and imported to France where it was sliced, packed and sold through supermarkets. Although tested samples had low counts (within regulations), the cheese was recalled. Samples from the production plant (cheese and surface swabs) revealed the same strain as had been isolated from patients. It was not, however, possible to link illness to reported consumption, owing to a poor response to a

food history questionnaire, which, in any case, was asking about consumption one to four months previously (Yde et al, 2009).

- An outbreak associated with Mexican-cheese, where 100% of cases had consumed the implicated cheese, compared with 60% of controls in the month before illness. Illness was linked to contamination from the processing environment (Jackson et al, 2011). This type of cheese is particularly popular with the Hispanic community.
- An outbreak in Quebec in 2008, with 38 cases, where a pasteurised milk cheese was implicated. 43% of cases had eaten the cheese, 43% more had bought other cheeses (made elsewhere) from retailers also selling cheese from the implicated plant. Ultimately contamination was traced back to one production plant, but there was also concern over cross-contamination at retail level (Gaulin et al, 2012).
- A large outbreak in 2007, in a tertiary care hospital in Norway. There were 17 cases, and all but two had underlying conditions or were receiving immunocompromising treatments. Most cases had symptoms of febrile gastroenteritis, but a few went on to develop possible CNS symptoms. The only food tested that was positive for *L. monocytogenes* was camembert made from pasteurised milk. Up to 6-million cfu/g were isolated from unopened packs (60g servings). The cheese had been served on three separate occasions. Other cases from outside the hospital had bought the same cheese at local markets or had attended a party where the cheese was served.
- An outbreak involving butter in a Finnish hospital. Case patients consumed four-times as much butter as controls, suggesting a continuous daily dose rather than a single exposure (Norton & Braden, 2007).
- Outbreaks have also occurred in Switzerland (2005), associated with locally made soft cheese and in the Czech Republic (2006), affecting a large number of people (78 cases) and associated with soft cheese (Allerberger & Wagner, 2009).

8.2. Outbreaks linked to sandwich fillings

Cases of listeriosis have predominately been linked to RTE foods, particularly meat (Awaisheh, 2013) and poultry products. These products have been implicated in both sporadic cases and outbreaks in Europe and in America (Mor-Mur & Yuste, 2010). In the UK, major sources of infection were multicomponent foods, finfish and beef products, both for foods affecting the general population and the over 60s (Little et al, 2010). Interestingly, the majority of outbreaks in hospitals have been linked to sandwiches, and mostly to sandwiches bought in ready-made. A number of cases and their root causes (where known) is given below.

- A UK hospital-acquired outbreak, involving three cases, all with underlying conditions. Patients were readmitted to the hospital with symptoms of listeriosis, having been in-patients in the previous month. Patients reported eating only hospital-provided foods whilst in-patients, and the food provided had been consumed immediately. The only common food exposures were pre-packed sandwiches and salads, although no single type of salad/sandwich was identified

as common. Pre-packed sandwiches had all come from the same supplier, but the salad had been prepared in the hospital kitchens. No isolates were recovered from the food from the kitchen. The sandwich manufacturer was following good manufacturing practice, with HACCP, and again no isolates were recovered when samples were taken. Breaches in temperature control were identified in the hospital, including accepting deliveries above 5°C and gaps in record keeping – especially at weekends. In addition, salads were not always washed properly and were given a two–three day shelf-life, rather than the recommended one day. These breaches were the most likely cause of the outbreak. Following the outbreak, food safety advice was reinforced and ward level storage, distribution and disposal practices reviewed (Coetzee et al et al, 2011).

- A chicken salad and chicken wrap sandwich were both found to contain *L. monocytogenes* isolates identical to those from two fatal cases. Both products were from the same producer (Marcus et al, 2009).
- An outbreak in a Manchester hospital involving sandwiches, in 2008. Food histories proved inconclusive in identifying a common source amongst five cases; three of which were in the hospital. *L. monocytogenes* was isolated from four sandwiches provided by an external supplier. Serotyping showed matching serotypes between most cases and tested sandwiches, except for one case where no match was found with food or environmental samples (HPA, 2008).
- An outbreak involving five pregnant women in the Swindon area in 2003. The only similarity in food histories (for the three months prior to symptoms), was that three of cases had eaten pre-packed sandwiches from a single retail outlet in the same hospital (during antenatal appointments). A fourth case was not sure if she had eaten the sandwiches, given she was being asked a long time after the event. Brie and cranberry sandwiches from a local supplier, and environmental samples from the manufacturing premises, including chopping boards, sink plug holes and a cleaning sponge, were positive for *L. monocytogenes*. The organism was not found at the premises of the supplier of meat and cheese for fillings (Dawson et al, 2006).
- Five cases of listeriosis in hospitals in New York. Those affected had a median age 62 years, and all had predisposing conditions. The only common food consumed/presumed consumed was cold, prepared tuna salad and sliced turkey. *L. monocytogenes* was isolated from the tuna salad and from the base of the can opener, as well as a drain. Tuna salad was prepared in the hospital in bulk and held for up to four days at 5°C before being plated and served. At this time a survey was also conducted amongst 53 hospital catering department. All served cold, prepared salads. 94% served them to pregnant women and 68-89% to other high risk groups. 81% served deli-style meats to any patient, with only 25% of these specifying heat treatment of the meat prior to serving (Cokes et al, 2011).
- Cases examined amongst three men, based on food histories only. One 48 year old man from Newcastle, receiving palliative chemotherapy and steroids, had eaten precooked chicken four weeks previously, but denied eating pate or soft

cheeses. A second man (79 years old) from Newcastle, being treated for carcinomas with palliative radiotherapy had eaten a sandwich from the hospital shop; A third 71 year old from Sunderland, being treated with steroids and beta$_2$-adrenoceptor stimulants and antibiotics, had eaten a sandwich from the hospital shop. All serovars isolated were 4b, as were isolates from a sandwich bought from the Newcastle hospital shop and from environmental samples at the caterers' premises (Graham et al, 2002).

- A hospital outbreak in Wales, in 2003, associated with an oncology unit affecting two patients, was linked to sandwiches from an outside supplier. Previous environmental contamination had been identified, and storage temperatures in the hospital sometimes exceeded 8°C, both in retail outlets and on the wards. Isolates recovered from patients were identical to those from sandwiches tested and from environmental samples taken in the supplier's premises (Shetty et al, 2009).
- Little et al (2012) looked at hospital-related cases between 1999 and 2008 (eight outbreaks). Sandwiches were implicated in all cases, with a variety of fillings – meat, cheese, fish, pate, salmon and egg. In all cases the underlying reason for contamination was cross-contamination at the sandwich manufacturer. In five outbreaks this was accompanied by storage above 8°C in the hospital (Little et al, 2012).
- An outbreak in a Northern Ireland hospital was linked to sandwiches, some of which had been brought to patients by family and friends, and which, it is suspected were stored in bedside lockers prior to consumption (HSCNI Outbreak Control Team, 2009).

9. Contamination during production

Todd & Notermans (2011) suggested that contamination from raw materials was generally associated with short term problems with the organism, whereas errors in facility maintenance, allowing the persistence of the organism, were associated with longer term problems.

Key reasons why foods present with high levels of *L. monocytogenes* include deficiencies in heat treatments (e.g. pasteurisation), post-process contamination, contamination of storage areas, introduction of *L. monocytogenes* into the environment through renovations or building works, contaminated raw ingredients, contamination of the processing environment (including specific steps, e.g. peeling/slicing, contamination of food-contact surfaces, surfaces covered by biofilm or visibly dirty (inadequate cleaning and sanitising)), adjustments made to deal with large demands (e.g. increasing production, but reducing disinfection time), long term colonisation of the environment, residues in packing machines, inadequate refrigerator space, temperature abuse in storage or distribution (of either ingredients or final product), and poor handling practices in manufacture and/or retail (Norton & Braden, 2007). Many of these are illustrated in the cases listed below, which also

highlight that it is usually more than one breakdown in food control procedures that results in a food causing illness. It is also worth noting that the long incubation time associated with invasive listeriosis means that many people are likely to have consumed the product before it is implicated and withdrawn from sale or recalled or corrective active taken.

- The same clonal type of *L. monocytogenes* was found in cold-smoked fish suspected to have caused an outbreak of listeriosis in Sweden. Fish residues were found in the packing machine. A previous routine health authority inspection carried out six months previously had found *L. monocytogenes* and these isolates were shown to have shared features with isolates found six months later. This suggested that the organism was part of the resident flora of the factory over a long time period (Tham et al, 2000).
- Following an outbreak associated with Mexican cheese, *L. monocytogenes* was isolated from a vat gasket in the post-pasteurisation section of the production line. As a result, an improved process flow was instigated, personnel were trained, and the faulty vat replaced. Later testing still found *L. monocytogenes*, and as a result the plant was closed. When the plant was stripped of equipment, an opening was found in the wall separating the raw milk processing room and the finished product room (Jackson et al, 2011).
- Following an outbreak in Quebec associated with cheese, samples from the dairy supplying the milk to make the cheese were found to be free of *L. monocytogenes* and the pasteurisation process adequate. However, 14.9% of the cheese sampled at the plant was positive for *L. monocytogenes*. The affected cheeses were largely soft-washed rind cheeses, and counts were in excess of 10^4cfu/g. Environmental samples showed 4.9% to be positive. Contamination was thought to be the result of using the same brine to wash cheeses over too long a period without replacing (Gaulin et al, 2012).
- Another Quebec outbreak associated with cheese was mainly associated with cross-contamination at retail level; 4.1% of retail environmental samples were positive, including knives, boards and the counter (Gaulin et al, 2012).
- An outbreak associated with sandwiches at a Manchester hospital; *L. monocytogenes* was isolated from the cutter blade in the processing environment, but no other problems were identified. However, insufficiently rigorous temperature control and storage in the hospital probably allowed *L. monocytogenes* to grow (HPA, 2008).
- Further outbreaks associated with sandwiches in a UK hospital in 2007. Several thousand sandwiches were withdrawn from hospitals in London and South East England when *L. monocytogenes* was isolated at high levels (above 100cfu/g) during routine sampling. The production environment at the supplying manufacturer was found to be positive for *L. monocytogenes*. Similarly, a suppliers' manufacturing environment was found to be positive for *L. monocytogenes* in another outbreak in 2004 where hospital sandwiches were implicated (HPA, 2008).

- An outbreak in South West England involving five pregnancy-related cases, in October 2003 where three (possibly four) cases were linked to the same hospital retailer. The same strains as had been isolated from patients were recovered from sandwiches and the sandwich suppliers' manufacturing environment (HPA, 2008).
- Two patients from an oncology unit in Wales had consumed sandwiches from a hospital canteen where there was insufficient control of storage temperature. The same strain was isolated from the sandwich factory, at low levels (HPA, 2008).
- Four cases involving immunocompromised patients in North East England in 1999. Patients developed listeriosis after eating sandwiches in the hospital. The same strain was isolated from the sandwich-manufacturing environment (HPA, 2008).
- A large outbreak in 2007, in a tertiary care hospital in Norway. *L. monocytogenes* was isolated from the floor, cheese cases and brine in the production plant (Johnsen et al, 2010).
- A hospital outbreak in 2003, in Wales, linked to sandwiches. Replacement of the floor of the factory was thought to be the source of the initial contamination, as well as use of the same cloth to clean floors and food-contact surfaces. The problem of contamination was exacerbated by poor temperature control in storage and display. This included staff on the wards removing sandwiches from chilled storage too far in advance of service (Shetty et al, 2009).
- A hospital outbreak involving German sausage showed the product to be contaminated. Evidence of contamination was also evident on worktops, in the vacuum filling machine, an apron of an employee and two drains (Winter et al, 2009).

10. Risk management and control

Control methods include the implementation of safety management systems, encompassing a number of controls, the application of physical methods, the use of antimicrobials, whether natural or synthetic and cleaning. Worryingly, a survey of hospital catering units in New York in 2009 (n=53), showed the majority had no food preparation policies or practices in place to minimise the risk of *L. monocytogenes* contamination (Cokes et al, 2011). Of importance is that management of (meat) safety risks involves all sectors, from producer, through processor, distributor, packer, retailer, food service worker and the consumer (Mor-Mur & Yuste, 2010). Thus those catering for vulnerable people in hospitals need to be confident in the quality of their raw materials and how they were produced, and also how the food is ultimately stored and served, as well as what happens in their kitchens. Thus industrial procedures have a role to play in minimising risks, dietary recommendations about avoidance of certain foods are also important, as is keeping control of, for instance, volunteer-run shops, where responsibilities for food hygiene practices may be less well-defined (Graham et al, 2002).

Many strategies have been investigated for the control of *L. monocytogenes*, including refrigeration, vacuum and MAP packaging and chemical additives (e.g. nitrite salts and nitrate, sodium lactate, sodium acetate and diacetate). However, the evidence suggests that these are not effective in fully controlling the organism (Awaisheh, 2013).

10.1. Food safety management

A number of safety management approaches are based on the principles of risk assessment and HACCP, which is particularly appropriate in the case of *L. monocytogenes*, where it is almost impossible to exclude the organism from the environment. Risk assessment relates to exposure to a hazard under particular conditions and the likelihood of adverse health effects, a process well established for chemical hazards (McLauchlin et al, 2004). For instance, the Canadian policy on *Listeria* contaminated foods is based on the principles of HACCP, based on a health risk approach involving industry controls, environmental and end product testing. It suggests sampling of food contact surfaces as being indicative of whether an establishment is operating under Good Manufacturing Practice (Farber et al, 2011). Swaminathan & Garner-Smidt (2007) also advocated the implementation of a food safety management system based on a stringent sampling system.

Hygiene standards and microbiological quality of RTE products has been found to be higher where management had food hygiene training and HACCP was in place (Little et al, 2009). A number of authors (Griffith, 2000; Seyler *et al.,* 1998; Noe and Schmitt, 1986) have noted that managers/supervisors have an important role in setting an appropriate culture within the work environment and providing conditions that facilitate behavioural change. In addition, in a study of food handlers in care homes, intention to perform safe food handling practices on all occasions was most strongly influenced by perceptions of what others thought they should do, emphasising the importance of other people in determining desirable behaviour (Seaman & Eves, 2008).

Recognising the capacity of an operation has also been shown to be important. Different levels and incidences of contamination were found in similar smoked salmon products made by different producers. High levels of contamination in one lot were thought to be related to the use of casual workers for slicing and packing operations when faced with increased processing activity (Cortesi et al, 1997). It is likely that the training and commitment of casual workers will be less than long term employees.

Examples of specific corrective actions include an interim purchase protocol to minimise the risk of contamination of sandwiches was put in place following the 2003 hospital outbreak in Wales. This included suppliers being required to provide evidence of registration with a Local Authority, current membership of a relevant trade association (e.g. the British Sandwich Association), copies of HACCP documentation, evidence of appropriate staff training, copies of quantitative

microbiological testing records and records of maintenance of temperature during transport. Suppliers were also required to inform the hospital if microbiological testing deviated from accepted levels (Shetty et al, 2009). Stringent temperature control has also been emphasised for sandwiches (HPA, 2008), and consumption as close to production as possible (Shetty et al, 2009).

Examples of practices to minimise the risk of listeriosis include steaming of RTE deli meats prior to service, not serving foods known to be high risk (or cooking them well, Elsner et al, 1997), using only cooked fruit and vegetables in cold salads or severely limiting the storage times for salads, development of evidence-based guidelines for shelf life of for instance, cold prepared salads, opened packets of deli meat not to be served to high risk patients, and restricting/forbidding the service of foods with a high risk of *L. monocytogenes* contamination to vulnerable groups (Cokes et al, 2011). HPA (2008) also noted giving advice to vulnerable groups about foods to avoid – including pate, smoked fish and mould-ripened soft cheeses. Buchholz & Mascola (2001) additionally mentioned that feta, brie, camembert and blue veined cheeses should be avoided, and leftover RTE foods should be heated until steaming hot, and that delicatessen meats should be either avoided or thoroughly reheated prior to consumption.

Recommendations for improving the safety of sandwiches include use of high quality ingredients, hygienic manufacture, appropriate shelf life and correct storage temperature (HPA, 2008), (Little et al, 2010). Also written food safety procedures should include ward kitchens, and ward fridges should be tested regularly to ensure they are running below 5°C. The authors also recommended that sandwiches should be consumed as close as possible to their production date (HPA, 2008).

Reduced shelf life has also been proposed. Swaminathan & Garner-Smidt (2007) predicted that if the storage time for deli meats was reduced from 28 days to 14 days, it would reduce the number of cases in the elderly by 13.6% – again emphasising that storage time is an important issue in the case of *L. monocytogenes*. This strategy was also employed after outbreaks in France, where the shelf life of RTE rillettes and jellied pork tongue was reduced from 48 days to 38 days, as there were concerns that the extended shelf life allowed *L. monocytogenes* to grow to potentially dangerous levels (Chan & Wiedmann, 2009).

10.2. Physical control methods

L. monocytogenes is easily inactivated by heat treatments (Goh et al, 2014) hence post-process heat treatments in pack would improve the safety of products. Heat resistance depends on for instance, the time/temperature ratio applied, the physio-chemical properties of food (pH, a_w, percentage of salt) and the food's organoleptic characteristics (Spanu et al, 2013).

FSIS-USDA (2003) recommends a 4-log cycle reduction of *L. monocytogenes* for successful thermal treatment of RTE meat products. The Food Safety Authority of

Ireland (2006) recommends 70°C for two minutes or more to eliminate any vegetative pathogenic microorganisms, and to achieve 6D reduction of *L. monocytogenes*. D values (minutes) reduced from 0.23–0.02 between heat treatments of meat slurry at 68–76°C respectively, which highlights the importance of time-temperature combinations. Thus 0.92 minutes at 68°C is equivalent to 0.08 minutes at 76°C (to give 4 $\log_{10}$ lethality), and 1.38 minutes at 68°C is equivalent to 0.12 minutes at 76°C to achieve a 6 $\log_{10}$ reduction in counts (Aguilar et al, 2013). The same authors noted that formulation can influence required time-temperature combinations, and thus lethality needs to be determined in the product of interest, not just in model systems, and also needs to be reviewed should formulations or other processing parameters change. They also found that fat seemed to have a protective effect during thermal processing, possibly as a result of lower heat conductance or reduced a_w in the fat portion.

Refrigeration is an important control measure, and some studies have investigated the difference in growth rates at temperatures commonly found in refrigerators, as well as below 5°C. These have shown that storage at lower temperature significantly reduces growth of *L. monocytogenes*. For some cheese products it is possible to apply lethal heat treatments after packaging. In the case of Ricotta, hot water pasteurisation reduced the level of contamination by 6 log in packaged products (Spanu et al, 2013). A similar immersion process has been shown to be effective for RTE deli-meats (Goh et al, 2014).

Thisted Lambertz et al (2012) reported that *L. monocytogenes* multiply substantially faster in naturally-contaminated smoked salmon at 8-10°C compared with 4°C. Ariahu et al (2010) also found that the organism multiplied more rapidly in soy milk at 10°C than 5°C in the presence of higher sugar concentrations and/or higher pH. Chan & Wiedmann (2009) found growth at 4°C was slow, with doubling times in the range 12 to more than 50 hours, and lag times from 59 – 477 hours (for vacuum packed and CO_2 packed roast beef respectively). Above 4°C, growth rate increases and lag time decreases, so storage of food able to support the growth of *L. monocytogenes* at 7–10°C greatly increases the risk that the organism could reach numbers that could cause human disease (Chan & Wiedmann, 2009). Similarly, Cortesi et al (1997) found that *L. monocytogenes* multiplied more rapidly at 10°C than 2°C, and Mahmoud (2012) pointed to a shorter generation time in smoked salmon at lower temperatures. In light of domestic refrigerators usually operating at temperatures well above the recommended 5°C, Swaminathan & Garner-Smidt (2007) estimated that if domestic fridges consistently operated at 7.2°C, the number of listeriosis cases would reduce by 69%. Storage time is also important, with the risk of *L. monocytogenes* growing to high numbers increasing with duration of refrigerated storage (Cokes et al, 2011). In particular, refrigerated storage prevents the growth of other microorganisms, allowing the selection of *L. monocytogenes,* which is usually a poor competitor (Lungu et al, 2011).

Interestingly, in products that also depend on the growth of lactic acid bacteria (producing lactic acid and lowering pH) to control pathogens, counts of *L. monocytogenes* drop more rapidly at higher storage temperature, as the lactic acid bacteria grow more rapidly and pH therefore reduces more quickly, controlling the growth of *L. monocytogenes*. This effect was seen in a mushroom sauce stored at 4°C and 8°C, but not in a cheese sauce, where the pH did not change so much during storage. In the cheese sauce, higher counts of *L. monocytogenes* were evident at 8°C (Grassi et al, 2013). The same effect has been seen as cheese matures. In cheese, as lactic acid develops and pH reduces, counts reduce, the rate of reduction being temperature dependent. Reductions occur more rapidly at higher temperatures, which promote the growth of lactic acid bacteria. Rate of destruction of *L. monocytogenes* in this way is also dependent on strain and initial counts, with the strain effects most apparent at low temperatures (4°C, Angelidis et al, 2013).

Another physical method of controlling *L. monocytogenes* is High Pressure Processing (HPP). HPP inactivates microorganisms and enzymes whilst maintaining nutrients and flavours. It inflicts lethal and sub-lethal injuries, mainly due to membrane damage. Sub-lethally injured cells are more susceptible to antimicrobials. In a study of fermented sausages, HPP alone had no listericidal effect, although previous studies have shown the effect to be strain dependent. Although the strains used to inoculate the sausage were strains previously shown to be susceptible to HPP in ham, food composition may have reduced effectiveness, as this has previously been shown to affect the lethality of HPP, for instance, low a_w protects against pressure-induced inactivation (Marco et al, 2013).

Ionising radiation markedly reduces numbers of *L. monocytogenes* (Mor-Mur & Yuste, 2010). X-ray irradiation at a dose of 1kGy removed *L. monocytogenes* to undetectable levels up to 25 days storage (Mahmoud, 2012). Electrolysed oxidising water can also reduce the number of *L monocytogenes* on RTE meat (Goh et al, 2014).

10.3. Antimicrobials in products

A wide range of antimicrobials have been tested for the control of *L. monocytogenes*. In particular there has been increased interest in natural antimicrobials as food preservatives, including plant essential oils. Essential oils have antibacterial, antifungal and antioxidant properties. Essential oils of clove, thyme, cinnamon, oregano, rosemary, sage and vanillin have been shown to be effective against various food pathogens. Awaisheh (2013) tested a variety of essential oils on strains of *L. monocytogenes* isolated from RTE meat. Essential oils of fir and qysoon had the greatest effect on all strains tested, with the effect increasing if the two essential oils were mixed. The essential oils did not eliminate *L. monocytogenes*, and the effect greater at high levels of contamination (3.15 log reduction compared to 1.4). The effect was most likely to relate to the content of alpha and beta pinene, 1, 8 cineol and borneol, which have been found to be active against *L. monocytogenes*.

Antimicrobials in packaging offers an alternative to post-packing operations, and have been suggested as more effective than adding antimicrobials to food as it localises antimicrobials on the product surface where the contamination usually is. In addition, there is lower inactivation by adsorption of the antimicrobial by the food constituents. The method can also be used in combination with other controlling processes e.g. HPP. Nisin in packaging induced pronounced reduction of *L. monocytogenes* counts during 90 days of refrigerated storage. Nisin inhibits the growth of a wide range of Gram negative organisms, including *L. monocytogenes*, in a number of food systems (Marco et al, 2013).

Chlorination is considered one of best ways to minimise transmission of pathogens and is the most commonly used sanitiser to treat fresh products. Sodium hypochlorite is a powerful disinfectant with oxidising properties, and is active against many organisms, but harmless to humans at low concentrations (Domenach et al, 2013). It has been shown to be effective against *L. monocytogenes*, decreasing counts by 1 – 2 logs on dipping in concentrations of 4 – 40ppm for an exposure time of one to 30 minutes. Both time and dose are significant. A concentration of 40ppm chlorine for 30 minutes was most effective (giving a 2 log reduction in cfu/g), with the main reduction occurring in the first 30 seconds (1 log). At home, the usual time for washing fresh produce in water with a concentration of 0.7ppm (tap water) is, however, only 10 seconds resulting in less effect (a reduction of only 0.6 log cfu/g). In all cases vegetables had been artificially inoculated to represent recent contamination. Under these circumstances organisms should be easier to remove as there has not been enough time for adhesion by bacteria to surfaces or the development of biofilms (Domenach et al, 2013). Thus in cases where time has allowed adherence of the bacteria, and biofilm development, the effects may be reduced.

10.4. Cleaning regimes

Given the high incidence of contamination of processing and preparation areas associated with outbreaks of listeriosis, effective cleaning is critical to control the organism and avoidance of cross-contamination.

Chlorine, iodine-based, acid-anionic and quaternary ammonium type disinfectants have been shown to be effective at eliminating *L. monocytogenes*, however, these are often tested in model systems. Efficacy can be significantly reduced in the presence of organic material, or when cells are dried onto, or attached to surfaces (Earnshaw & Lawrence, 1998). These authors tested a range of commercially available disinfectants intended for use in the food and beverage industry. When cells were in suspension, disinfectants at manufacturers' recommended concentration led to more than 5 $\log_{10}$ reduction in cfu/ml, with no difference between strains of *L. monocytogenes* or disinfectants. There were significant differences between strains using lower dilutions of the disinfectants; but if a strain was less resistant to one disinfectant it was generally sensitive to another, which highlights the need to rotate the use of disinfectants. Quaternary ammonium compounds were

found to be most effective against suspended cells, their action being due to reacting with cell membranes, denaturing essential cell proteins and inactivating cellular enzymes. Chlorine is thought to damage the cell membrane resulting in leakage of cell components. It also forms substitution products with proteins and amino acids and is very effective against unattached *L. monocytogenes* cells (Earnshaw & Lawrence, 1998).

The food industry uses a variety of sanitisers and cleaners to prevent accumulation of microbial cells and consequent biofilm formation. Some of these, however, are not very effective against some bacterial biofilms owing to differences in susceptibilities, and altered the physiological state of some cells in biofilms (Cruz & Fletcher, 2012). Earnshaw & Lawrence (1998) suggested that chlorinated alkaline detergents are more effective at removing biofilms. Failure to remove chemical and biological residues creates conditioning films for the initial stages of biofilm formation, facilitating cell attachment and becoming increasingly difficult to remove. Pathogens attached to surfaces can easily be transferred to food (Koo et al, 2013).

Preventing the formation of biofilms is through inhibiting initial adherence and colonisation, interfering with molecular signals modulating the growth of biofilms, and disintegration of the extracellular polymeric substance (EPS). This is mainly achieved through cleaning and sanitising (da Silva & Martinis, 2013). Conventional methods, however, are most effective against recently deposited organisms; mainly due to EPS protection of cells that are in established biofilms (da Silva & Martinis, 2013). Thus *L. monocytogenes* attached to surfaces is more resistant to disinfectants than planktonic cells (Koo et al, 2013). Enzyme-based cleaners that break down EPS are important for eradication of biofilms, and improved disinfectant efficacy. The use of bacteriocins may also help control initial adhesion and biofilm formation on abiotic surfaces (da Silva & Martinis, 2013).

Cruz & Fletcher (2012) tested 21 sanitizers on 20 strains of *L. monocytogenes* in suspension and in biofilms. The chemical groups of sanitisers were: acidified sodium chlorite, biguanide, chlorine dioxide, iodine, peroxyacetic acid, and quaternary ammonium compounds. They were tested at recommended levels and also dilutions. For cells in suspension, lower concentrations than recommended were required to achieve 5-$\log_{10}$ decrease in initial inoculum (consistent with Earnshaw & Lawrence (1998)). The required concentrations when cells were in biofilms increased measurably, independent of sanitiser type. Only acidified sodium chlorite, peroxyacetic acid (brand dependent) and chlorine dioxide products achieved a 99.999% reduction at recommended doses. Halogen based sanitisers were most adversely affected, requiring 10 – 18 times the recommended dose to achieve a 99.9999% reduction, independent of strain (Cruz & Fletcher, 2012). In this study the biofilm comprised a simple, mono-species; in nature the biofilm would be more complex, with mixture of organisms, and may thus be more resistant. Peroxyacetic-based sanitisers were thought to be effective as the small molecule was able to

penetrate the biofilm extracellular polymeric substance matrix, its mode of action and tolerance of moderate levels of organic matter (Cruz & Fletcher, 2012).

The age of the biofilm is also important and stresses the need to avoid the development of biofilms. Yang et al (2009) looked at the efficacy of commercial sanitising agents against biofilms of different ages on cutting boards. Effectiveness was significantly better against younger (seven day) biofilms than older ones (21 days) on smooth surfaces. For seven and 14 day biofilms, efficacy was higher on smooth rather than rough surfaces. Quaternary ammonium compound-based sanitisers were least effective.

The effectiveness of sanitisers is also affected by the food matrix. *L. monocytogenes* in fat-containing emulsions is less affected by sanitisers, as the fat protects the organism. However, in fish emulsions, unsaturated fats cause denaturation and oxidation of bacteria, and so enhance the effectiveness of sanitisers (Koo et al, 2013).

Cleaning cloths play an important role in the initial stages of removing pathogens from surfaces (Koo et al, 2013). The authors tested cleaning cloths (two blended cellulose/cotton of different thickness (0.18 and 0.23cm), microfibre, non-woven wipes, scouring pad and terry bar towel). The cloths led to significant reductions in *L. monocytogenes*; up to 2.62 and 3.44 log/cm^2 on stainless steel and Formica respectively. Cellulose/cotton and microfibre were most effective on stainless steel (up to 2.62 log/cm^2 reduction), and cellulose/cotton was most effective on Formica (up to 3.44 log/cm^2 reduction). Cloth thickness did not make a significant difference.

An alternative is the use of antimicrobials in cutting boards, e.g. Triclosan, an antimicrobial allowed in plastic food-contact materials. It has been shown to be effective against a wide range of pathogens. Similarly, boards containing silver have antibacterial activity (Goh et al, 2014).

10.5. Specifications

Development of specifications can be useful in minimising contamination of foods brought into an organisation, whether they are raw materials or finished products.

For fresh produce less than 100 cfu/g is given as a safety objective e.g. ICMSF (Ding et al, 2013). Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs states that RTE foods unable to support the growth of *L. monocytogenes* should not exceed 100 cfu/g at the end of shelf life (foods with a pH <=4.4 and a_w <=0.92; or pH <=5.0 and a_w <=0.94 with a shelf life of less than five days are considered to be RTE foods not able to support the growth of *L. monocytogenes; (*Angelidis et al, 2013, CFA, 2010). For foods that can support the growth of *L. monocytogenes* 100 cfu/g should not be exceeded which applies during the shelf life, unless the food business operator (FBO) cannot demonstrate sufficient evidence to prove this is the case, e.g. shelf life studies. In these cases absence in 25g is required before the food has left the control of the FBO. Norrung (2000) suggested lower levels may

need to be applied at port of entry in international trade, if the counts are not to be exceeded on consumption. They proposed a decision tree approach to determine sampling requirements and acceptable levels through to consumption taking account of the listericidal effect of processing, likelihood of recontamination, treatments applied immediately before consumption, and likelihood of multiplication of organisms during storage and distribution.

Other authors have suggested zero tolerance of *L. monocytogenes* for RTE products (Swaminathan & Garner-Smidt, 2007), and Coetzee et al (2011) recommended that food for hospital patients should be free of *L. monocytogenes* and other potential pathogens. The British Sandwich Association recommends less than 10cfu/g for *L. monocytogenes* in sandwiches (HPA, 2008).

11. Guidance

11.1. General guidance

Sources of guidance include standard operating procedures within food safety management systems based on HACCP, news reports, government produced fact sheets and industry codes of best practice.

The syntax, content and number of pages for guidance reviewed varied depending on the audience and contextual setting. Guidance for at risk groups is generally the easiest to read and understand with its clear messages for risks and prevention. The most detailed and technical information is generally found in guidance for dietitians, nurses and those responsible for HACCP within a food manufacturing setting. Most guidance provides background information on *L. monocytogenes* (e.g. sources, common food vehicles, etc.) and symptoms of listeriosis. All guidance outlines control measures on how to reduce the risk of infection.

11.2 Guidance available to vulnerable (at risk) groups

General guidance to enable at risk groups to make informed decisions is widely available in English speaking countries (UK, Ireland, USA, Canada, Australia, and New Zealand). Some information was available in Welsh (UK), Spanish (USA). Guidance can be given passively (HPA 2011) or discussed with those at risk by a healthcare professional (NZ Herald 2013). Information includes guidance on which foods pose the most risk, temperature control, cleaning and disinfection, expired perishable foods, symptoms and statistics of incidence for specific groups. There is also evidence of guidance produced by nutrition and dietetic services within NHS Trusts, including robust advice on high risk foods to avoid whilst neutropenic.

Failure to give vulnerable groups information about the risk to them of listeriosis has led to death. In one case in New Zealand, the husband of a woman who died of listeriosis in 2011 is demanding why his wife was not given an ‘Avoiding Listeria’ brochure during her treatment for Crohns disease. The husband believes his wife

would have definitely avoided certain foods if she had known the risk to her health (NZ Herald 2013).

The NHS website (2013) provides comprehensive guidance for those more vulnerable to listeriosis, but there seems to be a preference for pregnant women over other at risk groups. There is no further detailed information for those aged over 60 years or immunocompromised. This preference for guidance for pregnant women is a common theme for information available to the general public. Dr Bob Adak also suggests information on *Listeria* is given 'passively' and 'mainly to pregnant women' (HPA 2011). He also advocates more should be done for other groups to advise them on how to protect their health. The Centres for Disease Control and Prevention (CDC 2013) provide general factsheets (26 pages) on food safety for pregnant women, older adults, transplant patients, those with diabetes and persons with HIV/AIDS.

Most guidance for pregnant women states both the risk to mother and infant(s). Some recognise that pregnant mothers need to balance the risk of listeriosis with nutritional intake (PBCDH 2013) and therefore list different types of cheese that represent a low risk. The Food and Drug Administration (2013) states in its guidance Hispanic woman are more at risk from listeriosis, due to the consumption of traditional home-made cheeses made from unpasteurised milk. A report by the Health Protection Agency (HPA 2010) also suggests a higher incidence of listeriosis in pregnant women from ethnic minorities and those living in deprived areas. The reasons for this higher incidence include: established food safety messages not getting through, food safety controls not being followed and reliance on convenience and local shops. Cultural and economic factors are not usually stated in guidance for pregnant women.

Guidance for listeriosis in older adults generally refers to those over 60 years of age. There are no specific reasons in guidance aimed at the general public for why this group is more at risk other than being immunocompromised. FSA (FSA 2009) research findings in August 2009 suggest people over the age of 60 are more likely to take risks with 'use by' dates than younger people because they are reluctant to throw away food. Other research commissioned by the FSA (FSA 2009) suggest the over 60s were confused by fridge temperatures. Psychological factors are not addressed in general literature. General guidance is also available for other groups at risk including those whose immune system has been weakened by disease or illness. Examples include cancer, leukaemia, kidney or liver disease, AIDS, diabetes, and anyone on medication that can suppress the immune system (e.g. organ transplant patients). Some guidance states increased risks for specific groups: cancer patients (HPA 2010) persons with AIDS (CDC 2011) and kidney transplant patients (CARI 2010).

11.3 General guidance for healthcare setting

Those who work in a healthcare setting with a role in reducing listeriosis include catering staff, care workers dietitians, nurses and other medical staff. Guidance can be general or specific to the contextual setting or designated role. Some guidance also suggests relatives or friends of those staying in a healthcare setting must also follow food safety guidelines to reduce the risk of foodborne illness.

The FSA has guidance on its website entitled 'Preventing listeriosis in Hospitals and Nursing/Care Homes' (FSA 2013). This one page document was last updated in January 2013 and provides guidance for reducing the risk of listeriosis. Statistics and food safety law are used to emphasise the moral and legal obligations for food safety in a healthcare setting. Guidelines include fridge temperatures, use by dates, restricted foods and advice for relatives for not bringing in RTE foods. There is no guidance on cooking, reheating and cross-contamination. It is also unclear whether the document is aimed at management or food handlers as the depth of detail can vary, especially information signposted from the hyperlinks.

11.4. Guidance for hospital setting

Background information on *L. monocytogenes* including guidance on how to reduce the risk of listeriosis in a hospital setting does vary in scope, detail and complexity. Some hospitals include a dedicated section on listeriosis within documentation for the food management system with additional links to supplementary information and others have a specific policy on listeriosis. Despite this guidance outbreaks of listeriosis do occur in hospitals (HPA 2008).

The procedures produced by a NHS Trust in Manchester in 2012 provide comprehensive guidance on food safety in a ward and therapeutic kitchen. Within the document is an organisational structure for those accountable for food safety and meticulous descriptions for the duties in relation to control measures at each level. This organisational structure supports a positive food safety culture. Similar organisation structures are found within the food safety procedures of other NHS Trusts reviewed in the UK. Other similarities between NHS Trusts include fridge temperatures (at or below 5^{O}C), shelf life timescales, strict guidance on food brought in by visitors, food kept in patient lockers and use of ward kitchens by relatives. Guidance for bringing in food for patients outlined by one NHS trust in the southwest is particularly good. The website page states ethical reasons for restricting certain food for patients, the use of a well-trained nurse to check which foods are acceptable and the availability of a nurse in charge or dietitian for further advice. Other guidance includes a graded system for neutropenic diets (grades 1 and 2) to help maximise the food choice and minimise the use of unnecessary restrictions and guidance on storing staff food in ward kitchens.

Terminology is an import factor for caterers, dietitians and other healthcare professionals in assessing the risk to patients. Groups of people who have a higher risk of listeriosis infection are referred by the NHS as 'At Risk Groups'. Common

terms used for foods with low microbial content include neutropenic diet, sterile diet and clean diet. Any future guidance must take into account these terms to avoid confusion and to promote a consistent message.

The grey literature review also examined guidance for hospitals in USA, New Zealand and Canada. Food Standards New Zealand (2008) provides general guidance for vulnerable groups in various healthcare settings in Australia. Specific controls for *Listeria* include using freshly cooked meats instead of RTE cold meats, freshly prepared salads and storage limits for prepared foods not exceeding 24 hours. The EHA Consulting Group in the USA suggests food safety training commensurate for workers at all levels and advocates third party audits to identify problems and verify good practice. Canadian guidance produced by Fraser Health in 2010 states resident assessment and care plan should in place upon admission to a residential care facility or private hospital. It is also important to emphasise that some areas of the food premises may not be routinely cleaned and could be a reservoir for *L. monocytogenes* including drains, tin openers and changing facilities. This argument is supported by investigations conducted in the UK, USA and Ireland. Following a listeriosis outbreak at a hospital in New York a microbiological sampling survey revealed *L. monocytogenes* contamination in a floor drain in kitchen and a tin opener used for opening tuna (Infection Control Today 2011). Another survey conducted in 2011 by the Food Standards Authority Ireland highlighted serious lapses in cleaning and disinfecting meat slicers used to slice cooked meats: 'No FBO with a documented schedule had precise information on how the meat slicer should be cleaned and sanitised' (Food Standards Authority Ireland 2011).

11.5. Guidance for nursing/care home setting

The FSA produced Safer food, better business (SFBB) supplement for caterers and staff working in a small residential care homes to cover extra care, protecting food, gift food and mini kitchens (FSA 2013). This additional guidance acknowledges older adults are more at risk from foodborne disease rather an increased risk of listeriosis. There is a hyperlink to a factsheet for further information on listeriosis. The supplement asks management and food handlers to assess whether food donations and gifts have been handled safety before accepting them. The supplement suggests food handlers or care workers should give advice to family and friends about what food is safe to bring in as gift, but encourages lower-risk foods. There is no guidance on what to do if relatives insist on bringing restricted foods.

11.6. Guidance for sandwich bars and similar food service outlets

Sandwiches have been implicated in hospital acquired listeriosis (HPA, 2008). Outbreaks are often associated with lapses in temperature control during storage, accompanied by a product contaminated during production. The FSA (FSA 2013) has published a Food Industry Guide to Good Hygiene Practice: Sandwich Bars and Similar Food Service Outlets. This guidance may be useful for caterers in hospitals

and care/nursing homes that prepare and sell sandwiches open-served to be consumed directly by customers or wrapped and pre-packaged before they are sold.

The 93 page guide provides advice on achieving the principles of 'best practice' during the preparation of sandwiches and provides comprehensive information on: product handling, temperature control, pest control, staff training and the principles behind hazard analysis. There are specific references throughout the guidance on *L. monocytogenes*, including external links to temperature control, contamination, shelf life, microbiological testing etc. Appendix 11 within the guidance emphasises vulnerable groups in hospitals and stringent controls required to minimise the risk of listeriosis.

Outpatients may buy sandwiches from a restaurant or shop within the hospital (HPA, 2008). These sandwiches are likely to be displayed in a chilled cabinet. Additional guidance on the display of sandwiches can also be found in Food Hygiene Management (Springer 2012). This popular textbook used on Level 4 food safety courses provided some clear guidance on the storage of sandwiches in a chilled display cabinet. The cabinet should comply with BS 3035 and should be stocked with pre-chilled sandwiches below the load line, and sandwiches should not be stocked below fluorescent lighting as radiant heat can raise the surface temperature to unacceptable levels. Springer (2012) also lists ingredients used to make made-to-order sandwiches which must be pre-chilled before service to limit the growth of bacteria.

11.7. Guidance for food manufacturers

It is known that outbreaks of listeriosis in hospitals, nursing/care homes and the community have been linked to the consumption of contaminated RTE foods provided by an external supplier, including cooked meats, sandwiches, dairy products and prepared fresh produce.

Diversey and the University of Nottingham have produced a practical eight page guide for food manufacturers about the biology and behaviour of *L. monocytogenes* in the food production environment. The ‘Dealing with *Listeria*’ guide explains how this organism led to previous outbreaks of disease and gives illustrations of ways that the problem of *Listeria* can be addressed within the factory environment. It also states that even when all reasonable hygiene measures have been put in place, problems can still arise.

The document states long term strategies for dealing with *Listeria* spp. and requires managers to really understand the epidemiology of the organism. This is particularly important in maintaining the HACCP plan. The guidance examines key pieces of equipment known to be sources of contamination in a factory environment and contributory factors in large outbreaks in USA (melons 2011) and Canada (cooked meats 2008). It states the basic precautions required to control *Listeria* spp. are the same as those to control all foodborne pathogens. However, there are five key messages to control *L. monocytogenes* stated within the document:

- Maintain and clean the processing environment
- Establish good personal hygiene and clean working practices
- Clean food contact surfaces
- Prevent cross-contamination
- Control water.

Diversey and the University of Nottingham have also produced a practical seven page guide for food manufacturers on the risk of *Listeria* in fresh produce. The document includes background information on the characteristics of *Listeria* in fresh produce and examines the roots causes of previous outbreaks. There is some debate within the document about the internalisation of *L. monocytogenes* in plant via different routes of entry, e.g. hydroponic systems. Guidance for the control of *L. monocytogenes* in fresh produce include surface sanitisation of the fresh produce, general plant or factory sanitation and employee hygiene must be taken into consideration for effective control of *Listeria* spp.

12. Conclusions

The literature available on *L. monocytogenes* and listeriosis is extensive. There are many shared perspectives on subjects such as biological and behavioural characteristics relating to the pathogen, vulnerable groups, causal factors for outbreaks, common food vehicles and control measures to reduce risk. We are confident the triangulation of the literature on this subject is comprehensive and conclusive within the boundaries of the resources available.

The literature review has highlighted the importance of understanding the epidemiology of *L. monocytogenes*. It is clear that certain groups within the population are more at risk from listeriosis than others, particularly neutropenic patients, pregnant women, neonates and the over 60s. Health Protection Agency statistics show a significant decrease in reported infections for pregnant women in England and Wales. This may suggest the message about the risks of listeriosis is getting through to pregnant women – but not all. Studies suggest a higher incidence of listeriosis for pregnant women from ethnic groups due to cultural and economic reasons. The increase in reported cases of listeriosis in the over 60s is still in debate, but credible reasons include improved case recognition, surviving longer with chronic conditions, confusion over fridge temperatures and not disposing of food past its ‘use by’ date. Common food vehicles implicated in outbreaks are clearly identified. Soft and homemade cheeses, butter, cooked meats, pate, cantaloupe melons and vegetables have all led to incidents. Ready-made sandwiches is the food vehicle frequently linked to outbreaks of listeriosis in hospitals in England and Wales (HPA 2008). Guidance for vulnerable groups on which foods pose the higher risk is widely available. Literature on these high risk foods also states how to reduce the risk of contamination and multiplication.

Knowing the biology and behaviour of this pathogenic organism in the environment and how contamination can occur is clearly a pre-requisite for those responsible for the management of food safety and food handlers. Information commensurate to role should be included within the *Listeria* guidance.

Outbreaks are often associated with product contamination and lapses during storage and display. *L. monocytogenes* can survive as a resistant biofilm in food processing environments, thus effective cleaning and disinfection is critical. But investigation by food safety authorities demonstrate cleaning regimes are not always known or observed, particularly documented hot spots for *Listeria* contamination. *Listeria* guidance for hospitals and nursing/care homes should include information on effective cleaning and disinfection. Key areas may include cleaning of complex equipment, the selection and use of sanitisers, and cleaning cloths. Temperature abuse is a common factor in outbreaks of foodborne illnesses and management have a responsibility to ensure food handlers know the target levels and critical limits to control the growth of *L. monocytogenes.* It is clear in some catering establishments that management of appropriate fridge temperatures is an issue. The guidance should examine how to take a more direct approach to monitoring temperature, including checking records, observations and if necessary intervention to rectify problems in training and/or attitude.

Training is only effective when correct knowledge is put into practice and competence is verified on a regular basis. The trainer should be competent and the delivery of training must be inclusive, commensurate and contextual. The training of food handlers should be verified in the workplace and not just in the classroom. This can be done by asking food handlers questions on food safety controls and observing work practices. Training alone will not absolve the risk of incidence. It is clear that root causes of outbreaks across the world are also down to human error or deliberate violations. These psychological factors are important and should be addressed to reduce the risk of an incident.

Clearly monitoring and auditing do have important roles to play in reducing the risk of listeriosis, but incidents will occur if these processes are not carried out correctly. Guidance for management on monitoring and auditing should be included within the *Listeria* literature, with a particular emphasis on people, premises, product and process.

Suppliers of RTE products have been implicated in many outbreaks across the world. Many larger caterers will have robust protocols in place to select a safe supplier, including mandatory accreditation and site assessment. But some smaller caterers may not be so robust due a lack of knowledge or resources. The FSA does address suppliers within the SFFB pack and includes a checklist for certification and quality assurance, although a question does arise in measuring and benchmarking standards for certification and quality assurance. This is an area that should be addressed in the *Listeria* guidance.

13. Considerations for guidance on *Listeria* for hospitals and nursing/care homes

The literature review revealed expected and unexpected insights into *Listeria*, *L. monocytogenes* and listeriosis in a variety of contextual settings. Suggestions arising from the review for inclusion guideline are as follows:

- Guidance should be available for those responsible for the management of safety and food handlers.
- Guidance should be available for hospitals and nursing/care homes to reflect different contextual setting.
- Concise information for management on how to select a reputable supplier for RTE products. The content should refer to specific supplier accreditation, other quality management systems and trade memberships.
- Comprehensive information should be included on cleaning and disinfection procedures for the fabric of the food premises and catering equipment - especially complex equipment. Consideration should also be included for the design of food contact surfaces to avoid the build-up of biofilms. This information should be commensurate to management and food handlers.
- Guidance should cover how to clean and sanitise raw fruits and vegetables. Information should include recommendations on the most effective chlorine wash for *Listeria* spp. and other pathogens. Such guidelines are available within the FSA E-coli guidance (third edition).
- Guidance should state target and critical temperature limits for the control of *Listeria* throughout the chain of activities on site (delivery to consumption).
- Guidance should include clear guidance for shelf life for RTE products.
- Food production processes should be included in the guidance.
- Manufacturing specifications are a consideration for the guidance, but there will be debate on inclusion. For example should guidance go beyond the current legal limit and suggest RTE foods for vulnerable groups should be absent of the organism in 25g?
- Guidance should include whether foods should be restricted and those that require additional care to avoid listeriosis. The guidance should address the balance of the risk of listeriosis against nutritional intake and psychological factors.
- Information on the standards of delivery and content of training should be included within the guidance.
- The guidance should cover foods provided by visitors and safe handling of food gifts and donations.

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