Food Control 75 (2017) 1–13

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Review

A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments

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ARTICLE INFO

Article history:
Received 26 October 2016
Received in revised form
9 December 2016
Accepted 11 December 2016
Available online 12 December 2016

Keywords:
Listeria monocytogenes
Listeriosis
Outbreaks
Whole genome sequencing
Virulence
Dose response
Ecology
Growth/no growth
Risk assessment

ABSTRACT

Improved control measures starting in the 1990s have greatly reduced the prevalence of *L. monocytogenes* in many food categories, particularly in meats and meat products. However, the rate of listeriosis has remained constant during the last decade and the more severe, systemic (invasive) form of listeriosis is now recognized as occurring more frequently in small outbreaks than previously recognized. This review addresses the recent advances in epidemiology and virulence, in growth and modelling, and insights from the risk assessments. Recognition of recent outbreaks from food vehicles not traditionally associated with *L. monocytogenes* (celery, cantaloupe, mung bean sprouts, stone fruits, caramel apples and ice cream) was facilitated by PFGE and, increasingly, whole genome sequencing. The Key Events framework, an understanding of the key individual biochemical steps from ingestion to infection, provides a structure for relating new knowledge on strain variability, mutations, and host susceptibility to the probability of illness. Guidance for determination of the growth/no growth potential of a food has been issued by several regulatory authorities and the risk assessments indicate that prevention of growth remains a principle control element. The recognition of biofilm formation and the possible existence of dormant, non-dividing persister cells will require additional attention. The recent outbreaks underscored the individual characteristics of specific foods (melons vs all fruit; microenvironments in the caramel apples) and raised questions about the current understanding of infectivity of lower doses and the susceptibility of specific individuals. Advances have been made in these areas, but further research is clearly necessary to control this pathogen.

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http://dx.doi.org/10.1016/j.foodcont.2016.12.016

1. Introduction

Listeria monocytogenes remains a significant cause of foodborne illness. Even though the illness is in most cases expressed as a mild, febrile illness, it can also present as systemic (invasive) listeriosis with more severe symptoms and high hospitalization and case fatality rates. The incidence of listeriosis is low in the general population despite the wide distribution of the microorganism in the environment and the relatively high frequency of isolation in foods. The incidence of systemic listeriosis is much higher in susceptible populations, including pregnant women, the elderly and individuals with compromised immune systems.

Improved control measures starting in the 1990s have greatly reduced the prevalence of *L. monocytogenes* in many food categories, particularly in meats and meat products. However, the rate of illness has remained constant during the last decade. Furthermore recent outbreaks have challenged the conclusions of existing risk assessments and our understanding of the influence of virulence, host and food matrix on foodborne illness.

L. monocytogenes is widespread in the environment, and control of *Listeria* in food production facilities requires constant focus by risk managers. Therefore, better understanding of the characteristics of the microorganism, environmental impact, and interactions of virulence factors with host susceptibilities is necessary to devise better control measures to reduce the incidence of listeriosis.

2. Purpose of workshop and this paper

A workshop was hosted by the Joint Institute of Food Safety and Applied Nutrition (JIFSAN), University of Maryland and the Grocery Manufacturers Association Science and Education Foundation at the Greenbelt Marriott Hotel, Greenbelt, MD on *June 16-18th 2015*. The purpose of this workshop was to facilitate a discussion amongst experts to evaluate the latest information on risk factors of *L. monocytogenes,* to determine what additional information is needed to answer remaining questions on *L. monocytogenes* risks, and to facilitate the development of effective risk management strategies. In 2011, the Interagency Risk Assessment Consortium (IRAC) and the Joint Institute for Food Safety and Applied Nutrition (JIFSAN) sponsored a workshop that summarized the state of knowledge at that time about *L. monocytogenes* and foodborne listeriosis, and identified additional information that would be needed to improve assessment of *L. monocytogenes* risk (Hoelzer et al., 2013).

The 2015 workshop reviewed advances in our understanding to see if we addressed the gaps identified in the 2011 workshop and to see if there were any new information needs. The workshop asked additional questions that could be important to risk managers. Specific areas of interest were factors impacting virulence, criteria to evaluate whether a foodstuff supports the growth of *Listeria*, reviewing knowledge gained from recent outbreaks, evaluating the scope of existing risk assessments to determine what additional questions are relevant for risk managers (i.e. U.S. FDA/FSIS, 2003; FAO/WHO, 2004ab; U.S. FDA/FSIS, 2010).

The workshop participants addressed questions in three areas:

2.1. Recent advances in epidemiology and virulence

What do the recent outbreaks from food vehicles not traditionally associated with listeriosis tell us? Do differences among strains have an impact on virulence of specific immunocompromised subpopulations? What has been the impact of genetic tools such as Whole Genome Sequencing (WGS) and Pulsed field gel electrophoresis (PFGE) on outbreak identification and strain traceability?

2.2. Recent advances in growth & modelling

Based upon current understanding of public health impact, are any changes needed to the current approaches or focus on the management of the manufacturing of products in which *L. monocytogenes* cannot grow? Do we fully understand the ecology of growth and survival; in particular, are the definition criteria to define “products in which *L. monocytogenes* cannot grow” clear and scientifically sound? Can persister cells, which have enhanced survival capabilities, be present in foods?

2.3. Insights from risk assessments

Do recent incident/case investigations raise concerns about

L. monocytogenes that have not been previously addressed in risk assessments?

What are the gaps in currently available risk assessments? What information is needed to address questions of the public health impact and risk management strategies in food products in which the microorganism cannot grow? What are the risk management questions that need to be addressed by additional risk assessments? What are the data needs to address these gaps?

3. Recent advances in epidemiology, virulence and dose response

3.1. WGS/PFGE and impact on outbreak identification and strain traceability

Listeriosis has been a notifiable disease in the US since 2001. The development and implementation of molecular typing, PFGE, has allowed public health and regulatory agencies to track strains of pathogenic microorganisms, identify associations and outbreaks of foodborne illness from cases distributed geographically or through time, and support the link of clinical cases with individual food products. WGS, which determines the nucleotide sequence for the entire bacterial genome (in theory), is now technically feasible to use in routine epidemiological investigations and has provided greater precision in the information available to public health agencies.

A variety of bioinformatics tools have been developed to analyze and compare WGS data. Among them, the U.S. Food and Drug Administration's (FDA) Center for Food Safety and Applied Nutrition (CFSAN) Single Nucleotide Polymorphism (SNP) Pipeline compares differences in SNPs and the whole genome multilocus sequence typing (wgMLST) implemented by CDC compares differences in alleles (i.e. determines whether alleles are the same or where specific loci are different) to ascertain the relatedness of the different isolates. The U.S. Centers for Disease Control and Prevention (CDC) started implementing WGS in the *Listeria* initiative in 2013 and FDA, CDC, and FSIS have transitioned/are transitioning to routine use of WGS. FDA, in collaboration with other partners, initiated the GenomeTrakr Database, a public database of genome sequences that now contains thousands of sequences of *L. monocytogenes*, with that number rapidly increasing.

Food and environmental *L. monocytogenes* isolates collected by the U.S. Department of Agriculture Food Safety Inspection Service (USDA FSIS) and FDA are sequenced and evaluated for matches by CDC. This has identified case-food pairings beyond what was previously possible. As a result, more outbreaks have been identified with fewer cases and in foods not previously a focus of investigation. The CDC is currently undertaking a five year initiative of molecular surveillance to further link clinical and food isolates with the expectation of replacing traditional assays (Jackson et al., 2016). WGS can be used for both long term and short term global epidemiology of *L. monocytogenes* and can also be used to study virulence, evolution and population diversity of *L. monocytogenes*.

3.2. Food vehicles and outbreaks

Improvements in detection methodologies recent years have identified a larger number of outbreaks with fewer cases per outbreak. In some cases, implicated foods have not been considered by past experience and risk assessments to be likely vectors. In addition, expanding the definition of susceptible populations to children and considering the possibility of infection from consuming low doses were indicated by the recent outbreaks. Sections 3.2.1 and 3.2.2 summarize some of the recent findings.

3.2.1. The European Union

The EU has seen an increase in notifications (of both listeriosis outbreaks and sporadic cases). EFSA reported that in 2013, 1763 confirmed human cases of listeriosis were reported in 27 member states (EFSA, 2015). The EU notification rate was 0.44 cases per 100,000 population (an 8.6% increase compared with 2012). The vast majority of cases were reported to be domestically acquired. On average, 99.1% of the cases were hospitalized, which is the highest proportion of hospitalized cases of all zoonosis under EU surveillance. The EU case fatality rate was 15.6% among the 1228 confirmed cases for which this information was reported (69.7% of all confirmed cases). Seven food-borne outbreaks supported by strong evidence were reported by five Member States. Implicated Food vehicles were; crustaceans, shellfish and molluscs and products thereof (3), cheese (1), meat and meat products (1), pig meat and products thereof (1), and vegetables and juices and products thereof (mixed salad) (1) (EFSA, 2015).

3.2.2. US outbreaks

Regulatory initiatives and industry actions implemented between 1998 and 2008 have reduced outbreaks from ready-to-eat (RTE) red meats and poultry. In contrast, listeriosis outbreaks from dairy products showed no decrease in frequency (Cartwright et al., 2013). Since 2010, the U.S. has experienced a number of listeriosis outbreaks attributed to foods considered to be "moderate risk" or low risk" by the existing risk assessments, including fruit and vegetables (e.g., celery, lettuce, cantaloupe, sprouts, stone fruit and caramel apples), as well as ice cream.

3.2.2.1. Pre-cut celery, 2010. Ten cases of listeriosis were associated with machine cut, diced celery served in five different hospitals in Texas (Gaul et al., 2013). All of the patients were over 55, with a mean age of 80, having underlying health issues (all of them had been hospitalized prior to contracting listeriosis). Five of them died, with listeriosis attributed as the cause of death for three of the cases. All 10 patients had one or more immunocompromising conditions or were receiving corticosteroid or acid-reducing treatments that could have increased their susceptibility to invasive listeriosis. *L. monocytogenes* was isolated from diced celery and a chicken salad served in the hospitals. The outbreak strain of *L. monocytogenes* was detected at the processing facility and in several bags of diced celery retrieved from the manufacturing facility.

3.2.2.2. Ice cream, 2010–2015. An outbreak of listeriosis from consumption of ice cream was identified in March 2015 as a result of regular surveillance. Illness onsets during 2010–2014 were identified through a retrospective review of the PulseNet database and WGS. In all, there were nine cases associated with this outbreak (CDC, 2015a; Pouillot et al., 2016). All of the patients were hospitalized (at least eight consumed the product while hospitalized for a prior illnesses) and there were two deaths. This outbreak was unusual for a number of reasons. A very diverse collection of strains were associated with the outbreak. Three serotypes (1/2b, 3b, 1/2a) were associated with the food, the environment and the patients, which were dispersed into 17 PFGE patterns. In addition, extensive analyses of affected lots (produced between November 2014 and March 2015) by FDA showed a high prevalence of *L. monocytogenes* but at very low levels (the average was 8 MPN/g, maximum 357 CFU/g, 99.8% of samples < 100 MPN/g) (Chen et al., 2016; Pouillot et al., 2016). In the hospital some of the servings were made into milk shakes and it is possible that *Listeria* populations may have increased, although temperature abuse was not documented during the investigation. If growth of *L. monocytogenes* did not occur in the product prior to consumption, this indicates that

the patients may have consumed low levels of *L. monocytogenes*. However, an outbreak associated with a food having a low contamination level may reflect the non-zero probabilities associated with a large number of servings. If 200 ml milkshakes contained 50 cfu/g, the dose would be 10,000 cfu per serving occasion and there may have been multiple servings daily for potentially extended periods. This outbreak may also indicate that the underlying health of a patient, immune status and the medication that they are taking is more important that the dose. In a potentially similar scenario, contaminated ice cream mix made into shakes caused two hospital-acquired cases (Rietberg et al., 2015). The authors stated that dietary guidelines for high risk individuals may need to recognize that certain pasteurized products may involve potential risk.

3.2.2.3. Cantaloupe, 2011. This multi-state outbreak infected 147 persons (CDC, 2011). In total, there were 33 deaths and one miscarriage. The median patient age was 78 years; most ill people were over 60, and 99% of the patients were hospitalized. Seven of the cases were related to pregnancy or were newborns. There were five subtypes of *L. monocytogenes* involved. The outbreak was identified by PulseNet and typed by PFGE. FDA detected the outbreak strain(s) in the environment and in the food products. Strains 1/2a and 1/2b were involved. Most ill persons had purchased whole cantaloupes.

3.2.2.4. Mung bean sprouts, 2014. Five people became ill, all were hospitalized, and there were two deaths (CDC, 2015b). FDA detected *L. monocytogenes* in sprouts and irrigation water samples collected during a routine inspection and follow-up environmental samples also showed the presence of *L. monocytogenes*. WGS showed that all of the isolates (food, environmental and patient) were highly related. A subsequent inspection later that year showed that *L. monocytogenes* was still present in the production environment.

3.2.2.5. Stone fruit, 2014. In July 2014, a packing company in California recalled various stone fruits (whole peaches, nectarines, plums and pluots) due to concerns about *L. monocytogenes* contamination. In August, PFGE types from the stone fruits were uploaded into PulseNet; four exact PFGE matches from patients were detected. The subsequent investigation showed that two of these cases were linked to the recalled fruit, nectarines in one patient and nectarines and peaches in the other. WGS showed that the other two cases were not associated with the recalled fruit.

3.2.2.6. Caramel apples, 2014–2015. Thirty-five people were affected, 34 of them were hospitalized (CDC, 2015c). Eleven illnesses were pregnancy-related (occurred in a pregnant woman or her newborn infant), with one illness resulting in a fetal loss. Listeriosis contributed to at least three of the seven deaths reported. Three invasive illnesses (meningitis) were among otherwise healthy children aged 5–15 years. Unusual in this outbreak were the food vehicle and the serious illnesses in healthy children. In addition, co-infection was reported in one patient, where two isolates were detected in one patient. This outbreak was initially flagged, also by PulseNet, using WGS rather than PFGE. FDA isolated *L. monocytogenes* from the apple packing facility, as well as in the caramel apples. WGS showed that the isolates were highly related to those isolated from the patients. Subsequent research showed insertion of the stick into the apple may have created a local microenvironment at the apple-caramel interface that supports rapid growth, whereas the apple nor caramel alone do not support growth (Glass, Golden, Wanless, Bedale, & Czuprynski, 2015). This hypothesis was reiterated in the study of Salazar et al. (2016), who also observed increases in populations of lactic acid bacteria, yeasts and molds that they postulate might have an impact on the microenvironment supporting growth of the pathogen.

3.3. Strain variation & virulence

3.3.1. Strain variation in tolerance to quaternary ammonium disinfectants and to phage

Listeria monocytogenes can exhibit tolerance to quaternary ammonium disinfectants (quats), as well as temperature-dependent resistance to phage. Disinfectant tolerance can be gained through acquisition of new genes as well as mutations in existing genes. At least three efflux systems that appear to have been acquired by horizontal gene transfer are known to mediate tolerance to quats. Transposons mediating quat tolerance include Tn*6188*, which is primarily encountered in serotype 1/2a strains and harbors *qacH*, mediating efflux of quaternary ammonium disinfectants (Müller et al., 2013). A different transposon harbors *bcrABC*, which also mediates quat tolerance via efflux; *bcrABC* is typically found on plasmids harbored by strains of diverse serotypes and clonal groups (Dutta, Elhanafi, & Kathariou, 2013; Elhanafi, Dutta, & Kathariou, 2010). Other genes on the plasmid confer tolerance to toxic compounds such as heavy metals and triphenylmethane dyes and appear to have the potential to be co-mobilized with *bcrABC* (Dutta et al., 2013, 2014). Lastly, in a clone (CC8) that includes strains implicated in the 2008 deli meats outbreak of listeriosis in Canada, quat tolerance is mediated by *ermB*, harbored on a chromosomal island (Kovacevic et al., 2015).

Clonal complex 6 (CC6) strains, also designated epidemic clone II (ECII), were first recognized in the 1998–1999 US hot dog outbreak in the US (Cantinelli et al., 2013; Kathariou, 2002). Subsequent analysis revealed that CC6 is significantly more common among clinical isolates than among food or environmental isolates, suggesting that it may be a hypervirulent clone (Maury et al., 2016). A unique feature of CC6 strains is their temperature-dependent resistance to phage (i.e.) (Kim & Kathariou, 2009). These strains are completely resistant to all tested phages when grown at low temperatures but become susceptible to phage when grown at 37 °C. A gene cassette unique to this clone mediates such temperature-dependent phage resistance (Kim et al., 2012).

Genes and gene cassettes conferring tolerance to quaternary ammonium disinfectants and to phage appear to have been acquired from other bacteria. Strains harboring these genes may have enhanced fitness and persistence in manufacturing facilities. The potential impact of these determinants in virulence remains to be characterized.

3.3.2. Mutations in inlA, premature stop codon and virulence

L. monocytogenes strains are known to differ in virulence; for example, many outbreaks in the US are associated with serotype 4b. In addition, some strains show reduced infectivity in animal and cell culture models. The mechanisms leading to attenuated virulence are not yet completely clear. However, research has shown premature stop codons (PMSC) in the gene *inlA* of 1/2a, 12b and 1/2c strains that may play a role in the invasion of human epithelial cells (Lecuit et al., 2001). Epidemiological, animal and tissue culture data suggest that PMSC mutations in *inlA* result in isolates with reduced infectivity requiring 3 logs more cells to cause infection compared to fully virulent cells (Nightingale, Windham, Martin, Yeung, & Wiedmann, 2005; Chen et al., 2011; Cruz, Pitman, Harrow, & Fletcher, 2014). Furthermore, a large proportion of *L. monocytogenes* isolated from foods have PMSC mutations in *inlA*, which may in part explain why some strains are more frequently isolated from foods or processing environments compared to their linkage to clinical cases (Van Stelten, Simpson, Ward, & Nightingale,

2010). In serotype 1/2c, PMSC were found in inlA but not in inlB nor were deletions of *lapB, aut, flopA, ami* or *vip* genes found in any strains (Gelbíčová, Pantůček, & Karpíšková, 2016). Mutations in the *prfA* gene, although rare, have also been linked to attenuated virulence (Roche et al., 2005; Rupp et al., 2015).

3.3.3. Pathogenesis models

The prolonged time between the exposure to *L. monocytogenes* and the onset of illness, typically several weeks, makes it extremely difficult to get accurate dose information from human epidemiological data. Therefore, animal models have been used to determine dose-response relationships for listeriosis, and dose-response curves have been developed with mice, gerbils, guinea pigs and nonhuman primates as models for human exposure. These animal models may have differences in the mechanism of listeriosis infection as compared to humans; for example, the mouse receptor, E-cadherin, does not recognize the *L. monocytogenes* invasion protein, internalin A, and the guinea pig receptor, Met, does not bind an alternative invasion protein of *L. monocytogenes*, internalin B (Bonazzi, Lecuit, & Cossart, 2009). However, human gastrointestinal (GI) cells have receptors for both internalin A and B, which facilitates adhesion and internalization of *L. monocytogenes* (Bonazzi et al., 2009).

In comparing these models, the median lethal dose (LD_{50}) based on dose-response curves was similar for guinea pigs and nonhuman primates, at about 10^7 CFU (Williams, Castleman, Lee, Mote, & Smith, 2009). Following oral inoculation fetal deaths occurred in the guinea pig model at 10^6 CFU, and in the nonhuman primates at 10^3 CFU, while there were no fetal deaths at 10^7 CFU or below in the gerbil model (Roulo, Fishburn, Amosu, Etchison, & Smith, 2014; Smith et al., 2008; Williams et al., 2009). The LD_{50} values derived from the animal models compare favorably to the WHO human estimated LD_{50} of 1.9×10^6 CFU for human listeriosis (FAO/WHO, 2004a). Thus, the gerbil model was not more sensitive than either the guinea pig or the nonhuman primate model. The data also suggest that there may be multiple mechanisms for *L. monocytogenes* to enter intestinal epithelial cells.

In addition to mammalian models, the larvae of the greater wax moth, *Galleria mellonella*, has been increasingly employed as a model to study microbial pathogenesis, including that of *L. monocytogenes* (Joyce & Gahan, 2010; Mukherjee et al., 2010). The larvae are inexpensive and readily available, ethical issues and regulatory restrictions to ensure animal welfare with vertebrate models are avoided, and testing can be done at 37 °C, the temperature at which virulence factors of *L. monocytogenes* are expressed. Disadvantages associated with this model include the narrow window for doses that permit virulence assessments (10^6 CFU/larva) (Joyce & Gahan, 2010; Mukherjee et al., 2010) and the observation of unusually low virulence with certain outbreak strains which are presumed to be pathogenic to humans (Kuenne et al., 2013).

3.3.4. Key events framework

The Key Events Framework is an approach to understanding foodborne illnesses where the key individual biochemical steps from ingestion to infection are described as a conceptual structure for advancing the dose-response models (Buchanan, Havelaar, Smith, Whiting, & Julien, 2009). For example, in humans, the interactions of the *Listeria* surface proteins InlA and InlB with the human GI tract receptors E-cadherin and Met, respectively, are key factors in the infection process. However, these two attachment pairs do not appear to be sufficient to explain the diversity in infection nor fully explain what it means for an individual to be immunocompromised and more susceptible. There is evidence that pregnant monkeys exposed to *L. monocytogenes* exhibited high levels of fecal shedding (Smith et al., 2008), suggesting that colonization of the GI tract is another important factor in infection. A greater understanding of the genetic variation controlling host susceptibility, including the major histocompatibility complex, CMI and humoral immunity, and of the role of stress, T-cell status and immune state are needed to develop a model in which research scientists and public health officials can have a high degree of confidence.

4. Recent advances in ecology, persistence and growth

4.1. Ecology of growth and survival

L. monocytogenes is a bacterium that occurs widely in agricultural, aquacultural and food processing environments. A higher prevalence has been found in soils closer to water, soils with higher moisture, soils recently cultivated, irrigated or rained upon, and soils close to pastures (Strawn et al., 2013). Survival will also vary with soil type and conditions; moist and organic soils permit longer survival than dry, low-organic soils (McLaughlin, Casey, Cotter, Gahan, & Hill, 2011). *L. monocytogenes* is also a transitory resident of the intestinal tract in humans, with 2–10% of the general population being carriers of the microorganism without any apparent health consequences.

Foods are considered to be the major vehicle for listeriosis, which in the US is estimated to be 99% foodborne (Scallan et al., 2011). Of particular significance are ready-to-eat (RTE) foods, including processed foods that have been exposed to the processing environment after application of a listericidal process and prior to packaging. RTE foods are those that are normally eaten raw or handled, processed, mixed, cooked, or otherwise prepared into a form that is eaten without further listericidal steps. Sporadic cases and outbreaks of listeriosis have generally been associated with those RTE foods that are held for extended periods at refrigeration or chill temperatures which allow growth to high numbers at the time of consumption.

Many studies have shown that *L. monocytogenes* are widely distributed in food processing environments (Carpentier & Cerf, 2011; Ferreira, Wiedmann, Teixeira, & Stasiewicz, 2014; Tompkin, 2002). Listeria may enter food processing environment from raw materials or the movement of people or equipment and can persist due to ineffective cleaning and sanitation, poor design or condition of food equipment or environment or insufficient controls of movement of people or equipment. Its presence has also been reported in farm (Fox, Hunt, O'Brien, & Jordan, 2011), retail (Hoelzer, Pouillot, Dennis, Gallagher, & Kause, 2015; Wang, Ray, Hammons, & Oliver, 2015) and home environments (Evans & Redmond, 2015). Presence of *Listeria* in retail environments is of specific note, considering that deli meats alone, particularly if sliced and packaged in the deli, were previously estimated to be responsible for almost 83% of all human listeriosis cases in the US (U.S. FDA/FSIS, 2003). Notably, prevalence in the food environment may not necessarily be proportional with prevalence in the food product. For instance, despite the known high on-farm prevalence of *L. monocytogenes*, its presence in raw milk intended for farmstead cheese manufacture may be very low (D'Amico and Donnelly, 2010). This possibly is a result of factors inherent to small-scale artisan operations such as small herd and flock sizes, lack of extended milk holding, seasonal milking, and pasture-based feeding in addition to strict hygienic controls.

Transfer studies with cut produce have shown continuing conveyance from contaminated equipment (slicers) to uncontaminated product. Measurable transfer continued to occur after 10 slices with onions and tomatoes (Scollon, Wang, & Ryser, 2016; Wang & Ryser, 2016) and ham (Chaitiemwong, Hazeleger,

Beumer, & Zwietering, 2014). Fresh produce operations that use fluming water are very effective in transferring contamination and much less effective in removing contamination by draining or centrifuging (Buchholz, Davidson, Marks, Todd, & Ryser, 2012).

Many strains of *L. monocytogenes* are relatively resistant to a number of environmental conditions, such as high salt or acidity in food as well as low humidity or low oxygen in food environments. Although *Listeria* growth is significantly reduced at low temperatures, the organism has been found to grow and survive at refrigeration temperatures between −0.5 and 9.3 °C under laboratory conditions (Walker, Archer, & Banks, 1990). The potential for growth and survival in food is lower than that observed under laboratory conditions and is influenced by complex interactions between extrinsic and intrinsic physico-chemical growth promoting or by inhibiting parameters of the particular food or food environment as well as factors such as competitive microflora. Nevertheless, the ecological and physiological traits of *L. monocytogenes* allow it to colonize food plant environments, survive hurdles in processing/storage and proliferate in food products that support growth at low temperatures. Temperature abuse and fluctuations in temperature during commercial transport and retail sale may allow the organism to proliferate significantly. This has been demonstrated in produce where temperatures exceeded 45 °F (7.2 °C) during transport 0.24% of the time, backroom coolers 5%, and display coolers 5% (Zeng et al., 2014).

4.2. Persistence of cells in food environments

Presence of *L. monocytogenes* in the food processing environment is thought to be the primary source of post-processing contamination during food manufacturing and in retail or food service settings (Ferreira et al., 2014; Hoelzer et al., 2015; Malley, Butts, & Wiedmann, 2015). *Listeria monocytogenes* strains have been found to persist for years or decades in food processing plants (Ferreira et al., 2014) with specific strains having been isolated repeatedly over time in specific food operations. Such persistence may be due to the survival and growth of certain strains in niches within the food environment (e.g. cracks and crevices of surfaces, seals and gaskets that may be difficult to clean and disinfect), or repeated re-introduction of such strains from the external environment into food processing facilities over time. Most research on strain persistence has examined the role of biofilm formation and physiological tolerance to sanitation or processing hurdles. Another possible mechanism is the occurrence of particularly persistent cells.

Biofilm formation was recently reviewed (Carpentier & Cerf, 2011; da Silva & De Martinis, 2013; Ferreira et al., 2014). True biofilms consist of multiple cells and extracellular polymeric materials that protect individual cells from environmental stresses and foster interactions between cells in relation to nutrients, toxic metabolites and genetic material that may lead to enhanced survival and growth. Kadam et al. (2013) found that all of 143 strains of *L. monocytogenes* tested could form biofilms, although there was a very wide diversity between strains depending on medium composition as well as time and temperature. Bonsaglia et al. (2014) analyzed 32 strains (mostly isolates from food processing environments, milk and vegetables) and found almost all strains could form biofilms on stainless steel and glass surfaces, but strains differed in their ability to form biofilms based on the temperature and type of surface. Also Ferreira et al. (2014) reported that several strains investigated were capable of rapid attachment to surfaces used in food environments and food equipment but pointed out that strong evidence for true biofilm formation by *L. monocytogenes* in food operation environments is lacking. It's possible that *L. monocytogenes* is could occur as part of multispecies biofilms in food processing facilities.

Tolerance to disinfectants used to sanitize food environments, equipment and utensils has been considered as a possible mechanism for persistence and several studies have investigated the relative sensitivity of various *Listeria* strains to particular disinfection agents or the build-up of increased tolerance or resistance over repeated sublethal exposure to disinfectants (Moorman, Nettleton, Ryser, Linz, & Pestka, 2005). While some studies found persistent strains to be less sensitive than transient strains, others did not find such a correlation (Ferreira et al., 2014).

Carpentier and Cerf (2011) concluded that there are no *L. monocytogenes* strains with unique properties such as biofilm formation or stress resistance that lead to persistence. In contrast, Wang et al. (2015) investigated foods contaminated with persistent *L. monocytogenes* strains from the retail environment and concluded that such strains are likely to have wild-type virulence potential and may persist due to increased adhesion and biofilm formation capacity rather than sanitizer tolerance.

A third possible mechanism underlying persistence in food environments is the formation of persister cells (Knudsen, Ng, & Gram, 2013; Wen et al., 2011). The dormant, non-dividing state of persister cells enhances their ability to survive environmental stresses. Persister cells may represent a long-term survival (LTS) strategy for the organism, which changes its cellular morphology from bacilli to cocci during the transition to the LTS phase (Wen et al., 2011). Although the underlying mechanisms triggering this morphological and physiological transition remain largely unknown, such LTS cells have demonstrated increased tolerance to temperature and high pressure. Knudsen et al. (2013) reported that a range of *L. monocytogenes* strains, including clinical and outbreak-related strains, displayed the biphasic population response to antibiotics that is typical for populations with a persister cell subpopulation. The authors raised the possibility that a persister cell response by *L. monocytogenes* contributes to protection against cleaning and sanitation in food operations and also, during infection, may protect cells from the host immune system for extended periods of time, explaining why clinical symptoms of listeriosis often appear after a long incubation time. Abee, Koomen, Metselaar, Zwietering, and den Besten (2016) reviewed the heterogeneity of pathogen populations and analyses of the genotypic and phenotypic characteristics of persister subpopulations and stress-resistant variants. The molecular mechanisms underlying the generation of persister phenotypes were identified.

Several authors have concluded that it is virtually impossible to permanently eradicate *L. monocytogenes* from food environments because of its ubiquitous presence in the environment and many potential avenues for entry into the facility. Therefore, elimination and exclusion of the organism must be actively managed, for example by adequate hygienic design of a food premise and equipment, effective cleaning and sanitation, personnel practices and movement of people and materials into areas where food products are exposed. This includes the disassembly of equipment for deep cleaning, among other important control measures for operations producing RTE foods likely to be associated with sporadic illnesses or outbreaks of listeriosis (CAC, 2009).

4.3. Defining growth/no growth conditions

A variety of foods that support growth of the organism have been implicated in outbreaks and sporadic cases of listeriosis, such as processed meats, soft cheeses, smoked fish, butter, milk, and coleslaw. Since most products implicated in listeriosis have extended shelf-lives, time and temperature during transport and storage strongly contribute to the risk of listeriosis associated with RTE foods (CAC, 2009; Farber, Kozak, & Duquette, 2011). Conditions

at various interfaces, such as between oil and water or between particulates and fluid, may support growth that would not occur in the separate components. In addition, food rheology or structures within the food matrix may impact very locally the micro-ecological conditions and thus the ability of *L. monocytogenes* to proliferate.

The potential for *Listeria* to grow in a particular food during storage and distribution has been a key factor in determining the level of consumer risk and has been the basis of risk categorization by some regulatory authorities (Farber et al., 2011) and associated microbiological criteria (European Commission, 2005).

The Codex Alimentarius Commission (CAC) proposed the following criterion to characterize food products that support *L. monocytogenes* growth: "a RTE food in which there is a greater than average of 0.5 log increase in the level of the organism for at least the expected shelf-life (as labelled by the manufacturer) under reasonably foreseeable conditions of distribution, storage and use to consumption, including a safety margin" (CAC, 2009). This criterion was based on methodological considerations, with 0.5 log being two times the estimated standard deviation associated with the experimental enumeration using viable counting/plate counts (although not including sampling variation). The guidance does not specify the number of samples that would need to be tested to assess whether a food meets the criterion for growth, nor a suitable time/temperature margin to consider in the shelf-life test. Nevertheless, CAC was the first global organization to advocate a practical growth/no-growth criterion that can be evaluated experimentally and several governments have formally adopted the criterion (Health Canada, 2011; FSANZ, 2014). Although the European Commission has not formally adopted the criterion, a range of practical guidance documents (European Commission, 2013; EURL Lm, 2008, 20132014) have been produced to assist Member States and food industries within the European Union in assessing the growth potential of *L. monocytogenes*. These documents provide more detailed guidance on the number of samples to test, the design of the shelf-life tests and the development of strains suitable for challenge testing.

Shelf-life studies are not relevant when there is sufficient scientific basis that a food will not support growth. For example, typical intrinsic food conditions that are well documented to not support *L. monocytogenes* growth include a pH < 4.4, a_w < 0.92, or a combination of pH < 5.0 and a_w < 0.94, NaCl >16%, whereas freezing (−18 °C) is an effective extrinsic condition (CAC, 2009; European Commission, 2013). However, when there is variability in the pH, a_w or both over time or within the food product, control of growth in the particular product needs to be validated. Many resources on the impact of particular intrinsic or extrinsic factors on the growth of *L. monocytogenes* in foods (EURL Lm, 2013; ICMSF, 1996; FAO/WHO, 2004a,b) are available to identify particular properties of RTE foods that categorize them as no-growth foods.

Codex (CAC, 2009) suggests that shelf-life or validation studies be conducted by food business operators or by the appropriate product board, sector organizations or contract laboratories. Codex furthermore considers that the demonstration that *L. monocytogenes* will not grow in a RTE food can be based upon food characteristics, the study of naturally contaminated food, challenge tests, predictive modelling, information from the scientific literature and risk assessments, historical records or combinations of these. While Codex stresses that studies must be properly designed, it leaves it up to national governments to provide guidance on the specific protocols that should be employed for shelf-life studies.

The demonstration that particular foods support growth or not could be conducted in many different ways, which could lead to interpretations that are not necessarily consistent or comparable. Several governments have therefore provided more detailed guidance to food manufacturers and other relevant parties on validation studies specific to *L. monocytogenes* in RTE foods (CFA, 2010; European Commission, 2013; FSANZ, 2014; Health Canada, 2012). More general advice on conducting shelf-life studies is available from the National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 2005).

The European Reference Laboratory for *Listeria monocytogenes* (EURL Lm) has developed detailed guidelines for determining growth of *L. monocytogenes* in food, which include protocols for different shelf-life studies, calculation tools, examples and relevant background information. An early version was made public in 2008 (EURL Lm, 2008) and a revision has since been issued (EURL Lm, 2014) that complements a draft European Commission guidance document (European Commission, 2013). The latter provides more context to the use of the technical guidance of EURL Lm (2014) as well as decision trees on establishing a safe shelf-life and generating validation data through shelf-life evaluations. The decision tree on shelf-life evaluations concludes that such evaluations are not needed for a wide range of food products (Alvarez-Ordonez, Leong, Hickey, Beaufort, & Jordan, 2015). The EURL Lm and European Commission documents provide guidance on challenge testing (using artificially contaminated foods) and durability testing (using naturally contaminated foods) where the level and prevalence of natural contamination makes such testing relevant. The EURL Lm and the European Commission recommend evaluation of a moderately worst-case situation. For example, where prior information or models indicate a possibility that a product supports growth, it is recommended that samples from 3 different batches be evaluated to account for inter-batch variability. For each batch, the growth potential over the shelf-life is assessed and the batch with the most growth is taken to represent the ability of the food to support growth rather than a calculated average of growth. Augustin et al. (2011) concluded that an evaluation of three batches would provide useful information on the variable behavior of *L. monocytogenes* in a specific food. Considering inter-strain variability, a mixture of at least two strains of *L. monocytogenes* should be used for challenge testing (EURL Lm, 2014), although a mixture of three strains was previously recommended (EUR Lm, 2008). In the latter case, a reference strain was to be combined with two strains isolated from the same or a similar matrix as the food investigated. Notably, EUR Lm developed a set of reference strains for conducting challenge testing that was selected for its ability to grow faster under harsh conditions than others. These strains have since been made available to all National Reference Laboratories participating in EUR Lm (EURL Lm, 2013). In terms of temperature abuse in the food supply chain, EURL Lm (2014) advises the inclusion of different times and temperatures for different stages i.e., transport from the manufacturer to the retail display cabinet, during display in the retail cabinet and during consumer storage. It is recommended that either a temperature justified on the basis of specific survey data or a default value be used, adhering to temperature abuse conditions (12 °C) during retail and consumer storage.

The EURL Lm guidance documents have only advisory status and may not be applied broadly or consistently by different users. Alvarez-Ordonez et al. (2015) observed that food business operators may not have the specialized expertise, infrastructure or resources needed to conduct their own shelf-life studies or that there may be differences in the interpretation of challenge test outcomes between an a food business operators and the authorities.

The guidance provided in Canada on shelf-life testing (Health Canada, 2012) covers many of the practical, technical and scientific aspects that the European Union guidance covers and provides advice on experimental design, the selection and cultivation of

strains, etc. The Canadian approach is to use a mixture of at least three to five strains to conduct the challenge tests when there is good prior knowledge of how the organism grows or responds to a particular food commodity and (in accord to NACMCF, 2010) a mixture of up to ten different strains be used where prior knowledge is absent. The inoculum should include strains of serotypes 1/2a, 1/2b and 4b, with a preference to strains isolated from the same or similar foods to that being tested, and including strains related to outbreaks or sporadic cases, if available. Additional guidance on testing and validating growth/no-growth foods has been developed by Health Canada (2013). The guidance document identifies RTE food types/groups for which such validation testing is not required because they have intrinsic or extrinsic factors as indicated above. Specific protocols are described for the three different food categories that have been specified in the Canadian regulation (Health Canada, 2011).

The guidelines discussed above have been developed in the context of particular national or regional regulations. Although differences in both the breadth and depth of guidance provided exist, many of the underlying principles are the same. Ideally, recommendations and protocols for challenge/durability testing should be harmonized in order to foster consistency and comparability of outcomes. More research and guidance development in several areas may also further improve the value of the guidance currently available.

5. Insights from risk assessments

5.1. Background

Microbiological risk assessments acquire information about individual steps of a complex process and link them together to model (mathematically describe) the cell numbers or likelihood of illness for a specific process or even an entire industry (Ruzante, Whiting, Dennis, & Buchanan, 2013; Whiting & Buchanan, 2008). The data used may include contamination frequencies and levels, growth and inactivation models, process factors (dilution), storage times and temperatures, and consumer handling and consumption patterns to estimate probable consumption of the microorganism and the likelihood of illness (considering pathogenicity of microorganism and susceptibility of consumer). Risk assessments utilize the currently obtainable data and knowledge (Dennis, Kause, Losikoff, Engeljohn, & Buchanan, 2008). While they often lead to new insights about a multistep process, they do not create new knowledge and, therefore, may need to be revised with the availability of new information.

A risk assessment is commissioned by risk managers who have a need for the insights that the risk assessment can provide. The risk managers define a scope and specific objectives for the risk assessment that determine its structure, data needs and outputs. To understand relationships between various factors [parameters] and improve the control over *L. monocytogenes*, several risk assessments have been created by governments or international agencies (Codex) to provide guidance on where to improve industry practices and to focus regulatory action.

5.2. FDA-CFSAN/USDA–FSIS– 2003

The 2003 risk assessment, "Quantitative Assessment of Relative Risk to Public Health From Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods", conducted by FDA-CFSAN (Center for Food Safety and Nutrition) and USDA-FSIS (Food Safety and Inspection Service), was designed to determine the relative contribution to listeriosis cases by various RTE food categories in the United States (U.S. FDA/FSIS, 2003). Twenty-three food categories were defined and modeled using data on frequency and level of contamination at retail, growth during retail and home storage, consumption amounts and susceptibilities of three human populations (perinatal, elderly, intermediate-age). A dose-response model was developed that, given the determined frequencies and levels of *L. monocytogenes* at consumption, would predict the number of illnesses and deaths that were in the epidemiological data base of the CDC (Centers for Disease Control). Foods that supported the growth of *L. monocytogenes* (deli meats, frankfurters that were not reheated, pates and meat spreads, unpasteurized milk, smoked seafood, cooked crustaceans) had the highest risk of causing listeriosis per serving. High fat dairy products, soft unripened cheeses, pasteurized milk and fresh soft cheeses comprised the group next likely to cause listeriosis. The frequencies of consumption for the food categories affected the total number of cases of listeriosis resulting from that category. Leading categories were deli meats, pasteurized milk, high fat dairy products, and frankfurters not reheated. The next group included soft unripened cheeses, pate and meat spreads, unpasteurized fluid milk, cook crustaceans and smoked seafood. Food categories with low risks of causing listeriosis included hard cheeses, cultured dairy products, processed cheese, ice cream, and most deli salads which have added inhibitors, all foods that do not support the growth of *L. monocytogenes*.

The perinatal population, consisting of pregnant women and their fetus, were the most susceptible population, followed by the elderly and the intermediate-aged (the remaining population). Because of a lack of data to support further divisions, the latter group included individuals with various immune-suppressed conditions as well as fully immunocompetent individuals.

Scenario analyses were used to illustrate the importance of the opportunity for growth in a food. The ranking of a food category in terms of contributing to listeriosis risk was primarily governed by a food's composition, storage times and temperatures. The risk assessment concluded that nearly all cases of listeriosis resulted after a susceptible individual consumed a large dose of *L. monocytogenes* from a food that supports growth and had been time and/or temperature abused.

The risk assessment was used to develop guidance to more effectively control *L. monocytogenes* in foods and food environments, promote formulation of foods that limit the potential growth and focus regulatory activities toward the higher risk foods.

5.3. FAO/WHO - 2004

The Food and Agriculture Organization of the United Nations (FAO) and The World Health Organization (WHO) risk assessment, "Risk assessment of *Listeria monocytogenes* in ready-to-eat foods", was the first international microbiological risk assessment requested by the Codex Committee on Food Hygiene as a basis for developing one of its standards (FAO/WHO, 2004a; FAO/WHO, 2004b). The particular standard concerned was the "Guidelines on the application of general principles of hygiene to the control of *Listeria monocytogenes* in foods" (CAC, 2009). Specific objectives were to estimate the risk of illness at population level with the consumption of foods that contained specific maximum numbers of *L. monocytogenes* (0 CFU/25 g up to 1000 CFU/g), estimate the risk for consumers in different susceptible populations and estimate the difference in risk associated with foods that do and do not support growth on their likelihood of causing listeriosis.

The risk assessment found that nearly all cases of listeriosis result from the consumption of foods with high numbers of *L. monocytogenes* that would exceed both a zero tolerance limit (i.e. 0 CFU/25 g) and a 100 CFU/g limit. In terms of insights regarding control of the organism, it was found that control measures that

reduce the frequencies of contamination would have a proportional reduction in rates of illness and control measures that prevent the occurrences of high levels of contamination at consumption would have the greatest impact. In foods that don't support growth, reducing the occurrences at manufacture/retail would improve public health. In foods that support growth, better temperature control or limiting lengthy storage would reduce the risk.

Insights related to consumers, were that the relative susceptibilities of different subpopulations were greatly increased relative to the healthy (i.e. the under 60 year old population), for example with those over 65 years old being 7.5 times more susceptible and various cancer patients being 66 to 1364 times as susceptible.

Four food categories were chosen for the risk assessment based on their frequency of consumption and ability to support growth. They were pasteurized milk (rarely contaminated, supports growth and high frequency of consumption); ice cream (rarely contaminated, does not support growth and high consumption); smoked fish (often contaminated, supports growth and low consumption) and fermented meats (often contaminated, does not support growth and low consumption). The two foods that supported growth (milk and smoked fish) turned out to have 100- to 1000-fold greater risks than the respective two foods that did not support growth (ice cream and fermented deli meats).

These comparisons demonstrated the importance of high numbers at the time of consumption in the etiology of listeriosis, with those foods supporting growth of *L. monocytogenes* to be vehicles of particular concern.

5.4. FSIS – 2010 and follow-up risk assessments

After the FDA/FSIS 2003 risk assessment identified deli meats as the leading food category for causing listeriosis, FSIS developed a regulatory program to reduce consumer exposure to *L. monocytogenes* in these products. Three mitigation options explored by this risk assessment were provided to manufacturers: increasing the frequency of product testing for *Listeria*, utilizing a post-processing intervention, or adding a growth inhibitor to the product. The risk assessment determined that the latter two mitigations were likely to be most effective. Industry responded to this initiative and, with continuing improvements in sanitation, the presence of *Listeria* in RTE deli meats and poultry sampled by FSIS (within establishments) began to decline (U.S. FDA/FSIS, 2010).

However, the overall rate of listeriosis did not decrease despite this improvement. FSIS, therefore, undertook a risk assessment on the basis of an additional collection of data that focused on the retail deli to further understand the origins of contamination within this category (U.S. FDA/FSIS, 2010). The new/updated contamination data showed that deli meats sliced and packaged in the deli were contaminated five to seven times more frequently than deli meats sliced and packaged by a processor. This was accentuated in products that did not contain added inhibitors. Extensive testing of the environment in retail delis and observation of worker behavior found niches and cross contamination from a variety of food contact and non-food contact surfaces, lack of adequate sanitation, inadequate temperature control and glove/hand issues. This information was used by FDA and FSIS to create a "virtual deli" model and to generate six baseline situations and 22 scenarios (U.S. FDA/FSIS, 2013; Pouillot et al., 2015; Gallagher et al., 2016). Overall, the virtual deli indicated that the greatest risk is from contamination present in an incoming chub of a product that permits growth of *Listeria*. Contamination of a product that doesn't permit growth would be a lessor although still significant contributor to listeriosis, including from its contribution to environmental contamination and subsequent cross contamination to other products. Important environmental factors noted were workers, the slicer, trash handling and cleanup operations. The level of contamination at retail delis was found to directly affect the risk, for instance a two-fold decrease in contamination would result in a 20% reduction in illnesses. In terms of control measures, it was determined that when all products would have growth inhibitors there would be few cases of listeriosis attributable to deli meats. Gallagher et al. (2016) noted that control of temperature and storage time at the consumer's home led to the largest risk reduction (~99%) under the conditions of the simulation.

5.5. Dose-response modelling

The FDA/FSIS 2003 risk assessment used a combination of mathematical functions for the dose-response model although the most predominant was the logistic-exponential function. The FAO/WHO model (2004) used the exponential function because it had only one parameter, the r-value, which could be fitted to the limited data that were available. Both risk assessments created separate models with different parameter values for each of the three or two susceptibility groups, respectively. Given the low incidence of listeriosis in a population despite the frequent exposure to *L. monocytogenes*, these models presume that illnesses most frequently come from exposure to the high doses. The models further predict that at the low doses and at illness rates characteristic of listeriosis, the rate of infection is directly proportional to the dose.

Reducing the uncertainty of the dose-response models would improve the ability of risk assessments to provide useful information for risk managers. Improving the exponential model was accomplished by assuming that the r-value has a log-normal distribution reflecting the variability inherent in the dose, strain virulence, individual susceptibility, and food matrix (Pouillot, Hoelzer, Chen, & Dennis, 2014). This model estimates a higher risk for highly virulent strains and highly susceptible individuals.

6. Research and data needs

6.1. Outbreaks and virulence

Although great strides have been made regarding the knowledge of virulence and pathogenicity of *L. monocytogenes*, there is still much to learn. In order to better understand the virulence of *L. monocytogenes* it would be helpful to have a mechanism by which many strains could be investigated and compared at one time. Recent outbreaks in the U.S. have also raised the question the role of co-infection and the importance of multiple exposures. Further investigation is needed regarding the influence of microbiomes and medication on host susceptibility, as well as better method for assessing the degree of immune suppression in immunocompromised patients, are important topics that requires further characterization. There may be many additional deaths from listeriosis in nursing homes and other care facilities for elderly because causation of a systemic infection is seldom determined for these individuals.

6.2. Ecology and persistence

Further exploration is needed into mechanisms contributing to the persistence and recontamination of food processing environments with *L. monocytogenes*, including biofilm formation and retention in growth niches. Improvements in technical and practical measures to avoid contamination, control growth and persistence would be of value, especially for retail and food service operations. The development of specific hygiene measures and practices for such operations as well as additional communication

and training materials on risk and intervention strategies should be considered.

Investigations on the occurrence and role of persisters or long-term-survival (LTS) phase cells and specific stress resistant variants in both environmental niches and food are needed to determine whether these phenomena are important contributors to the risk of listeriosis. Moreover, the occurrence of such more durable cell forms in the human host should be investigated in more detail to understand their role and contribution to the risk of listeriosis. More research is needed on the statistical validity and the value of outcomes of the protocols that are currently used for growth/no-growth challenge studies. More rigorous protocols may be required to fully account for the variance in challenge tests and to detect an increase with sufficient confidence (Powell, 2009).

Existing guidelines for challenge or durability tests currently do not consider the impact of interfaces, food rheology or structures within the food matrix on the ability of *L. monocytogenes* to proliferate.

Accelerated shelf-life studies conducted at elevated temperatures for shorter periods of time have long been used in the food industry. While such approaches reduce the time and resources required for challenge/durability studies, they may put the organism in a situation that is too far from the real situation with the outcome no longer reflecting the situation being evaluated.

While current guidance documents consider the effect of the physiological state of the cells on lag phase, they may not deal sufficiently with the potential impact of delayed on-set of growth caused by various mechanisms and/or whether there is need to use reference strains that display shorter lag-phases.

The guidance available on challenge testing stresses the usefulness of predictive modelling. However, food business operators may find the use of predictive modelling particularly challenging in terms of skills and resources (Alvarez-Ordonez et al., 2015). Because microbiological growth kinetics may differ 2 to 4 log CFU/ml(g) between the most and least robust strains, the consequence of strain variability in quantitative modelling must be understood in order to generate realistic outcomes (Aryani, den Besten, Hazeleger, & Zwietering, 2015). More specific guidance and training may need to be developed on the use of predictive modelling in this context that is suitable for food business operators, testing laboratories and other possible target audiences.

6.3. Risk assessment and dose response

Past focus by regulatory agencies and industry was on foods traditionally associated with listeriosis, primarily deli meats and frankfurters, soft cheeses and smoked seafood and existing risk assessments reflect that emphasis. Recently, other foods have been identified as the vehicles for listeriosis including pre-cut fruits and vegetables, ice-cream, cantaloupe, mung bean sprouts, stone fruits and caramel apples. Some of these vehicles underscore need to recognize the individual characteristics of specific foods within the food groupings used in a risk assessment (e.g, widely differing environments associated with melons versus citrus fruit). Some outbreaks, particularly from ice cream, have raised questions about the current understanding of the infectivity of lower doses and, thereby, placing an emphasis on the susceptibility of the affected individual. In addition, the caramel apple case was unexpected in terms of our understanding of consumer susceptibility as otherwise healthy children were involved.

Outbreak investigations for listeriosis are difficult because of the sporadic nature of the illness and lengthy incubation period, which frequently makes identification and analysis of the causative food impossible. Advances in strain identification and prompt linking of sporadic cases through PFGE and WGS are recognizing more situations where investigations could yield valuable information. WGS may show many sporadic cases may, in fact, be part of an unrecognized outbreak with additional unlinked cases. Improvements in the currently available dose-response models will require better knowledge about the infection pathways and more detailed investigations of outbreaks. This will be the result of animal, cell culture, organ culture and other studies that combine to characterize strain virulence and mechanism of action that are probably important in determining the virulence of a strain and the susceptibility of an individual to infection. An individual's microbiome, for example, may be such a factor. A minimum threshold dose may exist and the pathways for a pregnant woman/fetus may be different from other individuals. Because the dose-response models must be extrapolated below observed dose-infectivity data, the shape of the curves is critical in the model's estimates and better biochemical understanding of the pathways will be necessary to better determine the best biologically plausible model. Because of the high uncertainty due to the wide differences in individual susceptibility, it may be advantageous to develop a series of dose-response curves for individuals with specific pathologies or treatment regimes that would have less uncertainty.

The Exponential dose-response model frequently used to model *L. monocytogenes* is simplified by having one parameter; however, the probability of illness is undoubtedly not the same for every *L. monocytogenes* strain or exposed individual. Replacing the r-value with a log-normal distribution (Pouillot et al., 2014) may be a better description of the host-pathogen relationship. To better define dose-response models and ultimately improve control, more thorough outbreak investigations are crucial. Obtaining sufficient contaminated foods that are in their original microbiological condition to quantitatively determine the frequency and prevalence of contamination is required. It should not be assumed that the cells of *L.* monocytogenes are uniformly distributed throughout the lot of implicated food and the enumerated samples fully characterize the individual servings. Individuals affected may have consumed particular servings containing high levels of the organism.

Determining the food's role in allowing growth of *L. monocytogenes* and possibly the food's effect on enhancing the microorganism's survivability are important. Individuals who have listeriosis are frequently identified, while healthy individuals who were also exposed are generally not identified. Better determination of the attack rate, health and other differences between individuals, exposure (number and size of servings, resolving whether frequent exposure increases or decreases probability of infection), and characterizing whether the infecting strain are examples of information that would improve our knowledge base.

7. Conclusions

Improvements in epidemiology and detection methods, particularly the advent of WGS, have led to the recognition of more frequent and smaller listeriosis outbreaks due to vehicles, including no-growth foods, that have not historically been associated with foodborne listeriosis Some outbreaks have prompted a re-evaluation of the significance of the consumption of low doses by susceptible individuals and the need to further investigate factors influencing virulence and host susceptibility. Additional understanding of the physiology and ecology of *L. monocytogenes* will assist risk managers to identify and strengthen strategies to manage this ubiquitous organism in food manufacturing and food service.

This workshop explored recent advances in our understanding of *L. monocytogenes* and identified further needs to assist risk assessors and risk managers to better understand the organism and its control. These areas include the microorganism's virulence

factors and mechanism of infection in different subpopulations so that risks are better understood. Control measures by the food industry potentially can be enhanced by knowledge about strain differences; better recognition of growth/no growth conditions, particularly in complex foods; elucidation of persister cells and their impact; and more comprehensive documentation of outbreaks leading to better understanding of the dose-response.

Funding

We would like to acknowledge the Grocery Manufacturers Association Science and Education Foundation, Patrizia Barone, Cargill, Inc., ConAgra Foods Inc., General Mills, Inc., Kraft Foods Inc., Nestlé USA, and Unilever for providing funding in order to conduct the workshop. In addition, we would like to thank Drs. S. Kathariou, R. Pouillot, E. Ryser and M. Zwietering for reviewing and editing sections of this manuscript.

Acknowledgements

The authors would thank all of the participants of the workshop for their contributions, especially those who presented (JIFSAN, 2015). We are grateful to the Joint Institute for Food Safety and Nutrition (JIFSAN) for hosting the workshop; specifically to Ms. Vernora Petty and Dr. Jianghong Meng.

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