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Ecology of *Listeria* spp. and *Listeria monocytogenes*

Significance in Dairy Production

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Ecology of *Listeria* spp. and *Listeria monocytogenes*

Significance in Dairy Production

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ABSTRACT

Listeria monocytogenes shows the highest versatility in foodborne disease outbreaks and is associated with a wide variety of foods. Recent outbreaks in deli meats in South Africa, frozen vegetables in Europe and ice cream in the USA have all linked contamination of the final product to the food-processing environment. Within the dairy context, historical control measures through heat (pasteurisation) have had a major impact on reducing the occurrence of listeriosis, but contamination of processed dairy products still occurs. More understanding of ecological niches within dairy manufacturing plants, in order to minimize the likelihood of recontamination events after critical control points, is still needed. The present review aims to summarise the different relevant actions in the food production process that need to be implemented to minimize the likelihood of unsafe final dairy product production in terms of *L. monocytogenes.*

Keywords: *Listeria monocytogenes, Listeriosis, Dairy Processing, Food Safety, Process Environment Monitoring, Control Measures*

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FOREWORD

Listeriosis is a foodborne disease caused by the pathogen microorganism *Listeria monocytogenes*. *L. monocytogenes* is now considered to be one of the major foodborne pathogens, not because of a high prevalence in foods, but due to the high morbidity / mortality of the disease, listeriosis, that it can cause.

Food manufacturers are expected to put in place good manufacturing practices, sanitation standard operating procedures and hazard analysis critical control point programmes to minimize environmental *L. monocytogenes* contamination and to minimise the likelihood of cross contamination in processing plants and in the retail environment.

The safety of the final manufactured dairy product cannot rely solely on final product testing. Instead, it must rely on robust hazard management systems which include a combination of prerequisite programmes, process control and process environment monitoring programmes (PEM) to monitor process environment contamination (including pathogens and hygiene indicator bacteria such as *Enterobacteriaceae* and / or coliforms).

The present review aims to summarise the different relevant actions in the food production process that need to be implemented to minimize the likelihood of unsafe final dairy product production in terms of *L. monocytogenes*.

The work on this bulletin was conducted by an Action Team leader by François Bourdichon (FR) with contributions from Kieran Jordan (IE) and Denise Lindsay (NZ) under the aegis of the IDF Standing Committee on Microbiological Hygiene (SCMH).

Caroline Emond
Director General
International Dairy Federation
October 2019

1 INTRODUCTION

Listeriosis is a foodborne disease caused by the facultative intracellular pathogen *Listeria monocytogenes*. The bacterium was first described in 1926 in laboratory rabbit zoonosis by E.G.D. Murray and was thought to only infect animals, until 1929 when the first human case of listeriosis was reported. *L. monocytogenes* was only recognised as a foodborne pathogen some 60 years later in an outbreak linked with coleslaw (1981) (Schlech et al., 1983). After that initial outbreak and the ones to follow, *L. monocytogenes* is now considered to be one of the major foodborne pathogens, not because of a high prevalence in foods, but due to the high morbidity / mortality of the disease, listeriosis, that it can cause (de Noordhout et al., 2014). Following the risk assessment performed by the Joint FAO/ WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) on *L. monocytogenes* (JEMRA, 2004), guidelines were published by the Codex Committee on Food Hygiene defining microbiological criteria in ready-to-eat (RTE) foods depending on the growth potential of *L. monocytogenes* (Codex Alimentarius, 2007). Codex member states have updated their regulations accordingly (FSANZ, 2014; Todd et al., 2011). Guidelines published by Health Canada (Health Canada, 2011) are also aligned to the proposed rationale, while the USDA-FDA published another approach of "zero tolerance" of *L. monocytogenes* in RTE foods (FDA, 2008) although the classification vs. growth potential was considered in their prior risk assessment (FDA, 2003). Following a recent foodborne outbreak with low level of contamination in the finished product, the position of the FDA is favouring a "zero tolerance" policy (Archer, 2018).

Listeriosis is now recognized to be almost exclusively foodborne. Food manufacturers are expected to put in place "good manufacturing practices, sanitation standard operating procedures and hazard analysis critical control point programs to minimize environmental *L. monocytogenes* contamination and to minimize the likelihood of cross contamination in processing plants and at retail" (EFSA, 2018; ILSI, 2005; JEMRA, 2004). The topic of the present review is focused on the dairy industry.

As *Listeria* spp. are ubiquitous, the safety of the final manufactured dairy product cannot rely solely on final product testing. Instead, it must rely on robust hazard management systems which include a combination of prerequisite programmes, process control and process environment monitoring programmes (PEM) to monitor process environment contamination (including pathogens and hygiene indicator bacteria such as *Enterobacteriaceae* and / or coliforms). *Listeria innocua*, considered to be the genetic ancestor of the genus, is a better ecological competitor than *L. monocytogenes* (Liu et al.,

2009). Searching for *Listeria* spp., in particular in the dairy processing environment, as discussed later, can better confirm the success of having different hygiene zones and the critical points identified by hazard analysis critical control plan (HACCP) to minimize the likelihood of contamination with *L. monocytogenes*, therefore giving better food safety assurance to the dairy business operator.

2

THE RISE OF A FOODBORNE PATHOGEN: *LISTERIA MONOCYTOGENES* IN THE DAIRY CHAIN

L. monocytogenes is well known for its presence in raw milk (Lovett et al., 1987; Paul et al., 2015) and in raw milk products which have resulted in foodborne disease outbreaks (Beckers et al., 1987; Verraes et al., 2015). In addition, *Listeria* spp., including *L. monocytogenes,* are well known persistent bacteria in food manufacturing environments, including the environment of the pasteurisation process of dairy products (Carpentier and Cerf, 2011). Data on numerous recalls of pasteurised dairy products in the last five years (see Table 1 for examples based on available online data) shows that *Listeria* is a continuing issue for processed dairy manufacturers. In addition, between 1985 and 2019, there have been 40 confirmed major outbreaks of listeriosis associated with commercially pasteurised dairy products which have been recorded in literature or have made new headlines (Table 2). In the majority of these cases (18/22 – 82%), where a source was identified, *L. monocytogenes* was found in niches in the dairy processing environment and contamination of final product occurred due to cross-contamination post the heating (pasteurisation) step.

Out of the numerous species of the *Listeria* genus, the three most common species associated with the dairy manufacturing environment are *L. innocua*, *L. monocytogenes* and *L. seeligeri* (Barancelli et al., 2014; McIntyre et al., 2015; Rückerl et al., 2014). Others which are also isolated include *L. ivanovii, L. welshimeri* and occasionally, *L. grayi* (Alvarez-Ordóñez et al., 2015; Barancelli et al., 2014; Silva et al., 2003). The presence of *L. innocua* in the processing environment is a good indicator that applied hygiene regimens are not adequate to mitigate for the presence of *Listeria* spp. in the plant and therefore, there is a likelihood of *L. monocytogenes* contamination (Ryser and Marth, 2007). This is because *L. innocua* and *L. monocytogenes* have been found to behave similarly in dairy products (Petran and Swanson, 1993; Ryser and Marth, 2007) and within dairy manufacturing environments (Lui et al., 2009). It is also commonly accepted that the presence of *L. monocytogenes* in dairy manufacturing plants is significant in wet areas, compared to those that remain dry (Redfern and Verran, 2017; Rückerl et al., 2014). In some cases, processing water itself can harbour *L. monocytogenes*. For example, in 2002, processing

water which had been contaminated by birds carrying *L. monocytogenes* resulted in an outbreak from consumption of cheese in Canada (Table 2) (McIntyre et al., 2015). In addition, when present, *L. monocytogenes* tends to be more common on non-food contact surfaces (non-FCS), compared to food contact surfaces (FCS) (Barancelli et al., 2014; Muhterem-Uyar et al., 2015). This, however, might be a bias of the process environment monitoring in place which tends to focus on the potential niches where *Listeria* spp. survives for a long time in the processing environment. Some examples of where *Listeria* has been found historically in dairy processing plants include, but are not limited to: holding tanks, conveyor belts, milk and brine fillers (Pritchard et al., 1995); crate and case washers, floor, foot baths and foot mats (Klausner and Donnelly, 1991; Kabuki et al., 2004). More recent studies have shown harbourage niches to include floors and floor drains, transport trolleys, pallets and tables (Rückerl et al., 2014); cheese brine, brining baths and associated brining equipment (Alessandria et al., 2010; Barancelli et al., 2014); cheese vats and cloths and curd cutters and storage coolers (Kousta et al., 2010). Non-FCS act as reservoirs for FCS contamination and subsequently, for final product contamination, as has been starkly illustrated in the recent outbreak of *L. monocytogenes* in Blue Bell ice cream in the USA (Table 2) (CDC, 2015a).

Regulatory approaches applied to *L. monocytogenes* in foods mainly focus on end-product testing, with a product classification in three main categories (ability to support growth, no growth demonstrated and infant/medical food). Different microbiological criteria apply considering the category of the food product.

Countries differ in their regulatory approach to the presence of *L. monocytogenes* in RTE food. Codex Alimentarius guidelines propose that for RTE foods in which growth will not occur, a rejection level is set at 100 colony-forming units (CFU) per gram of the products in one or more of five samples of 25g. In RTE foods in which growth could occur, absence in 5 x 25-grams product samples is required. If the ability to grow has not been determined, the ability to grow is presumed and the criterion of absence is applied (Codex Alimentarius, 2007).

Australia (FSANZ) and New Zealand (MPI) follow these criteria as set out by Codex (Anon, 2014). In the European Union, Commission Regulation (EC) No 2073/2005 sets limits which essentially align to Codex Alimentarius guidelines, with complementary introduction of criterion (absence in 10 x 25-grams product samples) for infant products and RTE foods for special medical purposes. In Canada, update of the "Policy on *L. monocytogenes* in RTE foods" has been completed in 2011 and is aligned to the European approach (Health Canada, 2011).

In the United States, the United States Department for Agriculture (USDA) maintains a policy of "zero-tolerance" for *L. monocytogenes* in RTE foods (Archer, 2018; FDA, 2008). Further details on regulations relating to *L. monocytogenes* can be found in a Special Issue of the journal, 'Food Control' (Todd, 2011), although there might have been some changes to this in the intervening years.

3

GROWTH CHARACTERISTICS OF *LISTERIA MONOCYTOGENES*

L. monocytogenes tolerates harsh conditions and could therefore survive or grow in different types of foods. The organism can grow at low temperatures (0.6 to 45°C) and in a wide pH range. It can also grow in salt concentrations of up to 14% and tolerates low water activity (Table 3).

L. monocytogenes has the ability to grow at refrigeration temperatures (4°C) (Lake et al., 2009). It survives under freezing conditions (Metzger et al., 2015) and has been found in ice cream, ice milk, sherbet and ice cream novelties of various types (El-Kest and Marth, 1992). *L. monocytogenes* is rapidly inactivated at temperatures above 70°C and the D-value at 72°C in milk has been estimated at between 0.9 – 2.7s (Sutherland and Porritt, 1997).

The pH optimum for growth is 7.0, with a range from 4.4 to 9.4 (Lake et al., 2009). Occasionally, some strains have shown potential for growth as low as pH 4.1 (Jay, 2005). These values are measured with HCl as the major acid source. For fermented dairy products, *L. monocytogenes* seems unlikely to grow below pH 5.2 (Sutherland and Porritt, 1997) due to the inhibition of lactic acid. Growth of *L. monocytogenes* was demonstrated not to occur at concentrations exceeding 6.35 mM undissociated lactate (Aryani et al., 2016; Wemmenhove et al., 2018). Tolerance to harsh acidic stress (pH 3.5) is induced in *L. monocytogenes* if exposed to mild acidity (pH 5.5) for a time (O'Driscoll et al., 1996). These acid adapted cells subsequently also survive better in fermented dairy products, such as cottage cheese, yogurt and whole-fat Cheddar cheese (Gahan et al., 1996).

L. monocytogenes grows optimally under microaerophilic conditions, but it also thrives well both aerobically and anaerobically (Müller-Herbst et al., 2014). *L. monocytogenes* can grow in atmospheres containing relatively high levels of CO_2, but inhibition occurs at elevated levels (Bennik et al., 1995). *L. monocytogenes* can grow even in relatively high (e.g., 30%) CO_2, but is inhibited under 100% CO_2. Growth was not retarded by a 5–10% CO_2 atmosphere (Lake et al., 2009).

The minimum water activity (a_w) permitting growth is considered as a_w = 0.92 with 11.5% NaCl and 0.93 with 40% sucrose (Jay, 2005). *L. monocytogenes* has the ability to survive in dry foods (a_w = 0.83) (Beuchat et al., 2011) and more recently has been shown to survive in flour (Taylor et al., 2018), so its potential to survive in low moisture dairy products should

not be underestimated. However, *L. monocytogenes* has been shown to progressively die-off in non-fat skim milk powder with time of storage (Doyle et al., 1985). Potassium sorbate, as an example of a commonly used preservative in food, inactivates *L. monocytogenes* at 2000 – 3000 ppm (pH 5.0) (Lake et al., 2009).

4

PATHOGENESIS OF *LISTERIA MONOCYTOGENES*

Listeria diseases have been described in more than 40 animal species. The mechanism of pathogenesis is conserved and generally leads to abortion in pregnancy, septicaemia, meningitis and encephalitis, as well as some reported cases of diarrhoea, skin infections and endocarditis (Farber and Peterkin, 1991). Thirteen serotypes have been identified for *L. monocytogenes*. All of these might be associated with human listeriosis; however, most human infection is associated with the serotypes 1/2a, 1/2b or 4b. The hospitalisation rate is over 90% and a death rate of about 24% of those infected (de Noordhout et al., 2014). A recent study demonstrated that *Galleria mellonella* insect larvae might be a useful animal model for studying the pathogenicity of *L. monocytogenes* (Martinez et al., 2017).

Two types of disease are associated with *L. monocytogenes*, non-invasive or invasive listeriosis.

Non-invasive listeriosis (referred to as febrile *listeria*l gastroenteritis) is the milder form. Illness occurs within 24h of ingestion and includes diarrhoea, fever, headache and myalgia (muscle pain). Outbreaks of this disease have generally involved the ingestion of high doses of *L. monocytogenes* by otherwise healthy individuals. The vast majority of these cases show no evidence of invasive disease beyond the intestine and it is usually self-limiting (2 days) (Ooi and Lorber, 2005).

Invasive listeriosis affects 'high-risk individuals' (YOPI – which includes the young, the old, pregnant women, neonates and immune-compromised adults) (McLauchlin et al., 2004). Invasive listeriosis can have an incubation time of between two weeks and three months (McLauchlin et al., 2004). Infection during pregnancy can occur at any stage, but is most often reported during the third trimester. The mother usually exhibits mild flu-like symptoms, but the unborn or newly born infant can develop severe systemic disease (McLauchlin et al., 2004). Stillbirths, premature termination of pregnancy or live birth of infected neonates is common. The immune systems of foetuses and new-borns are very immature and are extremely susceptible to these types of infections. Overall, this disease is characterised not only by the severity of the symptoms in susceptible individuals, but also by a high mortality rate.

The probability of *L. monocytogenes* in a food causing an illness varies and depends on several factors, including strain type and differences in host susceptibilities. While theoretically it takes one cell to cause listeriosis, the dose of *L. monocytogenes* cells causing illness in healthy individuals has been estimated at between 10^7 – 10^9 cells and 10^5 – 10^7 cells for high-risk people (Farber et al., 1996; Dalton, 1997). However, more recent outbreaks have occurred with much lower doses (<1000 CFU in some cases). For example, during a butter outbreak in Finland, it was estimated that the daily dose ranged from 10^1 – 10^5 CFU/day (Maijala et al., 2001) and the US ice cream outbreak in 2015 indicated an even lower potential dose (the lowest contamination level of the ice cream was estimated at 8 CFU/g, although temperature abuse and growth of *L. monocytogenes* in the milkshakes made from this ice cream causing the illness in hospital patients, could not be ruled out) (Pouillot et al., 2016). Further to this, a study by Chen et al. (2006) has also indicated that there is a wide variation of virulence associated with *L. monocytogenes* isolates, depending on serotype and strain, which will also influence the numbers of cells needed to cause illness.

5

SURVIVAL OF *LISTERIA* SPP. AND *L. MONOCYTOGENES* DURING THE DAIRY MANUFACTURING PROCESS

L. monocytogenes can be found in a large variety of habitats including soil, sewage, water, faeces of healthy animals and humans, vegetation and silage (Welshimer and Donker-Voet, 1971). Decaying plant material and silage are thought to be the main route of infection for farm animals. Farm animals are not considered to be the main reservoir for *L. monocytogenes*, although the organism can cause animal disease and infected dairy cattle can disseminate the organism throughout the farm environment (Nightingale et al., 2004). Infection of the milk from the udder in cases of clinical or sub-clinical mastitis can also occur (Jensen et al., 1996; Hunt et al., 2012). In terms of human infection, *L. monocytogenes* is carried asymptomatically in the faeces of 2–6% of the population and has been found in the nasal cavities and on hands of food workers (El-Shenawy, 1998), but human-to-human transmission is not recorded as a main route of infection (with the exception of mother to foetus transmission) (Lake et al., 2009). Ingestion of food is the main route of infection in humans (EFSA, 2018).

L. monocytogenes is frequently present in foods of animal (certain dairy products, various meats and meat products such as beef, pork, fermented sausages, seafood and fish products) and plant origin (fresh produce such as radishes, cabbage, celery, melons and cantaloupes, caramel apples, romaine lettuce and sprouts) and can become endemic in food processing environments (Gandhi and Chikindas, 2007). It might also be present in cooked foods as a result of post-process contamination or inadequate heat treatment, such as in ready-to-eat meats (Simmons et al., 2014). Dairy products which have been more frequently associated with *L. monocytogenes* include raw milk and soft cheeses, in particular white-moulded soft cheeses where growth can occur due to the consumption of lactic acid by the mould, resulting in an increase in pH on the surface. However, recent outbreaks have shown that *L. monocytogenes* can also be present and cause illness in other processed dairy products, such as ice cream and pasteurised milk products (due to post-heat recontamination events).

As previously mentioned, *Listeria* spp. and *L. monocytogenes* have been isolated from a variety of sites within dairy manufacturing plants, although these bacteria are most frequently found in moist environments or areas with condensation or standing water or food residues, including drains, floors, coolers, conveyors and case washing areas (for review, see Carpentier and Cerf, 2011). Biofilms within manufacturing plants, including in dairy processing, might serve as a source of *L. monocytogenes* for processed foods (Valderrama et al., 2013).

To minimize the likelihood of *L. monocytogenes* cross contamination in dairy manufacturing plants, monitoring the process environment, as suggested in FSMA (Food Safety Modernization Act, 2011 – USA), FSANZ (Australia and New Zealand, 2014) and EU regulation 2073/2005 (European Union, 2005), is carried out. For example, Article 5 of EU 2073/2005 stipulates "food business operators manufacturing RTE foods, which may pose a *L. monocytogenes* risk for public health, shall sample the processing areas and equipment for *L. monocytogenes* as part of their sampling scheme". Although this doesn't mention the frequency of sampling or the number of samples, the appropriate sampling schemes under responsibility of the food operator should be discussed with the relevant regulatory authorities to ensure that an appropriate finished products and processing environment sampling plan is implemented.

6

GROWTH POTENTIAL OF *LISTERIA* SPP. AND *L. MONOCYTOGENES* IN DAIRY PRODUCTS

In general, relatively high numbers of *L. monocytogenes* are required to cause disease. Due to intrinsic factors, such as undissociated lactate in some semi-hard cheeses or in acidic products such as yoghurts, not all dairy products could support the growth of *L. monocytogenes* to achieve the high numbers required (Aryani et al., 2016). Additionally, cross contamination from the processing environment is unlikely to result directly in such high numbers. Therefore, the ability of the dairy products to support the growth of *L. monocytogenes* is paramount. The EU and other jurisdictions have published guidelines for undertaking challenge studies to determine the ability of food to support the growth of *L. monocytogenes* (Anon, 2014; Beaufort et al., 2014; Health Canada, 2011).

Predictive modelling can be used as a pre-assessment to artificial contamination studies, to estimate the ability and extent of *L. monocytogenes* growth in a dairy product. *In silico* models such as "Combase" (Baranyi and Tamplin, 2004), Sym'Previus (Leporq et al., 2005) and "Pathogen Modelling Programme" (USDA, 2018) can be used for this purpose. If growth is predicted by the computer-based programmes, which are mainly based on broth systems, other characteristics of the dairy product not accounted for in the models, could potentially inhibit growth of *L. monocytogenes*, such as for example, competing microflora (Jordan et al., 2018). Using Combase, Schvartzman et al. (2011) predicted growth in the cheese matrix for 40% of cases, while no growth was actually observed.

The ability of dairy products to support the growth of *L. monocytogenes* must be determined for each food type, in particular if they differ in water content, pH profile during shelf-life, starter cultures applied and surface treatment, since different formulations and manufacturing have a significant impact on the growth potential of *L. monocytogenes*. Furthermore, any changes in the ingredients or process of a dairy product, either due to attempts to extend the shelf-life of a product, or to address consumer's expectations on new formulation, could lead to a change in the ability of *L. monocytogenes* to grow or die-off. Validation studies, including challenge studies where needed, might need to be undertaken with each newly developed dairy product. Extrapolation from one food type to another, or from generic models without validation of the specific cheese is not appropriate (Schvartzman et al., 2010).

In cases where growth potential is demonstrated, i.e., ≥ 0.5 log increase in numbers from day 0 to day end of the challenge study (cf. the EURL *L. monocytogenes* Technical Guidance document, Beaufort et al., 2014), the initial numbers present and the growth rate, will determine if the numbers will exceed the limit of 100 CFU/g during shelf-life. The EURL guidance document outlines a method to conduct artificial inoculation studies to determine growth rate. Challenge studies can either determine growth potential or growth rate of *L. monocytogenes* in a dairy matrix. According to these guidelines, when testing growth rate, each strain must be tested individually, sampling must be undertaken on at least ten occasions (for modelling purposes) and food storage must be carried out at a uniform temperature (Beaufort et al., 2014). Minimizing the growth of *L. monocytogenes* at retail level and further on in the distribution chain could lead to a 37% reduction in listeriosis cases (EFSA, 2018).

7

ANALYTICAL METHODS FOR DETECTION, ENUMERATION AND IDENTIFICATION

As defined in the Codex document, CAC GL 21/1997 modified in 2013 (Codex Alimentarius, 2013), analytical methods and their performance parameters are key components of microbiological criteria. Traditional culture-based methods have been continuously implemented since the early 1980s. Culture-based testing for *Listeria* spp. and *L. monocytogenes* is defined by the recently published standards ISO 11290 Parts 1 and 2 (ISO 2017a, and 2017b). However, in the dairy production business, the time taken to release final products due to waiting for pathogen results is a cost to the business. More rapid alternative methods are now widely available, based on immune-enzymatic assays or molecular detection, as long as the alternative method meets regulatory requirements such as those set out in EU 2073/2005: Article 5 – "The use of alternative analytical methods is acceptable when the methods are validated against the reference method in Annex I and if a proprietary method, certified by a third party in accordance with the protocol set out in ISO 16140 or other internationally accepted similar protocols, is used. If the food business operator wishes to use analytical methods other than those validated and certified as described in paragraph 3, the methods shall be validated according to internationally accepted protocols and their use authorised by the competent authority". ISO 16140 Part 2 (ISO, 2016) is usually used to validate such alternative microbiological methods. A further aspect which is important for dairy manufacturers is that an appropriate method should be implemented in a competent laboratory working under ISO 7218:2007 (ISO, 2007) and ISO 17025:2017 (ISO, 2017c).

Methodologies other than ISO methods are also available, such as GB 4789.30-2016 (GB, 2016), used in China and the USFDA BAM method for *Listeria monocytogenes* in foods and environmental samples (Hitchins et al., 2017), used in the USA. Alternative (proprietary) methods do not have a recognized validation and verification scheme in China, while in the United States, an ISO 16140 Part 2-based approach by AOAC is available, yet based on USFDA BAM as reference method and not ISO 11290 Parts 1 and 2 (ISO 2017a, and 2017b).

Alternative proprietary methods for *Listeria* spp. and *L. monocytogenes* typing can be found for Europe and ISO scheme on the AFNOR website (https://nf-validation.afnor.

org/en/food-industry/listeria-monocytogenes/), MICROVAL website (http://microval.org/en/issued-certificates/) and for the United States, the FSIS website (http://content.govdelivery.com/accounts/USFSIS/bulletins/842385). It is important to verify in the available validation dossier that the scope of the validation (food matrices) covers the analytes of concern and the sampling technique (e.g., pooling or not).

Typing methods are required for source tracking of contamination pathways of *Listeria* spp. and most specifically *L. monocytogenes*. For many years, pulsed field gel electrophoresis (PFGE), a restriction enzyme-based analysis which provides a fingerprint of a bacterial genome, has been used until most recently, as the gold standard for identification and clustering (determining clonality) of isolates. However, whole genome sequencing (WGS) was first introduced in the USA in 2008 and was instrumental in solving the Blue Bell ice cream *Listeria* outbreak. Since then, it has replaced PFGE to become the preferred FDA method for identification and tracking of pathogen isolates in food samples and in food manufacturing sites. Numerous food safety authorities now use the technique widely in other regions of the world and especially in the European Community (Hendriksen et al., 2018). WGS is an analytical technique which allows the determination of the complete genomic DNA sequence. Due to its accuracy in determining how bacteria are related to each other, WGS improves the detection, surveillance and response to foodborne diseases and outbreaks. The technology provides uniform typing systems across the environmental, animal, food and human sectors, and offers the potential to trace foodborne contamination back to its microbial sources beyond geographical differences (FAO-WHO, 2016).

WGS is more accurate than a serotype determination and more discriminatory than PFGE, ribotyping and RFLP techniques. It can show relationships between strains with the highest resolution and in a particularly limited time. WGS provides the ability to: differentiate sources of contamination, even within the same outbreak; determine which ingredient was originally contaminated by the pathogen associated with an illness outbreak and therefore narrow the search for the source of a contaminated ingredient; determine unexpected vectors for food contamination and provide information for root cause analysis and determine phenotypic properties of isolates, such as resistance or sensitivity to cleaning agents or adherence to surfaces (Kovac, 2017).

The identification can be shared and compared between laboratories across the world using open access international databases such as GenomeTrakr. Food microbiologists and clinical microbiologists can now pinpoint the origin of a foodborne contamination by comparison with clinical isolates. As a result, foodborne illnesses are being more easily tracked to a specific location. Regulatory bodies can now identify the origin of small outbreaks with a limited number of clinical isolates or identify long lasting contamination events causing limited numbers of cases in the population.

In time, it is predicted that WGS will become the new standard for isolate identification and replace other methods to accomplish this. In order to keep track of historical data when changing the identification technique, it is important to retain if not all isolates,

at least the type strains of each culture. Having a strain collection is important as WGS becomes more widely used and local / industrial / national initiatives should be taken to gather those isolates.

Currently, the major challenges with the application of WGS are the cost of this typing method in comparison to other typing methods, the lack of a WGS technical standard on methodology and no internationally agreed "cut-off" values that differentiate between strains (single nucleotide polymorphisms [SNP]). The acceptable number of SNPs can be specific to each microorganism. Interpretation of results does require bioinformatics specialists, while no internationally accepted approach is presently in place to protect WGS information stored in databanks from misuse. Food safety outbreaks or incidents cannot be solved solely based on WGS data, but must always be linked to epidemiological data for confirmation.

PFGE has been used as a gold standard for strain typing for more than 30 years. Due to capacity building (training, equipment, premises, data processing…), this technology can not be replaced overnight by a methodology that is not yet standardized. Also, in some situations where WGS would be too costly to set up, PFGE will continue to be a valuable tool for analysis.

For more information on using WGS in the food industry, a recent review by Jagadeesan et al. (2019) provides readers with a description of the various WGS technologies in use today, guidelines on determining SNP differences for various bacteria and which previously validated SNP-based tools to use, some of the validated databases recommended for use in comparing core-genome multi locus sequence typing (cgMLST) schemes for common foodborne pathogens, the most suitable references to use to provide guideline for phylogenetic tree analysis and a selection of the more commonly used bioinfomatics tools and pipelines needed for WGS analysis.

8

CONTROL MEASURES OF *L. MONOCYTOGENES* ALONG THE DAIRY MANUFACTURING PROCESS

Historically, heat treatment (pasteurisation of dairy products) is applied to raw materials to reduce the initial microbial contamination to acceptable levels. The current treatments are based on either industry guidance, codes of practice or regulation founded mostly on a historical approach established on the population reduction required. In terms of dairy products, an overall recognised pasteurisation treatment of raw milk for 15s at 72°C would result in a > 6-log reduction of *L. monocytogenes.* In addition, growth of *L. monocytogenes* in RTE food can be inhibited using one or more of the following control measures:

- pH less than or equal to 4.4 (EU 2073/2005, FDA, 2018)
- Water activity less than, or equal to 0.92 (e.g., milk powder in dairy manufacture) or less than, or equal to 0.94 in combination with a pH of less than, or equal to 5.0 (EU 2073/2005, FDA, 2018)
- Formulation containing one or more inhibitory substances which, alone or in combination, minimize the likelihood of growth of *L. monocytogenes* (e.g., biopreservation with food starters such as those used in hard type cheeses manufacturing (Wemmenhove et al., 2018) and undissociated lactic acid at concentrations above 6.35 mM (Aryani et al., 2016)
- Strict maintenance of cold chain (e.g., chilling of liquid milk in dairy manufacture)
- Minimizing the likelihood of cross-contamination (e.g., adherence to good manufacturing practices, GMP) and re-contamination (e.g., good hygiene practices, GHP) of heat-treated food products (e.g., separation of the raw and processed sides of the milk manufacturing environment).

Due to low levels occurring in most foods, final product testing is not effective. It is an important approach only where *L. monocytogenes* is likely to occur frequently. In cases where *L. monocytogenes* does not occur frequently, final product testing provides little information on product contamination, its origin and how to mitigate it. Any action should be focused on continuous and biased sampling of the processing environment, targeting areas with a positive result for additional hygiene measures, e.g., root cause

analysis. As it has been noted that contamination of non-FCS with *Listeria* spp., including *L. monocytogenes*, usually precedes contamination of FCS (GMA, 2014), a more beneficial strategy therefore, is to have in place a validated and effective PEM programme, including *Listeria* spp., as then the likelihood of cross-contamination to final dairy products is reduced.

As *Listeria* spp. are ubiquitous in the environment, complete elimination of the genus, and *L. monocytogenes* in particular, from the processing environment is an unrealistic aim. Control of *L. monocytogenes* in the processing environment is, however, an achievable aim. Achieving this control requires awareness of the ecology of *L. monocytogenes.* Since the other species of the *Listeria* genus are not pathogenic, their presence in the dairy manufacturing environment, while not constituting a public health risk, is considered to be a good indication for the potential presence of *L. monocytogenes* (Liu et al., 2009; Ryser and Marth, 2007). This is not an indicator as classically understood while performing testing of *Enterobacteriaceae* and /or coliforms for hygiene, but rather as an indication of the capacity of *L. monocytogenes* to survive in the processing environment and to anticipate its introduction. Testing for *Listeria* spp. in PEM and reacting to positive results as if they were *L. monocytogenes,* provides for a more sensitive and broader verification and control programme, than would testing for *L. monocytogenes* alone, particularly considering the expected very low prevalence of this pathogen in a well maintained, cleaned and sanitised dairy processing environment.

Sampling a dairy processing environment for *L. monocytogenes* is based on a seek and search (and destroy) bias to look for harbourage sites where contamination could occur, as opposed to selecting sampling sites for negative results to adhere to regulations. A well-designed PEM is one which actually finds the pathogen of concern, so that the data can be used to track any trends towards the increase or decrease of the organism in the dairy-processing environment. Molecular typing of isolates which are obtained during a well defined PEM can be expensive, but it does provide valuable information on the characteristics of the isolates obtained and on the nature of the contamination. For example, repeated isolation of a strain with the same molecular profile can indicate persistent contamination, which would require an alternative course of action than if each strain was different. In order to reduce molecular analysis costs, molecular typing need only be done for isolates obtained from high-risk areas.

Sampling of a processing environment should be based on product proximity, including looking for harbourage sites close to the end product. PEM for *L. monocytogenes* and *Listeria* spp. should be focused on the wet zones of the environment, e.g., the cleaning station, water condensates and drains (if any) (Carpentier and Cerf 2011, Valderrama et al., 2013), as compared to PEM for *Salmonella* which usually focuses on dry areas with Enterobacteriaceae as hygiene indicators. For example, one of the most common contaminated areas for *L. monocytogenes* are floor drains, as any contamination throughout the facility is likely to be washed through the drain where *L. monocytogenes* can persist in biofilms (for review, see Carpentier and Cerf, 2011). Drains should not necessarily be

considered as the only harbourage site, but they might also indicate the presence of a niche in the surrounding area. Note that monitoring the process environment is not monitoring the efficacy of cleaning procedures. Sampling of cleaned surfaces for *L. monocytogenes* should only be done to evaluate the efficacy of the cleaning procedures after detection of positives samples.

For an effective PEM, sampling should be done with a sponge-type swab or gauze swab, allowing sufficient surface area to be sampled according to the recommendations of ISO 18593-2018 (ISO, 2018). Adequate sampling will allow for a proactive approach to the food safety management system, where finished product testing is a reactive approach. *L. monocytogenes* contamination of final dairy products is a much more serious problem which requires significantly more intervention than contamination at the processing environment stage. Regulations on process environment sampling, as in article 5 of EU Regulation 2073/2005 or FSMA expectations should be duly implemented. The EU document on guidance for sampling provides recommendations with regard to timing of sampling, locations for sampling, among other things (EC, 2012). The most informative sampling sites can vary depending on the facility and the type of food produced. Information can be obtained from screening of FCS and non-FCS and it is important to have a balance between these different samples. Adequate sampling for *L. monocytogenes* will help identify an issue early and, thus enable it to be dealt with immediately. Such a programme should be reviewed regularly, depending on the results that are obtained.

In a medium to large processing facility, hygienic zoning such as establishment of critical control areas (CCAs) can be considered. CCAs should be clearly marked and hygiene barriers should minimize the likelihood that the CCAs will be contaminated through cross-contamination by inappropriate practices. Footbaths and change of personal protective clothing, the two most common zoning barriers, can be incorporated to reduce the likelihood of cross-contamination with *L. monocytogenes*. The definition of CCAs can facilitate targeted sampling in those areas and, therefore, different actions based on positive results from different CCAs. For example, the closer one gets to a final dairy product contact surface, the higher the likelihood of a positive result which could result in cross-contamination of processed dairy products. In small dairy processing facilities, the definition of CCAs with hygiene barriers might not be feasible, but the concept of CCAs should be considered, with restricted access for untrained staff to dairy product contact areas.

Sampling frequency should be assessed on a case-by-case basis for each processing environment. When a food-processing environment is being sampled for the first time, a broad sampling approach should be taken to identify contamination routes and potential harbourage sites. If a history of monitoring is available, or the contamination status is already known, a restricted number of sampling sites can be determined. Sampling frequency should be adapted to new sampling points if negative results are obtained, but should be increased again if positive results are obtained or if there are changes to the processing environment or manufacturing process (EU, 2005). Such changes in sampling should only be undertaken in consultation with external expertise, as appropriate.

During renovation or construction work, hygiene measures can be difficult to maintain. It can be difficult to recommend the use of hygiene protection (overshoes, overcoats) to craftsmen and building workers, or hygiene measures for building materials often stored outdoors before use. Products might still need to be produced in processing rooms adjacent to the construction area. It is important to be aware of the increased likelihood of cross contamination during such construction work. Construction of a physical barrier between the production area and the construction zone is important as a first step. Increased sampling to monitor the processing environment, combined with increased awareness, will also facilitate a reduction in the likelihood of cross-contamination. Recording of data obtained is critical for a dairy processor. As well as recording the results of a dairy processing monitoring programme, it is also important to record data on ingredients and raw materials so that any correlations can be identified, for example, processing environment contamination coinciding with a batch of raw material or a change in supplier. Equally as important to recording the data, is analysing the data. Processors should look for trends in their test results by either plotting them on a graph or putting them in a table. In the case of processing environment monitoring in particular, a graph of the results will help the producer see a trend towards unsatisfactory results and allow them to investigate and take appropriate actions to correct the situation.

Maintenance of a completely *L. monocytogenes*-free processing environment is relatively difficult to achieve as many different factors can affect occurrence. These can include, for example, contaminated incoming raw materials, staff members as carriers, insufficient cleaning strategies and sampling programmes, the facility design to minimize the likelihood of contamination, the location of the facility near a farm and so on. Another major factor in the occurrence of *L. monocytogenes* is management and staff member's awareness of the hazard and education of the processing facility and contamination issues therein. Lack of such awareness can lead to significant problems with end products which can result in product recalls, damage to company reputation, lawsuits, illnesses or even death. Thus, awareness, sampling and analysis are key trend factors in successful control of *L. monocytogenes*. If occurrence is detected it can be eliminated through targeted intervention measures, thus minimizing the likelihood of final product re-contamination (Leong et al., 2016).

In terms of chemical disinfection to control *L. monocytogenes*, the choice of sanitizers by food business operators is often limited by regulatory approval. The FDA recommends the use of sanitisers containing quaternary ammonium compounds (QACs, or QUATS) to be effective and, also because they have a residual antimicrobial effect (FDA, 2008). This recommendation correlates with studies which have shown that QACs may be more effective against *L. monocytogenes* biofilms than chlorine-containing sanitisers (Olszewska et al., 2016). Some studies have, however, shown that *L. monocytogenes* can develop a tolerance to treatment with QACs (Mereghetti et al., 2000; Olszewska et al., 2016). Studies have shown a two to four-fold increase in tolerance of *L. monocytogenes* cells to QACs after exposure for several hundred generations (Kastbjerg and Gram, 2012) and the presence of plasmids in some strains can further contribute to the tolerance (Naditz et al.,

2019). By contrast, no such increase in tolerance was found for hypochlorite or peracetic acid/hydrogen peroxide (Kastbjerg and Gram, 2012). Hence, judicious use of QAC sanitisers within dairy manufacturing plants should be practiced and sanitiser rotation implemented to minimize the likelihood of tolerant populations establishing themselves and for greater overall effectiveness of antimicrobial activity (FDA, 2008). Sanitisers can be rotated using, for example, peracetic acid/hydrogen peroxide, which have been shown to be effective at inactivating biofilm cells of *L. monocytogenes* (Belessi et al., 2011). To illustrate, a study by Costa et al. (2016) showed that a peracetic acid/hydrogen peroxide sanitiser effectively reduced the attached populations of 16 persistent strains of *L. monocytogenes* (from a Gorgonzola cheese manufacturing plant) by more than 4 log CFU.

As with other bacteria, planktonic cells of *L. monocytogenes* are readily inactivated by common chemical sanitisers used in dairy processing, compared to inactivation when attached as biofilms on stainless steel surfaces (Luque-Sastre et al., 2018). Several decades of studies have also demonstrated that populations of *L. monocytogenes* cells attached to different materials respond differently to applied sanitizers (Table 4 shows some examples). These results highlight the need to select a suitable sanitiser when sanitation programmes are implemented in the dairy manufacturing environment, as both the sanitiser type, time of application, concentration and surface type to be treated, seem to influence the likely success of control of *Listeria* spp (Skowron et al., 2018).

Sometimes however, no matter the efficacy of a sanitiser, *L. monocytogenes* is found to persist in manufacturing sites. It has been suggested that suitable growth conditions in harbourage sites, i.e., shelters due to unhygienic design of equipment and premises, or unhygienic or damaged materials, favour *Listeria* and that the pathogen becomes hard to eliminate (Belessi et al., 2011, Carpentier and Cerf, 2011). Thus, physical maintenance of equipment is also important for minimizing the likelihood of *L. monocytogenes* endemic population development in dairy manufacturing plants.

9 CONCLUSION

While it might be believed that sufficient information is known about *L. monocytogenes* to control this hazard in the dairy food chain, recent outbreak and recall events put in perspective the ability of this organism to colonize numerous ecological niches and remain dormant for many years in a processing environment. Finished product testing is not enough to ensure the safety of food production. Effective processing environment monitoring, through swabbing FCS and non-FCS, remains the most efficient pro-active approach to *L. monocytogenes* reduction. Recent advances in source tracking, especially with WGS, are helping to drive genomic approaches for a better characterization of resident strains, their resistance to cleaning agents and adherence to dairy product contact surfaces. Control measures can be customized for a better fit-for-purpose, with better hygienic design and a good rationale for the use of chemical cleaning agents. Based on the ICMSF equation for safe food ($H_0 - \Sigma R + \Sigma I \leq FSO$), good milking practices reduce the prevalence of *L. monocytogenes* in processed dairy (H_0), pasteurisation reduces the contamination (if any) of processed milk with *L. monocytogenes* (ΣR), intrinsic product factors and process environment monitoring (PEM) ensure no late (re-) contamination events with *L. monocytogenes* occur ($\Sigma I = \Sigma Growth + \Sigma Contamination$), thus fulfilling the food safety objective (FSO) for safe processed dairy products.

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Table 1. Examples of recalls of commercial pasteurised dairy products due to *Listeria monocytogenes* in the last 5 years.

Dairy product	Recall date	Type or Brand	Country	Involved in foodborne illness/outbreak
Butter	March 2016	Tesco flavoured butters	UK	No
	July 2017	Perron and Beure de Luc	Canada	No
	July 2017	St Laurent	Canada	No
	Jan 2018	Plaquette butter	Belgium	No
	June 2019	Bandon Co-Op butter	Ireland	No
Cheese	Jan 2015	Queseria Bendita soft cheese and sour cream	USA	Yes
	Sept 2015	Picnic Gourmet cheese spread	USA	No
	Sept 2015	Karoun dairies cheese	USA and Canada	Yes
	Oct 2015	Summer Fresh cheese dip	Canada	No
	Nov 2015	Inverloch cheddar cheese	Canada	No
	Dec 2015	Bothwell shredded cheese	Canada	No
	April 2016	Kopobbi whole milk ricotta cheese	USA	No
	April 2016	Brewer's Gold Irish soft cheese	UK	No
	Oct 2016	Kuster's cheese	USA	No
	Feb 2017	Fromi cheese	USA	No
	Feb 2017	Michigan Milk Producers Association (Saputo) Deutsch Käse Haus cheese	USA	No
	May 2017	Queso fresco cheese (Global Garlic Inc)	USA (from Nicaragua)	No
	Oct 2017	Little Milk Co cheese	Ireland	No
	Jan 2018	Creamed cheese containing fish	USA	No
	Jan 2018	Panera Bread cream cheese	USA	No
	April 2018	Fromagère de la Brie brand, l'Explorateur soft ripened cheese	USA (from France)	No
	Nov 2018	Green Cedar Ackawi cheese	USA	No
	Nov 2018	Sprout Creek Margie cheese	USA	No
	Jan 2019	Yorkshire Fine Cheese - Barncliffe Brie	UK	No
	Feb 2019	JOD Food - Irish Cheddar cheese with chilli	Ireland	No
	April 2019	Société Fromagère de la Brie cheese	France (and other countries)	Yes
	June 2019	Lactalis McLelland Galloway Coloured Medium Grated Cheddar	UK/Scotland	No
	July 2019	Damse Mokke Koe	Belgium/EU	No

Dairy product	Recall date	Type or Brand	Country	Involved in foodborne illness/outbreak
Cream	Aug 2015	UK pasteurised double cream – affecting several companies	UK	No
Dairy dessert	June 2019	Cadbury UK (Müller UK)	UK	No
Ice cream	Jan 2015	Full Tilt ice-cream	USA	No
	Jan 2015	Pink's ice-cream	USA	No
	March 2015	Blue Bell ice-cream	USA	Yes
	April 2015	Jen's Splendid ice cream	USA	No
	July 2016	Agave ice cream	USA	No
	Sept-Oct 2016	Blue Bell ice cream 2nd recall	USA	No
	Oct 2016	Nestle ice cream	USA	No
	Oct 2016	Publix ice cream	USA	No
	Oct 2016	Blue Bunny ice cream	USA	No
	Oct 2016	Chocolate Shoppe ice cream	USA	No
	Nov 2016	Weight watcher's cookie dough sundae	USA	No
	Nov 2016	Ashbey's Sterling ice cream	USA	No
	Nov 2016	Cedar Crest ice cream	USA	No
	Nov 2016	AC Creamery ice cream	USA	No
	Nov 2016	Agave ice cream	USA	No
	Nov 2016	L.A. Creamery ice cream	USA	No
	Nov 2016	McConnell's ice cream	USA	No
	Dec 2016	Foxy's ice cream	USA	No
	Dec 2016	Snow Monkey ice cream	USA	No
	April 2017	Wholesome foods vanilla cup	Canada	No
	Jan 2018	Fieldbrook Foods ice cream	USA	No
	Sept 2018	Reilly Craft Creamery ice cream	USA	No
	Oct 2018	Working Cow Homemade ice cream	USA	Yes
Liquid milk	June 2016	Neilson chocolate milk	Canada	Yes

Table 2. Outbreaks of *L. monocytogenes*, available in literature and online, associated with pasteurised commercial dairy products (since 1985).

Date	Dairy Product	Country	Pathogen serotype	Source of pathogen if known	Numbers ill	Number of deaths	Reference
1985	Mexican-style soft cheese	USA	*L. monocytogenes*	Unknown	142	52	Linnan et al., (1988)
1987	Butter	USA	*L. monocytogenes serotype 1/2a*	Unknown	11	0	Mascola et al., (1999)
1989 - 1990	Blue cheese/hard cheese	Denmark	*L. monocytogenes*	Unknown	26	6	Jensen et al., (1994)
1994	Chocolate milk	USA	*L. monocytogenes*	Unknown	52	0	De Buyser et al., (2001)
1994	Milk	USA	*L. monocytogenes* 1/2b	Post-pasteurisation contamination	45	Not specified	Dalton et al., (1997)
1998-1999	Butter	Finland	*L. monocytogenes* 3a	Persistent strain in processing plant, post-process contamination	25	6	Lyytikäinen et al., (2000)
2002	Soft ripened cheese	Canada	*L. monocytogenes* 4b	Environmental transmission likely occurred from farm animals to workers to culture solutions used during cheese production.	49	0	McIntyre et al., (2015)
2002	Soft ripened cheese	Canada	*L. monocytogenes* 4b	Birds were identified as likely contaminating the dairy plant's water supply and cheese during the curd-washing step.	86	0	McIntyre et al., (2015)
2003	Butter	UK	*L. monocytogenes* type V, serotype 4b, phage type A	Butter isolate also isolated from drain in the plant	17	0	ACMS and Advisory Committee on the Microbiological Safety of Food (2003)
2004	Morra bocconcini cheese	Canada	*L. monocytogenes*	Inadequate milk pasteurisation	1	0	News Ontario (2004)
2006	Cheese	USA	*L. monocytogenes*	Unknown	3	1	CDC NORS (2018)
2006-2007	Cheese (acid curds)	Germany	*L. monocytogenes*	Unknown	189	Not specified	Koch et al., (2010)
2007	Milk (flavoured and plain)	USA	*L. monocytogenes*	Environmental cross-contamination (especially floor drain) in the finished product area	5	3	CDC NORS (2018)

Date	Dairy Product	Country	Pathogen serotype	Source of pathogen if known	Numbers ill	Number of deaths	Reference
2008	Mexican style queso fresco cheese	USA	*L. monocytogenes*	Post-pasteurisation equipment contamination	13	2	CDC NORS (2018)
2008	Cheese	Canada	*L. monocytogenes*	Processing plant cross-contamination (brine hypothesised as potential source but unproven)	38	5	Gaulin et al., (2012)
2008	Blue Stilton (Cropwell)	Canada	*L. monocytogenes*	Unknown	1	0	Canadian Food Inspection Agency (2008)
2008	Mexican style cheese	USA	*L. monocytogenes*	Unknown	8	0	CDC NORS (2018)
2009 - 2010	Camembert cheese (made from pasteurised milk)	Norway	*L. monocytogenes*	Unknown	17	3	Johnsen et al., (2010)
2009 - 2012	Queijo fresco cheese	Portugal	*L. monocytogenes* IVb	Problems in cheese processing plant, likely cross-contamination	30	11	Magalhães et al., (2015)
2009-2010	Quargel cheese (cheese curd)	Austria / Germany/ Czech Republic	*L. monocytogenes* 1/2a	Insect contamination of process equipment	34	8	Schoder et al., (2014)
2009	Mexican style cheese	USA	*L. monocytogenes*	Unknown	18	0	CDC NORS (2018)
2009	Mexican style cheese	USA	*L. monocytogenes*	Unknown	8	0	CDC NORS (2018)
2010	Mexican style queso fresco and other soft cheeses (Queseria Soft cheeses)	USA	*L. monocytogenes*	Unknown	5	1	CDC NORS (2018)
2010	Mexican style cheese	USA	*L. monocytogenes*	Unknown	6	1	CDC NORS (2018)
2011	Hard cheese	Belgium	*L. monocytogenes* 1/2a	Unknown	12	4	Yde et al., (2012)
2012	Latin-style fresh cheese	Spain	*L. monocytogenes*	Post-pasteurisation and preparation contamination	2	0	de Castro et al., (2012)
2012	Camembert and Brie cheese (made from pasteurised milk)	Australia	*L. monocytogenes*	Unknown (source not found)	26	4	Ross (2013)

Date	Dairy Product	Country	Pathogen serotype	Source of pathogen if known	Numbers ill	Number of deaths	Reference
2012	El Rancho del Sur soft cheese	USA	*L. monocytogenes*	Unknown	1	0	Food Safety News (2012)
2013	Cream puffs (profiteroles)	Australia	*L. monocytogenes*	Post-pasteurisation equipment contamination	3	1	NSW Communicable Diseases Report (2013)
2013	Cheese	USA	*L. monocytogenes*	Inappropriate handling during labelling	6	2	CDC (2013)
2013	Mexican style cheese	USA	*L. monocytogenes*	Unknown	9	1	CDC NORS (2018)
2014	Semi-soft, Latino-style cheese (Caujada en Terron)	USA	*L. monocytogenes*	Cross-contamination and poor hygiene practices at the plant, badly leaking roof, rusting and deteriorating equipment and food residues on equipment even after cleaning	11	1	USFDA (2016)
2014	Semi-soft, Latino-style cheese called quesito casero (Oasis Brand)	USA	*L. monocytogenes*	Manufacturing plant environmental cross-contamination	5	1	CDC (2014)
2014	Ice-cream	USA	*L. monocytogenes*	Cross-contamination at manufacturing factory – found in processing equipment	2	0	Food Safety News (2014)
2015	Varieties of soft cheese (Queseria Bendita brand)	USA	*L. monocytogenes*	Unknown	3	1	USFDA (2015)
2015	Blue Bell ice cream	USA	*L. monocytogenes*	Equipment at manufacturing plant linked to outbreak strain, chronic cleaning and sanitizing problems identified in plants, outbreak strain found in carton fillers and a cleaning tub. Coliform numbers indicated an issue (>100 CFU/g). (Outbreak thought to have started in 2010 – retrospective tracing)	10	3	CDC (2015a)
2015	Pasteurised soft cheese varieties (Karoun Dairies)	USA	*L. monocytogenes*	Cases reported since 2010 traced. Likely cross-contamination from the manufacturing plant, but actual source (e.g., equipment) not reported.	30	3	CDC (2015b)

Date	Dairy Product	Country	Pathogen serotype	Source of pathogen if known	Numbers ill	Number of deaths	Reference
2016	Partially skimmed chcolate milk (Saputo Inc)	Canada	*L. monocytogenes*	Environmental sampling confirmed the presence of the *L. monocytogenes* outbreak strain on post pasteurisation equipment used for chocolate milk, as well as on non-FCS	34	0	Canadian Food Inspection Agency (2016)
2018	Ice-cream	USA	*L. monocytogenes*	Unsanitary manufacturing conditions. Same strain of *Listeria* in manufacturing environment and patients.	3	0	USFDA (2019)
2019	Cheese	France	*L. monocytogenes*	Not stated but may be cross-contamination as company manufactures both raw and pasteurised milk cheese products.	2	2 (adult/ foetus) (raw milk cheese)	Food Safety News (2019)

Table 3. Summary table of the growth parameters for *L. monocytogenes*.

Growth Conditions	
Temperature	Range: 0.6 – 45°C Optimum: 37˚C
pH	Range: 4.4 – 9.4 (minimum of 5.2 in fermented dairy)
Atmosphere	Facultative anaerobe
Water Activity	Minimum a_w = 0.92
Toxin or infection	Infection
Inactivation/survival dynamics	
Inactivation Temperature	Inactivated (D-value) at 72°C / 0.9 – 2.7s in milk Survives under freezing condition (-18 to -20°C)
pH	Tolerance to severe acid stress (pH 3.5) has been shown to be induced if exposed to mild acidity (pH 5.5) for a time
Water Activity	Survives $a_w \leq 0.83$
Inhibition	Inactivated by potassium sorbate (2000 – 3000 ppm)

Table 4. Examples of inactivation (log reductions) of *L. monocytogenes* biofilms (48 – 72h) when treated with commonly used sanitisers on different surface types (compiled from Korany et al., 2018; Krysinski et al., 1992; Ronner and Wong, 1993; Skowron et al., 2018).

Sanitiser tested	Log reduction (stainless steel)	Log reduction (rubber)	Log reduction (polyester / polyurethane)	Log reduction (polystyrene)
Chlorine (sodium hypochlorite)	(1 – 5 min, 0.5%) 1.97 – 3.55 (2 min, 100ppm) 4.5 (10 min) 1.3	(1 – 5 min, 0.5%) 1.79 – 2.21	(10 min) <1	(1 min, 200ppm) 2.57
Peracetic acid (with or without hydrogen peroxide)	(1 – 5 min, 0.5%) 6.63 (10 min) (>4)	(1 – 5 min, 0.5%) 5.10 – 5.70	(10 min) 1.4	(1 min, 200ppm) 3.85
Acid anionic	(2 min, 200ppm) 4 – 5 (10 min) (>4)	(2 min, 200 ppm) <1	(10 min) <1	-
QAC	(1 min, 200ppm) 4 (1 – 5 min, 0.5%) 4.06 – 5.01 (10 min) (>4)	(1 – 5 min, 0.5%) 1.72 – 3.14	(10 min) 1.4	(1 min, 400ppm) 2.20

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