The problem of Listeriosis and ready-to-eat products: prevalence and persistence

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Despite efforts made by different regulatory agencies throughout the world, listeriosis is still one of the most serious foodborne diseases of our society due to the severity of manifestations (septicaemia, meningitis and foetal death) with a case-fatality rate ranging from 20% to 50%. *Listeria monocytogenes* is a food-borne pathogen widely distributed throughout the natural environment and consequently, it is present in many animals and plants. Its extended distribution and psychrotrophic character allow the pathogen to persist and grow in different types of refrigerated ready-to-eat products (RTE). The increase in the consumption of RTE products (which do not require cooking before consumption and with an extended shelf-life at refrigeration temperatures), together with the expanding population of highly susceptible people, could be the reasons for the observed increase of listeriosis in European countries. In this chapter, the epidemiology of listeriosis and the problems related to RTE products will be discussed.

Keywords Listeria monocytogenes; Listeriosis; outbreaks; cross contamination; ready-to-eat

1. Species of the genus *Listeria*

Members of the genus *Listeria* are gram-positive rods that are found as single units or in short chains. *Listeria* does not produce spores and capsules are not formed. The genus usually grows well in most commonly bacteriological medium, in aerobic or anaerobic conditions. Colonies are small (1 to 2 mm after 24h/37ºC), smooth and blue-gray on nutrient agar when examined with obliquely transmitted light. The organisms are motile at 28ºC by means of one to five peritrichous flagella but are less motile at 37ºC. The optimum growth temperature is between 30ºC to 37ºC, but the pathogen is able to multiply at refrigeration temperatures (4ºC).

The genus *Listeria* contains species of low GC content Gram-positive bacteria closely related to the genus *Bacillus*, *Clostridium*, *Enterococcus* and *Staphylococcus* [1, 2]. Currently, the genus contains six species: *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria innocua*, *Listeria seeligeri*, *Listeria welshimeri*, and *Listeria grayi* [3]. However, in 2009, a new species has been isolated from the natural environment of Finger Lakes National Forest, USA [4], and identified as *L. marthii*.

Six of the virulence factors responsible for key steps of *L. monocytogenes* intracellular parasitism (prfA, plcA, mpl, hly, actA, and plcB) are physically linked in a chromosomal island formally known as the hly or PrfA-dependent virulence cluster or LIPI-1 (*Listeria* pathogenicity island 1). The genus *Listeria* includes three hemolytic species: *L. monocytogenes*, *L. ivanovii*, and *L. seeligeri*, all of which contain LIPI-1 (Table 1). But only two of these species (*L. monocytogenes* and *L. ivanovii*) are potentially pathogenic. Despite the presence of the virulence gene homologues, *L. seeligeri* has been rarely linked to disease in humans or animals.

Table 1. Biochemical identification of *Listeria* species

Species	Beta - hemolysis	CAMP test reaction:		Acid production from:	
		S. aureus	*R. equi*	L - Rhamnose	D - Xylose
L. welshimeri	-	-	-	+/-	+
L. innocua	-	-	-	+/-	-
L. monocytogenes	+	+	-	+	-
L. seeligeri	+	+	-	-	+
L. ivanovii	++	-	+	-	+
L. grayi	-	-	-	-	-
L. marthii [a]	-	-	-	-	-

[a] 16S ribosomal RNA sequence analysis and DNA-DNA hybridation confirmed their close phylogenetic relationship to *L. monocytogenes* and *L. innocua* and their more distant relationship to *L. welshimeri, L. seeligeri*, *L. ivanovii*, and *L. grayi*

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2. Listeriosis

The infectious disease caused by *L. monocytogenes* is known as Listeriosis, a disease transmitted by food consumption that can affect both humans and animals. The two pathogenic species, *L. monocytogenes* and *L. ivanovii*, are able to invade host cells. *L. monocytogenes* is able to infect humans and animals [5], while *L. ivanovii* is an animal pathogen (predominantly linked to disease in ruminants). Although human listeriosis is mainly caused by *L. monocytogenes*, some cases of human infections in the United Kingdom and France have been linked to *L. ivanovii* [6, 7].

2.1. Ecology of *Listeria monocytogenes*

Listeria species are widely disseminated throughout the environment. They have been isolated from soil, decaying vegetable material, silage, sewage, water, animal feed, food-processing environments and asymptomatic human and animal carriers [8-10]. Nevertheless, the primary habitats of *L. monocytogenes* are considered to be the soil and decaying vegetable matter where this pathogen survives and grows saprophytically [11]. *L. monocytogenes* is able to colonize industry environment and consequently, processed food [12-14].

2.2. Pathogenicity of *Listeria monocytogenes*

L. monocytogenes is a facultative intracellular pathogen able to survive in macrophages and to invade a variety of non-phagocytic cells, such as epithelial cells, hepatocytes and endothelial cells [15]. After ingestion of contaminated food with *L. monocytogenes*, the gastrointestinal tract is thought to be the primary site of entry of pathogenic cell into the host. During invasion, *Listeria* becomes enveloped within a phagocytic vacuole; then, phagosome membrane disruption is mediated by the hemolysin of the bacteria. Once in the cytosol, *Listeria* is able to multiply, indicating that the cytosoplasmic compartment is permissive for *Listeria* proliferation. Later, bacteria are surrounded by a fibrillar material composed of actin filaments which rearranges to form an actin tail, which allows movement until the cell periphery comes into contact with the membrane, pushing it and leading to the formation of finger-like protrusions with a bacterium at the tip. These pseudopods penetrate uninfected neighbouring cells which, in turn, are engulfed by phagocytosis, resulting in the formation of a secondary phagosome delimited by a double membrane. Bacteria escape rapidly and initiate a new intracellular cycle [16]. Once *L. monocytogenes* crosses the intestinal barrier, cells are dispersed into the host carried by the lymph or blood, reaching the mesenteric lymph nodes, the spleen and the liver [17].

The minimum dose required to cause clinical infection in humans has not been determined but the large numbers of *L. monocytogenes* detected in food responsible for epidemic cases of listeriosis suggest that it is high, 10^6 CFU/g. Nevertheless, low doses can not be excluded in so far as they too may cause infection, especially in the high risk group. Levels of contamination as low as 10^2 or 10^3 CFU/g have been linked with clinical cases. The infection dose may vary depending on the virulence of the strain and the host factors. Evidence comes from the fact that only 3 of the 13 known serotypes of *L. monocytogenes* (1/2a, 1/2b and 4b) are responsible for the majority of human and animal cases of listeriosis worldwide [18-20]. However, serotypes 1/2a, 1/2b and 1/2c are the most frequently found in food products [21, 22].

2.3. Types of listeriosis

Contaminated food is the major source of infection in both epidemic and sporadic cases [23, 24]. The groups at risk for listeriosis are pregnant women and neonates, elderly (60 years or older) and immunocompromised adults with underlying disease. In most cases, in non-pregnant adults, listeriosis is associated with at least one of the following conditions: malignancies (leukaemia, lymphoma), chemotherapy, immunosuppressant therapy (organ transplantation or corticosteroid treatment), chronic liver disease (alcoholic or cirrhosis), kidney disease and diabetes.

The infection caused by *L. monocytogenes* can appear in two forms:

1) Non-invasive listeriosis is a gastroenteritis disease with the typical symptoms of fever, diarrhea and vomits. In general, all of the population exposed to the pathogen is capable of developing the disease. The clinical course of this type of infection usually begins approximately 20 h after ingestion of heavily contaminated food [25].

2) Invasive listeriosis is recognized as a serious food-borne disease due to the severity of its symptoms and the high case-fatality rate, 20% to 30% [18, 26]. The incubation period for the invasive illness is generally much longer than the gastrointestinal form, between 20 to 30 days [27, 28]. In both perinatal listeriosis and invasive listeriosis in the adult patient, the predominant forms of presentation correspond to disseminated infection (bacteremia or septicaemia) or to local infection in the central nervous system (meningitis or meningoencephalitis). There are other atypical forms of the disease such as endocarditis, myocarditis, arteritis, localized abscesses or osteomyelitis [29-31]. In the case of fetomaternal and neonatal listeriosis, the infection normally invades the fetus via placenta [32]. Manifestations of pregnant women are dramatic: abortion, birth of a stillborn foetus, granulomas in multiple internal organs (baby with generalized infection) or meningitis in the neonate. Infected women usually remain asymptomatic or may present a flu-like syndrome with chills, fatigue, headache and muscular pain approximately 2 to 14 days before miscarriage.

2.4. Incidence of human listeriosis

Since the year 2000, there has been a reported increase in the cases of listeriosis in several European countries (EU) [33-35]. Ingestion of *L. monocytogenes* is likely to be very common, given the ubiquitous distribution of this bacteria and the high frequency of contamination of raw and industrially processed foods. However, the incidence of human listeriosis is low when compared with other bacteria such as *Salmonella* or *Campylobacter*.

Human listeriosis notification in the European Union (EU) was 0.3 cases per 100000 inhabitants in 2007 [36], with the highest notification rates reported in Denmark (1.1), Finland (0.8), Sweeden (0.6) and Luxembourg (0.6). This data should be observed with care due to different surveillance systems among countries. For this reason, we believe that the incidence of listeriosis could be higher due to underreported cases in some European countries. Two such examples are Spain and Italy. A collaborative surveillance study of *Listeria* infections in Europe described an incidence of 0.15 cases per 100,000 inhabitants in the year 2000 for Spain (based on information from voluntary reporting), while for that same period, the incidence detected by Garrido *et al*. was 0.91 cases per 100000 inhabitants [21]. In a similar way, the active surveillance performed in Italy which involved distributing clinical and food questionnaires to hospitals, combined with the characterization of the isolated strains, resulted in a higher number of cases of listeriosis than that which was reported by mandatory notifications [37].

Overall, 1558 cases of listeriosis from 27 Member States of the EU were notified in 2007. The most affected groups were aged persons and children, with an incidence of 1 and 0.51 per 100000 inhabitants, respectively. Among the group of children (0-4 years old), 85% of the cases occurred in newborns. Fatality rate in the EU was estimated to be approximately 20% (Of the 795 cases of disease outcome reported, 160 persons died). Nevertheless, it should be noted that the 66.8% case fatality rate was observed among aged persons (107 deaths) [36].

2.5. Outbreaks of listeriosis

The study of the listeriosis outbreak in the Maritime Provinces of Canada (1981) provided, for the first time, conclusive evidence of foodborne transmission of *L. monocytogenes*. The outbreak was detected because of the unusually high number of perinatal listeriosis observed at a Nova Scotia hospital over a 3 month period. The most likely vehicle of infection was coleslaw. The microbiological confirmation was possible because of the isolation of the same strain of *L. monocytogenes* from coleslaw obtained from the refrigerator of a patient, and from unopened packages of coleslaw from the manufacturer [38].

Although the majority of listeriosis cases are sporadic, the investigation of outbreaks has high priority because it offers the opportunity to identify the source of contamination (which is very difficult to do in sporadic cases). It is important to identify the contaminated food vehicle in order to remove it from the market and to warn consumers.

Many categories of food have been related with outbreaks and sporadic cases in the past 30 years: milk, soft cheeses and dairy products, patê, sausages, smoked fish, salads, delicatessen and ready-to-eat products [39-44]. Table 2 shows the reported outbreaks over the past decade.

Table 2. Listeriosis outbreaks (2000 to 2010)

Year	Country	Food	No. of cases	Serotype	Reference
2000 [a]	New Zealand	Delicatessen RTE meats	31	1/2	[45]
2000	USA	Delicatessen turkey meat	30	1/2a	[46]
2000-2001	USA	Mexican style cheese	13	4b	[47]
2001 [a]	Sweeden	Dairy products	42	1/2a	[48]
2001 [a]	Japan	Cheese	38	1/2b	[49]
2002 [a]	USA	Delicatessen turkey meat	16	4b	[50]
2002	Canada	Cheese	17	4b	[51]
2003	USA	Cheese made from raw milk	12	4b	[52]
2003	UK	Sandwich	4	1/2	[53]
2005	Switzerland	Tomme cheese	10	1/2a	[54]
2006	Czech Republic	Cheese and salad	75	1/2b	[55]
2007	USA	Pasteurized milk	5	NA b	[56]
2006-2007	Germany	Scalded sausages	16	4b	[57]
2008	Canada	Ready- to- eat meats	57	1/2a	[58]
2008	Chile	Brie and Camembert Cheese	119	NA b	[59]
2009	Denmark	Beef meat	8	NA b	[60]
2008	Austria	Jelly pork	19	4b	[61]
2009-2010	Germany, Austria and Czech Republic	Curd Cheese	34	1/2a	[62, 63]

[a] Gastrointestinal listeriosis. [b] Not available

It is interesting to note that the majority of detected outbreaks were related with processed food: delicatessen ready to eat meats and cheeses. Two of the outbreaks referred to in Table 2 were caused by the consumption of food delivered to a hospital (UK, 2003 and Germany, 2006-2007). These outbreaks demonstrate the importance of active HACCP establishment and risk management in hospital kitchens and patient food supply.

The characterization of the isolates using serological and molecular subtyping is a useful tool for epidemiological studies and for relating a suspicious food to a given pathogen [18, 64]. But it is also necessary to perform periodically repeated surveys of risk food products in order to compare food and clinical isolates. This is evidenced in the last outbreak detected in Europe (2009 to 2010). Researchers had previously reported an outbreak of listeriosis in Austria and Germany due to consumption of "Quargel" cheese, with 14 patients from June 2009 to January 2010. Microbiological investigation in "Quargel" samples led to confirming the presence of the identical Pulse-Field Gel Electrophoresis (PFGE) pattern and serotype 1/2a (clone 1), isolated from food and clinical cases [62]. Further investigation revealed another PFGE pattern (clone 2); this second outbreak clone of *L. monocytogenes* serotype 1/2a accounted for 20 cases (13 Austrian cases, 6 German cases and one Czech case) [63].

As pointed out by several authors [18, 43, 64, 65], most cases of listeriosis are caused by serotype 4b, while food isolates mainly belong to serogroup 1/2. However, recent studies observed a variation of this classical distribution with an increase of serotype 1/2a among clinical cases [37, 66], as can be observed in Table 2.

3. Ready-to-eat products

Ready-to-eat products (RTE) are defined as a "food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level micro-organisms of concern" [67]. The extended distribution throughout the environment and the psychrotrophic character of *L. monocytogenes*, appear to be the main cause of the high prevalence in different kinds of refrigerated RTE products [68]. Despite the fact that low temperatures can reduce the growth rate of this pathogen, it is known that *L. monocytogenes* is able to grow at refrigerating temperatures [69]. Because these products are usually cooked during manufacture and consumed without further heating, they present high risks to the consumer due to possible cross-contamination with the pathogen and further growth. In this sense, the increase of RTE consumption due to changes in lifestyle and the difficulties to control temperature in the global trade distribution of these products could be some of the reasons for the observed increase of listeriosis over the past few years [33].

Governments need risk assessment in order to select risk management measures for food trade at national and international levels, preventing risks to the consumers [70]. In 2003, in collaboration with the Food Safety and Inspection Service of the US Department of Agriculture (FSIS) and the Centre for Disease Control and Prevention (CDC), the US Food and Drug Administration (FDA) released the results of a risk assessment for predicting the potential relative risk of listeriosis from eating certain RTE foods among three age-based groups of people: perinatal group (16 weeks after fertilization to 30 days after birth), elderly (60 years of age and older), and intermediate-age (general population, less than 60 years of age). This assessment evaluated foods within 23 categories considered to be principal potential sources of *L. monocytogenes*. Deli meats were classified in the "very high risk" category while cheeses were situated at "moderate risk" [71]. However, numerous outbreaks have been related with cheeses over the past 10 years [49, 51, 52, 63]. On the other hand, 29.9 % of listeriosis cases in the EU (2007) were of unknown origin [36], thereby showing the importance of performing active surveillance of the pathogen in the high risk categories (RTE meats).

3.1. Legislation

In order to assure the protection of public health, governments improve legislation with specific laws for controlling food placed on the market. In the European Community, food safety criteria for *L. monocytogenes* in RTE food are established in the (EC) Regulation No. 2073/2005 [67], later modified by the (EC) Regulation No. 1441/2007 [72] and the (UE) Regulation No. 365/2010 [73]. Briefly, this Regulation classifies RTE products into three groups:

1) RTE products intended for infants or medical purposes: absence of pathogen in 10 samples of 25 g of product placed on the market during their shelf-life.
2) RTE not able to support the growth of *L. monocytogenes*: 5 samples of 25 g during their shelf-life must have less than 100 CFU/g of *L. monocytogenes*.
3) RTE able to support the growth of *L. monocytogenes*: once the product leaves the industry, 5 samples must have absence of pathogen in 25 g, and during shelf life the concentration in 5 samples must be lower than 100 CFU/g.

3.2. Incidence of *L. monocytogenes* in ready-to-eat meats

The psychrotrophic nature of *L. monocytogenes* makes it difficult to control the pathogen in RTE products due to refrigerated presentation at the point of sale. There are high varieties of RTE meats available at the market (ham, turkey and chicken), types of presentations (vacuum, modified atmosphere, sliced at the counter) and numerous brands. These facts, together with the elevated consumption of these products, make the control of *L. monocytogenes* one of the common goals of Governments and Industry.

The prevalence of pathogen in ready-to-eat meat products has been studied by numerous researchers. Different studies have shown that the prevalence of *L. monocytogenes* in RTE meat products may vary from 0 to 72%, with different levels of CFU per gram upon expiry of shelf life [74]. In a study carried out in Spain [21], researchers analyzed different brands available at the market and two types of presentations: opened deli meats and vacuum packaged. A total of 601 samples of sliced deli-meats and patê were analyzed, with a low prevalence in vacuum patê (0.8%). Significant differences in prevalence were found in sliced deli-meat depending on the presentation, showing a higher incidence in in-store-packaged products (8.5%) with respect to products packaged by manufacturers (2.7%), suggesting that the slicing equipment could be the source for the pathogen dissemination (cross contamination).

3.3. Persistence of *L. monocytogenes*

When raw products enter into a production line, they could be already contaminated by *L. monocytogenes*, but with the proper treatment (cooked, smoked), the pathogen could be eliminated, providing a safe final product. Sometimes the treatment to eliminate *L. monocytogenes* fails and the pathogen is able to establish itself and survive for long periods of time. The persistence of well-adapted clones has been suggested by numerous researchers [12, 76, 77], using different typing methods. Among the molecular typing methods, pulse-field gel electrophoresis (PFGE) is widely used, because of its excellent discriminatory power and reproducibility [75]. The combination of serology and PFGE is a useful tool for studying the distribution and transmission of the pathogen along the food chain. In this sense, repeated pulsotypes (genomic fingerprint using PFGE) were detected periodically from two manufacturers of smoked fish [21].

L. monocytogenes is able to attach to different surfaces forming biofilms and consequently, it is able to spread and survive. Biofilm formation by *L. monocytogenes* is associated with its persistence in food environments and higher resistance to antimicrobials used in food environment. Periodically repeated surveys are needed for determining the prevalence and clones of *L. monocytogenes* in food manufacturing environment [21]. In the aforementioned study, possible cross contamination during slicing procedures of meat products was investigated by consecutively purchasing two different sliced products in each establishment. Identical *L. monocytogenes* strains were observed in each of the two products (6 out of 11 butcher shops), suggesting that the slicing equipment could be the source of pathogen dissemination. The implementation of HACCP systems or other food safety programs at retail level, as well as adequate disinfection strategies for controlling persistent strains will contribute to prevent cross contamination [78].

3.4. The risk of listeriosis by consumption of RTE products: could consumers prevent it?

Although consumers are considered to be responsible for proper food-handling practices, it has been reported that they are frequently unaware of their role in the prevention of food-borne disease [79]. The majority of consumers fail to recognize the significant risk and mechanisms of bacteria growth associated with food-borne disease [80]. Due to the lack of knowledge observed, consumers need more information regarding safe handling practices; most important, persons at risk of listeriosis (aged people, immunocompromised and pregnant women) should be aware of the risk of consuming RTE foods [81].

Numerous studies on refrigeration temperatures of retail and home refrigerators show temperatures above the 4ºC recommended [82-84], allowing pathogen growth during the shelf life of the product. Temperature of home refrigerators ranged between 0.6ºC to 14.5ºC [82]. A drop in temperature is the key factor for *L. monocytogenes* growth reduction within the shelf-life of product, with special emphasis once the product is purchased and remains under the responsibility of consumers [85]. Therefore, there is a need to improve consumer education regarding food storage practices, first, emphasizing the importance of storing RTE at low temperatures (≤4ºC) and taking into account the maximum date of consumption. Secondly, it is necessary to inform consumers regarding the importance of being aware of their fridge temperature and to advise them to store RTE products in the coldest place of their refrigerator.

In conclusion, for a better control of *L. monocytogenes* in RTE products, improvement should be made in labelling RTE products at risk with regard to time and temperature of storage, as well in consumer education regarding food storage practices.

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