FRI FOOD SAFETY REVIEWS

Review of Epidemiology of Foodborne Listeriosis

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INTRODUCTION

Listeria monocytogenes is an important foodborne pathogen not because it causes large numbers of symptomatic cases but because of its relatively high case–fatality rate. About 94% of listeriosis cases are hospitalized and about 16% die. Despite the widespread occurrence of *L. monocytogenes* in the environment, relatively few exposed people become ill. Average annual incidence in the U.S. is estimated to be 1,591 cases. This estimate is based on the actual number of cases identified in a year multiplied by factors to correct for underreporting and under-diagnosis (*222*). Some outbreaks of listeriosis resemble other foodborne illness, with symptoms of gastro-enteritis and fever occurring after a median incubation period of 24 hours. *Listeria* concentrations in implicated food may be quite high (10^4–10^9cfu/g), and cases often do not have well-established risk factors (*78;198*). In other outbreaks, most victims are elderly, immunocompromised, or pregnant and *Listeria* is invasive, causing bacteremia, meningitis, or illness or death in a fetus or newborn infant. In these cases, median incubation periods are 2, 9, and 27.5 days, respectively (*94*). In one case associated with the 2011 cantaloupe outbreak, an 88-year-old woman lost vision in one eye due to invasive listeriosis (*112*).

Although the incidence of cases of *L. monocytogenes* per 100,000 population at FoodNet sites declined significantly since the baseline years of 1996–1998 (when it ranged from 0.43 to 0.53), there has been little progress during the past 10 years (2002–2011), when incidence in the U.S. fluctuated between 0.26 and 0.32, with an incidence of 0.28 in 2011. Preliminary data for 2012 indicate an incidence of 0.25. We have not yet reached the Healthy People 2010 goal for listeriosis of 0.24 or the 2020 goal of 0.20 (*37*).

Listeriosis appears to be primarily a foodborne infection and is particularly a problem on foods that are not cooked just prior to consumption, including ready-to-eat (RTE) meats, soft cheeses, and unpasteurized dairy products, as well as sprouts, salad vegetables, and fruit. Thermal processing of milk and meat will destroy *L. monocytogenes* but post-processing contamination does occur. Because this pathogen grows during refrigeration, simply keeping foods cold does not ensure their safety.

L. monocytogenes that contaminates foods may originate in soils from farms and pastures (*147;245*), in slaughtering facilities (*20*), in food processing plants (*16*), and in slicers and other equipment in delicates-sens (*107*). Elimination of *Listeria* from many environ-mental sources can be challenging because of the resistant, persistent biofilms formed by these pathogens (*50*).

Several high-profile outbreaks, with high fatality rates, in the 1980s were attributed to cole slaw, milk, and Mexican-style cheese. In the 1990s, there were several outbreaks and recalls of meat products due to the presence of this pathogen. RTE deli meats were identified as vehicles of infection in 31% of the outbreaks (1998–2011) having known etiology listed by CDC, with dairy products accounting for another 41.4%. However, only one of the outbreaks tabulated by CDC occurring after 2006 was associated with meat (*36*). More recent outbreaks (2010–2012) in the U.S. and other countries have been attributed to soft cheese in Australia (*242*), imported ricotta cheese in the U.S. (*35*), hog head cheese (an RTE meat) in Louisiana (*32*), packaged sliced ham in Switzerland (*111*), chopped celery in Texas (*252*), and cantaloupe in the U.S. (*34*).

HUMAN ILLNESS

Outbreaks and Cases

Older adults (>60 years), pregnant women and their fetuses and newborn infants, and other persons with reduced immune function are particularly at risk for invasive listeriosis. According to the *Listeria* Initiative surveillance system, 71% of the 590 invasive cases reported in the U.S. in 2011 occurred in the elderly and 9.7% of cases were pregnant women. At least 74% of nonpregnant cases aged <65 years were immuno-compromised. Compared to listeriosis incidence in the general population (0.28/100,000), incidence rates for adults ≥65 years, pregnant women, and pregnant Hispanic women were 4 times, 10 times, and 24 times higher, respectively. Overall case–fatality rate was 20.5% (*39;40*). FoodNet Surveillance data for 2011 (a subset of U.S. data) indicated a case–fatality rate of 19.3% for listeriosis as compared to only 0.37% for reported cases of salmonellosis. Preliminary data for 2012 indicate case–fatality rates of 10.74 and 0.42 for *Listeria* and *Salmonella*, respectively (*37*).

Surveillance data on foodborne disease from the EU indicated that there were 1,476 confirmed cases of listeriosis in 2011, a decline of 7.8% from the previous year. Overall in the EU, reported incidence of listeriosis was 0.32 cases/100,000. However, there was significant variation among individual countries, from 0.88 in Denmark and 0.80 in Finland to 0.31 in Austria and 0.26 in the U.K. Overall case–fatality rate was 12.7% for reports indicating this information (*63*).

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Data from other countries indicate:

- A total of 93 cases of listeriosis reported in Australia in 2012. This translates to an incidence rate of 0.41 (*8*)
- A total of 132 cases of listeriosis reported in 2011 in Canada (0.38/100,000) (*8*)
- A total of 26 cases in New Zealand in 2011 (0.6/100,000) (*141*)

Surveillance data on prevalence of human listeriosis in many Asian countries and in less developed countries are not readily available and may be reported only in national languages. A small number of cases have been reported, for example, in Singapore (*126*) and India (*12*), and *Listeria* has been detected in foods in China (*233*) and Africa (*174;183*).

Economic Costs

A recent analysis of health costs of foodborne infections estimated that the average cost of a case of listeriosis using a basic cost-of-illness model was $1,272,279 (in 2010 U.S. dollars). The estimate from this basic model includes costs for medical care (2008 estimates for care), productivity losses, and mortality and is likely an underestimate for actual costs in 2013. With an estimated average annual number of cases of 1,591, this cost estimate would total $2.025 billion, annually. Estimated average health costs for illness caused by some other pathogens, including nontyphoidal *Salmonella* and norovirus, are much lower: $4,312/case and $530/case, respectively. But total estimated annual costs are higher for these pathogens because they each cause >1,000,000 cases/year (*224*).

Important Species and Strains of Listeria

Several different species of *Listeria* have been described and have been detected in soil and water samples (*220*), on foods, including RTE fish products (*132*), and in human fecal samples (*225*). *L. monocytogenes*, however, is the pathogen of most concern for human illness. There are four evolutionary lineages of *L. monocytogenes*, of which III and IV are uncommon and primarily isolated from animals. Lineage II includes serotype 1/2a and 1/2c, which are widespread in the natural environment, including foods, and have caused human outbreaks. Lineage II strains often have many plasmids conferring resistance to metals, bacteriocins, and other antimicrobial substances, but some strains have low virulence for humans. Lineage I strains include serotypes 4b and 1/2b and are associated with most human listeriosis outbreaks (*188*). A comparison of 300 isolates from around the world found that there were a few frequent clones (4b, 1/2b, and 1/2c) found globally and some other clones that appeared to be limited to certain geographical areas (*44*).

Multiple serotypes of *L. monocytogenes* have been implicated in some large outbreaks of listeriosis, including the 2011 multistate outbreak linked to cantaloupe, which included serotypes 1/2a and 1/2b (*135*). Complete genome sequencing of numerous serotypes of *L. monocytogenes* revealed a highly similar pan-genome but also prophages, transposons, mobilizable islands, and hyper-variable hotspots, which allow evolution of cells to adapt to different niches and changing conditions (*133*). Indeed, an examination of 33 strains of *L. monocytogenes* revealed differences in virulence and adaptation to cold (*130*). Strains also vary in their ability to form biofilms and persist in food processing plants (*264*).

FOOD ATTRIBUTION

Outbreak Data

Soft cheeses, RTE meats, fresh produce, and seafood are commonly cited vehicles of infection for listeriosis. Selected outbreaks of listeriosis, illustrating this, are listed in **Table 1**. Food vehicles reported in data from the CDC outbreak database (1998–2011, plus two 2012–2013 cheese outbreaks in the U.S.) are depicted in **Figure 1**. Meat (primarily RTE) and dairy products each account for about a third of the outbreaks. Salads included potato, taco, and tuna; produce included cantaloupe, celery, and sprouts. Meat and produce caused larger than expected shares of cases, however, most likely because of the wide distribution of some RTE meat products and of contaminated cantaloupe during the large 2011 outbreak. On the other hand, many of the cheese outbreaks were attributed to Mexican-style cheeses that were produced in smaller amounts and distributed locally, and therefore a smaller number of people were exposed. In other countries, smoked fish is an important vehicle for listeriosis. But the only two outbreaks in the CDC list that involved fish were attributed to tuna salad and sushi.

A study using data from U.S. outbreaks (1999–2008) attributed foodborne listeriosis mainly to dairy products (30%), deli and other meats (35%), and complex foods (15%). Seafood and produce accounted for a total of 10%. Expert elicitation attributed more illness to deli meats (54%) and seafood (8.7%) and less to dairy products (23.6%) (*15*).

Another review of listeriosis outbreaks during this time period noted changes in characteristics of outbreaks from earlier to later years. While outbreaks caused by dairy products do not appear to be decreasing in frequency, there has been a marked decrease over time in outbreaks associated with RTE

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meat and poultry. Other novel vehicles, such as fresh produce, have recently been identified as vehicles (*30*). Data on 31 outbreaks (domestic and foreign) during the past 5 years described in the literature (*see* Table 1) reinforce the continuing importance of cheese and the declining, but still significant, role of RTE meat products. Of these outbreaks, 20 were attributed to cheese; 5 to meat, most identified as RTE; 4 to produce; and 2 to combination foods containing fish.

Sporadic Cases

An important factor to consider in addressing the problem of listeriosis is that a majority of cases are sporadic, not outbreak-related. These individual cases may not be investigated thoroughly and we may be underestimating (or not even considering) some foods that may be important vectors for *Listeria*. An Australian investigation into factors associated with sporadic listeriosis found that living in a non-English-speaking household was correlated with perinatal listeriosis. Among non-perinatal cases, age (>60 years), prior hospitalization, use of gastric acid inhibitors, and eating Camembert cheese were identified as risk factors (*51*).

Surveillance of Foods

RTE deli meats have been implicated in a number of listeriosis outbreaks. A risk assessment by FSIS in 2003 indicated that deli meats accounted for about 64% of foodborne listeriosis (*206*). Data from the FSIS microbiological testing program for RTE meat and poultry products for *L. monocytogenes* demonstrate a decline in positive samples over the past 20 years, from 4.61% in 1990 to 1.45% in 2000, and to 0.27% in 2011 (*74*).

However, this decrease in prevalence of *Listeria* in tested meats has not resulted in a significant decrease in incidence of listeriosis as reported by CDC in the past decade (*37*) (*see* **Figure 2**). Part of the explanation for this discrepancy may be that consumers are purchasing more deli meats that have been sliced at retail facilities. Recent studies indicate that these retail-sliced meats account for approximately 83% of the listeriosis cases associated with deli meat, and a high proportion of deaths resulted from meats that did not contain microbial growth inhibitors (*64*). Other studies of sliced ham, turkey, and roast beef also demonstrated that products without growth inhibitors and those sliced at retail were a greater risk for listeriosis (*201;202*). Data on *Listeria* in pork and pork products from a large number of studies all over the world are summarized in a recent review (*10*).

Other vehicles for listeriosis have become more prominent in recent years, including fresh produce and a continuing problem with certain dairy products. FDA recently issued a draft assessment of the risk for listeriosis from soft-ripened cheese. It was estimated that soft cheese made from raw milk poses 50–160 times the risk for listeriosis as cheese made from pasteurized milk (*73*).

As yet there is not very good surveillance data for prevalence of *Listeria* in many foods. A very large market basket survey is now underway testing three categories of FSIS-regulated foods and 12 categories of food regulated by FDA (deli meats, salads containing meat, dried/fermented sausage, smoked seafood, raw milk, soft cheeses, and low-acid cut fruits). More than 24,000 food items have been sampled so far. Some preliminary results have been reported (*149*).

Data gathered by the EU indicate that fermented sausages, soft/semi-soft cheeses, and fishery products had higher rates of non-compliance with *Listeria* regulations than other RTE meats and other foods (*63*). A 2010–2011 survey of 3,053 packages of smoked or gravad fish, 3,530 heat-treated meat products, and 3,452 soft or semi-soft cheeses from 27 European countries detected *L. monocytogenes* in 10.3% of fish, 2.07% of meat, and 0.47% of cheese. Contamination levels were generally low but at least one sample in each category exceeded 100 cfu/g at the end of shelf life (*66*). Results of other recent surveillance studies are presented in **Table 2.**

FOOD CONTAMINATION

Contamination Pathways

Listeria is widely present in the environment, and there are many potential pathways by which food may be contaminated. Contaminated soil or water may introduce *Listeria* to produce in the field. Food-producing animals may carry *Listeria*, often without symptoms, and be a source of contamination for milk and meat. Biofilms containing *Listeria* in food production and processing facilities may constitute a persistent, ongoing, sometimes sporadic source of bacteria (*241*). Employees handling food may also spread *Listeria* and facilitate cross-contamination in production facilities and food preparation areas. A great deal has already been written on this subject (*215*) and the sections below will describe information gathered from recent outbreaks or unusual/unexpected routes of contamination.

Condensate in food processing plants has been suspected as a possible source of pathogenic bacteria contaminating food during production. However, recent analyses of 2,281 condensation samples (overhead pipes, dripping pans) from harvest, fabrication, and RTE meat processing environments found low levels of aerobic bacteria, coliforms, yeast,

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and molds. *Listeria* spp. was detected in only two samples (*25*).

A study to determine the extent of attachment of *L. monocytogenes* to six different types of conveyer belts found that the bacteria adhered best to the four plastic belts and least to the two stainless steel belts. This suggests that the plastic belts pose a greater risk for cross-contamination (*267*).

Several outbreaks of listeriosis have been traced to machinery in food processing plants, raising the question as to whether *L. monocytogenes* can survive or grow in the lubricants used in this equipment. Tests with eight artificially inoculated H1 lubricants (seven greases, one oil) found that there was no growth, and viable counts decreased by >99.9% within 7 days in all materials (*275*).

Floor drains in poultry processing plants and other plants can be colonized by *L. monocytogenes* and the biofilms can make the drains difficult to clean. An experiment to determine whether a 2-second spray of water into a contaminated drain would result in aerosolization of *L. innocua* demonstrated that cells could be detected on the floor 4 m away from the drain and on walls 2.4 m above the floor and 4 m from the drain (*16*). Using a similar protocol (2-second spray into a contaminated drain) resulted in low level contamination of raw chicken fillets left on a table 2.4 m from the drain (*17*).

Several models have been developed to predict the importance of different pathways for contamination of RTE foods. Some recent models include: (*a*) surface transfer of *L. monocytogenes* during slicing of salami (*234*); (*b*) estimation of transfer of *L. monocytogenes* during slicing and removal during sanitation (*106*); (*c*) public health impact of cross-contamination of RTE meats with *L. monocytogenes* in retail environments (*200*); and (*d*) risk assessment modeling of *L. monocytogenes* contamination of RTE meats (*79*).

L. monocytogenes can produce extracellular polymers that aid in attaching to a variety of surfaces and protect the cells from cleaning and sanitizing agents. Once a biofilm is established, it can serve as an ongoing source of contamination. *L. monocytogenes* strains vary in their ability to form biofilms (*47;76*). Of 29 isolates of *L. monocytogenes* isolated from bovine carcasses and beef processing facilities, 4 were determined to have a strong ability for adhesion, 8 were weak, and the others had a moderate ability to adhere to surfaces (*80*). One persistent strain of *L. monocytogenes* isolated from a cold-smoked fish processing plant in Japan was found to produce greater amounts of biofilm with more extra-cellular polysaccharide than a transient strain from the same facility. Cells in the biofilm were about 150 times as resistant to benzalkonium chloride as cells of the transient strain (*178*). Persistent strains also appear to recover better than other strains after damage by some antimicrobials, such as chitosan (*187*).

Important aspects of biofilms were discussed in recent reviews. Effects of environmental factors on biofilm formation, including temperature, acidity, sodium chloride, and other compounds present in foods, were explained and methods for studying biofilm formation were described. Strategies for prevention and control of biofilms were evaluated (*50;58;241*).

Animals, abattoirs, and raw meat processing

Relatively few studies have surveyed the prevalence of *Listeri*a in live poultry, cattle, and pigs. Reported incidence of *Listeria* in fecal samples is generally much lower than reports on some other pathogens such as *Salmonella*, *Campylobacter*, and *E. coli*.

Poultry. A recent survey of pasture-raised broilers in Tennessee found 7 cecal samples from 399 birds tested positive for *L. monocytogenes*. This bacterium was also found in some soil and grass samples from the environment where they lived. No data are available on *Listeria* in poultry raised in large, conventional systems in the U.S. but some data from other countries indicate a low level of contamination in live birds (*164*).

Approximately 38% of neck skin samples from chickens slaughtered in four processing plants in northern Greece tested positive for *L. monocytogenes* (*216*). However, sampling for *Listeria* on chickens coming into U.S. slaughter plants (feathered carcasses, pre-scald) found that only 2.5% carried *L. monocytogenes* (*18*).

No *Listeria* were present in a commercial chicken further-processing plant when it first opened. However, within 4 months *L. monocytogenes* was detected in floor drains even after cleaning and sanitizing. Evidence indicated that the source of *Listeria* was raw product (deboned chicken) entering the plant (*19*). Investigations into *L. monocytogenes* contamination on mechanically deboned chicken in Italy determined that the source of contamination was the drying–cooling tunnel the birds passed through after slaughter and before deboning. Small feathers from carcasses collected on evaporative cooling pads in the tunnel and provided a niche for *Listeria* survival and growth (*20*).

A large survey for *Listeria* in Thai factories producing frozen cooked chicken detected *Listeria* spp. in 3.2% and *L. monocytogenes* in only 1% of 12,833 meat and environmental samples. The most common surfaces contaminated were the freezer drain, the metal detector conveyer belt, and the liquid nitrogen chiller exhaust pipe (*121*).

Cattle, sheep, goats. *L. monocytogenes* is also present in feces of ruminants and may be secreted

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into milk if a cow (*166*) or goat or ewe (*108*) has mastitis. A trace-back investigation of contamination of raw ewe's-milk cheese in Austria found that ewes with mastitis were shedding an average of 3×10^4 cfu *L. monocytogenes*/ml of milk (*228*).

A survey of *L. monocytogenes* contamination in Chinese beef processing plants detected these bacteria in 26.4% of samples, with hides being most frequently contaminated (*284*). *L. monocytogenes* and other foodborne pathogens were readily transferred from inoculated beef fillets passed through a mincing machine to all six non-inoculated fillets passed sequentially through the same machine (*193*).

Pigs. Data on *Listeria* in pigs on the farm from several studies in Europe and the U.S. are summarized in a recent review. Prevalence in rectal swabs is <4% in most studies but some environmental samples from pens revealed more widespread contamination (*10*). However, two studies from Italy tracing *Listeria* from pigs through production of ham and sausage found that strains of *Listeria* found on pig carcasses were different from those detected in meat later in the manufacturing process (*161;204*).

Other animals. A survey of ducks on commercial farms in Malaysia found that 2.8% were positive for *L. monocytogenes* (*1*).

Deli meats

Although RTE meats have been previously cooked, they may be recontaminated with *Listeria* during subsequent handling, particularly during slicing and packaging. Meats sliced in a retail establishment are considered a greater risk than those sliced and packaged in a processing facility. However, some outbreaks have been traced to contamination at processing plants. Sources of contamination in factories producing fermented sausage in Italy (*161*) and Parma ham (*204*) appeared to be within the processing plants, and frequently the same strains would be detected over a long period of time.

Because deli meats have been a recognized vehicle for listeriosis for a number of years, numerous papers report on investigations into cross-contamination and reservoirs where *L. monocytogenes* may persist. Slicers have been a major focus of contamination studies as *Listeria* have been shown to be transferred from slicers to a variety of meats (*142*). Swab samples from slicers in restaurants were found to harbor a variety of microbes: pseudomonads were the dominant strains, and enterobacteriaceae and lactic acid bacteria were also commonly present. The blade guard of the slicer had the greatest diversity of bacterial species (*128*). Meat residues that accumulated in slicing machines at a processing plant were identified as the likely source of bacteria in the large 2008 Canadian outbreak of listeriosis (*22*). Cooked ham was the vehicle for a 2011 Swiss outbreak but contamination did not occur in the plant producing the ham. This company outsourced slicing and packaging to another establishment, and this is where the outbreak strain was detected (*111*). Mathematical models have been developed to describe transfer of listeriae from slicers to meat (*106;236*).

Prevalence of *L. monocytogenes* in retail environments varies, and it appears that some retail delis are more widely contaminated than others. There is more handling of foods in these stores as products are sliced, moved around in display cases, and weighed as consumers purchase them. Temperature control may not be as strict. In one study, listeriae were detected mainly on nonfood contact surfaces, such as floors, sinks, and the dairy case; in another study, listeriae were detected on a greater variety of surfaces and it was noted that the same strain of *L. monocytogenes* persisted in some establishments for over a year. Contamination problems were greater in larger establishments and in stores with a poor inspection history (*107;221*).

Listeria contaminating one area of a deli can be transferred to other surfaces and foods. A structured elicitation, involving 41 experts from the retail food industry and state regulatory agencies, identified hands and gloves as major potential sources of contamination. However, there is not much data documenting transfer of bacteria from these sources. Other sites that may be involved in contamination pathways in retail deli stores include cutting boards, scales, deli preparation areas, floor drains, and knife racks (*104*). A recent study tracked cross-contamination pathways through a mock retail deli market using GloGerm, a product that glows under UV light. In separate experiments, six sites (meat chub, slicer blade, preparation table, employee's gloves, employee's hands, and floor drains) were inoculated with 20 ml GloGerm. After about 10 min of work doing standardized deli tasks, several locations were found to be contaminated, including deli case door handle, deli case shelf, and prep table sink. Contamination spread from all source sites (except floor drains) but contamination was not consistent across all trials (*153*). Another study examined cross-contamination occurring when 21 participants sliced deli meats, one of which was coated with a fluorescent compound. Elevated levels of the fluorescent compound were consistently found on gloves, the slicer's meat grip, and the outside wall of the carriage tray. There was variability in the extent of cross-contamination among the participants, which may also be true for employees in a deli (*88*).

Dairy products

Investigations at a dairy farm that recently had positive tests for *L. monocytogenes* in bulk tank milk revealed

the presence of this bacterium in 66% of milk filters, 16% of bulk tank milk samples, and 6% of water samples. It appeared that there was a common source of contamination and the most likely sources were believed to be an asymptomatic cow shedding *L. monocytogenes* or some site within the milking machine where *L. monocytogenes* could survive and grow (*192*).

A 2009–2010 outbreak of listeriosis in Europe was traced to an acid curd cheese called quargel. Examination of the recalled lots of cheese found *L. monocytogenes* levels of 10^2 to 3×10^7 cfu/g of cheese in 14 positive lots of red smear cheese. Other lots of mold-ripened cheese, manufactured during the same time period, tested negative for *Listeria*. Therefore, it appeared that the red smear, usually a culture of *Brevibacterium linens*, was the source of contamination (*227*).

A widespread, cheese-associated listeriosis outbreak in Canada in 2008 was traced to a single cheese-producing plant. Investigations at the plant indicated that the pasteurization process was adequate and samples from the dairy herd providing milk all tested negative. However, analyses of environmental samples from the plant detected the outbreak strain in five different locations. Since the soft washed-rind cheese was the only type associated with the outbreak, it appeared that the brine solution, used to frequently wash the cheeses during maturation, was contaminated with *L. monocytogenes* (*84*).

As noted in other processing plants, floor drains can be a significant source of contamination in cheese factories. A survey of 34 cheese factories in Italy found 19 were contaminated with *Listeria* spp. and 7 contained *L. monocytogenes*. *L. monocytogenes* was detected in 18.8% of floor drains, 4.9% of food contact surfaces, and 2.4% of cheese samples (*194*).

Seafood

A survey of facilities producing ready-to-eat foods in Canada detected *L. monocytogenes* in food from 5 of 12 plants processing fish but not in meat and dairy products. However, this bacterium was present in the environment of all types of plants (*131*). Contamination pathways in a cold-smoked salmon plant in Italy were investigated over a period of 6 years. Although *L. monocytogenes* was present on some of the incoming fish, PFGE analysis of *Listeria* strains from fish and the plant environment demonstrated that the contaminants detected on the final smoked fish products were *L. monocytogenes* strains that had persisted in the plant environment for several years (*59*).

Sources of *L. monocytogenes* contaminating blue crabs in processing plants have also been investigated. *L. monocytogenes* was isolated from 4.5% of raw crabs, 0.2% of cooked crabs, and 2.1% of environmental samples. The receiving dock and raw crab coolers were the most frequently contaminated sites (*191*).

Eggs

Very little information has been published on contamination of eggs with *L. monocytogenes*. Eggs have not generally been associated with listeriosis, and contamination levels appear to be low according to a survey of commercial eggs in Mexico (*97*). A recent investigation of five egg-breaking plants in France found *L. monocytogenes* on 2% of egg shells, 8% of environmental samples, 8.5% of raw egg samples, and 0% of pasteurized eggs. A much higher prevalence was observed for *Listeria* spp., and 1.8% of pasteurized eggs tested positive for *Listeria* spp. (*211*).

Produce

An assessment by FDA cited several factors as contributing to contamination of cantaloupe in the 2011 multi-state outbreak. Low levels of *L. monocytogenes* in the field were described as the likely ultimate source. Conditions in the packing facility allowed easy dispersal and growth of bacteria. There was water pooling on the floor and neither the floor nor the packing equipment was easily cleanable. No pre-cooling step was used to remove field heat from the fruit before cold storage (*72*).

Sanitation issues were identified as contributing to contamination of celery in the 2010 Texas outbreak. Inspectors found a condensation leak over a food preparation area, soil on a food preparation table, and hand washing issues in the plant that produced the chopped celery and other produce (*252*).

Survival and Growth in Foods

Listeria is well known for its ability to grow in cold temperatures. It can also adapt to changes in acidity and high salt concentrations. Under stressful conditions *L. monocytogenes* can form long filaments, which may result in an underestimation of risk in certain foods (*262*).

A risk assessment tool has been developed to aid in determining risk for significant *Listeria* levels in cold foods that are transported without adequate temperature control. Data from ComBase and FDA were used to assess risks posed by holding cold foods at warmer temperatures for certain periods of time in supermarkets, delis, restaurants, and during transport to stores to homes (*223*).

Numerous investigations, over many years, have recorded data on growth and survival of *Listeria* spp. in a variety of foods. The following sections will report results of recent research in the past 4–5 years.

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Meat

Raw meat is usually not considered a risky food for listeriosis because cooking will kill these bacterial pathogens. However, low populations of *Listeria* could increase during refrigerated storage, particularly if there were some periods of temperature abuse, and some bacterial cells in a highly contaminated piece of meat could survive light cooking and grow during further refrigerated storage or be a source of cross-contamination in a kitchen. Data on growth of *Listeria* on raw chicken (*203*) and pork (*55;281*) at different temperatures have been used to construct models that may be used for risk assessment.

Refrigerated storage periods of up to 6 months prior to slicing did not affect growth of *L. monocytogenes* on slices of cook-in-bag turkey and ham formulated with lactate/diacetate. After slicing, meat was inoculated with approximately 1.45 log CFU/cm^2 of bacteria, vacuum packaged, and stored at 4°C for 13 weeks. Cell counts increased to 1.5–2.3 (ham) and 2.3–2.5 (turkey) log CFU/cm^2 (*87*).

Goetta, an uncured sausage-like meat product, does support the growth of *L. monocytogenes* at both 12 and 4°C. Cooking for 5 min/side resulted in a 5-log reduction in *Listeria* populations (*199*).

Cured meats such as ham, bacon, and frankfurters contain several added ingredients that restrict microbial growth, including salt, lactate, and nitrate/nitrite. Natural and organic foods do not contain added nitrite and some other antimicrobials, and *L. monocytogenes* has been reported to grow better in these products. Moisture levels, protein content, and salt concentrations also affect growth of this pathogen (*250*). Thirty days of drying of a fermented sausage, chourico de vinho, reduced water activity sufficiently to destroy all pathogens (*61*).

RTE turkey deli meat does support the growth of *L. monocytogenes.* A recent analysis of the transcriptome produced during this growth indicates that transcription of genes coding for virulence factors was not significantly changed during growth on turkey, but some genes important for adaptation to environmental conditions were upregulated (*9*). This may explain the observation that *L. monocytogenes* grown on deli turkey is significantly more resistant to synthetic gastric fluid than cells grown on culture media (*197*).

Experiments to measure listerial growth on different deli meats during simulated home storage (4, 7, or 10°C) found, as expected, that growth was greater at higher temperatures. Lactate and/or diacetate added to some meats totally inhibited growth of *L. monocytogenes* in roast beef, but only partially inhibited growth in turkey and ham (*282*).

Cheese

Queso fresco, a Mexican-style cheese, has been identified as the food vehicle for listeriosis in numerous outbreaks. Data on growth of *L. monocytogenes* inoculated onto slices of this cheese or into curd before forming cheese blocks demonstrated that this pathogen grew faster at 10°C than at 4°C, but after 20 days of storage at these temperatures populations reached 7.8 log regardless of temperature or inoculation method (*137*). Several studies reported development and validation of predictive models for survival or growth in different types of cheese: (*a*) process cheese at 4, 12, and 22°C (*57*); (*b*) Camembert and blue cheeses (*146*); and (*c*) goat's milk and production of goat's milk cheese (*4*).

Fish

Several outbreaks of listeriosis have been traced to cold-smoked fish, and recent research has investigated the importance of freeze–thawing and type of cure (wet or dry) on growth of *L. monocytogenes* in smoked fish. Wet-cured salmon had a higher pH and water activity than dry-cured fish and this resulted in a lag phase of <1 day after inoculation for wet-cured compared to a lag of 3.7–11.2 days for dry-cured fish. Freeze–thawing did not significantly affect growth of other endogenous bacteria on salmon but did allow more rapid growth of *L. monocytogenes*. These processing steps will affect the safety of cold-smoked salmon (*120*).

Fresh produce and salads

Little data is available from experimental studies on growth of *L. monocytogenes* on many kinds of fresh produce. Categories of produce appear to differ in the growth rates and population densities of *Listeria* that they will support. But there is not enough information to make reliable estimates in many cases (*105*). Some factors identified as affecting the growth of *L. monocytogenes* on greens and salads include: (*a*) presence of other bacteria (natural flora) (*155;185*); (*b*) acidity of dressing, for example in mayonnaise salads (*2*); and (*c*) temperature (*219;254*). *L. monocytogenes* populations inoculated onto fresh-cut celery decreased by about 1 log during 7 days at 4°C, increased by about 0.5 log during 7 days at 12°C, and increased by about 0.3 log during 2 days at 22°C (*266*). Models have been developed to describe growth of *Listeria* under different conditions on lettuce (*218*) and cabbage (*273*).

Investigations into the growth of *Listeria* on cantaloupe were designed to understand the deadly 2011 listeriosis outbreak. Whole cantaloupes were inoculated on the surface with a suspension of *L. monocytogenes* and then pieces were cut and incubated at 5, 10, and 20°C for up to 3 days. Enrichment was

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required to detect *Listeria* on fresh-cut pieces but significant growth was observed during incubation at the higher temperatures (*258*). In another study fresh-cut cantaloupe pieces were inoculated with three main serotypes of *L. monocytogenes* and incubated at different temperatures (4 to 43°C). No differences were observed in growth of the three serotypes, and experimental data were used to develop kinetic models of growth for use in estimating shelf-life of cut fruit and conducting risk assessments (*68*).

Other foods

Hummus (pH 4.50–4.52) proved to be a poor substrate for growth of *L. monocytogenes*, although ≥1 log cfu/g of the initial concentration of 1.86–2.23 log cfu/g survived for 27 days at 4 and 10°C. *Listeria* populations were significantly lower in hummus without added sodium (*2*).

Fresh pasta sauces, one containing cheese (pH 5.68) and the other mushrooms (pH 5.25), were inoculated with *L. monocytogenes* and stored for 31 days (shelf life of this product) at 4 and 8°C. Although *Listeria* survived in the sauces, there was little or no growth, presumably because of the presence of lactic acid bacteria which did grow, competing with *Listeria* and further reducing the pH (*95*).

Fermented black olives (pH 3.95; NaCl 6.02%), inoculated with several strains of *L. monocytogenes* and incubated aerobically, did not support growth of this pathogen but some cells did survive for up to 15 days at both 4 and 20°C. Neither *Salmonella* nor *E. coli* were viable after 1 day under the same conditions (*96*).

Walnut kernels were inoculated with 3–10 log cfu of *L. monocytogenes*/g, dried, and then stored at 23°C for 3 weeks to a year. *Listeria* survived better during storage than *E. coli* and *Salmonella*, and the calculated decline was 1.1–1.3 log cfu/month (*23*).

Although listeriosis has not been associated with fresh mushrooms, the cool, moist environments in which mushrooms are grown could potentially support the growth of *Listeria* spp. However, a survey of five production zones in a mushroom production facility detected *L. monocytogenes* in just 1.6% of samples and these were all from phase 1, the raw material composting area (*268*).

STRATEGIES FOR CONTROLLING LISTERIA

Numerous interventions have been instituted by manufacturers of RTE meat and poultry products to control *L. monocytogenes*. Preventing contamination by using effective cleaning and sanitation procedures, equipment that is more easily cleaned and has fewer niches where pathogens might survive and grow, and better designed plant facilities are important first steps. "Seek and destroy" monitoring programs are used to detect growth niches of bacteria in a production facility and determine effective procedures for dealing with these harborage sites (*70*). It has been estimated that these strategies have significantly decreased the likelihood of recontamination after thermal processing in the past 10 years (*165*). Safety has been further enhanced by formulating some RTE meats with salt, nitrite, lactate/diacetate mixtures, and some other antimicrobial compounds to inhibit growth of *Listeria* (*89;90;145;165;244*).

Efficacy of methods for decontamination of fresh produce was recently reviewed (*92*). Other recent papers describing strategies for controlling *Listeria* are summarized below.

Human Factors—Education, Monitoring

Current online food safety training materials often fail to address concerns specific to workers at delis. Therefore, an expert consensus method was used to identify baseline food safety training practices that could be used as guidelines for food safety instructors for retail delis (*124*). As an example, interventions to reduce recontamination may not be as rigorous in many retail establishments selling RTE deli meats. One study reported that compliance with hand washing recommendations was low among employees in retail deli departments in Maryland and Virginia (*148*). Two recent articles discussed various aspects of food safety culture and employee education at retail food establishments. It is not enough to simply provide food safety information to employees. Both written instructions and demonstrations on the use and cleaning of equipment are needed (*180*). Managers should encourage safe practices and make it clear that food safety is a priority even when employees are very busy (*181;246*).

For some high-risk populations, such as pregnant women, the elderly, and those in hospitals or nursing homes, it may be prudent to avoid serving certain foods known to present a greater risk for foodborne illness. Following an outbreak of listeriosis in a hospital traced to tuna salad prepared on site, a survey of 54 acute-care hospitals in New York City found that most served RTE deli meats and RTE salads to patients (*45*). A survey of long-term-care facilities found that 9% served soft cheeses made from unpasteurized milk and most facilities served deli meats. However, only a few places reported always heating the deli meats just before serving them (*182*).

Physical Processes

Heat

In-package pasteurization of RTE meat and poultry products was recently reviewed, with discussions on time–temperature combinations and product configuration in packages (*109*). Post-packaging thermal processing of fully cooked chicken breast in 60–90°C water was evaluated for destruction of *L. innocua*. Data were used to construct a model to aid poultry processors in determining appropriate thermal treatments (*138*).

The presence of several ingredients in a food can affect the heat sensitivity of *L. monocytogenes*. Apple polyphenols added to ground beef reduced heat resistance of *L. monocytogenes*. Data suggest that commercial and home processors of meat could add apple polyphenols to meat, reduce sodium chloride concentrations, and produce a safe product at lower temperatures (*118*). Another model was developed to predict inactivation of *L. monocytogenes* in liquid food products. In addition to temperature, other factors, including pH, sugar and sodium chloride levels, and temperature of growth or storage before inactivation, were significant variables affecting effectiveness of thermal treatments (*265*).

Aerated steam treatment for 300 sec reduced *L. monocytogenes* levels on mung bean and alfalfa seeds by more than 5 logs but did not completely inactivate this pathogen. This treatment did not significantly affect germination of mung beans but did lower the germination rate of alfalfa seeds by 11.9% (*247*).

A new method for cooking foods using highly controlled radio frequency energy was not very effective in inactivating *L. monocytogenes* in meatballs although it significantly reduced populations of *E. coli* and *Salmonella* (*226*).

Near-infrared heating for 50 sec reduced the number of *L. monocytogenes* cells on ham slices by 3.38 log with little effect on sensory qualities. In comparison, convective heating required 180 sec and caused changes in color and texture (*98*). Treatment of cooked chicken breast meat for up to 8 min with near-infrared heating to temperatures of 62–75°C reduced *L. monocytogenes* levels by 0.35–1.6 log/min. This was more effective than a hot water immersion process for inactivating this pathogen (*110*).

Microwave heating of foods to ensure safety has been reported to yield mixed results. Such is the case with the most recent reports monitoring *L. monocytogenes*. While a new "smart" microwave oven reduced *L. monocytogenes* counts on catfish fillets within 2 min of 1250 W heating, there was noticeable degradation of the fillet structure (*235*). Microwaving at manufacturers' recommended levels significantly inactivated *L. monocytogenes* on chicken breast but was less effective on chicken patties. Both product type and level of contamination affect success of this method (*173*).

Reheating of inoculated cooked chicken breast meat by microwaving, domestic oven, or stove top cooking reduced *L. monocytogenes* populations by 2–5 log cfu/g. However, inoculated products stored in the refrigerator for several days had higher cell counts, and fairly high numbers survived reheating (*75*).

Irradiation

Irradiation doses of ≥1 kGy significantly reduced viable *L. monocytogenes* cells inoculated on smoked salmon to undetectable levels immediately after treatment. However, during subsequent storage of the salmon at 5°C for 35 days some *L. monocytogenes* cells exposed to 1 kGy gradually recovered; cells exposed to 2 kGy did not recover during this time (*152*). Use of a pectin–nisin film to cover RTE turkey during and after irradiation did significantly reduce proliferation of surviving *L. monocytogenes* during subsequent refrigerated storage (*115*).

High pressure

High pressure processing inactivates bacteria by increasing membrane permeability, generating reactive oxygen species, and disrupting protein–lipid interactions at the cell surface. Many cells die as a result of this treatment but other, damaged cells may recover during storage at cooler temperatures. Therefore some research has investigated the use of antimicrobial compounds in conjunction with high pressure processing to effectively control *L. monocytogenes*. Examples include the use of essential plant oils (*86*), nisin on dry cured ham (*100*), KCl and K lactate as substitutes for NaCl in smoked dry cured ham (*243*), nitrite as the usual chemical addition and from celery (*53;176;177*), and the lactoperoxidase system on cold-smoked salmon (*169*).

Models have been developed to describe inactivation of *L. monocytogenes* during high-pressure processing. Data on inactivation of *L. monocytogenes* on sliced ham at 300–800 MPa were used to construct models suitable for setting process criteria to ensure safety (*101*). An enhanced quasi-chemical kinetics model was developed to evaluate the non-linear inactivation kinetics of *L. monocytogenes* in a protein food system for several combinations of pressure (207–414 MPa) and temperature (20–50°C). This model has four steps to more accurately predict inactivation of various foods (*62*).

Electrolyzed water

Electrolyzed water was reported to reduce *L. monocytogenes* on raw chicken by 1.5–2.3 logs following dipping for 10 min (*208*). However, it was not very

effective on raw fish, and *L. monocytogenes* continued to grow after treatment if the fish were stored under refrigeration (*157*).

Ultraviolet light and pulsed light

Ultraviolet light (UV) damages DNA in bacteria, often resulting in inactivation. *L. monocytogenes* on the surface of chicken frankfurters was exposed to different doses of pulsed UV light and the resulting survival curve was nonlinear. The Weibull model more accurately estimated inactivation of these bacteria on poultry than the log-linear model (*122*).

Intense pulsed light (IPL) using a broad spectrum white light induces damage to bacterial cell structures, including DNA. A comparison of the effects of UV and IPL on *L. monocytogenes* demonstrated that a 7-log reduction could be achieved by treatment with 376 and 455 W/m^2 for 60–180 seconds whereas a 4-log reduction was achieved with 1200 seconds of UVC exposure (*42*).

Pulsed light can inactivate *L. innocua* in protein-containing solutions. However, effectiveness depends on type and concentration of proteins (*7*). Some negative sensory effects were observed in raw fish and beef after treatments with 8.4 and 11.9 J/cm^2 of pulsed light, which reduced *L. monocytogenes* counts by about 1 log (*102*). However, treatment of a cured RTE meat product with 11.9 J/cm^2 had little effect on sensory qualities while reducing *L. monocytogenes* by 1.5–1.8 logs (*82*).

Pulsed light also inactivates *L. innocua* on several types of packaging materials. Effectiveness depends on surface roughness and surface reflectivity (*210*).

Carbon dioxide

The use of supercritical carbon dioxide to inactivate *L. monocytogenes* was evaluated using cells suspended in media. Depressurization rate and the ratio of cell mass to carbon dioxide were the most important variables affecting inactivation of the pathogen (*240*). *L. monocytogenes* inoculated on dry cured ham to a concentration of 10^7 was completely inactivated by supercritical carbon dioxide at 12 MPa, 50°C, for 15 min. Less stringent conditions were effective for lower levels of contamination. Color and other sensory attributes were slightly affected by the treatment (*69*).

Ozone

In-package ozonation to a concentration of 1000 ppm reduced *L. monocytogenes* concentrations on stem scar areas of tomatoes by about 4 logs and on tomato surfaces to undetectable levels (*67*).

Antimicrobial Compounds

Antimicrobial compounds added to foods present another hurdle to limit growth of *L. monocytogenes*. Two risk assessments estimated that contaminated retail deli meats without growth inhibitors were responsible for about 70% of deaths from listeriosis (*64;201*). Various growth inhibitory substances have been tested and found to have some efficacy in inhibiting growth of *L. monocytogenes.* Some examples published in the past 5 years are listed below.

- **Nitrate and nitrite** are traditionally used in cured meat products as effective antimicrobials. However, during the past decade there has been an increasing interest in natural and organic foods and the use of celery with a naturally high nitrate content as a natural curing agent. A variety of ingredients and processes have been developed and were recently reviewed (*230*). Naturally cured meat products have lower nitrite levels, and the addition of clean label antimicrobials can enhance the safety of these products. Challenge tests with ham (*158;249*), roast beef (*158*), and deli-style turkey breast (*158*) demonstrated that vinegar-lemon-cherry powder blend, cultured sugar-vinegar blend, and buffered vinegar delayed growth of *L. monocytogenes* by 2–4 weeks. Antimicrobial effects were greatest on roast beef.
- **Organic acids** (sorbate, benzoate, propionate) have been shown to inhibit *Listeria* in RTE meat and poultry products (*89;90*). Combinations of lactate and diacetate, incorporated into product or used as a surface treatment, also inhibit growth of *L. monocytogenes* on deli meats (*117;144;145;202;244*). These compounds were more effective in low-fat formulations than in high-fat products (*196*).
- **Antimicrobial dips** have been investigated as a means of ensuring safety of frankfurters during refrigerated storage. Organic acids, particularly lactate and diacetate, significantly inhibited growth of *L. monocytogenes* (*143*), and some plant-derived compounds (carvacrol, trans-cinnamaldehyde, β-resorcylic acid) in combination with hydrogen peroxide were also inhibitory (*259*). Several anti-microbials (citric acid, acidified sodium chlorite, trisodium phosphate) used as dips for chicken legs suppressed growth of *L. monocytogenes* (*3*). Trisodium phosphate as a dip was shown to significantly reduce populations of *Salmonella* but not of *L. monocytogenes* on catfish fillets (*209*) and fresh vegetables (*248*).
- **Non-sodium, inorganic chlorides and sulfates** have been suggested as replacements for NaCl in order to reduce sodium levels in foods. Some challenge tests with cooked ham and white sauce found that

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reductions in NaCl levels by about 30% did not result in increased growth of *L. monocytogenes*. Tests in broth, using equivalent molalities, found that calcium and magnesium chlorides exerted a greater antilisterial effect than NaCl, whereas KCl and magnesium sulfate had about the same effect as NaCl (*217*).

- **Bacteriocins**: Nisin was reported to be an effective antilisterial compound on fresh, refrigerated vacuum-packaged shrimp (*272*) and minced tuna and salmon roe (*251*). Bacteriocins from *Pediococcus acidilactici* inhibited *L. monocytogenes* on cold-smoked salmon (*170*). A novel bacteriocin, amysin, reduced growth of *L. monocytogenes* on refrigerated, sliced bologna (*119*). Novel applications of bacteriocins in food preservation were recently reviewed (*11*).
- **Essential oils** have a low solubility in water, making it difficult to evenly disperse them throughout foods. Thymol (*231*) and eugenol (*232*) were enclosed in nanocapsules and effectively mixed in milk and cider, inhibiting growth of *L. monocytogenes*. Peppermint oil in nanoemulsions exhibited prolonged antibacterial activity against *L. monocytogenes* as compared to bulk peppermint oil (*140*). Oregano oil was found to act synergistically with nisin in inhibiting *L. monocytogenes* (*257*). Although several essential oils exhibited antilisterial effects on fresh-cut vegetables, they adversely affected product appearance (*229*).
- **Cranberry powder** at 3% significantly reduced growth of *L. monocytogenes* on hot dogs but, at this concentration, there were adverse organoleptic effects (*279*). Cranberry compounds in several column fractions more effectively inhibited *L. monocytogenes* than *E. coli* (*27;134*).
- **Cinnamon bark extract**, encapsulated in nanoparticles made of poly(D,L-lactide-co-glycolide), was successfully released in aqueous media to inhibit *L. monocytogenes*. These nanocapsules improved delivery of a hydrophobic antimicrobial (*103*).
- **Liquid smoke** added to chicken-pork hot dogs at levels of 2.5 and 5% inhibited growth of *L. monocytogenes* without significant detrimental effects on flavor or texture (*172*).
- **Mixtures and multiple ingredients** have been tested for antimicrobial activity. Green tea extract and grape seed extract were effective as partial antilisterial replacements for lactate and diacetate in hot dogs (*195*). Apple skin extract, oregano, and olive juice powder were reported to have antilisterial effects (*77*).

Competitive Cultures

Lactic acid bacteria (LAB) are generally recognized as safe and are well known biopreservatives in some dairy and fermented foods. In experiments using a commercial preparation of three LAB strains (Lactiguard®) on frankfurters formulated with or without lactate/diacetate, LAB bacteria reduced growth of *L. monocytogenes* by 1–3 logs (*127*). A bacteriocin-producing *Lactobacillus* decreased populations of *L. monocytogenes* by 2.4 log in a meat sausage model held at 22°C for 15 days (*60*). Lactic acid bacteria have also been used with modified atmosphere packaging to control *L. monocytogenes* on chicken legs and extend shelf life (*160*).

L. monocytogenes biofilms on surfaces and equipment in food processing and preparation areas have also been targeted with competitive exclusion bacteria. LAB strains were able to significantly reduce attachment of *L. monocytogenes* to stainless steel when added before or simultaneously with the pathogen. A concentration of 10^6 cfu LAB/ml was required to inhibit 10^3 cfu *Listeria*. LAB (10^8 cfu/ml) were also able to displace *Listeria* that had been preinoculated on stainless steel (*179*). Two species of probiotic bacteria (*Lactobacillus*) reduced numbers of *L. monocytogenes* biofilm cells by more than 3 log during competition, exclusion, and displacement assays (*277*). Experiments in a poultry processing plant found that a mixture of *Lactococcus* and *Enterococcus* that had previously been shown to inhibit *Listeria* on a variety of surfaces eliminated detectable *Listeria* in 5 of 6 floor drains after 4 treatments in a week. The drains remained *Listeria*-free for 13 weeks thereafter (*283*).

Packaging

Recent reports on smart or active packaging systems including antimicrobial substances discussed their applications for meat and poultry products and other foods:

- A **polylactic acid (PLA) film coated with 0.07% lauric arginate** reduced *L. monocytogenes* levels on ham by 2–3 logs after 7 days of storage. Higher coating concentrations had a greater antimicrobial effect but reduced transparency of the film (*253*).
- Antimicrobial packaging containing **oxygen scavengers or carbon dioxide generators** was found to reduce *L. monocytogenes* by 1.1 to 4.76 logs, with greater reductions occurring at a higher temperature of 22°C. A packaging structure containing an **allyl isothiocyanate generator** significantly inhibited *L. monocytogenes* at 22°C but not at lower temperatures (*43*).

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- A **pp/EVOH film containing 5% oregano essential oil** reduced spoilage flora and inhibited pathogens on packaged salads (*175*).
- Incorporation of **4% green tea extract into a chitosan-coated plastic film** inhibited growth of *L. monocytogenes* on ham during storage at 4 and 20°C (*269*).
- **Chitosan coating combined with organic acids** caused up to a 5.38 log reduction in *L. monocytogenes* on RTE shrimp stored at 4°C for 16 days (*139*).
- Essential oils of **oregano and thyme added to LDPE films** during extrusion (*263*) and to edible films used for packaging fish (*113*) inhibited growth of *L. monocytogenes*.
- Two reviews discussed **antimicrobial biopackaging for meat and poultry** (*46*) and **smart packaging** of muscle-based food products (*123*).

Modified atmosphere packaging can also reduce pathogen levels. Several types of packaging reduced *L. monocytogenes* populations by 1 log after 24 hours and by 1.5–2.4 logs after 72 hours on shelf-stable meat snacks (RTE turkey tenders and kippered beef steak strips) during storage at 23°C. Packaging treatments included vacuum, nitrogen flushed with oxygen scavenger, heat sealed with oxygen scavenger, and heat sealed without oxygen scavenger (*260*).

Cleaning and Sanitation

Minimizing contamination of food- and nonfood-contact surfaces in processing plants and retail environments is complicated by the ability of *L. monocytogenes* to form biofilms attached to surfaces. This protects the bacteria from many antimicrobial substances that may be lethal or inhibit growth. One approach to preventing biofilm formation is the modification of equipment and food contact surfaces to prevent attachment of bacteria. N-halamine modification of stainless steel (*13*) and low-density polyethylene (*14*) significantly reduced survival of *L. monocytogenes*. Five to six layers of poly(acrylic acid) and branched polyethyleneimine were immobilized on the surfaces of these materials and provided a potentially rechargeable antimicrobial surface. In other tests, a coating of nitrogen-doped titanium dioxide on glass and stainless steel increased destruction of *L. monocytogenes* exposed to UV light. However, this treatment failed to achieve a 3-log reduction in cell numbers and would not be considered an effective disinfection method (*213*).

Data on efficacy of cleaning and sanitation methods utilizing different physical, chemical, or biological agents are summarized below.

- Some tests found that three **commercial sanitizers** used at the manufacturers' recommended levels were unable to completely destroy free *L. monocytogenes* cells, and even at concentrations of 4 times the recommended levels could not remove cells attached in biofilms (*28*). Other tests on microtiter plates in the lab found that commercial sanitizers tested at the manufacturers' recommended levels were sufficient to kill planktonic cells but not all were effective against biofilms (*49*). Numerous strains of *L. monocytogenes* carry a large plasmid with genes encoding resistance to benzalkonium chloride (*285*). Use of the bacteriocin enterocin AS-48 was reported to enhance the antilisterial activity of several commercial sanitizers (*26*).
- **Neutral electrolyzed water (NEW)** reduces populations of *L. innocua* on cutting boards (hardwood and bamboo) by about 4 logs, which is similar to the reduction achieved with sodium hypochlorite (*167*). NEW was reported to be an effective bacteriocide for free listerial cells at 30 ppm for 0.5 min. However, listerial biofilms required 10 min treatment with 65 ppm NEW to achieve a similar reduction in *L. monocytogenes* populations (*6*).
- **EDTA** at 0.1 mM when administered at the beginning of biofilm formation efficiently prevented biofilm formation without inhibiting planktonic cell growth. EDTA appears to inhibit cell-to-surface and cell-to-cell interactions, thereby preventing aggregation and attachment of bacteria to surfaces (*41*).
- **Chlorine dioxide** at a concentration of 2 mg/L inactivated *L. monocytogenes* by 5 log after a 30-minute treatment of a commercial meat slicer and an industrial hot dog peeler (*256*).
- **Infection of bacteria with specific viruses (phages)** can cause their death and has been proposed as a sanitation method. Experiments with an *L. monocytogenes* biofilm demonstrated that after 8 hours of treatment the biofilm began to break up and cell counts decreased. However, viable cells were still present after 48 hours. Phage treatment may be useful in combination with other sanitation procedures (*168*).
- Biofilms are composed of living cells, extracellular polymeric substances, and nutrients that accumulate there. But the exact composition of listerial biofilms has not as yet been determined. Tests to determine whether enzymes could degrade biofilm components found that several proteases (**papain, proteinase K, trypsin**) were most effective in reducing listerial biofilms (*271*).

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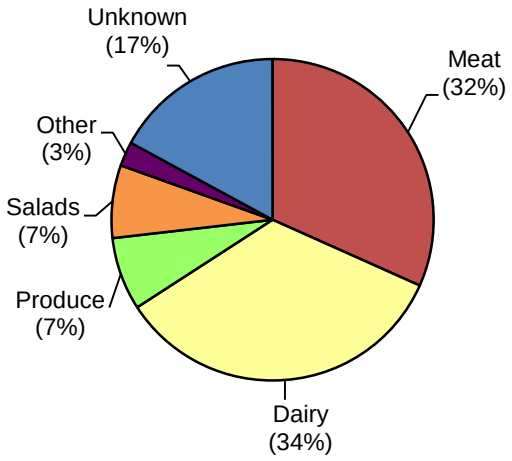
Figure 1. Food vehicles associated with outbreaks and cases reported by CDC, 1998–2011 (*36*).

Outbreaks



Cases

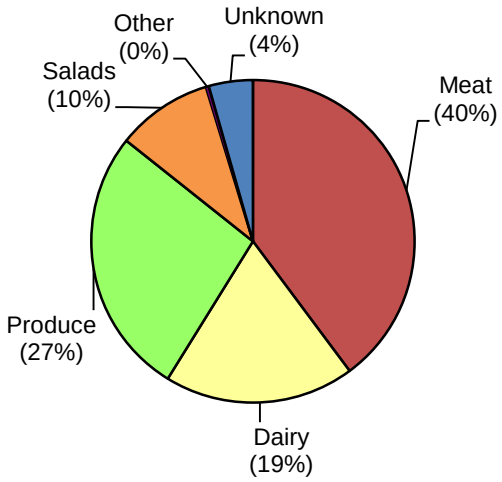


Figure 2. Comparison of the decline in human listeriosis cases as compared to decline in percentage to RTE meat samples testing positive for *Listeria* (*33;74*).

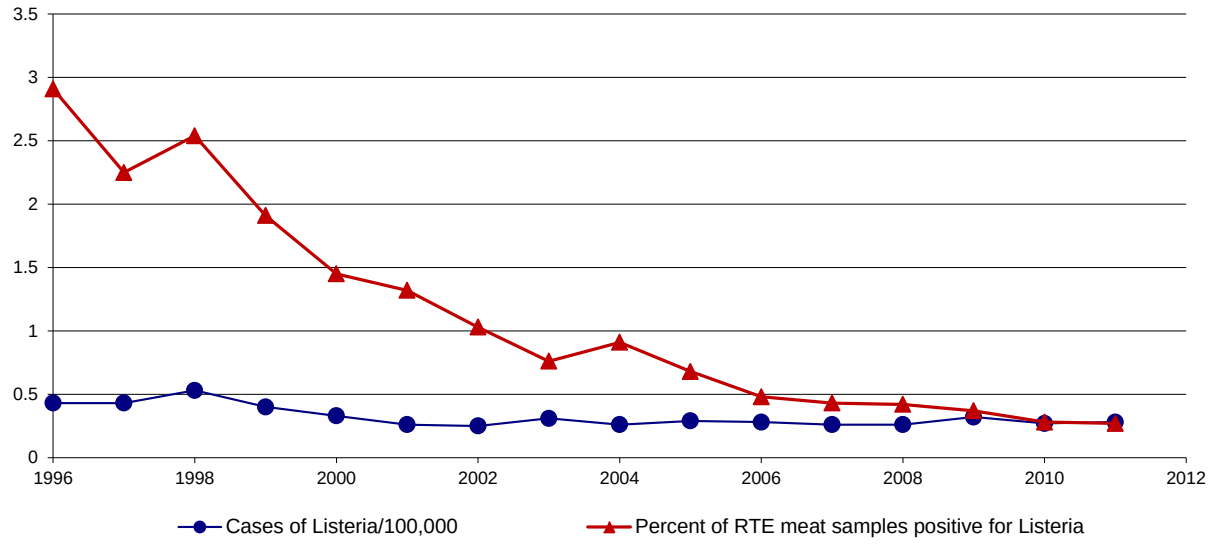


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Table 1. Selected outbreaks of listeriosis with known vehicles of infection.

Year	Food	Cases	Deaths	Location	Reference(s)
2013	Cheese, soft	5	1	U.S. (4 states)	(*38*)
2012–13	Cheese, soft	26	3	Australia	(*242*)
2012	Cheese, ricotta salata	22	4	U.S. (14 states)	(*35*)
2012	Cheese, Latin-style, fresh	2	0	Spain	(*54*)
2011	Cantaloupe	147	33	U.S. (28 states)	(*34*)
2011	Cheese, Mexican-style, pasteurized	2	0	U.S.: New Jersey	(*36*)
2011	Cheese, blue-veined, unpasteurized	15	1	U.S. (multistate)	(*36*)
2011	Cheese, pasteurized	2	1	U.S.: Michigan	(*36*)
2011	Cheese, hard, pasteurized	12	2	Belgium	(*280*)
2011	Ham, sliced, packaged	11		Switzerland	(*111*)
2010	Hog head cheese (RTE meat)	8	2	U.S.: Louisiana	(*32*)
2010	Celery	10	5	U.S.: Texas	(*36*)
2010	Sushi	2	0	U.S.: Washington	(*36*)
2010	Cheese, Mexican-style, pasteurized	6	1	U.S. (multistate)	(*36*)
2010	Melons	9		Australia	(*190*)
2009–10	Cheese, acid curd (quargel)	34	8	Europe	(*227*)
2009	Beef	8	2	Denmark	(*239*)
2009	Cheese, Mexican-style, pasteurized	8	0	U.S. (multistate)	(*36*)
2009	Cheese, Mexican-style	18	0	U.S. (multistate)	(*36*)
2009	Cheese	45	9	Chile	(*278*)
2008–09	Cheese, Mexican-style (asadero), pasteurized	7		U.S. (5 states)	(*114*)
2008	Cheese, Brie, goat-milk	91	5	Chile	(*5*)
2008	Cheese, pasteurized	38		Canada	(*84*)
2008	RTE meats	57	23	Canada	(*22*)
2008	Pork, jellied	13	0	Austria	(*198*)
2007	Milk, pasteurized	5	3	U.S.: Massachusetts	(*31*)
2007	Cheese, Camembert, pasteurized	17	3	Norway	(*116*)
2006–07	Cheese, acid curd	189		Germany	(*125*)
2006–07	Unknown (hospital kitchen)	6	5	Brazil	(*156*)
2006–07	Sausage, RTE, scalded	16	5	Germany	(*276*)
2006	Cheese	75	12	Czech Republic	(*270*)
2005	Cold meats	3		Australia	(*189*)
2005	Cheese, soft (Tomme)	12	3	Switzerland	(*21*)
2003	Sandwich (cheese?)	5	0	U.K.	(*52*)
2002	Cheese, unpasteurized	18	0	Canada	(*85*)
2002	Turkey, deli meat	54	8	U.S. (8 states)	(*93*)
2001	Turkey, sliced	16	0	U.S.: California	(*78*)
2001	Cheese, raw-milk	48	0	Sweden	(*29*)
2001	Cheese	38	0	Japan	(*154*)
2000	Meat (ham, corned beef), RTE	31	0	New Zealand	(*237*)
2000	Cheese, Mexican-style, unpasteurized	13	0	U.S.: North Carolina	(*151*)
2000	Turkey, sliced	30	7	U.S. (11 states)	(*186*)
1999–2000	Meat, RTE paté (rillettes)	10	3	France	(*56*)
1999–2000	Pork tongue, jellied	32	5	France	(*56*)
1999–2000	Fish, vacuum-packed	10	4	Finland	(*99*)
1998–99	Frankfurters	108	21	U.S. (24 states)	(*159*)
1998–99	Butter	25	6	Finland	(*150*)
1998	Fish, cold-smoked trout	5	0	Finland	(*163*)
1994–95	Trout, cold-smoked or gravad	9	2	Sweden	(*65*)
1992	Pork tongue in jelly (RTE)	279	63	France	(*212*)

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Table 2. Results of recently published (2009–2013) surveillance studies for *L. monocytogenes* in food.

Food	Country	# Samples	% Positive	Strains	Concentrations	Reference(s)
Beef, ground	Argentina	40	37%			(*71*)
Beef, raw	China	107	10.3%	1/2b, 4b		(*274*)
Chicken breast, raw, skin on	Canada	187	34%	1/2a		(*48*)
Chicken breast, raw, skin off	Canada	99	15%	1/2a		(*48*)
Chicken breast, raw	Malaysia		20%		<3 to16 MPN/g	(*91*)
Chicken, raw	China	106	13.2%	1/2b, 4b		(*274*)
Chicken, ground	Canada	254	44.5%			(*205*)
Chicken nuggets, frozen	Canada	306	21.2%			(*205*)
Turkey, ground	Canada	251	35.5%			(*205*)
Poultry products, RTE	Germany	300	1%	1/2a	<10 cfu/g (94% of samples) >100 cfu/g (1% of samples)	(*162*)
Pork, raw	China	100	20%	1/2b, 4b		(*274*)
Meats, cooked, sliced, RTE	U.K.	1686	1.53%		<100 cfu/g	(*205*)
Pâtés, RTE	U.K.	1648	0.32%		<100 cfu/g	(*205*)
Meat products, non-cooked, imported	Japan	77	7.8%		<100 cfu/g (4 samples) 100–400 cfu/g (2 samples)	(*184*)
Meat products, RTE	Sweden	507	1.2%		<100 cfu/g	(*136*)
Meat, cooked	Belgium	639	1.1%			(*261*)
Meat, cooked	Algeria	94	3.2%			(*24*)
Meat, raw	Iran	1107	2.4%			(*207*)
Meat, vacuum-packed	Spain	340	2.7%	1/2a, 1/2b, 1/2c, 4c	100–1000 cfu/g (2 samples	(*83*)
Meat, not vacuum packed	Spain	241	8.5%	1/2a, 1/2b, 1/2c, 4c	100–1000 cfu/g (7 samples)	(*83*)
Milk, bulk tank	Finland	183	5.5%		<1 to 30 cfu/ml	(*214*)
Cheese, soft, semi-soft	Sweden	525	0.4%		>100 cfu/g (1 sample)	(*136*)
Cheese, soft	Algeria	39	5.1%			(*24*)
Cheese, Mexican-style, fresh	Mexico	200	15%			(*255*)
Cheese, imported	Japan	70	0			(*184*)
Fish, smoked	Sweden	558	12%		>100 cfu/g (3 samples)	(*136*)
Fish, smoked	Belgium	148	27.8%			(*261*)
Fish, smoked	Spain	142	25%	1/2a, 4c	>1000 cfu/g (7 samples)	(*83*)
Fish, RTE	Canada	40	20%	1/2a, 1/2b	<100 cfu/g	(*132*)
Seafood, RTE	Italy	38	23.7%		>100 cfu/g (3 samples)	(*81*)
Seafood, raw	China	109	13.8%	1/2b, 4b		(*274*)
Anchovy, raw	Turkey	50	2%	1/2b, 4b		(*238*)
Anchovy, salted	Turkey	50	12%	1/2b, 4b		(*238*)
Mussel, raw	Turkey	50	2%	1/2b, 4b		(*238*)
Vegetables (fresh, fresh-cut, and frozen)	Spain	191	4.2% 10.5%	1/2a, 4b	<100 cfu/g by culture 4.97 log(10)/g by PCR	(*171*)
Salads, mayonnaise-based	Belgium	1187	6.7%			(*261*)
Egg shells, pooled samples	Mexico	65	4.6%			(*97*)
Bakery products, sandwiches	Greece	479	8.7%			(*129*)

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