COMPLIANCE POLICY GUIDE (CPG)

CPG Sec. 555.320 Listeria monocytogenes

Final

Contains Nonbinding Recommendations
Draft - Not for Implementation

Compliance Policy Guide
Guidance for FDA Staff
Sec. 555.320
Listeria monocytogenes

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Regulatory Affairs
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I. INTRODUCTION:

The purpose of this Compliance Policy Guide is to provide guidance to FDA Staff on FDA's enforcement policy for *Listeria monocytogenes* (*L. monocytogenes*) in foods.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are

cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND:

L. monocytogenes is a pathogenic bacterium that is widespread in the environment and may be introduced into a food processing facility. *L. monocytogenes* can contaminate foods and cause a mild illness (called listerial gastroenteritis) or a severe, sometimes life-threatening, illness (called invasive listeriosis). Foods that have been implicated in outbreaks of invasive listeriosis have been foods that are ready-to-eat (RTE).

RTE foods can be contaminated if ingredients in the foods are contaminated with *L. monocytogenes* and are not treated to destroy viable cells of this pathogen, or if *L. monocytogenes* is allowed to contaminate the RTE food because of improper sanitary conditions or practices. Most RTE foods do not contain detectable numbers of *L. monocytogenes*. For many RTE foods, contamination with *L. monocytogenes* can be avoided – *e.g.*, through the application of current good manufacturing practice requirements that establish controls on ingredients, listericidal processes, segregation of foods that have been cooked from those that have not, and sanitation. Sanitation controls include effective environmental monitoring programs designed to identify and eliminate *L. monocytogenes* in and on surfaces and areas in the plant.

In 2003, FDA and the Food Safety and Inspection Service of the United States Department of Agriculture, in consultation with the Centers for Disease Control and Prevention of the United States Department of Health and Human Services, released a quantitative assessment (the Risk Assessment) of relative risk associated with consumption of certain categories of RTE foods that

had a history of contamination with *L. monocytogenes*, or that were implicated epidemiologically with an outbreak or a sporadic case of listeriosis. The Risk Assessment estimated that the risk of listeriosis would vary widely among these food categories.

According to the Risk Assessment, foods estimated to pose the highest risk of being associated with listeriosis are RTE foods that support the growth of *L. monocytogenes*. Examples of RTE foods that support the growth of *L. monocytogenes* include:

- Milk;
- High fat and other dairy products (*e.g*., butter and cream);
- Soft unripened cheeses (greater than 50 percent moisture) (*e.g*., cottage cheese and ricotta cheese);
- Cooked crustaceans (*e.g*., shrimp and crab);
- Smoked seafood (*e.g*., smoked finfish and mollusks);
- Raw seafood that will be consumed as sushi or sashimi;
- Many vegetables (such as broccoli, cabbage, and salad greens);
- Non-acidic fruit (such as melon, watermelon, and papaya); and
- Some deli-type salads and sandwiches (particularly those containing seafood and those prepared at retail establishments without acidification and/or the addition of antimicrobial substances).

In contrast, the foods estimated to pose the lowest risk of being associated with listeriosis are foods that, because of intrinsic factors, extrinsic factors, and/or processing factors do not support the growth of *L. monocytogenes*. Intrinsic factors include chemical and physical factors

that are normally within the structure of the food, *e.g.*, pH and water activity. Extrinsic factors are those that refer to the environment surrounding the food, e.g., storage temperature. Processing factors include substances added to adjust the pH of food (e.g., acids) and substances that, alone or in combination with other substances, have antimicrobial properties (e.g., sorbates and benzoates). It is well established that *L. monocytogenes* does not grow when:

- The pH of the food is less than or equal to 4.4;
- The water activity of the food is less than or equal to 0.92; or
- The food is frozen.

Foods may naturally have a pH or water activity that prevents growth of *L. monocytogenes* or processing factors may be deliberately used to achieve those characteristics (*e.g.*, by adding acid to deli-type salads to bring the pH to less than or equal to 4.4). At pH values above 4.4, processing factors generally are used in combination to prevent the growth of *L. monocytogenes* (e.g., sorbates or benzoates may be used in combination with organic acids such as acetic acid, lactic acid, and citric acid in foods such as deli-type salads). The effectiveness of a particular listeristatic control measure in preventing growth in a particular RTE food generally is determined case-by-case, for example, using the results of growth studies specific to the food matrix.

Examples of RTE foods that generally are considered to not support the growth of *L. monocytogenes* include:

- Fish that are preserved by techniques such as drying, pickling, and marinating;
- Ice cream and other frozen dairy products;
- Processed cheese (*e.g.*, cheese foods, spreads, slices);

- Cultured milk products (*e.g.*, yogurt, sour cream, buttermilk);
- Hard cheeses (less than 39 percent moisture) (*e.g.*, cheddar, colby, and parmesan);
- Some deli-type salads, particularly those processed to a pH less than 4.4 and those containing antimicrobial substances such as sorbic acid/sorbates or benzoic acid/benzoates under conditions of use documented to be effective in preventing the growth of *L. monocytogenes*;
- Some vegetables (such as carrots); and
- Crackers, dry breakfast cereals, and other dry foods.

Fruits, vegetables, and cheeses (e.g., soft and semi-soft cheeses) not listed in this CPG may include some products that support growth as well as other products that do not support growth.

III. **POLICY:**

FDA will review the available evidence on a case-by-case basis to determine if a food is a RTE food that supports growth or a RTE food that does not support growth.

A. **Ready-to-Eat Food**

"Ready-to-eat food" (RTE food) means a food that is customarily consumed without cooking by the consumer, or that reasonably appears to be suitable for consumption without cooking by the consumer.

A food may be considered to be suitable for consumption without cooking by the consumer, and thus a RTE food, even though cooking instructions are provided on the label. For examples, fresh and frozen crabmeat and individually quick frozen (IQF) peas and corn

may be RTE foods. Some consumers eat such products without cooking, because they appear to be ready-to-eat.

B. Ready-to-Eat Foods that Support Growth of *L. monocytogenes*

Generally, we intend to consider that a RTE food will support the growth of *L. monocytogenes* if it does not meet the characteristics of a RTE food that does not support growth, as indicated in section III.C.

FDA may regard a RTE food that supports growth of *L. monocytogenes* to be adulterated within the meaning of section 402(a)(1) of the Federal Food, Drug, and Cosmetic Act (the Act; the FD&C Act) (21 U.S.C. 342(a)(1)) when *L. monocytogenes* is present in the food based on the detection method indicated in section IV.A.

C. Ready-to-Eat Foods that Do Not Support Growth of *L. monocytogenes*

A RTE food does not support the growth of *L. monocytogenes* if the food:

- Has a pH that is less than or equal to 4.4; or
- Is customarily held and consumed in a frozen state; or
- Has a water activity that is less than 0.92; or
- Is processed using an effective listeristatic control measure (e.g., an antimicrobial substance or a combination of factors such as pH, water activity, and antimicrobial substances).

FDA may regard a RTE food that does not support the growth of *L. monocytogenes* to be adulterated within the meaning of section 402(a)(1) of the Act (21 U.S.C. 342(a)(1)) when *L. monocytogenes* is present at or above 100 colony forming units per gram of food (cfu/g)

IV. **REGULATORY ACTION GUIDANCE:**

A. **Ready-to-Eat Foods that Support Growth of *L. monocytogenes***

The following represents criteria for recommending legal action to CFSAN/Office of Compliance/Division of Enforcement (HFS-605):

- *L. monocytogenes* is detected in one or more subsamples of a RTE food that supports the growth of *L. monocytogenes*.

Use Bacteriological Analytical Manual Online, Chapter 10 - "*Listeria monocytogenes*," "Detection and Enumeration of *Listeria monocytogenes* in Foods" as the method for detecting and confirming presence of *L. monocytogenes* (available at http://www.cfsan.fda.gov/~ebam/bam-10.html (http://www.cfsan.fda.gov/~ebam/bam-10.html)).

B. **Ready-to-Eat Foods that Do Not Support Growth of *L. monocytogenes***

Consult with CFSAN/Office of Compliance/Division of Enforcement (HFS-605) before recommending legal action for RTE foods that do not support the growth of *L. monocytogenes*. Use ISO 11290-2:1998(E) "Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration method" as the method for enumerating *L. monocytogenes*. (ISO 11290-2:1998/Amd. 1:2004(E) "Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration method AMENDMENT 1: Modification of the enumeration medium" amends ISO 11290-2:1998(E). The amendment uses ALOA agar instead of PALCAM agar. If ALOA agar is not commercially available in the United States, use PALCAM according to ISO

11290-2:1998(E)). ISO methods are available from the International Organization for Standardization at http://www.iso.org/iso/en/ISOOnline.frontpage (http://www.iso.org/iso/en/ISOOnline.frontpage) (http://www.fda.gov/about-fda/website-policies/website-disclaimer).

Use rapid biochemical test kits according to the Bacteriological Analytical Manual Online, Chapter 10 – "Detection and Enumeration of *Listeria monocytogenes* in Foods" Section E-11 (available at http://www.cfsan.fda.gov/~ebam/bam-10.html (http://www.cfsan.fda.gov/~ebam/bam-10.html)), instead of ISO 11290-2:1998(E) Section 9.5, for confirmation of *L. monocytogenes* isolates.

C. **Foods that are Not RTE Foods**

Consult with CFSAN/Office of Compliance/Division of Enforcement (HFS-605) when *L. monocytogenes* is present in a food that is not a RTE food.

D. **Other Considerations**

The criteria in this guidance do not establish an acceptable level of *L. monocytogenes* in food. FDA may choose to take legal action against adulterated food that does not meet the criteria for recommending legal action to CFSAN.

Further, the criteria in this guidance do not excuse violations of the requirement in section 402(a)(4) of the Act (21 U.S.C. 342(a)(4)) that food may not be prepared, packed, or held under insanitary conditions or the requirements in FDA's good manufacturing practices regulation (21 CFR part 110). As set out in 21 CFR 110.80, food manufacturers must take "[a]ll reasonable precautions ... to ensure that production procedures do not contribute contamination from any source."

V. **SPECIMEN CHARGES:**

A. **Domestic Seizure**

The article of food was adulterated when introduced into and while in interstate commerce and is adulterated while held for sale after shipment in interstate commerce within the meaning of the Act, 21 U.S.C. 342(a)(1), in that it bears and contains a poisonous or deleterious substance, namely *Listeria monocytogenes*, which may render it injurious to health.

B. **Import Detention**

The article of food is subject to refusal of admission pursuant to section 801(a)(3) of the FD&C Act in that it appears to be adulterated within the meaning of section 402(a)(1) of the FD&C Act in that it bears and contains a poisonous or deleterious substance, *Listeria monocytogenes*, which may render it injurious to health.

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Research Paper

Growth of *Listeria monocytogenes* in Thawed Frozen Foods

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ABSTRACT

The growth characteristics of *Listeria monocytogenes* inoculated onto frozen foods (corn, green peas, crabmeat, and shrimp) and thawed by being stored at 4, 8, 12, and 20°C were investigated. The growth parameters, lag-phase duration (LPD) and exponential growth rate (EGR), were determined by using a two-phase linear growth model as a primary model and a square root model for EGR and a quadratic model for LPD as secondary models, based on the growth data. The EGR model predictions were compared with growth rates obtained from the USDA Pathogen Modeling Program, calculated with similar pH, salt percentage, and $NaNO_2$ parameters, at all storage temperatures. The results showed that *L. monocytogenes* grew well in all food types, with the growth rate increasing with storage temperature. Predicted EGRs for all food types demonstrated the significance of storage temperature and similar growth rates among four food types. The predicted EGRs showed slightly slower rate compared with the values from the U.S. Department of Agriculture Pathogen Modeling Program. LPD could not be accurately predicted, possibly because there were not enough sampling points. These data established by using real food samples demonstrated that *L. monocytogenes* can initiate growth without a prolonged lag phase even at refrigeration temperature (4°C), and the predictive models derived from this study can be useful for developing proper handling guidelines for thawed frozen foods during production and storage.

Key words: Frozen and thawed foods; Growth model; *Listeria monocytogenes*; Modeling; Ready-to-eat food

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Listeria monocytogenes is a gram-positive, rod-shaped bacterium that causes the foodborne disease listeriosis in humans. Listeriosis can manifest as an invasive disease that can result in meningitis, pneumonia, septicemi, and death. Listerosis mainly affects the elderly, the immunocompromised *(20, 23, 27),* and pregnant women, who may develop flulike symptoms and experience miscarriage or stillbirth *(20, 23, 27)*. Although listeriosis is relatively rare, the mortality rate is high, and most patients are hospitalized. Scallan et al. *(33)* estimated that 1,600 cases of listeriosis occur annually in the United States, of which 250 cases are fatal. Although this pathogen is ubiquitous in the environment *(32),* it can be readily inactivated by pasteurization and cooking *(5)*. *L. monocytogenes* can grow at refrigeration temperatures, and refrigerated ready-to-eat foods that support the growth of *L. monocytogenes* have been associated with listeriosis outbreaks *(6, 14, 17, 20, 21, 40, 42)*. In the 1980s, the U.S. Food and Drug Administration and U.S. Department of Agriculture (USDA) Food Safety and Inspection Service established a "zero-tolerance" policy for *L. monocytogenes* in ready-to-eat foods *(35)*. Since then, several risk assessments have been conducted to better understand the risk of consuming food contaminated with *L. monocytogenes (14, 29, 39, 42)*.

Freezing is an effective control to prevent the growth of pathogens, including *L. monocytogenes*. However, once a frozen food is thawed, it may be able to support the growth of *L. monocytogenes,* if present. Cooked and frozen shrimp and crabmeat, along with frozen green peas and corn, may be thawed and held refrigerated before consumption, and some consumers may eat them without cooking or reheating. Because *L. monocytogenes* can grow at refrigeration temperatures, holding these foods for extended periods may allow this pathogen to grow to levels that represent a public health concern. A survey of frozen vegetables conducted in Portugal showed that 14.8 to 22.6% of frozen vegetable samples were positive for *L. monocytogenes (24)*. Another investigation demonstrated that 26% of frozen seafood samples, including frozen cooked shrimp, cooked crabmeat, and raw seafood, were positive for *L. monocytogenes* overall *(43)*.

There is a gap in the knowledge concerning the growth kinetics of *L. monocytogenes* in frozen foods that are then thawed and held at refrigeration temperatures. The 2013 Food Code requires that foods that fall under the category of "time-temperature control for safety" be stored at <5°C for up to 7 days, based on limiting the growth of *L. monocytogenes* (to an increase of no more than 10-fold or 1 log) *(41)*. However, refrigerated temperature control can present a challenge in both retail and the consumer home setting. In a survey of product temperatures at retail locations, it was shown that 30.7% of products in retail

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display and 9.4% of backroom refrigerators had temperatures higher than 5°C *(9)*. Cold salad bar settings may provide further opportunity for temperature abuse. For example, one study showed that the food surface of potato salad had temperatures of 13 to 16°C at salad bars, even though the units were set to the coldest setting *(37)*. The same study also showed food handling practices that might lead to prolonged display and storage of food items: for example, mixing fresh food into old batches of leftover food on the salad bar *(37)*. This study highlights the difficulties of controlling the food temperature in salad and food bar settings and in monitoring how long food products have been exposed to potential temperature abuse. Temperature abuse can also occur within the home; the abovementioned study showed that 16.8% of products tested within consumers' homes were stored at temperatures exceeding 5°C *(9)*.

The objective of this study was to investigate the growth kinetics of *L. monocytogenes* in thawed frozen foods (corn, green peas, crabmeat, and shrimp) stored at 4, 8, 12, or 20°C. The temperatures reflect recommended refrigeration temperature (4°C), elevated "abuse" refrigeration temperature (8 or 12°C), and room temperature (20°C). Furthermore, the growth curves derived were used to develop predictive models for the lag-phase duration (LPD) and the exponential growth rate (EGR) in those food types at 4 to 20°C. Knowledge of the length of the lag phase of this organism could provide more accurate handling guidance for frozen foods that are thawed and, subsequently, held at refrigeration temperatures.

MATERIALS AND METHODS

***L. monocytogenes* strains and culture conditions.** Twelve strains of *L. monocytogenes* from the Grocery Manufacturers Association culture collection (Washington, DC) were used in this study: N-7351 (1/2b, isolated from deli meat), N-7389 (1/2b, isolated from deli meat), N-7391 (1/2c, isolated from deli meat), N-7427 (4d, isolated from deli meat), N-7292 (4b, clinical isolate), N-7293 (4b, clinical isolate), N-7447 (1/2c, isolated from seafood salad), N-7497 (4b, isolated from seafood salad), N-7503 (1/2a, isolated from seafood salad), N-7601 (1/2b, isolated from seafood salad), N-7295 (4b, clinical isolate), and N-7296 (4b, clinical isolate). Working cultures were made from glycerol-frozen or lyophilized stocks stored in a −80°C freezer and maintained on tryptic soy agar (TSA; Difco, BD, Sparks, MD) with 0.6% yeast extract (YE; Difco, BD) slants at 4°C and transferred every 6 months. Before inoculation, a loopful of each strain was transferred in 10 mL of tryptic soy broth (TSB; Difco, BD) with 0.6% YE (TSB+0.6% YE) and grown aerobically at 35°C for 24 h (stationary-phase culture).

Preparation of inocula. One hundred microliters of each stationary-phase culture, approximately 10^9 CFU/mL, was transferred to an individual test tube containing 10 mL of sterile TSB+0.6% YE and incubated at 4°C for 7 days for cells to adapt to the cold *(32)*. After the 7-day incubation, each culture reached approximately 10^8 CFU/mL. All 12 strains of refrigeration temperature–adapted cultures were combined into a cocktail (2 mL of each culture) in a centrifuge tube. The cocktail, containing approximately 10^8 CFU/mL of *L. monocytogenes* cells, was serially diluted in 0.1% peptone water (pH 7.0; Fisher Scientific, Fair Lawn, NJ) to a desired inoculation level.

Source and inoculation of food. Four types of frozen food samples, blanched individually quick frozen corn, individually quick frozen green peas, cooked snow crabmeat, and cooked peeled shrimp, were obtained from a local grocery store and by mail order. Food samples were obtained frozen and held at −18°C prior to and during inoculation. Crabmeat from frozen cooked snow crab with shell was aseptically removed from shell as a part of sample preparation before the weighing process. Prior to each individual growth experiment, random samples from the four types of thawed frozen foods were tested for being *L. monocytogenes* negative by using VIDAS LMO2 (bioMérieux, Marcy l'Etoile, France). Aerobic plate counts were also performed with TSA plates incubated at 35°C for 48 h to obtain counts for background micoflora in each product, and the pH was determined by using a pH meter (Accumet Research AR 20, Fisher Scientific).

Test samples were weighed (25 g) into stomacher bags (Whirl-Pak, Nasco, Fort Atkinson, WI) while they were still frozen and inoculated with 100-μL aliquots of the culture cocktail that was distributed over the product surface by using a pipette. The initial inoculation level was approximately 10^3 CFU/g (confirmed immediately after the inoculation by plating, as described in the following). The inoculated product was stored frozen at −18°C for 7 days. Following frozen storage, the inoculated food samples were taken out of the freezer and transferred to air incubators set at 4, 8, 12, or 20°C. The initiation of the growth curves (time zero) was the time the food sample was transferred to 4, 8, 12, or 20°C (i.e., not the time the food sample reached temperature) as imitating consumer practices or practices potentially seen at food bars.

Enumeration of *L. monocytogenes*. At predetermined time intervals (established by preliminary experiments), samples were removed from incubation, and the samples were enumerated for *L. monocytogenes*. Samples were diluted 1:10 in buffered peptone water (3M, St. Paul, MN) and pulsified for 30 s (Pulsifier, Microgen Bioproducts, Ltd., Camberley, UK). If required, further decimal dilutions of samples were made with peptone water. The diluted samples were plated onto polymyxin–acriflavin–lithium chloride–ceftazidime–aesculin–mannitol agar (PALCAM; Difco, BD) by using a spiral plater (model AP 4000, Spiral Biotech, Norwood, MA). Preliminary experiments indicated that resuscitation steps for injured cells were not necessary. Plates were incubated for 48 h at 35°C. Cell counts were obtained by using a Q count system (model 510, Spiral Biotech). Three independent growth experiments, with one sample per replicate, were conducted for each food type at each storage temperature.

Curve-fitting and primary model. Data for each replicate were converted to log CFU per gram and iteratively fit to the two-phase linear growth equation *(4, 36)* to generate LPD and EGR by minimizing the residual sum of squares using the Solver function in Microsoft Excel, Version 1997 (Microsoft Corporation, Redmond, WA; worksheet provided by Dr. Richard Whiting [Exponent, Inc., Knoxville, TN]), in which an if–then statement defines the model:

$$N = N_0 + \mathrm{IF}[t < \mathrm{LPD}, N_0, \mathrm{EGR} \times (t - \mathrm{LPD})] \quad (1)$$

where N is the log CFU/g at time t, N_0 is the initial log CFU/g, LPD is the lag-phase duration (h), EGR is the exponential growth rate ([log CFU/g]/h), and t is the elapsed time (h).

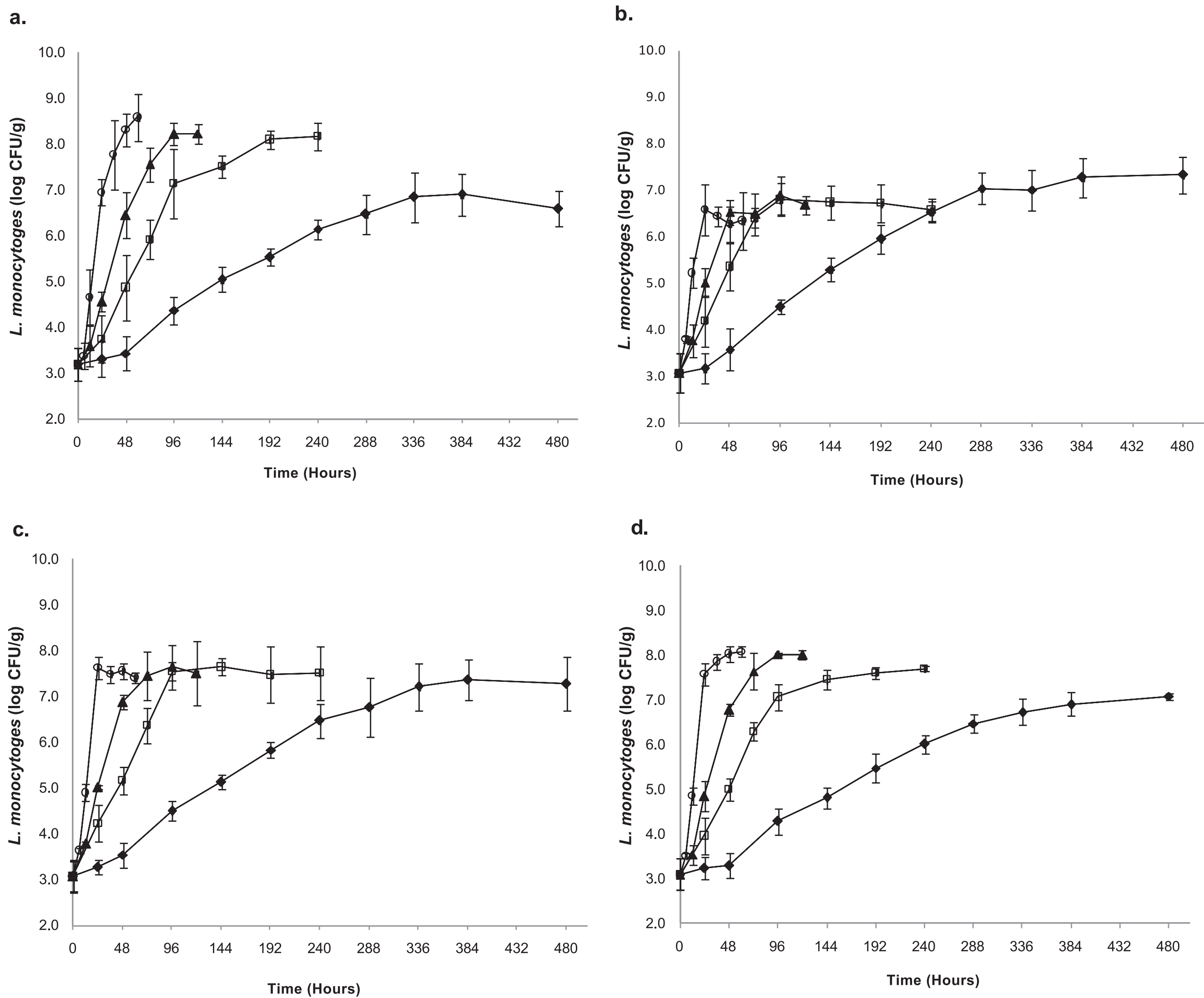

FIGURE 1. L. monocytogenes *growth curves of thawed frozen food samples, (a) crabmeat, (b) corn, (c) green peas, and (d) shrimp, performed in triplicate at four storage temperatures (♦, 4°C; □, 8°C; ▲, 12°C; ○, 20°C). The error bars indicate standard deviation.*

Secondary model for EGRs. To integrate the effect of storage temperature, EGRs were further calculated using data from the primary model (equation 1) with the square root model *(8, 30)*. The curve fitting was performed with the Excel Solver.

$$\sqrt{\text{EGR}} = a(T - T_{\min}) \tag{2}$$

where a is the constant, T is the temperature, and $T_{\min}$ is the theoretical minimum temperature at growth that no growth is possible.

Secondary model for LPDs. To incorporate the effect of storage temperature on the LPD, the quadratic model was used to calculate LPD predictions *(31)*. LPDs were calculated by using data from the primary model (equation 1) with the quadratic model.

$$\text{LPD} = p_1 + p_2T + p_3T^2 \tag{3}$$

$p_i = (i = 1, \ldots, 10)$ are coefficients to be estimated and T is the temperature.

Data analysis. The fit of models was evaluated by the residual mean squares (R^2) based on regression analysis *(15, 44)*. LPDs and EGRs derived from the secondary model were compared against a calculation on predictions made from the USDA Pathogen Modeling Program (PMP) *(38)* by using pH (7.0), NaCl (0.5%), and $NaNO_2$ (0%). These parameters were selected based on sample characteristics. For NaCl (percentage) and $NaNO_2$ (percentage), nutrient descriptions on the product label of each product were used.

RESULTS

Growth of *L. monocytogenes* in four types of thawed frozen foods. Frozen corn, green peas, crabmeat, and shrimp were obtained, and the pH values of the products were 7.2, 6.8, 7.2, and 7.5, respectively. Representative uninoculated samples were tested for *L. monocytogenes,* which was not detected. The products were inoculated with a cocktail of *L. monocytogenes* and held at −18°C for 7 days. Then, the inoculated samples were incubated at 4, 8, 12, or 20°C, and growth was monitored for up to 20 days.

L. monocytogenes grew to stationary phase in all products at all temperatures, as shown in Figure 1a through 1d. Growth of *L. monocytogenes* occurred much more

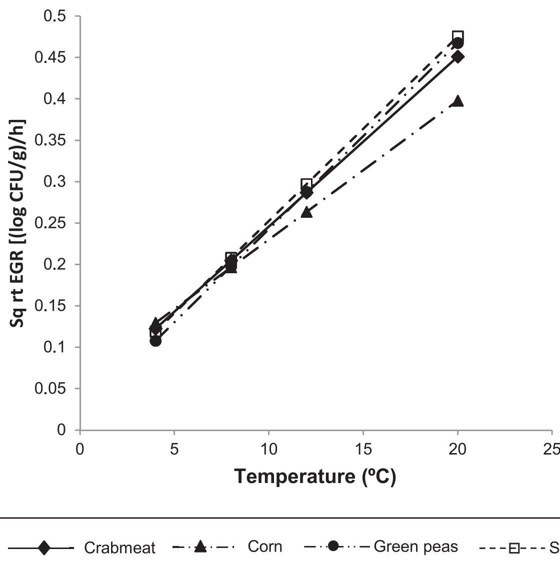

FIGURE 2. *Square root model predictions of the exponential growth rate (EGR; [log CFU per gram]/hour) calculated from the two-phase linear (primary) model for* L. monocytogenes *in four types of thawed frozen foods (crabmeat, corn, green peas, and shrimp) over the temperature range of 4 to 20°C.*

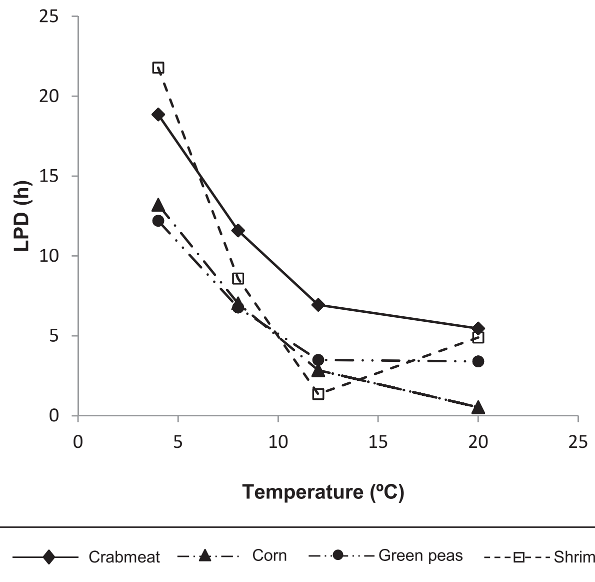

FIGURE 3. *Quadratic model predictions of the lag-phase duration (LPD; hours) calculated from the two-phase linear (primary) model for* L. monocytogenes *in four types of thawed frozen foods (crabmeat, corn, green peas, and shrimp) over the temperature range of 4 to 20°C.*

rapidly as the storage temperature increased for all foods. For example, *L. monocytogenes* reached stationary phase, when the growth curves appeared plateaued, after approximately 288 to 380 h in all four food types stored at 4°C, whereas the stationary phase was reached within 24 to 48 h, when samples were stored at 20°C. For all food types, the lag phase became shorter as the temperature increased. For instance, LPD was approximately 48 h for food samples stored at 4°C, whereas LPD was less than 12 h for food samples stored at 20°C. The final cell density was different in four types of foods. *L. monocytogenes* appeared to reach higher numbers in crabmeat and shrimp compared with corn and green peas overall, although statistical analysis was not conducted.

Calculation of LPD and EGR by using primary and secondary models. The LPD and EGR for *L. monocytogenes* in each food type at each storage temperature were generated by using two-phase linear models (equation 1), based on the log growth data of each replicate. Then, an EGR value was further calculated by using linear regression analysis of the square root model (equation 2), based on the outcome from equation 1, to incorporate the effect of storage temperature. The results revealed the relationship with storage temperature on EGRs of *L. monocytogenes,* with the square root of the EGRs becoming greater, indicating faster growth, as the growth temperature was increased for all food types (Fig. 2). The fit of the secondary model was good ($R^2 > 0.98$), as shown in Table 1.

For LPD, the quadratic model was used as the secondary model. Storage temperature had an impact on LPD, which generally decreased as the temperature increased in all food types (Fig. 3). However, the fit of the model was not ideal; R^2 values ranged from 0.23 to 0.71 (Table 1), due to high variability among replicates, indicating the model does not describe the lag phenomenon in these food samples precisely. Still, the model may be able to describe average LPD. The shape of the curve for shrimp was concave, with increasing the predicted LPD at 20°C, and high variability among replicates at 20°C.

Development of predictive models for *L. monocytogenes* in thawed frozen foods. A linear regression equation derived from the regression analysis of equation 2 or 3 for

TABLE 1. *Residual mean square* (R^2) *values and formulas for each thawed frozen food sample (crabmeat, corn, green peas, and shrimp), based on linear regression analysis for the predicted LPDs and square root of EGRs from square root models and actual* L. monocytogenes *growth data*

	LPD		Square root of EGR	
	R^2	Linear regression equation	R^2	Linear regression equation
Crabmeat	0.3748	$LPD = 28.73 - 2.792temp + 0.0814temp^2$	0.9839	$\sqrt{EGR} = 0.0205temp + 0.0403$
Corn	0.7117	$LPD = 21.41 - 2.300temp + 0.0628temp^2$	0.9816	$\sqrt{EGR} = 0.0168temp + 0.0623$
Green peas	0.3921	$LPD = 19.77 - 2.164temp + 0.0673temp^2$	0.9812	$\sqrt{EGR} = 0.0225temp + 0.0178$
Shrimp	0.2369	$LPD = 41.00 - 5.550temp + 0.1872temp^2$	0.9945	$\sqrt{EGR} = 0.0223temp + 0.0296$

TABLE 2. *LPD and EGR predictions for* L. monocytogenes *in each thawed frozen food calculated from the predictive models as compared with USDA PMP predictions calculated by using parameters similar to food samples*[a]

	Predicted LPD (h)				Predicted EGR ([log CFU/g]/h)			
	4°C	8°C	12°C	20°C	4°C	8°C	12°C	20°C
Crabmeat	18.86	11.60	6.94	5.45	0.015	0.042	0.082	0.203
Corn	13.21	7.03	2.85	0.53	0.017	0.039	0.070	0.159
Green peas	12.20	6.77	3.49	3.40	0.012	0.039	0.083	0.219
Shrimp	21.79	8.58	1.36	4.89	0.014	0.043	0.088	0.226
USDA PMP	62.03	32.00	17.56	6.35	0.027	0.056	0.107	0.303

[a] Aerobic, broth culture, pH (7.0), NaCl (0.5%), and $NaNO_2$ (0%).

each type of food is presented in Table 1. The aim of these equations is to predict EGRs or LPDs of *L. monocytogenes* in each type of the thawed frozen foods over the temperature range of 4 to 20°C. However, the LPD models were not reliable, which will be discussed subsequently.

Predicted EGRs and LPDs and comparison with PMP predictions. Predicted values of EGRs and LPDs for each food type from 4 to 20°C were calculated by using equations (established models: Table 1). A higher EGR value means a faster growth rate. Predicted EGR values demonstrated similar trends for all food types, with EGR increasing as storage temperature rose. The EGR values were slightly higher in crabmeat and shrimp than in green peas and corn. The predicted LPD values showed that a trend in which shorter LPDs were observed on the vegetables than the seafood, especially at lower temperatures (4 or 8°C). For example, thawed frozen vegetables had a predicted LPD of less than 13.2 h, and thawed frozen seafood had a predicted LPD of about 18 to 21 h at 4°C. These predicted LPDs and EGRs values were compared with the growth rates and lag phase derived from the USDA PMP (Table 2). At all four temperatures, the PMP predicted more rapid EGRs than were determined in the current study. However, the PMP predicted longer LPDs than those LPDs derived in this study.

Aerobic plate counts of uninoculated food samples. Corn and green pea samples had higher initial aerobic plate counts (time zero), with 4.05 and 2.87 log CFU/g, respectively, while the crabmeat and shrimp began with 1.65 and 2.18 log CFU/g, respectively.

DISCUSSION

Thawed frozen corn, green peas, crabmeat, and shrimp supported the growth of *L. monocytogenes* at each incubation temperature (4, 8, 12, or 20°C). The lag phase at 4°C was shorter than PMP predictions; however, the growth rates were consistent with PMP predictions and results in other publications. For example, Farber *(13)* showed 2- to 3-log growth of *L. monocytogenes* in 7 days in cooked shrimp and crabmeat incubated at 4°C; in the current study, a 2-log increase was seen in the same time frame. Hughey et al. *(18)* demonstrated 2 log of growth of *L. monocytogenes* in fresh corn and green beans in 9 days, which appears to be slightly less than the observation from the current study (i.e., approximately 3 log in 9 days in corn or green pea samples). These studies and our own results indicate that *L. monocytogenes* grows more rapidly in seafood products than in vegetables overall. There have been various studies on developing predictive models for growth of *L. monocytogenes* in laboratory media or foods *(7, 19, 22, 44),* and predictive models for growth of the organism in smoked salmon have been investigated extensively, as explored in Giménez and Dalgaard *(16)*. However, there is no study on a development of predictive models for the growth of this organism in thawed frozen foods. In the current study, growth parameters, LPDs and EGRs, of *L. monocytogenes* in thawed frozen foods were first calculated by using a two-phase linear model as a primary model based on experimental growth data. The two-phase model is a modified version of the three-phase linear model and allows the calculation of LPD and EGR without data from the stationary phase *(4)*. Several studies have been published using the two-phase linear model *(4, 10, 11, 25, 26)*. Secondary models were applied to incorporate the effect of storage temperature to EGRs and LPDs. In the current study, the square root model and quadratic model were used for prediction of EGR and LPD, respectively, based on the data obtained from a primary model. These models are simple and expandable to incorporate other factors and have been used in many published studies, as discussed in Ross and Dalgaard *(31)*.

The goodness of fit for EGR predictions was high based on R^2 values; hence, the equations derived from the regression analysis can be used to predict EGRs of *L. monocytogenes* between 4 to 20°C for those samples. On the contrary, the fit of model for LPDs was not ideal. Several models were used to fit data to predict LPDs, such as square root model and reciprocal model. None of the models provided an ideal fit. The quadratic model is one of empirical models, describing a set of data from experiments in a simple mathematical correlation *(31)*. However, the equations derived for predicting LPDs in this study are not adequate and are not reliable to predict precise LPD. Therefore, they should not be used to predict LPD.

A possible reason for the undesirable fit and outcome could be that not enough data points were collected during the growth experiment, particularly during the lag phase. This resulted in "no lag time (0 h)" calculation at the primary model step for some samples and caused high variability among replicates. In comparison to the development of growth rate models, creating lag time models

that estimate accurate lag phases are more difficult because the lag phenomenon is still not clearly understood *(1)*. There are many factors influencing lag behavior such as (i) adaptation mechanisms to a new environment, (ii) character and phenotype of the bacterium, (iii) physiological state of cells, (iv) physiological history of the cells, (v) inoculum size, or (vi) distribution condition in samples *(36)*. Therefore, whichever model is used, it is important to consider that models can only describe the simplified form of real phenomena and the imprecision of lag-time predictions *(1)*.

Despite being unable to adequately model the LPD, the growth curves showed that the lag phase was relatively short at each temperature, considering that the thawing process was included. Before conducting the experiment, it was hypothesized that freezing of the cultures may create an extended lag phase for this organism once the foods were thawed and held at refrigeration temperatures. However, this was not observed in the results. The short lag phases may indicate that there was no obvious effect of freezing and thawing to initiate growth of *L. monocytogenes* in thawed frozen foods incubated at 4 to 20°C. *L. monocytogenes* is known to be resistant to injury due to freezing in food and broth systems *(12, 28)*. Beauchamp et al. *(2)* also found that various methods of thawing frozen hot dogs had little effect on survival and growth of *L. monocytogenes* during refrigerated storage. Furthermore, the short lag phase observed here may have been due to the use of inocula that were acclimated to refrigeration temperatures by growing to stationary phase at 4°C prior to freezing in the food. Usage of environment-acclimated organisms when conducting laboratory challenge studies is recommended because those organisms may better replicate a real-world scenario *(34)*.

The predicted EGRs were compared with those from the USDA PMP. The values were in the same order of magnitude, but the PMP did produce slightly higher (i.e., rapid) EGR values. One reason why the PMP predictions were higher may be because the current study used actual food samples as growth media, which may be less supportive in nutrient composition for growth of this organism compared with laboratory broth media used to build the PMP predictions. Furthermore, the competing effect of background microflora is not incorporated in PMP predictions. Several researchers investigated inhibitory effect of spoilage organisms against *L. monocytogenes*. Buchanan and Bagi *(3)* demonstrated that growth of *L. monocytogenes* was inhibited due to coinoculation with *Pseudomonas fluorescens* in brain heart infusion broth with sodium chloride (5 and 25 g/L) at 4°C. In a study by Giménez and Dalgaard *(16)*, growth of *L. monocytogenes* was inhibited due to a cocktail of spoilage organisms (lactic acid bacteria, *Enterobacteriaceae,* and *Photobacterium phosphoreum*) in vacuum-packaged cold-smoked salmon slices at 5, 10, 17.5, or 25°C. General prediction models established, based on laboratory conditions (i.e., broth culture), may display predictions different from predictive models derived from data in real food having a complex matrix with competing microflora *(34)*.

The data generated in this study show that thawed frozen corn, green peas, crabmeat, and shrimp support the growth of *L. monocytogenes* in the temperature range of 4 to 20°C. Under the current experimental conditions, there was a relatively short lag phase, especially at the three higher temperatures (8, 12, and 20°C). Creating growth curves and subsequent predictive growth models of *L. monocytogenes* in these foods over a wide range of temperatures could aid in the development of specific handling and holding guidelines for the foods after thawing. Conducting additional research to obtain more data to develop predictive models for LPD would be highly desirable. Investigations of the prevalence and contamination level of *L. monocytogenes* in certain frozen foods could assist the industry to improve food safety and provide a better indication of the risk to public health.

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Infectious Dose of *Listeria monocytogenes* in Outbreak Linked to Ice Cream, United States, 2015

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The relationship between the number of ingested *Listeria monocytogenes* cells in food and the likelihood of developing listeriosis is not well understood. Data from an outbreak of listeriosis linked to milkshakes made from ice cream produced in 1 factory showed that contaminated products were distributed widely to the public without any reported cases, except for 4 cases of severe illness in persons who were highly susceptible. The ingestion of high doses of *L. monocytogenes* by these patients infected through milkshakes was unlikely if possible additional contamination associated with the preparation of the milkshake is ruled out. This outbreak illustrated that the vast majority of the population did not become ill after ingesting a low level of *L. monocytogenes* but raises the question of listeriosis cases in highly susceptible persons after distribution of low-level contaminated products that did not support the growth of this pathogen.

Understanding the likelihood of developing invasive listeriosis after ingesting a given number of *Listeria monocytogenes* cells (dose-response relationship) is important in managing risks linked to this pathogen in food. Nevertheless, several challenges hamper characterization of this dose-response relationship, including the lack of an appropriate animal model, the relative rarity of outbreaks, long incubation periods that impede the collection of well-preserved implicated food samples, and heterogeneity of the initial contamination level (*1*).

In early 2015, an outbreak of invasive listeriosis linked to ice cream products was identified in the United States (*2*). A total of 10 case-patients with listeriosis related to this outbreak were reported from Arizona and Oklahoma (1 case each); Texas (3 cases); and Kansas (5 cases, all in inpatients of 1 hospital) (*2*). *L. monocytogenes* isolates from 4 of the Kansas case-patients were indistinguishable by pulsed-field gel electrophoresis from isolates recovered from ice cream made in 1 plant of the implicated company (factory 1). The isolate from the fifth Kansas case-patient did not match any isolate recovered in this outbreak investigation. *L. monocytogenes* isolates from patients in other states were linked to ice cream products manufactured in another facility (factory 2) of the same company (*2*). The US Food and Drug Administration (FDA) collected a large volume of ice cream from factory 1 for microbiological testing.

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This outbreak provided a unique opportunity to assess exposure levels to *L. monocytogenes* from implicated ice cream products among infected persons and the overall population. Because ice cream has a long shelf life and *L. monocytogenes* does not grow but survives for long periods in frozen products (*3*), the level of *L. monocytogenes* in implicated products manufactured during the outbreak, although collected after the outbreak, was likely to be representative of levels in products eaten by exposed persons. We assessed the outbreak data to gain insight into contamination levels among products from 1 factory implicated in the outbreak, the number of *L. monocytogenes* cells ingested by specific subpopulations during this outbreak, and the dose-response relationship for *L. monocytogenes*.

Materials and Methods

Framework for Dose-Response Derivation

In microbial dose-response frameworks, it is generally assumed that as few as 1 independently acting cell that survives host defense measures can initiate infection (1-hit theory [*4,5*]). This minimal infective dose of 1 cell is associated with a probability (r) of infection. Assuming r is low and constant within a subpopulation (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/12/16-0165-Techapp1.pdf), r can be estimated by the ratio of the number of invasive listeriosis cases in a

subpopulation (X_p), by the estimated number of *L. monocytogenes* cells ingested by the subpopulation Dp; that is, r = Xp / Dp. In addition to using this classical derivation of r, we estimated in this study r values using the *L. monocytogenes* dose-response model of Pouillot et al. (6) (online Technical Appendix).

Listeriosis Cases

This study considers only the 4 hospitalized Kansas case-patients whose illnesses were confirmed to be linked to ingestion of products manufactured in factory 1. Illness onset dates ranged from January 2014 through January 2015 (Figure). All 4 were >67 years and <84 years of age. Medical records review indicated all 4 had underlying medical conditions that contributed to compromised immune function before exposure to *L. monocytogenes* in milkshakes. Food histories were available for 3 of the Kansas case-patients. All patients with food histories ate product 1 from factory 1 through milkshakes. One patient had 2 milkshakes (1 day at lunch and the following day at dinner); another had 2 milkshakes (1 day at dinner and 6 days later at dinner), and the remaining patient had 3 milkshakes (1 day at dinner and 4 and 9 days later at dinner and lunch, respectively). Two serving units of product 1, each weighing ≈80 g, were used to prepare each milkshake. Strains of *L. monocytogenes* isolated from the 4 patients were indistinguishable by pulsed-field gel electrophoresis to strains recovered from product 1.

Number of *L. monocytogenes* Cells Ingested by the Population

The factory 1 production line linked to the Kansas cases made 8 different types of ice cream products (products 1–8) (*7*). (The website for this reference identifies 10 universal product codes corresponding to 8 different types of ice cream products; 2 products were sold individually and grouped in larger packages). FDA collected and counted *L. monocytogenes* cells in samples of products 1–3 (*8*; L.S. Burall, unpub. data). We characterized the variability of *L. monocytogenes* levels in products 1–3 (online Technical Appendix).

No samples of products 4–8 were collected. In a low-exposure scenario, products that were not tested were assumed to be uncontaminated. In a medium-exposure scenario and in a high- exposure scenario, contamination levels were predicted on the basis of the processes used to produce these products. Specifically, we specified in these scenarios that contamination levels were similar for products 1 and 4 and were similar for products 2 and 5–8 because the process used to produce product 4 was similar to that used for product 1, whereas production processes for products 5–8 were similar to that for product 2.

The number of *L. monocytogenes* cells ingested by the population was then estimated by multiplying the average number of *L. monocytogenes* organisms per serving by the number of servings distributed in the various subpopulations. The number of ice cream servings distributed in the

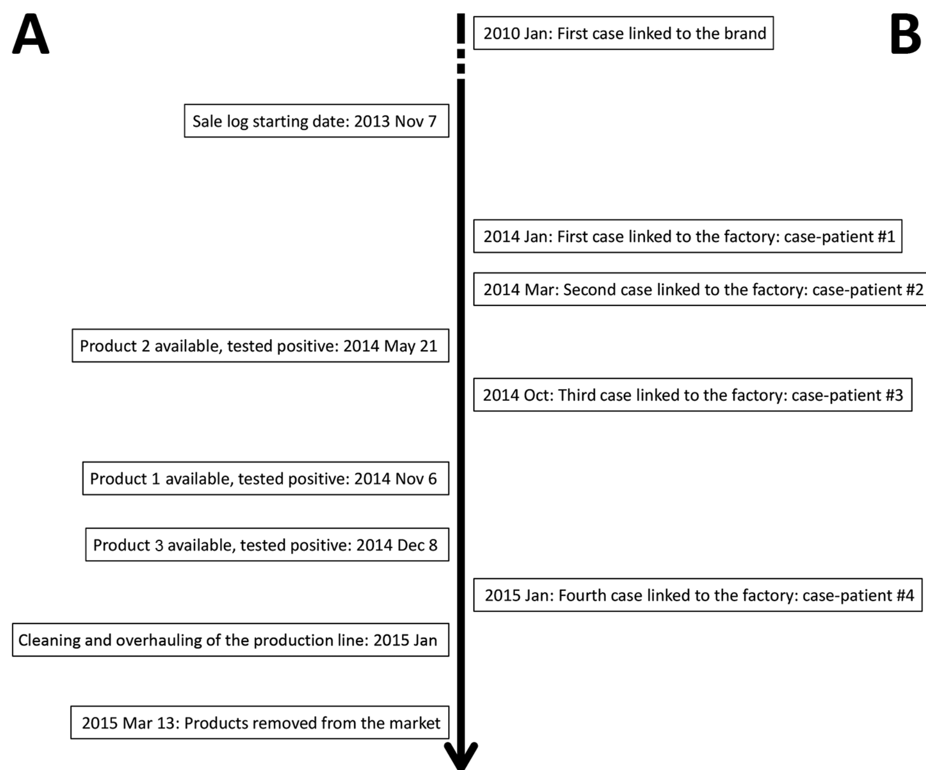

Figure. Timeline of listeriosis outbreak linked to ice cream, United States, 2015. A) Data for products produced in factory 1 (*2*); B) data for outbreak start and 4 case-patients at 1 hospital in Kansas.

various subpopulations was estimated from product distribution records for factory 1.

We do not know when contamination of the production line at factory 1 began. We isolated *L. monocytogenes* from a product manufactured on this line on May 21, 2014, but we had no samples manufactured before this date. Although the first known case associated with the brand of ice cream occurred in January 2010, the first case-patient specifically linked to factory 1 was hospitalized in Kansas on December 24, 2013, and listeriosis was diagnosed in January 2014 (patient 1, Figure). In the low-exposure scenario and medium-exposure scenario, we assumed the date at which contamination began at factory 1 was December 1, 2013, that is, a few weeks before hospitalization of the first case-patient whose illness was linked to ice cream produced at this facility. Contamination could have begun earlier than this date given that 1 listeriosis case-patient whose illness was linked to the same brand, but produced at factory 2, became ill in 2010. In the high-exposure scenario, we assumed contamination began 2.5 years before the outbreak was recognized, that is, midway between 2010 and the date the outbreak was recognized.

To estimate the proportion of servings that reached inpatients deemed to be highly susceptible to listeriosis, we multiplied the proportion of ice cream distributed to hospitals for patient consumption by the overall proportion of intensive care unit (ICU) beds in these hospitals (i.e., 10%) as a surrogate of the proportion of inpatients deemed to be highly susceptible to invasive listeriosis. To estimate the proportion of servings potentially eaten by pregnant women, ≥ 65 y. and ≥ 75 y. persons, we assumed that the implicated brand was eaten by different subpopulations similarly to other brands of ice cream (online Technical Appendix).

To understand why 4 cases of ice cream–associated listeriosis clustered at a single hospital, we created 2 indices for the hospitals that received contaminated product(s) from factory 1 at least 1 time during November 7, 2013–March 16, 2015. The first index ascertained the severity of patient illness at each hospital (illness score) and was calculated by determining the percentage of total beds constituting ICU beds (scale: 0%–4.9%, 1 point; 5%–9.9%, 2 points; 10%–14.9%, 3 points; and ≥15%, 4 points). Hospitals were contacted by telephone and queried about the total number of beds licensed and the number dedicated to treatment of patients in ICU (medical, surgical, pediatric, neonatal, and burn). To quantify the availability of contaminated products at each hospital (supply score), we divided the total number of servings shipped to each facility during the recorded distribution period (16 months) by the total number of hospital beds (scale: <1 serving per bed, 1 point; 1–3.99, 2 points; 4–6.99, 3 points; and >7, 4 points). Using the 2 indices, we summed scores for all hospitals (maximum possible score 8) as an overall measure of patient illness and potential product exposure.

Results

Number of *L. monocytogenes* Cells per Serving

All tested samples of product 1 manufactured before the outbreak was recognized were positive for *L. monocytogenes* (*8*). Assuming the 5 lots of product 1 tested were representative of all lots of contaminated product 1, we estimated the mean number of *L. monocytogenes* cells in each 80-g unit of product 1 at 620 CFU (95% credible interval [CrI] 380–2,100 CFU). From the distribution of contamination level inferred from the model, we estimated that 0.1% of servings of product 1 had a dose >7,400 CFU (95% CrI 4,400–58,000 CFU) (see Table 1 for other statistics). *L. monocytogenes* was recovered from 80% of 294 units of product 2 (unit size 70 g) tested (mean 310 CFU/serving [95% CrI 55–11,000 CFU/serving]). Of the 95 units of product 3 tested, 45% yielded *L. monocytogenes* (mean 0.12 CFU/g).

Table 1. Estimated contamination level of *Listeria monocytogenes* per gram and per serving unit of 3 products in a multistate outbreak of ice cream–associated listeriosis, United States, 2015

	Estimate (95% credible interval)		Quantile (95% credible interval)			
Product/dose	Mean	SD	90%	99%	99.9%	99.99%
Product 1						
Per g	8 (5–26)	10 (6–62)	17 (10–60)	46 (27–270)	92 (55–730)	160 (97–1,500)
Per 80-g serving	620 (380–2,100)	760 (460–4,900)	1,300 (820–4,800)	3,700 (2,200–22,000)	7,400 (4,400–58,000)	13,000 (7,800–120,000)
Product 2						
Per g	5 (1–160)	200 (17–35,000)	2 (1–10)	48 (11–620)	520 (91–12,000)	3,600 (470–140,000)
Per 70-g serving	310 (55–11,000)	14,000 (1,200–2,500,000)	140 (43–710)	3,400 (800–43,000)	37,000 (6,400–840,000)	250,000 (33,000–9,800,000)
Product 3						
Per g	0.12 in 45% of products					
Per 160-g serving	8.64 in 45% of servings					

Number of *L. monocytogenes* Cells Consumed by the Population

Sales data suggested widespread distribution of contaminated products to hospitals and the general population (e.g., schools, grocery stores, restaurants). We estimated that the general population ingested a total of 1.5×10^9 (low-exposure scenario) to 1.4×10^{10} (high-exposure scenario) *L. monocytogenes* cells (Table 2). We estimated that, overall, the highly susceptible population ingested 7.2×10^6 (low-exposure scenario) to 3.3×10^7 (high-exposure scenario) *L. monocytogenes* cells.

Among hospitals that received ≥1 products from the production line of factory 1 known to produce contaminated ice cream, the median percentage of total beds constituting ICU beds (severity of illness score) was 8.7% (range 0%–70.7%; mean 10%). The median number of servings per bed (supply score) over the recorded distribution period (16 months) was 2 (range 0.1–93.7; mean 4.3). The Kansas hospital with the 4 cases of ice cream–associated listeriosis had 62.2 servings of the implicated products per bed (13.5% of beds in the hospital were ICU beds); the servings per bed value for the hospital was exceeded by only 1 other hospital (93.7 servings/bed; 6.5% ICU beds). After combining the severity of illness and supply scores for each hospital, we found the median value was 5 (range 2–7; mean 4.6); a combined score of 7 was achieved by 9% of hospitals, of which 1 was the Kansas hospital with the 4 cases (the hospital with 93.7 servings/bed had a combined score of 6).

Probability of Infection after Ingestion of 1 Cell

Under the low-exposure scenario, we estimated that the probability of infection, *r*, after ingestion of 1 bacterium in the overall population was

$$r = \frac{4}{1.5 \times 10^9} = 2.6 \text{ x } 10^{-9}$$

Using this same approach, we determined the value of *r* for the overall population was $r = 6.5 \times 10^{-10}$ under the medium-exposure scenario and $r = 2.9 \times 10^{-10}$ under the high-exposure scenario (Table 2). The integration of the model by Pouillot et al. (*6*), considering a normal distribution of the $\log_{10}$ of the *r* parameter in the population rather than a constant one, led to a distribution with a mean −9.38 and an SD of 0.88 for the overall population under the lower-exposure scenario, a mean of –10.0 for the medium-exposure scenario, and a mean of –10.3 for the high-exposure scenario (Table 2).

We also assessed persons at greatest risk for invasive listeriosis, including pregnant women, highly susceptible persons (e.g., those with compromised immune function), persons ≥65 years of age, and persons ≥75 years of age (Table 2). Because no ice cream–associated cases were reported among pregnant women, we used an estimate of 0.5

Table 2. Probability of invasive listeriosis after ingestion of ice cream products contaminated with *Listeria monocytogenes*, United States, 2015

Exposure scenario/model	Population, no. cases in population				
	All, n = 4	Highly susceptible, n = 4	Pregnant, n = 0*	Age ≥65 y, n = 4	Age ≥75 y, n = 2
Lower†					
r constant					
No. *L. monocytogenes* cells consumed	1.5×10^9	7.2×10^6	2.2×10^7	2.3×10^8	1.2×10^8
Estimated *r* parameter	2.6×10^{-9}	5.5×10^{-7}	$<2.3 \times 10^{-8}$	1.7×10^{-8}	1.7×10^{-8}
Corresponding to 1 case every… servings‡	37,867	181	>4,363	5,756	5,832
$\log_{10}(r)$ normally distributed					
Estimated μ parameter	–9.38	–6.19	<(–7.92)	–8.00	–8.02
Estimated σ parameter	0.88	0.24	0.54	0.54	0.54
Medium§					
r constant					
No. *L. monocytogenes* cells consumed	6.2×10^9	1.5×10^7	8.9×10^7	9.4×10^8	4.8×10^8
Estimated *r* parameter	6.5×10^{-10}	2.7×10^{-7}	$<5.6 \times 10^{-9}$	4.3×10^{-9}	4.2×10^{-9}
Corresponding to 1 case every… servings‡	154,612	375	>17,812	23,501	23,811
$\log_{10}(r)$ normally distributed					
Estimated μ parameter	–10.0	–6.40	<(–8.49)	–8.60	–8.62
Estimated σ parameter	0.88	0.24	0.54	0.54	0.54
High¶					
r constant					
No. *L. monocytogenes* cells consumed	1.4×10^{10}	3.3×10^7	2.0×10^8	2.1×10^9	1.0×10^9
Estimated *r* parameter	2.9×10^{-10}	1.2×10^{-7}	$<2.6 \times 10^{-9}$	1.9×10^{-9}	1.9×10^{-9}
Corresponding to 1 case every… servings‡	339,153	816	>39,071	51,552	52,230
$\log_{10}(r)$ normally distributed					
Estimated μ parameter	–10.3	–6.80	<(–8.83)	–8.97	–8.97
Estimated σ parameter	0.88	0.24	0.54	0.54	0.54

*0.5 used for computation.
†Products 1–3 contaminated beginning 2013 Dec 1; products 4–8 not contaminated.
‡Corresponding to 1 case every… servings, including 10,000 *L. monocytogenes* cells.
§Products 1–8 contaminated beginning 2013 Dec 1.
¶Products 1–8 contaminated beginning 2012 Jun 1.

cases and provided only an upper limit value for *r*. (This value was chosen arbitrarily. A Poisson process with mean 0.5 would have led to 0 cases in 90% of occurrence.)

Discussion

This outbreak investigation provided unique data to characterize the dose-response relationship between *L. monocytogenes* in general and susceptible populations. Multiple factors compelled us to estimate as precisely as possible doses of *L. monocytogenes* ingested by consumers of contaminated products. First, the number of samples microbiologically tested was by far the largest ever reported from an outbreak setting (*8*). Second, because ice cream preserves the viability of *L. monocytogenes* but does not support its growth, levels of contamination were likely to have been accurately measured and have remained relatively constant over the extended shelf lives of the products. Finally, an exceptionally stable level of contamination within product types minimized variability in exposures. Hospital records indicated that patient 4 drank milkshakes made with product 1 on 3 different days during January 11–19, 2015, before sepsis caused by *L. monocytogenes* infection was diagnosed on January 23. This patient could have eaten ice cream from lots we enumerated. Only 4 (0.2%) of 2,320 samples of product 1 yielded a concentration >100 CFU/g, equivalent to a dose of ≥16,000 *L. monocytogenes* cells per milkshake (2 servings of 80 g × 100 CFU/g, assuming the 2 servings were >100 CFU/g). Inferences on the interlot, interbox, and intrabox variability helped us define precisely the distribution of contamination levels from serving to serving and confirmed that a very high concentration of *L. monocytogenes* cells in any given serving unit was not likely. The estimated mean dose per milkshake is 1,240 *L. monocytogenes* cells (95% CrI 760–4,200 *L. monocytogenes* cells). We estimate that 1 of 10,000 milkshakes would have a load >26,000 *L. monocytogenes* cells (95% CrI 15,600–240,000 *L. monocytogenes* cells). Assuming there was no initial contamination of the milkshake machines and no growth of the pathogen in the milkshakes, the mean contamination level of *L. monocytogenes* in the milkshakes (8 cells/g of ice cream) was relatively low compared with contamination levels in some other outbreaks (*9–12*). However, in the absence of leftovers from the actual implicated milkshakes, we cannot rule out the possibility that the 4 susceptible patients received some of the highest contaminated products from the factory line, triggering infection. Experimental trials of *L. monocytogenes* growth in milkshakes made from these naturally contaminated ice cream samples held at room temperature showed an absence of growth during 8 hours and an average population level increase after 14 hours limited to 1.14 log CFU/g (*13*). We cannot exclude the possibility that variations in procedures used to clean the milkshake machines might have enabled isolated microbial growth on ≥1 machines. We believe the extremely high prevalence of contamination of product 1 might have inoculated ≥1 machines with repeated preparations over the long period during which contaminated products were distributed; however, no *Listeria* was isolated from samples collected from these machines after the outbreak was recognized (Charles Hunt, Kansas Department of Health and Environment, pers. comm., 2016 Jun 27).

Although the 4 cases of ice cream–associated listeriosis in a single hospital raise the possibility of a systematic problem within the hospital, it is also possible that the combination of severely ill patients, including some with specific risk factors for listeriosis such as hematologic cancers (*14*), in a setting in which a large amount of contaminated ice cream was served contributed to this series of infections. Medical staff at the hospital also might have had a heightened suspicion of listeriosis after diagnosis of the initial case, which might have increased the likelihood of detecting cases. Overall, the Kansas hospital received 55% of all product 1 sold to hospitals. Thus, observing the 4 cases in this specific hospital was not improbable. (The probability to observe 4 successes out of 4 trials is 9% when the independent probability of success is 55%.)

Although precise quantification of exposure to *L. monocytogenes* ingestion through contaminated ice cream is difficult to infer for specific persons, an assessment of exposures among populations is more feasible. Despite the relatively low levels of contamination of ice cream products in this listeriosis outbreak, the exceptionally high prevalence of contaminated products, combined with the protracted duration of contamination of the production line (at least 1 year and possibly longer), contributed to exposure of many persons to *L. monocytogenes*. This finding suggests that widespread distribution of contaminated products with low-dose contamination by *L. monocytogenes* in a product that does not support growth of *L. monocytogenes* might lead to only a limited number of reported infections. We focused our study on 1 cluster of outbreak-related cases, the one for which FDA was able to collect samples of ice cream for microbiological testing. Five other cases of ice cream–associated invasive listeriosis were identified in states other than Kansas; these cases were linked to another production factory operated by the same company, expanding further the quantity of contaminated ice cream sold to the public.

The Food and Agriculture Organization of the World Health Organization (FAO/WHO) (*15*) estimated an *r* parameter of 3.2×10^{-7} in a well-documented listeriosis outbreak involving immunocompromised patients in Finland in 1998–1999 (*16,17*); in this outbreak, the median estimated dose ingested was 8.2×10^{3} *L. monocytogenes*. Our estimate of the *r* parameter for the susceptible population is in the same order of magnitude (1.2×10^{-7} to 5.5

$\times 10^{-7}$). In the population of pregnant women, FAO/WHO (*15*) estimated a r parameter of 2.6×10^{-11} on the basis of an outbreak of cheese-associated listeriosis involving pregnant Hispanic women in Los Angeles County, California, USA, in 1985 in which the estimated dose was 1.7×10^{7} *L. monocytogenes* (*10*). More recently, Imanishi et al. (*18*) estimated an attack rate of 1 case/10,000 exposed pregnant women in Colorado, USA, during a 2011 multistate outbreak of listeriosis linked to contaminated cantaloupe (*19*); no enumeration data were available in this outbreak. Studies have shown that cut cantaloupe supports the growth of *L. monocytogenes* (*20,21*), suggesting that some exposures could have been high during this outbreak. In the ice cream–associated outbreak described here, no cases were reported among pregnant women despite presumably widespread exposures among this subgroup of susceptible persons. Specifically, a large number of contaminated ice cream products were presumably ingested by pregnant women during the long duration of contamination of the production line. From the expected number of *L. monocytogenes* cells ingested by this subpopulation, we estimate, under the various assumptions used in this study, a value of $r < 2.6 \times 10^{-9}$ to $r < 2.3 \times 10^{-8}$. In summary, estimates for r derived in the present study are comparable in order of magnitude with estimates derived from previous outbreaks, a finding that is noteworthy in light of the low levels of contamination of ice cream products and the fact that these products did not support growth. Although other outbreaks were linked to higher level of contamination per serving than in the present study, the number of contaminated servings was much lower in those outbreaks than in the present one.

On the other hand, estimates for r obtained in the present study are higher than those estimated by using epidemiologic data (*6,15,17*). Using epidemiologic data, FAO/WHO (*15*) estimated that the probability of infection after consumption of 1 *L. monocytogenes* cell is in the order of $r = 5 \times 10^{-12}$ for susceptible persons (immunocompromised persons, pregnant women, and elderly persons), and 5×10^{-14} for nonsusceptible persons (*15*). These values predict the occurrence of 1 listeriosis case for every 20 million exposures to 10,000 *L. monocytogenes* cells in the susceptible population (10,000, which was chosen arbitrarily, would correspond to the dose after ingestion of 100 g of a product contaminated at 100 CFU/g) and 1 case of listeriosis for every 2 billion exposures to 10,000 *L. monocytogenes* cells in the nonsusceptible population. The estimates obtained in our study were much higher than these values: 1 case expected for every 339,200 servings of 10,000 bacteria per serving, such as for the general population in the high-exposure scenario. Similarly, using the model of Pouillot et al. (*6*), we estimated that values from the ice cream outbreak data are ≈2 $\log_{10}$ higher than those based on epidemiologic data. A possible explanation for these differences is that a particularly virulent strain of *L. monocytogenes* was present in ice cream. Differences in r estimates obtained from outbreak investigations versus epidemiologic data also could result from observation bias, wherein recognition of cases instigates a study, leading to high number of cases for equation input and thus higher estimates for r. In contrast, situations where contaminated products are distributed but no cases are recognized are underrepresented in such evaluations.

This outbreak of ice cream–associated listeriosis recognized in 2015 demonstrates that illnesses can occur when products with low-level contamination that do not support growth are distributed widely to the public, even though it is not possible to conclude with certainty whether the cases were linked directly to the products or indirectly after a growth step on a milkshake machine. The outbreak also illustrates that even when the distribution of products contaminated with *L. monocytogenes* is widespread, most consumers of the products will not become ill when contamination levels are low and no growth is facilitated. Finally, this outbreak adds yet further evidence of the risk for listeriosis faced by persons with weakened immune systems and calls for effective risk management to mitigate infections (*22*).

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