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

Régis Pouillot, Karl C. Klontz, Yi Chen, Laurel S. Burall, Dumitru Macarisin, Matthew Doyle, Kären M. Bally, Errol Strain, Atin R. Datta, Thomas S. Hammack, Jane M. Van Doren

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.

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

Author affiliations: Food and Drug Administration, College Park, Maryland, USA (R. Pouillot, K.C. Klontz, Y. Chen, D. Macarisin, M. Doyle, E. Strain, T.S. Hammack, J.M. Van Doren); Food and Drug Administration, Laurel, Maryland, USA (L.S. Burall, A.R. Datta); Via Christi Hospitals, Wichita, Kansas, USA (K.M. Bally)

DOI: <http://dx.doi.org/10.3201/eid2212.160165>

subpopulation (X_p), by the estimated number of *L. monocytogenes* cells ingested by the subpopulation D_p ; that is, $r = X_p / D_p$. 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

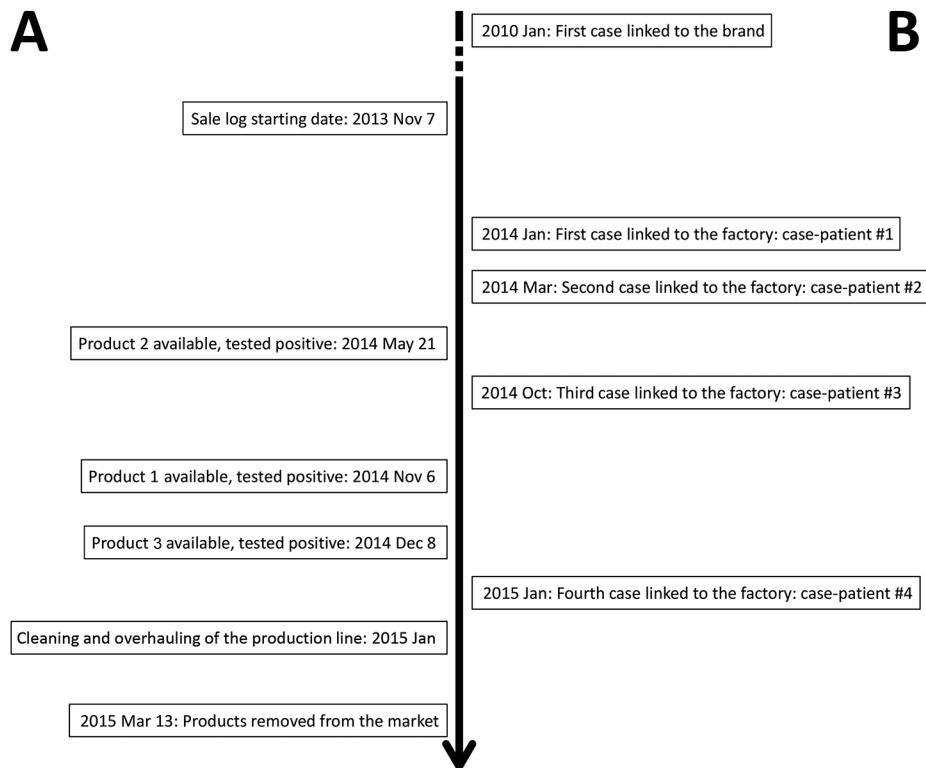


Figure. Timeline of listeriosis outbreak linked to ice cream, United States, 2015. A) Data for products produced in factory 1 (2); B) data for outbreak start and 4 case-patients at 1 hospital in Kansas.

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

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

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

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
Lowert†					
<i>r</i> constant					
No. <i>L. monocytogenes</i> cells consumed	1.5×10^9	7.2×10^6	2.2×10^7	2.3×10^8	1.2×10^8
Estimated <i>r</i> 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§					
<i>r</i> constant					
No. <i>L. monocytogenes</i> cells consumed	6.2×10^9	1.5×10^7	8.9×10^7	9.4×10^8	4.8×10^8
Estimated <i>r</i> 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¶					
<i>r</i> constant					
No. <i>L. monocytogenes</i> cells consumed	1.4×10^{10}	3.3×10^7	2.0×10^8	2.1×10^9	1.0×10^9
Estimated <i>r</i> 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 $\geq 16,000$ *L. monocytogenes* cells per milkshake (2 servings of 80 g \times 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 mi-

crobial 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 $\approx 2 \log_{10}$ higher than those based on epide-

miologic 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).

Acknowledgments

We acknowledge the outbreak response partners whose contributions to the listeriosis investigation resulted in identification of the contaminated products, distribution records, and clinical case records vital to this study. We thank the state and local partners from the Alabama Department of Public Health, Kansas Department of Agriculture, Kansas Department of Health and Environment, Oklahoma Department of Agriculture, Food and Forestry, South Carolina Department of Health & Environmental Control, Texas Department of State Health Services, and Tulsa Health Department for their help in the outbreak investigation. We also thank the federal partners at the Centers for Disease Control and Prevention's Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases; FDA's Office of Regulatory Affairs, especially the Dallas, Kansas City, Atlanta, and New Orleans District Offices; FDA's Center for Food Safety and Applied Nutrition; and FDA's Coordinated Outbreak Response and Evaluation Network. We thank Libby Wei and Sara Kreshpanji, each supported by a University of Maryland/Joint Institute for Food Safety and Applied Nutrition student internship, for helping to gather and analyze data. This study used the high-performance computational capabilities of the Scientific Computing Laboratory at the FDA Center for Devices and Radiological Health. We thank Mike Mikailov, Brian Fitzgerald, Stuart Barkley, Luo Fu Jyh, Lohit Valleru, and Stephen Whitney for their invaluable contributions to supercomputing.

Dr. Pouillot is a visiting scientist in the Risk Analysis Branch within the Division of Risk and Decision Analysis, Center for Food Safety and Applied Nutrition, FDA. His research interest is using data analysis and models to understand and evaluate the risk for foodborne illnesses.

References

- Hoelzer K, Chen Y, Dennis S, Evans P, Pouillot R, Silk BJ, et al. New data, strategies, and insights for *Listeria monocytogenes* dose-response models: summary of an interagency workshop, 2011. *Risk Anal.* 2013;33:1568–81. <http://dx.doi.org/10.1111/risa.12005>
- Centers for Disease Control and Prevention. Multistate outbreak of listeriosis linked to Blue Bell Creameries products (final update). 2015 [cited 2015 Dec 10]. <http://www.cdc.gov/listeria/outbreaks/ice-cream-03-15/>
- Palumbo SA, Williams AC. Resistance of *Listeria monocytogenes* to freezing in foods. *Food Microbiol.* 1991;8:63–8.
- Haas CN, Rose JB, Gerba CP. Quantitative microbial risk assessment. New York: Wiley; 1999.
- Teunis PF, Havelaar AH. The Beta Poisson dose-response model is not a single-hit model. *Risk Anal.* 2000;20:513–20. <http://dx.doi.org/10.1111/0272-4332.204048>
- Pouillot R, Hoelzer K, Chen Y, Dennis SB. *Listeria monocytogenes* dose response revisited—incorporating adjustments for variability in strain virulence and host susceptibility. *Risk Anal.* 2015;35:90–108. <http://dx.doi.org/10.1111/risa.12235>
- BlueBell.com. For the first time in 108 years, Blue Bell announces a product recall. 2015 [cited 2016 Jan 7]. http://www.bluebell.com/BB_withdrawal
- Chen Y, Burall L, Macarasin D, Pouillot R, Strain E, De Jesus A, et al. Prevalence and level of *Listeria monocytogenes* in ice cream linked to a listeriosis outbreak in the United States. *J Food Prot.* 2016;79:1828–932.
- Johnsen BO, Lingaas E, Torfoss D, Strøm EH, Nordøy I. A large outbreak of *Listeria monocytogenes* infection with short incubation period in a tertiary care hospital. *J Infect.* 2010;61:465–70. <http://dx.doi.org/10.1016/j.jinf.2010.08.007>
- Linnan MJ, Mascola L, Lou XD, Goulet V, May S, Salminen C, et al. Epidemic listeriosis associated with Mexican-style cheese. *N Engl J Med.* 1988;319:823–8. <http://dx.doi.org/10.1056/NEJM198809293191303>
- Pérez-Trallero E, Zigorraga C, Artieda J, Alkorta M, Marimón JM. Two outbreaks of *Listeria monocytogenes* infection, northern Spain. *Emerg Infect Dis.* 2014;20:2155–7. <http://dx.doi.org/10.3201/eid2012.140993>
- Heiman KE, Garalde VB, Gronostaj M, Jackson KA, Beam S, Joseph L, et al. Multistate outbreak of listeriosis caused by imported cheese and evidence of cross-contamination of other cheeses, USA, 2012. *Epidemiol Infect.* 2016;144:2698–708. <http://dx.doi.org/10.1017/S095026881500117X>
- Chen Y, Allard E, Wooten A, Hur M, Sheth I, Laasri A, et al. Recovery and growth potential of *Listeria monocytogenes* in temperature abused milkshakes prepared from naturally contaminated ice cream linked to a listeriosis outbreak. *Front Microbiol.* 2016;7:764. <http://dx.doi.org/10.3389/fmicb.2016.00764>
- Goulet V, Hebert M, Hedberg C, Laurent E, Vaillant V, De Valk H, et al. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. *Clin Infect Dis.* 2012;54:652–60. <http://dx.doi.org/10.1093/cid/cir902>
- Food and Agriculture Organization/World Health Organization. Risk assessment of *Listeria monocytogenes* in ready to eat foods. Technical report. Report no.: Microbiological Risk Assessment Series 5. Rome: The Organizations; 2004 [cited 2016 Jan 28]. <ftp://ftp.fao.org/docrep/fao/010/y5394e/y5394e.pdf>
- Lyytikäinen O, Autio T, Majjala R, Ruutu P, Honkanen-Buzalski T, Miettinen M, et al. An outbreak of *Listeria monocytogenes* serotype 3a infections from butter in Finland. *J Infect Dis.* 2000;181:1838–41. <http://dx.doi.org/10.1086/315453>
- Food and Drug Administration. Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods [cited 2016 Jan 28]. <http://www.fda.gov/downloads/Food/FoodScienceResearch/UCM197330.pdf>
- Imanishi M, Routh JA, Klaber M, Gu W, Vanselow MS, Jackson KA, et al. Estimating the attack rate of pregnancy-associated listeriosis during a large outbreak. *Infect Dis Obstet Gynecol.* 2015;2015:201479. <http://dx.doi.org/10.1155/2015/201479>
- Centers for Disease Control and Prevention. Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe—United States, August–September 2011. *MMWR Morb Mortal Wkly Rep.* 2011;60:1357–8.
- Danyluk MD, Friedrich LM, Schaffner DW. Modeling the growth of *Listeria monocytogenes* on cut cantaloupe, honeydew and watermelon. *Food Microbiol.* 2014;38:52–5. <http://dx.doi.org/10.1016/j.fm.2013.08.001>
- Fang T, Liu Y, Huang L. Growth kinetics of *Listeria monocytogenes* and spoilage microorganisms in fresh-cut cantaloupe. *Food Microbiol.* 2013;34:174–81. <http://dx.doi.org/10.1016/j.fm.2012.12.005>
- Silk BJ, Date KA, Jackson KA, Pouillot R, Holt KG, Graves LM, et al. Invasive listeriosis in the Foodborne Diseases Active Surveillance Network (FoodNet), 2004–2009: further targeted prevention needed for higher-risk groups. *Clin Infect Dis.* 2012;54(Suppl 5):S396–404. <http://dx.doi.org/10.1093/cid/cis268>

Address for correspondence: Régis Pouillot, US Food and Drug Administration, 5001 Campus Dr, College Park, MD 20740, USA; email: Regis.Pouillot@fda.hhs.gov

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

Technical Appendix

Framework for Dose-Response

Mathematically, the resulting data from an exposed population can be represented as infection indicator x_i for each individual, which take a value of 1 if infection is observed and a value of 0 otherwise (I). For a serving i , under the 1-hit theory assumptions (2,3) we have

$$x_i \sim \text{Bernoulli}(\pi_i) \text{ with } \pi_i = 1 - (1 - r_i)^{d_i}$$

where π_i is the probability that the individual got infected after ingesting a specific serving i , d_i is the dose (number of *Listeria monocytogenes* cells) ingested through the serving i and r_i is the average probability that a pathogen from serving i survive the host-pathogen response and initiate infection (I). For the population, the observed number of cases is a realization of all those independent Bernoulli processes. We have:

$$\text{(Eq. 1) } E(X) = \sum_{i=1}^m (1 - (1 - r_i)^{d_i})$$

where, additionally, $E(X)$ is the expected number of cases and m is the number of exposed persons. The characterization of the dose-response thus requires the knowledge of the probability that the pathogen survive the host-pathogen response (r_i , variable, from serving to serving, notably because of individual susceptibility) and the individual doses (d_i , variable, from serving to serving), considered as independent from r_i . Some authors reported that the r parameter could be considered as constant within a given subpopulation (e.g., susceptible and nonsusceptible subpopulation within [4]). Under this assumption, (Eq. 1) simplifies to

$$\text{(Eq. 2) } E(X_p) = \sum_{i=1}^{m_p} (1 - (1 - r)^{d_i}).$$

where X_p is the number of cases and m_p is the number of persons in the subpopulation p and the distribution of the dose only has to be known. Moreover, because r is usually very small for *L. monocytogenes*, $1 - (1 - r)^d \approx rd$. Under this limit, the equation simplifies to

$$\text{(Eq. 3)} \ E(X_p) = \sum_{i=1}^{m_p} r d_i = r \times m_p \times \bar{d}$$

where \bar{d} is the mean number dose ingested in the population. From (Eq. 3), r can be then evaluated as

$$\text{(Eq. 4)} \ \hat{r} = \frac{X_p}{m_p \bar{d}} = \frac{X_p}{\widehat{D}_p},$$

where \widehat{D}_p is the estimated number of *L. monocytogenes* ingested by the population.

To better characterize variability in *L. monocytogenes* dose-response, Pouillot et al. (5) used a log-normal distribution to describe r_i , rather than a constant, that is $\log_{10}(r_i) \sim \text{normal}(\mu_p, \sigma_p)$, with negligible probability that $r > 1$. During an outbreak, σ_p characterize only the within subpopulation variability in susceptibility because strain virulence variability can be neglected (5,6). Following assumptions used in Pouillot et al. (5) and Food and Drug Administration/Food Safety and Inspection Service (6), we considered a high variability in susceptibility within the overall population (90% of the individual variability in r may be contained within a range of $2.9 \log_{10}$, leading to $\sigma = 0.82 \log_{10}$), a medium variability in susceptibility within the pregnant women population and the older adult populations (90% of the individual variability in r may be contained within a range of $1.8 \log_{10}$, leading to $\sigma = 0.55 \log_{10}$), and a low variability in susceptibility in the highly susceptible population (90% of the individual variability in r may be contained within a range of $0.8 \log_{10}$, leading to $\sigma = 0.24 \log_{10}$). With an assumption of a log-normal distribution of r_i , (Eq. 1) cannot be simplified and the equation should be integrated numerically over the distribution of r_i and d_i . Integrations were performed using R software (7).

Derivation of the Contamination Level Distributions

Briefly, 2,320 samples of ice cream product 1 (80 g each), 295 samples of product 2 (70 g), and 96 samples of product 3 (160 g) were microbiologically tested. *L. monocytogenes* cells were enumerated in these products by using the most probable number (MPN) method from

dilution assay results. Microbiological methods and summary statistics are described in Chen et al, (8) and Burall et al (unpub. data).

Product 1 samples were collected from 7 lots. All tested products from the 5 first lots were contaminated (2,020 positive samples of 2,020 tested). After the first reports of contaminated products, the production line was reportedly cleaned and overhauled in factory 1 on January 30, 2015 (9). Ninety-six percent (287 positive samples of 300) of products tested from 2 later lots, manufactured after the cleaning, were contaminated. *L. monocytogenes* contamination levels were extremely homogeneous among products within boxes, boxes within lots, and across lots (8). The observed mean concentration of *L. monocytogenes* in product 1 before the cleaning of the line was 9 MPN/g of product.

From the experimental design (8) and from the raw results, we characterize in this study the variability in *L. monocytogenes* levels across lots (lot to lot; data from 5 lots), across boxes within a lot (box to box; 8–53 boxes tested per lot), and across servings within a box (serving to serving; 10–20 servings tested per box). We restricted the analysis to lots manufactured before cleaning and overhauling of the production line. To evaluate the lot-to-lot, box-to-box within lots, and serving-to-serving within box variability, a hierarchical Bayesian framework was developed based on the results of the 2,020 dilution assays conducted on product 1. The model is written $\lambda_l \sim \text{Normal}(\mu, \sigma_1)$; $\lambda_{b,l} \sim \text{Normal}(\lambda_l, \sigma_2)$; $\lambda_{s,b,l} \sim \text{Normal}(\lambda_{b,l}, \sigma_3)$; $p_{s,b,l} \sim \text{Binomial}(n_s, 1 - \exp(-10^{v_s \lambda_{s,b,l}}))$; where l stands for lot, b stands for box, and s stands for serving, p , n , and v are the number of positive tubes, the number of tested tubes and the “grams” of tested product for each tubes in the dilution assay experiment. Uninformative prior distributions $\mu \sim \text{Normal}(-4, 10)$ and $\sigma_x^{-2} \sim \text{Uniform}(0, 10)$ were used.

Product 2 samples were collected from 10 lots manufactured before the cleaning of the manufacturing line. Both the prevalence of contamination and the number of *L. monocytogenes* cells detected in product 2 samples were lower and more variable than respective values for product 1 (L.S. Burall et al., unpub. data). The prevalence was estimated to be 80% (234/294 samples, L.S. Burall et al., unpub. data). The observed overall mean contamination (using a value of one half the limit of quantification, that is, 0.016 MPN/g for negative samples) for product 2 was 3.4 MPN/g. We derived a Bayesian model similar to the one for product 1 to evaluate variability in *L. monocytogenes* levels across lots (lot-to-lot; data from 10 lots), across

boxes within a lot (box-to-box; 1 to 6 boxes tested per lot), and across servings within a box (serving-to-serving; 5–10 servings tested per box).

Ninety-five samples of product 3 from 5 lots manufactured before the cleaning of the manufacturing line were tested. Forty-three (45%) were positive for *L. monocytogenes*. The mean *L. monocytogenes* contamination level for positive samples was lower, estimated to 0.12 MPN/g and the standard deviation to 0.14 MPN/g).

For product 1, from the Bayesian model, the mean of the \log_{10} concentrations is estimated 0.70 \log_{10} CFU/g, with an interlot variability of 0.21 \log_{10} CFU/g, an interbox variability of 0.14 \log_{10} CFU/g, and an intrabox variability of 0.33 \log_{10} CFU/g (Technical Appendix Table 1). The lot-to-lot variation is not known as precisely as the other levels of variability because fewer lots than boxes or products were examined.

From these results, we simulated the production of the manufacturing line using the empirical posterior distributions from the Bayesian analysis (Technical Appendix Figure). Under the model (assuming that the 5 lots are representative of all lots), Table 1 in the main document reports estimations for different quantiles of the distributions.

From the Bayesian model, the mean of the *L. monocytogenes* \log_{10} concentrations in product 2 in the lots examined was estimated as $-1.43 \log_{10}/g$, with an interlot variability of 0.38, an interbox variability of 0.99, and an intrabox variability of 0.82 (Technical Appendix Table 1). The credible interval are much larger than those obtained for product 1, reflecting the wider variability and the lower number of tested samples. The mean dose for one 70-g serving of product 2 (the serving size of this product) was estimated as 310 cfu (95% credible interval [CrI] 55–11,000 CFU). Table 1 in the main text provides additional estimates for various quantiles in the distribution.

Because the number of tested samples for product 3 was low ($n = 95$), we did not derive a distribution for this product but considered that, as observed in the tested sample, 45% of these product 3 were contaminated and that the average concentration of *L. monocytogenes* in contaminated products 3 was 0.12 *L. monocytogenes* cells per gram.

In further estimation of the prevalence of contaminated products, 100% will be used for product 1 and product 1 like, 80% for product 2 and product 2 like, and 45% for product 3.

Estimation of the Proportion of Ice Cream Eaten by Various Subpopulations

Demographic data were estimated from data provided by the Centers for Disease Control and Prevention (10) and by the US Census Bureau (11). Per capita consumption of ice cream for pregnant women and for the overall population were estimated by using the FARE software (Food Analysis and Residue Evaluation, v.11.10, leased from Exponent, Inc, Washington, DC, USA.) from the 1999–2012 National Health and Nutrition Examination Survey/What We Eat In America (NHANES/WWEIA) (12), considering any ice cream consumption the first day of survey (Technical Appendix Table 2). Comparable estimates were done for persons ≥ 65 years and ≥ 75 years of age (Technical Appendix Table 2).

References

1. Schmidt PJ, Pintar KD, Fazil AM, Topp E. Harnessing the theoretical foundations of the exponential and beta-Poisson dose-response models to quantify parameter uncertainty using Markov chain Monte Carlo. *Risk Anal.* 2013;33:1677–93. [PubMed http://dx.doi.org/10.1111/risa.12006](http://dx.doi.org/10.1111/risa.12006)
2. Haas CN, Rose JB, Gerba CP. Quantitative microbial risk assessment. New York: Wiley; 1999.
3. Teunis PF, Havelaar AH. The beta Poisson dose-response model is not a single-hit model. *Risk Anal.* 2000;20:513–20. [PubMed http://dx.doi.org/10.1111/0272-4332.204048](http://dx.doi.org/10.1111/0272-4332.204048)
4. Food and Agriculture Organization/World Health Organization. Risk assessment of *Listeria monocytogenes* in ready to eat foods. Technical report. Report no.: Microbiological Risk Assessment Series 5. Rome: The Organizations; 2004 [cited 2016 Jan 28]. <ftp://ftp.fao.org/docrep/fao/010/y5394e/y5394e.pdf>
5. Pouillot R, Hoelzer K, Chen Y, Dennis SB. *Listeria monocytogenes* dose response revisited—incorporating adjustments for variability in strain virulence and host susceptibility. *Risk Anal.* 2015;35:90–108. [PubMed http://dx.doi.org/10.1111/risa.12235](http://dx.doi.org/10.1111/risa.12235)
6. Food and Drug Administration. Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods [cited 2016 Jan 28]. <http://www.fda.gov/downloads/Food/FoodScienceResearch/UCM197330.pdf>
7. R Development Core Team. R: a language and environment for statistical computing. 2008 [cited 2012 Mar 20]. <http://www.R-project.org>

8. Chen Y, Burall L, Macarasin D, Pouillot R, Strain E, De Jesus A, et al. Enumeration of *Listeria monocytogenes* in ice cream products linked to a multistate listeriosis outbreak. J Food Prot. In press 2016.
9. Centers for Disease Control and Prevention. Multistate outbreak of listeriosis linked to Blue Bell Creameries products (final update). 2015 [cited 2015 Dec 10].
<http://www.cdc.gov/listeria/outbreaks/ice-cream-03-15/>
10. Curtin SC, Abma JC, Ventura SJ, Henshaw SK. Pregnancy rates for U.S. women continue to drop. NCHS Data Brief. 2013;(136):1–8. [PubMed](#)
11. US Census Bureau. Annual estimates of the resident population for the United States, regions, states, and Puerto Rico: April 1, 2000 to July 1, 2009. 2015 [cited 2016 Jan 28].
<https://www.census.gov/popest/data/state/totals/2009/tables/NST-EST2009-01.xls>
12. US Department of Agriculture and US Department of Health and Human Services. What we eat in America [cited 2016 Jan 29]. <https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/wweianhanes-overview/>

Technical Appendix Table 1. Results from the Bayesian analysis of the contamination variability for product 1 (2020 MPN results) and Product 2 (294 MPN results)*

Parameter	Symbol	Product 1, mean (95% CrI)	Product 2, mean (95% CrI)
Overall mean of the log ₁₀ concentration (log ₁₀ MPN/g)	μ	0.70 (0.46–0.91)	–1.43 (–2.03 to –0.89]
Interlot standard deviation	σ_1	0.21 (0.09–0.53)	0.38 (0.02–1.25)
Interbox standard deviation	σ_2	0.14 (0.11–0.16)	0.99 (0.68–1.45)
Interproduct standard deviation	σ_3	0.33 (0.32–0.35)	0.82 (0.74–0.91)

*CrI, credible interval; MPN, most probable number.

Technical Appendix Table 2. Estimation of the proportion of ice cream eaten by various subpopulations*

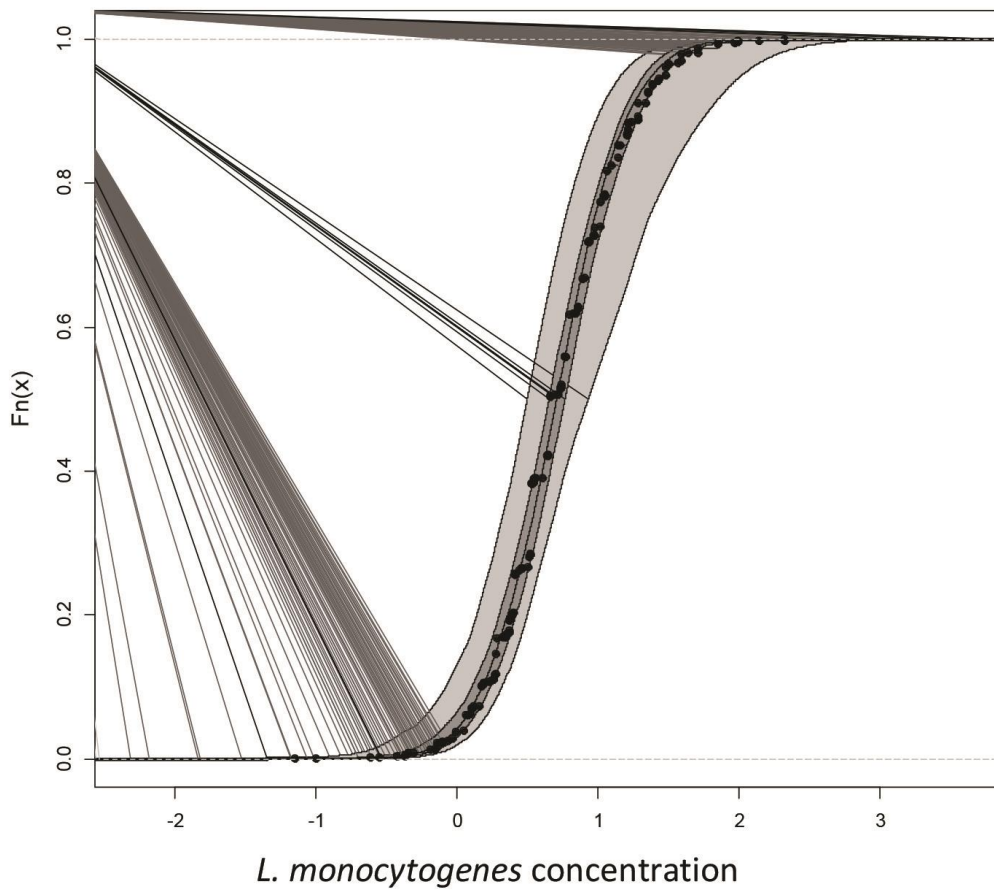
Variable	United States	Pregnant women	≥65 y	≥75 y	Source
Population size	307,006,550†	3,513,205‡	39,570,590†	18,778,523†	(10,11)
Per capita estimate of ice cream eaten per day	20.62 g	25.98 g	24.29 g	25.82 g	1999–2012 NHANES/WWEIA (12)
Proportion of ice cream eaten by the specific population§	100%	1.44%	15.2%	7.7%	

*NHANES, National Health and Nutrition Examination Survey; WWEIA, What We Eat in America.

†On July 1, 2009.

‡Evaluated from (10) as (4,131,000 births in 2009 in the United States × [9 mo of 30 d] + 1,152,000 abortions in 2009 in the United States × [2 mo of 30 d] + 1,087,000 fetal losses in 2009 in the United States × [3 mo of 30 d]) / 365

§Evaluated as (population in subgroup × per capita consumption for that subgroup)/(total US population × per capita consumption in the United States).



Technical Appendix Figure. Simulated and observed (points) cumulative density function of the *Listeria monocytogenes* contamination for product 1 associated with a listeriosis outbreak, United States, 2015. The envelopes represent the 95% uncertainty interval (light gray) and the 50% uncertainty interval (dark gray).