Risk Analysis, Vol. 35, No. 1, 2015 DOI: 10.1111/risa.12235

Listeria monocytogenes Dose Response Revisited—Incorporating Adjustments for Variability in Strain Virulence and Host Susceptibility

Régis Pouillot*, Karin Hoelzer, Yuhuan Chen, and Sherri B. Dennis

Evaluations of *Listeria monocytogenes* dose-response relationships are crucially important for risk assessment and risk management, but are complicated by considerable variability across population subgroups and *L. monocytogenes* strains. Despite difficulties associated with the collection of adequate data from outbreak investigations or sporadic cases, the limitations of currently available animal models, and the inability to conduct human volunteer studies, some of the available data now allow refinements of the well-established exponential *L. monocytogenes* dose response to more adequately represent extremely susceptible population subgroups and highly virulent *L. monocytogenes* strains. Here, a model incorporating adjustments for variability in *L. monocytogenes* strain virulence and host susceptibility was derived for 11 population subgroups with similar underlying comorbidities using data from multiple sources, including human surveillance and food survey data. In light of the unique inherent properties of *L. monocytogenes* dose response, a lognormal-Poisson dose-response model was chosen, and proved able to reconcile dose-response relationships developed based on surveillance data with outbreak data. This model was compared to a classical beta-Poisson dose-response model, which was insufficiently flexible for modeling the specific case of *L. monocytogenes* dose-response relationships, especially in outbreak situations. Overall, the modeling results suggest that most listeriosis cases are linked to the ingestion of food contaminated with medium to high concentrations of *L. monocytogenes*. While additional data are needed to refine the derived model and to better characterize and quantify the variability in *L. monocytogenes* strain virulence and individual host susceptibility, the framework derived here represents a promising approach to more adequately characterize the risk of listeriosis in highly susceptible population subgroups.

KEY WORDS: Dose response; *Listeria monocytogenes*; risk assessment

1. INTRODUCTION

Listeria monocytogenes is one of the leading causes of hospitalization, fetal loss, and death due to foodborne illnesses in the United States.[1] Derivations of *L. monocytogenes* dose-response relationships, though crucially important for risk assessment and risk management, are impaired by the difficulties of collecting adequate data from outbreak investigations or sporadic cases, by the lack of appropriate animal models, and by the inability to use volunteer studies due to ethical and practical concerns.[2,3]

Two well-accepted *L. monocytogenes* dose-response models have been developed by U.S. agencies[4] and an international expert panel,[5] both scaled to epidemiological data. In 2003, the Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services and the Food Safety and Inspection Service (FSIS) of the U.S.

*Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, 5100 Paint Branch Pkwy, HFS-005 MD 20740, USA; Regis.Pouillot@fda.hhs.gov.

Department of Agriculture published a joint risk assessment for *L. monocytogenes* in 23 selected categories of ready-to-eat (RTE) foods.[4] The risk assessment evaluated the risk of invasive listeriosis and death due to listeriosis for the total U.S. population as well as for three separate population subgroups: (i) neonates infected in utero through contaminated food consumed by their mothers; (ii) the intermediate-age population; and (iii) older adults. One dose-response relationship (i.e., modeling mortality in humans following the ingestion of *L. monocytogenes)* was initially developed and different multipliers were subsequently applied to generate models for invasive listeriosis for each of the population subgroups. To derive the dose-response relationship for mortality in humans, five dose-response models (i.e., probit, exponential, logistic, multihit, and Gompertz-log) were initially fitted to data obtained in mice challenged with a single *L. monocytogenes* strain. These models were weighted and used simultaneously to characterize uncertainty in the shape of the dose-response curve, with the best-fitting exponential model receiving the greatest weight. A distribution of median lethal dose values (LD_{50}) observed in mice challenged with different *L. monocytogenes* strains was subsequently incorporated in the dose-response model to characterize *L. monocytogenes* strain variability in virulence and its uncertainty. Variability and uncertainty in host susceptibility within the three population subgroups were estimated based on observations in mice and epidemiological data, and incorporated in the dose-response model as well. Finally, because the derived model considerably overestimated the expected number of invasive listeriosis cases, surveillance data on the incidence of listeriosis in the United States were used to scale the dose-response relationship to reflect differences in susceptibility between humans and mice.[4]

In 2004, an international expert panel of the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) developed another dose-response model based on a data subset extracted from the exposure estimates and the estimated annual number of cases used to derive the draft FDA/FSIS dose-response model published in 2001. The FAO/WHO dose-response model for invasive listeriosis is an exponential dose-response model.[6] The exponential dose-response model is a "single-hit" model:[6,7] it assumes that the probability of a given bacterial cell causing the adverse effect is independent of the number or characteristics of other ingested pathogens, so that a single ingested microorganism is sufficient to cause the adverse effect with some probability greater than zero. The exponential dose-response model further assumes that the bacterial cells are randomly distributed in the food, hence the dose per portion follows a Poisson distribution, and that the average probability, r, that one pathogen, within a given exposure of a particular consumer to a specific population of pathogens, will survive the host-pathogen interaction to initiate infection and cause illness is constant.[8]

If the virulence of pathogens or the susceptibility of consumers varies from exposure to exposure, then r may vary and may be represented by a random variable with distribution $f(r)$. [8] Challenges remain regarding how best to quantify the distribution of r in relation to the host, the bacterial strain, and the exposure scenario. To account for differences in host susceptibility for *L. monocytogenes*, the FAO/WHO group of experts assumed the existence of two distinct values for r, applicable to the general population and population subgroups with increased susceptibility, respectively. The two r parameters (i.e., one value for each of the two population subgroups) were estimated from epidemiological[9] and food exposure[10] data obtained in the United States. The estimated r parameters were extremely low (i.e., approximately 10^{-12}–10^{-13} for the population with increased susceptibility and 10^{-13}–10^{-15} for the general population), translating into a very low probability of illness following the ingestion of a low dose of bacteria. This dose-response model or some adaptations of the model have been used in various risk assessments.[11-14]

Since 2004, new scientific data have become available, demonstrating the considerable variability in virulence among *L. monocytogenes* strains and molecular subtypes.[15–18] New data have, for example, shown that the entry of *L. monocytogenes* into certain human epithelial cells is primarily receptor mediated, depending on specific interactions between internalins on the bacterial surface and their respective host cell receptors.[19–22] Therefore, point mutations in the *inlA* gene can lead to virulence attenuation of *L. monocytogenes* strains.[16,23,24] New data are also available regarding the variability in susceptibility among individuals with different predisposing conditions such as pregnancy, old age, or other underlying conditions.[25–28] The relative risk of listeriosis for pregnant women, for example, has been estimated to be approximately 100 times higher than

that for nonpregnant women.[25–27] Relative risks higher than 1,000 have been reported for individuals with chronic lymphocytic leukemia when compared to a reference population of individuals <65-year old without any known underlying conditions.[26]

Because of the challenges in developing adequate dose-response models of listeriosis, an interagency expert workshop was held in the United States in 2011, with the goal of identifying new data, strategies, and insights for *L. monocytogenes* dose-response modeling. Short-term strategies identified during this workshop included updating the dose-response model developed by FDA/FSIS[4] by incorporating new data and insights about differences in strain virulence and *L. monocytogenes* pathophysiology. A key-events approach to dose-response modeling[29] was identified as a promising though extremely challenging, data-intensive, and potentially unachievable framework for future microbial dose-response models.[2]

Current dose-response models linked to epidemiological data tend to agree that a low dose of *L. monocytogenes* leads to an average low probability of invasive listeriosis in the general population as well as in broadly defined populations with heightened susceptibility.[4,5,30] However, a more nuanced evaluation of *L. monocytogenes* dose response for *L. monocytogenes* strains with different virulence and for different human population subgroups at heightened risk of listeriosis is needed to adequately characterize the listeriosis risk in different population subgroups, including those with highest susceptibility. Such nuanced models would allow for more in-depth inference about the listeriosis risk posed to highly susceptible population subgroups by highly virulent *L. monocytogenes* strains, and may become instrumental for evaluating key risk management issues such as the potential public health threat associated with the ingestion of a given dose of *L. monocytogenes*.

In this article, the existing exponential *L. monocytogenes* dose-response model[5] for invasive listeriosis is being revisited. A mathematical framework for considering variability in *L. monocytogenes* virulence and in host susceptibility is derived and applied to currently available epidemiological data, including data from one well-documented listeriosis outbreak.[4,5,31] Unlike other foodborne pathogens such as *Salmonella*,[32–34] *Campylobacter*,[35] or norovirus,[36,37] *L. monocytogenes* is characterized by an extremely low probability of illness at low exposure doses when averaging across the total population or broadly defined population subgroups[4,5,30] and by extreme variability in the probability of infection among population subgroups with different predisposing risk factors.[5,26,27,38] Two dose-response models are evaluated and compared here in light of the unique challenges associated with modeling *L. monocytogenes* dose response.[2,4,5,29] The first evaluated model uses beta distributions to characterize variability in r from exposure to exposure, resulting in an "exact beta-Poisson" dose-response relation[6] (also known as "hypergeometric"[7] or "actual beta-Poisson"[8] dose-response relation), which may be simplified to an approximate "beta-Poisson" model if certain conditions are met.[7,39] The second model, a newly developed "lognormal-Poisson" model, characterizes variability in r due to variability in strain virulence and host susceptibility using lognormal distributions. As will be illustrated in this article, the lognormal distribution was found appropriate and useful for modeling the special case of *L. monocytogenes* dose response whereas the beta-Poisson model showed insufficient flexibility to adequately model one of the well-described *L. monocytogenes* outbreaks.

2. FRAMEWORK, MODEL, AND DATA

2.1. General Derivation of the Evaluated Dose-Response Models

A single-hit model is assumed.[6,7] The probability of acquiring the adverse effect under study (i.e., invasive listeriosis) if a dose of d bacterial cells is ingested in a certain serving is given by:

$$P(\text{ill}; d, r) = 1 - (1 - r)^d, \tag{1}$$

where "ill" stands for "illness" (here, invasive listeriosis) and r is the probability of developing invasive listeriosis from the ingestion of a bacterial cell in a given, specific serving. Note that r may be seen as constant for that serving,[6] or as an average probability that one cell of the specific population of pathogens present in the meal will survive and initiate the infection and illness of this specific consumer.[8] Assume that each serving is specific to a given context, determined by the individual i (characterized by the presence of a given set of predisposing risk factors at the time of consumption) consuming the food and by the *L. monocytogenes* strain s present in the ingested food (with a certain set of given virulence determinants at

the time of consumption). In this study, r is considered constant for this particular serving, but variable across servings, with its variability determined by the variation in susceptibility across individuals and the variation in virulence across strains.

Assume further that the *L. monocytogenes* dose in a given serving is Poisson distributed and the distribution of r across a given population of servings is described by a random variable with density function $f(r;\boldsymbol{\theta})$. Then the marginal probability of infection for an average dose d is described by:[6]

$$P(\text{ill};d,\boldsymbol{\theta}) = \int_0^1 (1-\exp(-rd))\, f(r;\boldsymbol{\theta})\, \mathrm{d}r. \quad (2)$$

Any probability density function with practical domain [0; 1] can be chosen for f. A beta distribution is a convenient choice for modeling variability in r because its domain is restricted to [0,1], it provides flexibility over the domain, and the simplified beta-Poisson model is easy to implement.[6] The exact and simplified beta-Poisson dose-response models have been repeatedly used for modeling illnesses from other foodborne pathogens such as norovirus,[37,40] *Salmonella*,[33,34] or *Campylobacter jejuni*.[8] The beta-Poisson model was also used to model *L. monocytogenes* dose-response from animal data.[41] If a lognormal (base 10) distribution is chosen for f, that is, $\log_{10}(r) \sim \text{normal}(\mu,\sigma)$, with negligible probability that $r \geq 1$, Equation (2) leads to:

$$P(\text{ill};d,\mu,\sigma) = \frac{\log_{10}(e)}{\sigma\sqrt{2\pi}} \int_0^1 \left(\frac{1}{r}(1-\exp(-rd)) \times \exp\left(-\frac{(\log_{10}(r)-\mu)^2}{2\sigma^2}\right)\right)\mathrm{d}r. \quad (3)$$

Equation (3) has no closed form and requires numerical integration. However, it simplifies to an exponential dose-response model for any given value r.

$$P(\text{ill};d,r) = 1-\exp(-rd) \quad (4)$$

In this study, we investigated a beta distribution and a lognormal distribution to characterize the distribution of r from meal to meal, using data from multiple sources, including human surveillance and food survey data. The derivation using the beta-Poisson model can be found in the Appendix, which shows that this model is inappropriate for the special case of modeling *L. monocytogenes* dose response in humans, most notably because it could not adequately model extreme situations such as outbreaks. The lognormal distribution was eventually chosen because its heavy-tail property was deemed useful for modeling the special case of *L. monocytogenes* dose response, and because its infinitively divisional property allowed for mathematically relatively simple separation of different sources of variability in dose response.

2.2. Dose-Response Model Within Populations Subgroups

The probability of developing listeriosis after ingesting a given dose of *L. monocytogenes* is highly variable from meal to meal, and considerably impacted by the *L. monocytogenes* strain and the presence and nature of underlying host conditions such as pregnancy, old age, or certain diseases and conditions.[25–27] The variability in r may be separated into three sources: variability in susceptibility across mutually exclusive population subgroups with a shared predisposing risk factor, variability in susceptibility across individuals within a given population subgroup, and variability in virulence among *L. monocytogenes* strains with different virulence determinants.

For a given population subgroup g, the marginal dose response can be rewritten as:

$$P(\text{ill};d,\boldsymbol{\theta}_g) = \int_0^1 (1-\exp(-rd))\, f(r;\boldsymbol{\theta}_g)\, dr,$$

where $\boldsymbol{\theta}_g$ is characteristic of the subgroup g. The distribution $f(r;\boldsymbol{\theta}_g)$ represents the remaining individual (within group) susceptibility variability and strain virulence variability in r.

The resulting distribution of r across all population subgroups can be expressed as a mixture of distributions for individual population subgroups, weighted by the relative size of each population subgroup in the total population:

$$g(r) = \sum_g \pi_g f(r;\boldsymbol{\theta}_g), \quad (5)$$

where π_g, $\sum_g \pi_g = 1$, is the proportional size of the population subgroup g within the total population.

Substituting $f(r)$ by $g(r)$ in Equation (2) leads to the dose response for the total population. This dose-response relationship integrates, in addition to those factors accounted for by the subpopulation-specific dose-response model, the variability in mean susceptibility across population subgroups.

2.3. Specification of μ_g and σ_g from Surveillance Data

Let c_g equal the number of invasive listeriosis cases in a given population subgroup g and $M_{d,g}$ equal the number of servings with a given mean dose d ingested by the population subgroup g. Then, the expected value of c_g is given by:

$$E[c_g] = \int_0^\infty M_{d,g} P(\text{ill}; d, \boldsymbol{\theta}_g)\, \mathrm{d}d. \tag{6}$$

Estimating c_g from epidemiological data and $M_{d,g}$ from food exposure data generates an infinite number of solutions for the ordered pair (μ_g, σ_g). However, if a measure of variability of r_g is known, the problem simplifies to a root-finding problem. As an example, if we are able to characterize Q_{90}, the $\log_{10}$ of the ratio between the 5th and the 95th percentile of $f(r; \boldsymbol{\theta}_g)$, we can estimate $\boldsymbol{\theta}_g$ for estimated $E[c_g]$ and Q_{90} using some iterative solver routine.

2.4. Characterization of Variability

2.4.1. Specification of σ_g

Under limited assumptions, the infinitively divisional property of lognormal distributions allows for a characterization and separation of interindividual and interstrain variability. The potential of a given *L. monocytogenes* strain to cause disease (i.e., strain virulence determined by a given set of transient and fixed virulence factors) may be considered independent of the susceptibility of a given host to listeriosis (i.e., host susceptibility due to a given set of comorbidities and other factors impacting individual susceptibility such as genetic predisposition).

In this study, r is defined as the probability of infection for a given individual following the ingestion of one given *L. monocytogenes* cell during a given serving. Note that r may be considered for our purpose as the product of two independent probabilities: the probability p_i, linked to events controlled by host factors that ultimately lead to a failure to stop infection, and p_s, which reflects bacterial factors that control virulence and pathogenicity:

$$r = p_i \times p_s. \tag{7}$$

We assume that p_s and p_i follow lognormal distributions. Because the product of two independent lognormally distributed random variables is itself a lognormal random variable, r is also lognormally distributed. Let $p_i \sim \text{lognormal}(\mu_i, \sigma_i)$ for all $i \in g$, and let $p_s \sim \text{lognormal}(\mu_s, \sigma_s)$ for strains s. Based on Equation (7) we see that for a given population subgroup and strain,

$$r \sim \text{lognormal}\left(\mu_i + \mu_s, \sqrt{\sigma_i^2 + \sigma_s^2}\right), \tag{8}$$

and the marginal density across all strains can therefore be found by $\mu_g = \mu_i + \mu_s$ and $\sigma_g = \sqrt{\sigma_i^2 + \sigma_s^2}$.

$Q_{90,i}$ is defined as the $\log_{10}$ of the 90% individual within-group susceptibility variability range. Note that σ_i can be estimated as $\sigma_i = (Q_{90,i}/2)/\Phi^{-1}(0.95)$ where Φ^{-1} denotes the inverse of the standard normal cumulative density function. Here, σ_s can be estimated using the same rationale for the interstrain variability. If $Q_{90,s}$ is the $\log_{10}$ difference between the 5th and the 95th percentile, $\sigma_S = (Q_{90,s}/2)/\Phi^{-1}(0.95)$.

The subroutine must find (μ_g, σ_g) solution of:

$$E[c_g] = \int_0^\infty M_{d,g} P(\text{ill}; \mu_g, \sigma_g)\, \mathrm{d}d, \tag{9}$$

where

$$\sigma_g = \frac{\sqrt{{Q_{90,i}}^2 + {Q_{90,s}}^2}}{2\Phi^{-1}(0.95)}. \tag{10}$$

2.4.2. Intragroup Variability $Q_{90,i}$

Due to a variety of factors, such as genetic predisposition, susceptibility to infection differs across individuals, even after accounting for underlying comorbidities, albeit with considerably decreased variability. To derive estimates for our model, we used the estimates of variability in susceptibility presented in FDA/FSIS.[4] In FDA/FSIS,[4] three distributions that encompass the range of susceptibility observed in animal studies were used to adjust the $\log_{10}$ cfu of the effective dose for populations with low, medium, and high variability.[4] Assuming exponential dose response in animal studies, the range of variation in the $\log_{10}$ LD_{50} translates into the range of variation in the $\log_{10}$ r parameter.[1] Therefore, we represented the variability in the probability of illness from a single cell (in $\log_{10}$ r) using the variability in the $\log_{10}$ cfu that had been used to modify the effective dose in FDA/FSIS.[4] According to FDA/FSIS (Table IV-8 in Ref. 4), 90% of the individual variability within the population group with low, medium, and high

[1] We have, for an exponential dose response, $r = \frac{-\ln(.5)}{\text{LD}_{50}}$. The LD_{50} is inversely proportional to r. A variation of $\pm x$ $\log_{10}$ in $\log_{10}$ LD_{50} corresponds to a similar variation of $\pm x$ $\log_{10}$ in $\log_{10}$ r.

variability in susceptibility may be contained within a range of 0.8 $\log_{10}$, 1.8 $\log_{10}$, and 2.9 $\log_{10}$, respectively. FDA/FSIS[4] used the medium variability distribution for neonatal populations and high variability for intermediate-age and elderly subpopulations. In this study, we divided the population into 11 population subgroups with similar underlying conditions (Table I), essentially as described previously.[11,42] Assuming that our 11 subpopulations would be more precisely defined with regard to predisposing risk factors and therefore less variable in susceptibility than the broadly defined "elderly" and the "intermediate-age" population subgroups defined by FDA/FSIS,[4] we used FDA/FSIS[4] "medium variability" estimates for all of the 11 groups, that is, $Q_{90,i} = 1.8 \log_{10}$.

2.4.3. Interstrain Virulence Variability $Q_{90,S}$

In the FDA/FSIS assessment, variations in host susceptibility and in strain virulence were represented by distributions that modified the effective dose for individual servings.[4] The distribution for strain virulence was estimated notably by the observed variation in LD_{50} (in $\log_{10}$ cfu) among different *L. monocytogenes* strains in mouse experiments.[4] According to FDA/FSIS (Table IV-6 in Ref. 4), 90% of the strain variability ranges within a 5 $\log_{10}$, leading to $Q_{90,s} = 5 \log_{10}$.

Substituting these values in Equation (10) generates $\sigma_g = 1.62 \log_{10}$.

2.5. Integration of the dose-response Models

2.5.1. Exposure Data

The *L. monocytogenes* concentration distribution reported by Chen *et al.*[30] was used for exposure estimates. This distribution was obtained by fitting data from a survey of more than 31,000 RTE retail food samples, representing eight RTE categories sampled in the years 2000 and 2001 in two states of the United States.[43] *L. monocytogenes* was not detected in 98.2% of the samples. The $\log_{10}$ concentration ($\log_{10}$ cfu/g) in the remaining contaminated products followed a four-parameter beta distribution[2] with parameters $\alpha = 0.29$, $\beta = 2.68$, $a = -1.69$, and $b = 6.1$.[30] A 50 g serving size was assumed in this study. The number of servings of these eight RTE categories consumed by the U.S. population was estimated at 1.23×10^{11} servings per year based on the FDA/FSIS risk assessment.[4] As considered in previous risk assessments,[4,5] we made the assumption of an identical distribution of *L. monocytogenes* doses and strains for all population subgroups.

[2] x follows a four-parameter beta distribution with parameters (α, β, a, b) if $(x\text{-}a)/(b\text{-}a) \sim \text{Be}(\alpha, \beta)$

2.5.2. Epidemiological Data

To allow comparisons across population subgroups g with similar underlying conditions, we identified population subgroups with specific predisposing risk factors (e.g., different types of illness, old age, pregnancy), and evaluate variability in susceptibility within and across these subgroups.

Goulet *et al.*[26] published data on the relative risk of listeriosis in France for 36 mutually exclusive susceptible population subgroups, each consisting of individuals sharing a specific underlying condition. Because the data were too scarce to derive dose-response models separately for 36 mutually exclusive subgroups, the 36 subgroups identified by Goulet *et al.*[26] were combined (where appropriate) and regrouped into 11 subgroups based on underlying pathophysiology and expected degree of T-cell inhibition, essentially using a grouping scheme as previously described.[11,42]

We assumed that the relative risk of listeriosis for a given population subgroup and the relative size of each evaluated population subgroup would be comparable between France and the United States. The number of cases in each subgroup had to be normalized to the listeriosis burden estimates from the United States to allow extrapolation of the data (Table I). We evaluated two estimates of the total listeriosis cases in the United States, the first based on 1996–1997 data[9] and the second on 2005–2008 data[1] from FoodNet surveillance. We chose the latter, i.e., 1,591 cases per year, as input to derive the dose-response relationship because the 2000–2001 timeframe for the food survey[43] corresponded to the timeframes for the listeriosis estimates and, more importantly, the latter listeriosis estimate was based on new and improved methods for estimating overall foodborne illness in the United States.[1]

2.5.3. Sensitivity Analysis

As will be discussed below, we identified two major assumptions needed to use the data described above. To evaluate the impact of these assumptions

Table I. Number of Persons with Underlying Conditions and Number of Cases of Invasive Listeriosis Observed in France, 2001–2008;[26] Expected Number of Invasive Listeriosis Cases per Subgroups in the United States; See Text for Underlying Assumptions and Ref. 26 for a More In-Depth Description of the Population Subgroups

Population Subgroup	Number of Individuals in France (from and Adapted from Ref. 26)	Listeriosis Cases During an 8-Year Period in France (from and Adapted from Ref. 26)	Relative Risk (CI 95%)[a]	Expected Number of Listeriosis Cases in the United States (Based on 1,591 Cases from Ref. 1)
Less than 65 years old, no known underlying condition (i.e., "healthy adult")	48,909,403	189	Reference group	153
More than 65 years old, no known underlying condition	7,038,068	377	13.9 (8.6, 23.1)	306
Pregnancy	774,000	347	116 (71, 194.4)	282
Nonhematological cancer	2,065,000	437	54.8 (34.2, 90.3)	355
Hematological cancer	160,000	231	373.6 (217.3, 648.9)	188
Renal or liver failure (dialysis, cirrhosis)	284,000	164	149.4 (82, 270.1)	133
Solid organ transplant	25,300	16	163.7 (26.3, 551.5)	13
Inflammatory diseases (rheumatoid arthritis, ulcerative colitis, giant cell arteritis, Crohn's disease)	300,674	68	58.5 (25.2, 123.4)	55
HIV/AIDS	120,000	22	47.4 (10.5, 140.4)	18
Diabetes (type I or type II)	2,681,000	79	7.6 (3.5, 15.6)	64
Heart diseases	1,400,000	29	5.4 (1.5,14.4)	24
Total population	63,757,445	1,959		1,591

[a]Estimated using a Poisson regression without adjustment. These 95% CIs should be considered only as indicative but suggest that all those groups have a risk of listeriosis significantly higher than the reference group.

on the generated risk estimates we conducted the following sensitivity analyses for these two assumptions. (i) Due to the lack of sufficient data, we assumed equal exposure to contaminated food for all population subgroups. This assumes that outreach targeted at minimizing foodborne exposures of high-risk population subgroups is ineffective. As a sensitivity analysis, the model was tested with the alternative assumption that the number of servings containing a given number of bacteria for all of the more susceptible subgroups are one-tenth of that for "healthy adults" (i.e., the <65 years of age without any known underlying conditions). (ii) The exposure data we used in deriving the dose-response model did not consider bacterial growth from retail to consumption, and considered a maximum level of contamination of 6.1 $\log_{10}$ cfu/g. Because growth in the consumer home has been identified as a potentially important risk factor in previous risk assessments, we conducted a sensitivity analysis to evaluate the impact of this assumption. The model was tested using the four-parameter beta distribution of $\log_{10}$ concentration described in Section 2.5.1, with a maximum parameter increased from $b = 6.1$ to $b = 8.1$ $\log_{10}$ cfu/g. This distribution leads to an average concentration in contaminated products of 20,545 cfu/g as compared to 390 cfu/g for the baseline scenario.

2.6. Dose-response Relationship Using Outbreak Data

It was assumed that a single food item and *L. monocytogenes* strain are involved in an outbreak affecting a specific population subgroup g, thus eliminating the impact of strain-to-strain variability in the dose-response evaluation. The virulence of the outbreak strain, $p_{s(\text{outbreak})}$, is then fixed but unknown. We used the lognormal-Poisson model (and the beta-Poisson model; see the Appendix) to analyze a well-documented listeriosis outbreak, the butter outbreak that occurred in Finland in 1998–1999,[31] as re-examined by FDA/FSIS[4] and FAO/WHO.[5] This outbreak was characterized by a relatively high attack rate among immunocompromised individuals (mostly hematological or organ transplant patients) for a relatively low dose of *L. monocytogenes*.[4,5] The FAO/WHO panel derived an r value of 3.15×10^{-7} from data collected during this outbreak.[5]

The lognormal dose-response properties help to evaluate the dose response during outbreaks. As can be inferred based on Equation (7), r is the product of a fixed value $p_{s(outbreak)}$ and a lognormally distributed variable p_i. Thus:

$$r \sim \text{lognormal}\left(\mu_g + \log_{10}\left(p_{s(outbreak)}\right), \sigma_i\right). \quad (11)$$

Given that $p_s \sim lognormal\,(\mu_S, \sigma_S)$, the jth quantile of p_s is given by $p_s\,(j) = 10^{\left(\mu_s + \Phi^{-1}(j)\times\sigma_s\right)}$. Assuming that $p_{s(\text{outbreak})} = p_s\,(j)$:

$$r \sim \text{lognormal}\left(\mu_i + \mu_s + \Phi^{-1}\,(j) \times \sigma_s, \sigma_i\right). \quad (12)$$

Substituting μ_g for $\mu_i + \mu_s$gives for r:

$$r \sim \text{lognormal}\left(\mu_g + \Phi^{-1}\,(j) \times \sigma_s, \sigma_i\right). \quad (13)$$

Percentiles of interest can now easily be estimated using the parameters derived above.

All numerical integrations and optimizations of the models were performed using the R software.[44] The code is available from the corresponding author on request.

3. RESULTS

3.1. Estimation of *r* for Different Population Subgroups Using Food Exposure and Epidemiological Surveillance Data

Solutions for the ordered pair (μ_g, σ_g) for all 11 population subgroups, based on numerical integration, are presented in Table II. Notably, estimates of $\mu_g$varied widely across population subgroups, ranging from $\mu_g = -14.1$ for those less than 65 years of age without any known underlying conditions (i.e., "healthy adults") to $\mu_g = -11.0$ for individuals with hematological cancer. These estimates translate into mean values of r equaling 7.9×10^{-12} and 9.6×10^{-9}, respectively. The corresponding 99.9th percentiles equal 7.7×10^{-10} and 9.3×10^{-7} for healthy adults and individuals with hematological cancer, respectively, indicating that the risk of illness per ingested cell generally remains relatively low for most population subgroups and most types of exposure. The variation in dose response across population subgroups is illustrated in Fig. 1, highlighting in particular the comparison among the total population, pregnant women, and healthy adults. As expected, the marginal dose-response model for the total population more closely resembles that for healthy adults than those for the most susceptible population subgroups.

The probability of illness and the expected numbers of cases for a variety of population subgroups and ingested doses are presented in Table III. For healthy adults <65 years old, the mean probability of illness remains below 1:10,000 if doses below 7.5 $\log_{10}$ cfu/serving are ingested. However, for those with hematological cancer, ingestion of doses in the range of 5.5 $\log_{10}$ cfu/serving translates into a mean probability of illness around 1:1,000. Considering this dose-response relationship and the exposure to *L. monocytogenes* through food consumption, most of the 1,591 cases analyzed in this study are expected to be due to foods contaminated with doses between 3.5 and 7.5 $\log_{10}$ cfu/serving (Table III). Notably, 20% of the 188 expected cases among those with hematological cancer are expected to be due to contamination with doses ≤5 $\log_{10}$ cfu/serving. Doses of 4 $\log_{10}$ cfu/serving or lower are estimated to be responsible for 2% of cases among healthy adults, but an estimated 4% of cases among pregnant women and an estimated 5% of cases among individuals with hematological cancer are expected to be caused by such relatively low doses.

As shown above in Equation (4), for a fixed value of r, the dose-response model simplifies to an exponential dose-response model. Fig. 2 illustrates the dose-response relationships for the total population for the 0.01st, 0.1st, 1st, 50th, 99th, 99.9$^{\text{th}}$, and 99.99th percentiles of the distribution (including group-to-group, individual within-group, and strain-to-strain variability) of r. This figure also overlays the marginal lognormal-Poisson model from this study with the exponential dose-response models reported previously by FAO/WHO[5] for the susceptible population as well as the one by Chen *et al.*[24] for *L. monocytogenes* strains with genes encoding a full-length *inlA* for the 25% higher-risk population. Notably, the dose response for the total population derived here results in a higher risk of infection for low doses than either of the two published dose-response models (Fig. 2). The dose-response model obtained in this study for the least virulent strains, however, leads to a considerably lower risk of illness at low doses than either of the published models.

3.2. Sensitivity Analyses

When the model was tested with the alternative assumption that the number of servings including a given number of bacteria for all of the more susceptible subgroups equals one-tenth of that for

Table II. Parameters of the Lognormal-Poisson Dose-response Model for Invasive Listeriosis Following the Ingestion of *L. monocytogenes* in Different Population Subgroups and Resulting Statistics for *r*, the Probability of Illness Following the Ingestion of One Cell of *L. monocytogenes*; The Distribution of *r* Includes the Individual Within-Group and the Strain Variability

	Estimates of a Log_{10} Normal Distribution[a] of *r*		Estimates of *r*			
Population Subgroup	μ	σ	Mean	50th Percentile	99th Percentile	99.9th Percentile
Less than 65 years old, no known underlying condition (i.e., "healthy adult")	−14.11	1.62	7.90×10^{-12}	7.82×10^{-15}	4.48×10^{-11}	7.68×10^{-10}
More than 65 years old, no known underlying condition	−12.83	1.62	1.49×10^{-10}	1.47×10^{-13}	8.44×10^{-10}	1.45×10^{-8}
Pregnancy	−11.70	1.62	2.01×10^{-9}	1.99×10^{-12}	1.14×10^{-8}	1.95×10^{-7}
Nonhematological cancer	−12.11	1.62	7.76×10^{-10}	7.68×10^{-13}	4.40×10^{-9}	7.54×10^{-8}
Hematological cancer	−11.02	1.62	9.60×10^{-9}	9.51×10^{-12}	5.44×10^{-8}	9.33×10^{-7}
Renal or liver failure (dialysis, cirrhosis)	−11.56	1.62	2.79×10^{-9}	2.76×10^{-12}	1.58×10^{-8}	2.71×10^{-7}
Solid organ transplant	−11.51	1.62	3.14×10^{-9}	3.11×10^{-12}	1.78×10^{-8}	3.06×10^{-7}
Inflammatory diseases (rheumatoid arthritis, ulcerative colitis, giant cell arteritis, Crohn's disease)	−12.08	1.62	8.43×10^{-10}	8.35×10^{-13}	4.78×10^{-9}	8.19×10^{-8}
HIV/AIDS	−12.19	1.62	6.50×10^{-10}	6.44×10^{-13}	3.69×10^{-9}	6.32×10^{-8}
Diabetes (type I or type II)	−13.13	1.62	7.47×10^{-11}	7.39×10^{-14}	4.23×10^{-10}	7.26×10^{-9}
Heart diseases	−13.30	1.62	5.01×10^{-11}	4.96×10^{-14}	2.84×10^{-10}	4.86×10^{-9}
Whole population	N/A[b]	N/A	1.19×10^{-10}	1.56×10^{-14}	2.47×10^{-10}	6.87×10^{-9}

[a]The log_{10} normal distribution is parameterized as $x \sim \text{lognormal}(\mu, \sigma)$ if $\log_{10}(x) \sim \text{normal}(\text{mean: } \mu, \text{standard error: } \sigma)$.
[b]Nonapplicable: the dose response for the whole population uses a mixture of log_{10} normal distribution (see text for details).
[c]The distribution of *r* includes the individual and the strain variability.

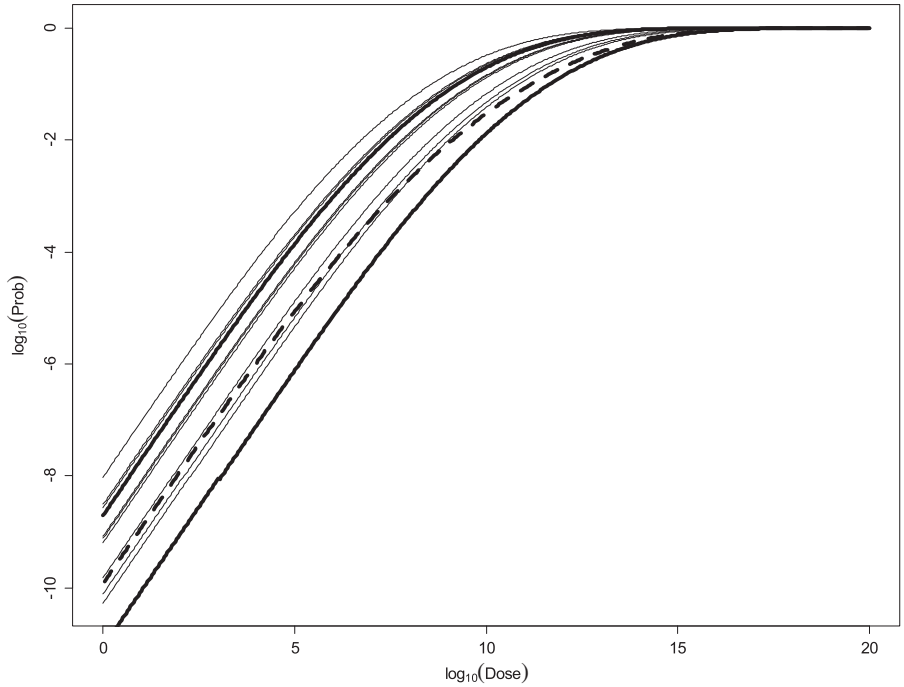

Fig. 1. Marginal (over strains and individuals within subgroups) lognormal-Poisson dose-response models for the 11 population subgroups (thin lines), emphasizing (thick lines) the dose-response relationship for those <65 years of age without known underlying conditions ("healthy adult"; bottom thick line) and for pregnant women (top thick line). Marginal (over strains and individuals) lognormal-Poisson dose response for the total population (thick dashed line).

"healthy adults," the dose response was shifted to the left for the susceptible groups. In this case, the overall expected number of cases for servings containing ≤ 4 $\log_{10}$ cfu equaled less than 6% of all cases as compared to 3% of all cases in the baseline scenario. The assumption of equal food consumption across population subgroups therefore only had a modest impact on our analysis. When the model was tested with a maximum *L. monocytogenes* level of 8.1 $\log_{10}$ cfu/g, a shift of the corresponding dose response to the right was logically obtained: with this maximum level, 0% of the cases would be predicted for a dose of 4 $\log_{10}$ cfu/g and 4% for a dose of 6 $\log_{10}$ cfu/g for the total population.

3.3. Application of the Dose-response Framework to Listeriosis Outbreaks

Fig. 3 compares the published exponential dose-response model[5] estimated from the Finnish butter outbreak data ($r = 3.15 \times 10^{-7}$)[5,31] to the dose-response model for transplant recipients derived in this study, showing both the prediction averaged across individual strains and for individual percentiles of the virulence distribution p_s. Fig. 3 suggests that the dose-response model from this study is able to predict the data observed in the Finnish outbreak, and that the strain was highly virulent, as the corresponding dose-response overlays that of a strain with a level of virulence close to the 99.9th percentile of r.

4. DISCUSSION

4.1. The New Framework for *L. Monocytogenes* Dose-response, Adjusted for Variability in Host Susceptibility and Strain Virulence

The FAO/WHO[5] dose-response model can be considered as a marginal dose-response model for a population exposed to a cross-section of *L. monocytogenes* strains. As such, this model averages across numerous individuals with differing levels of susceptibility and multiple *L. monocytogenes* strains with varying levels of virulence. While such evaluations can be highly informative for many purposes they may be inappropriate to evaluate certain rare but potentially highly relevant events, such as the

Table III. Marginal Probability of Illness and Expected Number of Cases in Selected Population Subgroups and in the General Population as a Function of the Dose, Considering Individual Susceptibility Within Groups, Strain Variability, and Dose Variability for a Given Mean Dose

	Marginal Probability of Illness for Individuals						Expected Number of Cases					
Log_{10}(Dose)	<65-year old	>65-year old	Pregnant women	Hematological cancer	Solid organ transplant	Whole population	<65-year old	>65-year old	Pregnant women	Hematological cancer	Solid organ transplant	Whole population
0.0	8.8×10^{-12}	1.5×10^{-10}	2.0×10^{-9}	9.5×10^{-9}	3.1×10^{-9}	1.2×10^{-10}	0	0	0	0	0	0
0.5	2.8×10^{-11}	4.8×10^{-10}	6.2×10^{-9}	3.0×10^{-8}	9.8×10^{-9}	3.8×10^{-10}	0	0	0	0	0	0
1.0	8.8×10^{-11}	1.5×10^{-9}	2.0×10^{-8}	9.5×10^{-8}	3.1×10^{-8}	1.2×10^{-9}	0	0	0	0	0	1
1.5	2.8×10^{-10}	4.8×10^{-9}	6.3×10^{-8}	3.0×10^{-7}	9.9×10^{-8}	3.8×10^{-9}	0	0	0	0	0	1
2.0	8.8×10^{-10}	1.5×10^{-8}	2.0×10^{-7}	9.3×10^{-7}	3.1×10^{-7}	1.2×10^{-8}	0	0	0	0	0	1
2.5	2.8×10^{-9}	4.7×10^{-8}	6.2×10^{-7}	2.8×10^{-6}	9.6×10^{-7}	3.7×10^{-8}	0	0	1	1	0	3
3.0	8.8×10^{-9}	1.5×10^{-7}	1.9×10^{-6}	8.6×10^{-6}	2.9×10^{-6}	1.1×10^{-7}	0	1	1	1	0	6
3.5	2.5×10^{-8}	4.6×10^{-7}	5.8×10^{-6}	2.5×10^{-5}	8.9×10^{-6}	3.5×10^{-7}	1	2	3	3	0	14
4.0	7.8×10^{-8}	1.4×10^{-6}	1.7×10^{-5}	7.2×10^{-5}	2.6×10^{-5}	1.0×10^{-6}	2	4	6	5	0	29
4.5	2.5×10^{-7}	4.3×10^{-6}	5.0×10^{-5}	2.0×10^{-4}	7.5×10^{-5}	3.0×10^{-6}	4	9	12	10	1	60
5.0	7.6×10^{-7}	1.3×10^{-5}	1.4×10^{-4}	5.2×10^{-4}	2.0×10^{-4}	8.6×10^{-6}	8	19	22	17	1	115
5.5	2.4×10^{-6}	3.8×10^{-5}	3.7×10^{-4}	1.3×10^{-3}	5.4×10^{-4}	2.4×10^{-5}	15	35	38	28	2	200
6.0	7.1×10^{-6}	1.1×10^{-4}	9.5×10^{-4}	3.1×10^{-3}	1.3×10^{-3}	6.3×10^{-5}	27	58	56	38	3	308
6.5	2.1×10^{-5}	2.9×10^{-4}	2.3×10^{-3}	7.0×10^{-3}	3.2×10^{-3}	1.6×10^{-4}	39	77	68	42	3	389
7.0	6.1×10^{-5}	7.5×10^{-4}	5.2×10^{-3}	1.5×10^{-2}	7.1×10^{-3}	3.9×10^{-4}	41	73	56	33	3	344
7.5	1.7×10^{-4}	1.8×10^{-3}	1.1×10^{-2}	2.9×10^{-2}	1.5×10^{-2}	9.1×10^{-4}	17	27	19	10	1	121
8.0	4.5×10^{-4}	4.3×10^{-3}	2.3×10^{-2}	5.3×10^{-2}	2.9×10^{-2}	2.0×10^{-3}	0	0	0	0	0	0
8.5	1.1×10^{-3}	9.3×10^{-3}	4.3×10^{-2}	9.2×10^{-2}	5.4×10^{-2}	4.2×10^{-3}	0	0	0	0	0	0
9.0	2.7×10^{-3}	1.9×10^{-2}	7.6×10^{-2}	1.5×10^{-1}	9.4×10^{-2}	8.5×10^{-3}	0	0	0	0	0	0
9.5	6.1×10^{-3}	3.7×10^{-2}	1.3×10^{-1}	2.3×10^{-1}	1.5×10^{-1}	1.6×10^{-2}	0	0	0	0	0	0
10.0	1.3×10^{-2}	6.6×10^{-2}	2.0×10^{-1}	3.2×10^{-1}	2.3×10^{-1}	3.0×10^{-2}	0	0	0	0	0	0
10.5	2.6×10^{-2}	1.1×10^{-1}	2.9×10^{-1}	4.3×10^{-1}	3.3×10^{-1}	5.1×10^{-2}	0	0	0	0	0	0
11.0	4.8×10^{-2}	1.8×10^{-1}	3.9×10^{-1}	5.5×10^{-1}	4.4×10^{-1}	8.5×10^{-2}	0	0	0	0	0	0
Total							153	306	282	188	13	1591

[a]No serving with >7.8 log_{10} *L. monocytogenes* cells is expected from the set of exposure data, leading mathematically to no expected cases at or above 8.0 log_{10}.

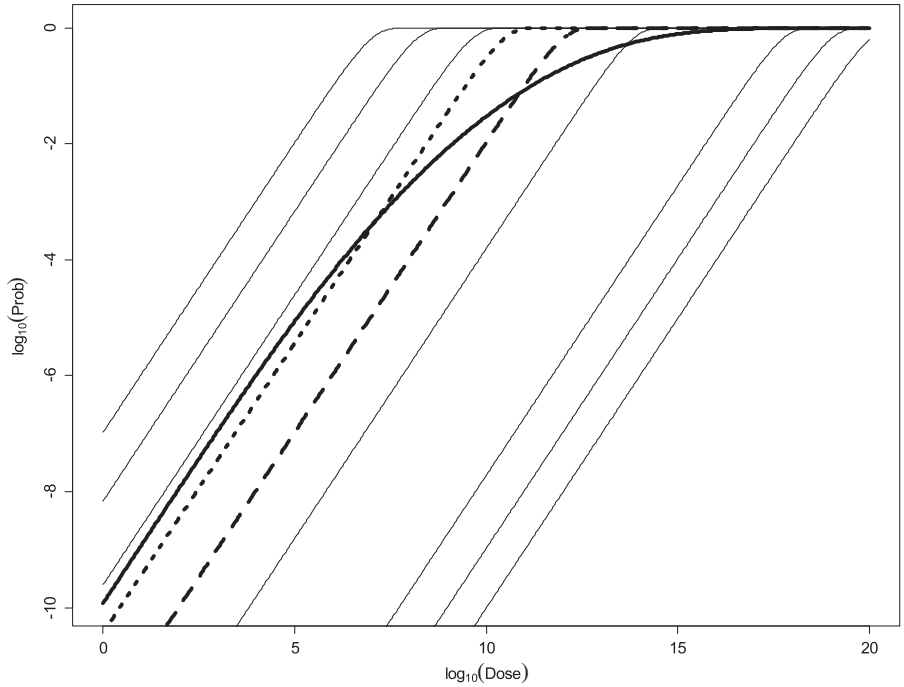

Fig. 2. Marginal lognormal-Poisson dose-response model for the total population (black solid line) and exponential dose-response model for r in the 0.01st, 0.1st, 1st, 50th, 99th, 99.9th, and 99.99th percentiles of the strain and individual distribution (thin black lines, from right to left). These estimates are compared to the dose-response relationships generated by FAO/WHO[5] for invasive listeriosis in the fraction of the population with increased susceptibility ($r = 1.06 \times 10^{-12}$; see Ref. 5, p. 56) (dashed line) and by Chen *et al.*[24] for *L. monocytogenes* with genes encoding a full-length *inlA* for the 25% higher-risk population ($\log_{10}(r) = -10.44$; dotted line).

ingestion of a highly virulent *L. monocytogenes* strain by a highly susceptible individual. Moreover, small population subgroups with extremely high susceptibility may not be adequately reflected in such dose-response relationships, potentially explaining at least in part why traditional exponential dose-response models of *L. monocytogenes* could so far not be reconciled with outbreak data.

The lognormal-Poisson dose-response models derived here extend and advance *L. monocytogenes* dose-response modeling to explicitly consider variability in strain virulence and in susceptibility across population subgroups. As such, the extended model more accurately captures the risk of listeriosis in those population subgroups at highest risk of listeriosis. Because the relative risk of listeriosis has been shown to vary by as much as 1,000-fold across population subgroups with clearly defined risk factors,[26] the ability to accurately characterize the listeriosis risk for different population subgroups is of paramount importance for risk management and for a comprehensive characterization of the listeriosis risk posed by different RTE food items. Similarly, strains differ considerably in virulence. Chen *et al.*[24] found a 2–3 $\log_{10}$ difference in the marginal exponential dose-response parameters r for *L. monocytogenes* subtypes encoding a full length or truncated version of *inlA,* respectively. In a guinea pig model, Van Stelten *et al.*[23] found more than a 1 $\log_{10}$ increase in median infectious dose for a *L. monocytogenes* strain carrying a premature stop codon (PMSC) in *inlA* compared to that for an epidemic clone. Accounting for variability in strain virulence is therefore clearly of great importance. The variation in virulence used in this study (i.e., variability of 5 $\log_{10}$ based on inter 5th–95th percentiles) is higher than the differences in strain virulence that would be expected based on the data for strains with and without PMSCs in *inlA*. However, other virulence factors likely also contribute to virulence differences among *L. monocytogenes* strains.[21,45] Therefore, the true variability in strain virulence is likely larger than that estimated solely based on differences in *inlA* alleles. In addition, food matrix effects were implicitly accounted for in the variability in strain virulence, thus likely also increasing

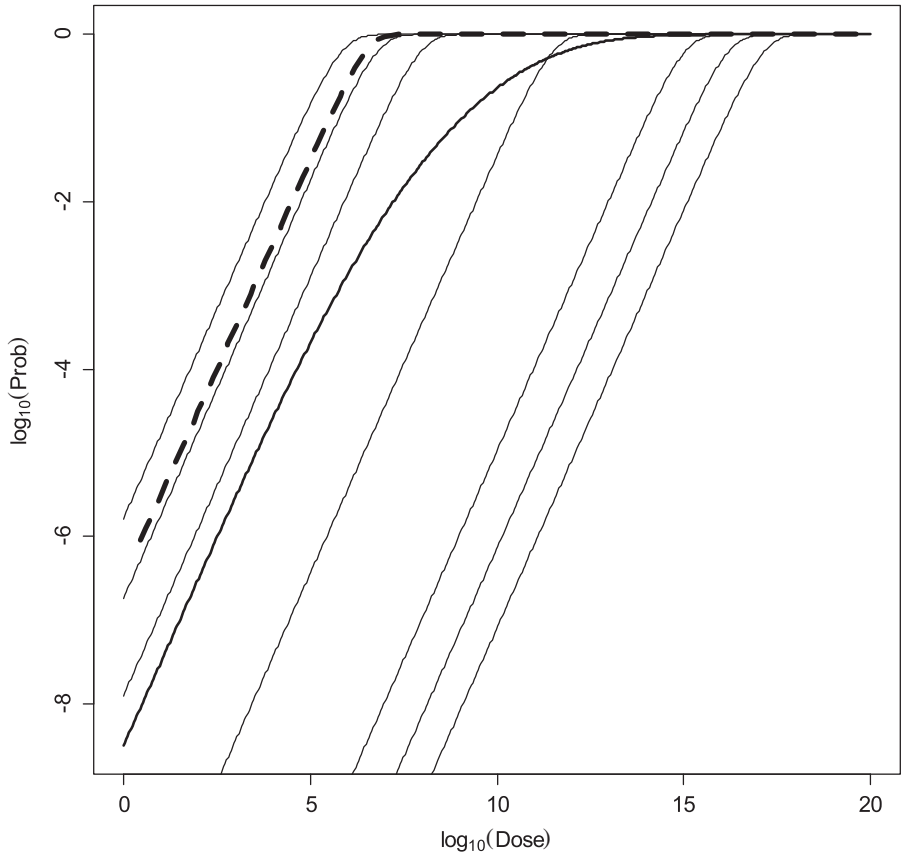

Fig. 3. Lognormal dose-response relationships for invasive listeriosis following the ingestion of *L. monocytogenes*, comparing the marginal dose response for the transplant recipient population (solid thick line), the dose response for individual strains with virulence in the 0.01st, 0.1st, 1st, 50th, 99th, 99.9th, and 99.99th percentiles of the virulence distribution (thin lines, from right to left), and the exponential dose-response model for invasive listeriosis based on a butter outbreak in Finland, 1998–1999,[31] as reexamined by FAO/WHO ($r = 3.15 \times 10^{-7}$,[5] p. 34; dashed line).

variability. Despite the progress that has been made in recent years, a better understanding of virulence differences among *L. monocytogenes* strains and, in particular, experimental data evaluating the potential impact of food matrix effects, is clearly needed to further refine *L. monocytogenes* dose-response models.

4.2. Beta-Poisson vs. Lognormal-Poisson Dose Response

The beta distribution was introduced as a pragmatic choice to model the variability in r.[6,39] It offers a great amount of flexibility on the [0; 1] domain,[6] but a mechanistic basis for the choice of beta distributions is lacking. In the case of *L. monocytogenes*, the expected value of r is extremely low when averaging over the general population or even over relatively broadly defined susceptible population subgroups, leading to extremely high values of parameter β. The shape of the beta distribution when used with such extreme parameters does not allow sufficient flexibility, making it impossible to fit the model to certain epidemiological listeriosis data, including the Finnish outbreak data, as illustrated in the Appendix. Therefore, even though the beta-Poisson represents a useful and often-used choice for modeling a number of foodborne pathogens, it appears suboptimal for the unique case of *L. monocytogenes* as evaluated here. Interestingly, if a gamma distribution with $r \sim \text{gamma}(\alpha, 1/\beta)$, with a negligible probability of $r > 1$, would be used to describe r variability, the associated probability of infection would also lead to the beta-Poisson dose-response model (Equation (12)).[8,46] Our result thus suggests that the use of a gamma distribution to model r would

similarly not be suitable for the unique case of *L. monocytogenes* dose response.

We used a lognormal distribution to model variability in host susceptibility and strain virulence, leading to a "lognormal-Poisson" dose-response relationship. Importantly, the resulting lognormal-Poisson dose-response equation does not simplify to a simple mathematical formula and requires numerical integration, thus making the use of this model mathematically more challenging. The domain of the lognormal distribution is defined as $[0; \infty)$. Yet, in this study we found that even for the most susceptible population subgroup (i.e., hematological cancer patients) the probability of r exceeding 1 is estimated at 4.5×10^{-12}, thus in the order of 1 in a trillion, and therefore *de facto* negligible. Because the probability of r exceeding 1 is *de facto* zero, r is theoretically $[0; \infty)$ but practically distributed on the domain $[0; 1]$ in the considered *L. monocytogenes* case. Importantly, this is most likely not true for pathogens other than *L. monocytogenes.* For other foodborne pathogens, the probability of illness after ingestion of a single cell is usually much higher than that for *L. monocytogenes* and the probability of $r > 1$ would be nonnegligible, which would make it incorrect to use the lognormal-Poisson dose response. The lognormal distribution is a heavy-tail distribution. Using heavy-tail distributions is an appropriate modeling assumption if the objective is to describe extreme events such as the ingestion of a highly virulent *L. monocytogenes* strain by a highly susceptible individual. Importantly, the lognormal-Poisson dose-response model was able to predict a well-described outbreak of listeriosis where traditional models of *L. monocytogenes* dose response failed to do so, indicating the potential usefulness of this model.

4.3. Limitations of the Currently Available Data

Whenever possible, health-protective assumptions that would lead to estimating a higher probability of infection for low doses were preferentially chosen in this study. However, the potential impact of some assumptions is more difficult to evaluate than for others. For instance, French data were used as the basis of extrapolations of the expected number of listeriosis cases per population subgroup in the United States. This extrapolation appears appropriate for several reasons. One key finding of the FAO/WHO[5] risk assessment of *L. monocytogenes* in RTE foods is a lack of evidence for differences in the risk of listeriosis after consumption of a given *L. monocytogenes* dose by a member of given population subgroup across countries. Similarly, epidemiological studies have shown that the relative risk of listeriosis for pregnant women appears to be comparable between France and the United States.[26,27] Unfortunately, data on the relative risk of listeriosis is currently lacking for other population subgroups in the United States.[27] It was estimated that for each case of invasive listeriosis, 1.1 cases were not diagnosed in the United States.[1] This figure might be higher in neonatal and elderly cases as compared to other subpopulations.[47] Due to a lack of information, we have not addressed this uncertainty in the partitioning of the total number of cases in the United States among the different population subgroups.

In addition, the French relative size of population subgroups was directly extrapolated to the U.S. population. Even though certain indicators, such as the proportion of individuals with diabetes, are not the same in France and in the United States,[48] some major demographic parameters relevant in this study appear comparable between these countries, such as the proportion of people under 65 year of age, the proportion of people living with cancer, the fertility rates, and life expectancies.[49,50] Actually, the estimation of the relative size of population subgroups in the French study is based on a rigorous, specific, and complicated method designed to avoid duplicated counts.[26] Therefore, it appears preferable to use the French estimates directly rather than further adjusting the estimates to the relative size of U.S. populations with similar comorbidities.

For every risk assessment anchored to human surveillance data—such as our risk assessment presented here—the assumptions used to estimate exposure data highly influence the dose-response model and prediction. If it is estimated that only a small number of bacteria are consumed, any dose-response scaled to epidemiological data will mathematically be shifted to the left (i.e., toward a higher risk at low dose). We used data from Chen *et al.*,[30] which was the most extensive food survey in the United States on record. However, even this large of a study may not capture the true variability in the numbers of *L. monocytogenes* in RTE foods, particularly for the high end of the concentration distribution, and thus may be considered as underestimating exposure. Using these data leads to three implicit assumptions: (i) all bacterial cells consumed

in the population originate from only eight RTE food categories (i.e., fresh soft cheeses, bagged salad, blue veined cheeses, mold ripened cheeses, seafood salads, smoked seafood, luncheon meats, and deli salads) even though other products, such as low acid cut fruits[51,52] or vegetables,[53] could also be nonnegligible sources of *L. monocytogenes*; (ii) no growth is considered to occur between retail and consumption even though postretail growth has been shown to be one important factor increasing the risk for listeriosis[4,5]—these data have the advantage of being actual observed *L. monocytogenes* levels originating from a market basket survey[43] and not relying on predictive modeling that may overestimate the bacterial growth in products; and (iii) the maximal achievable concentration of *L. monocytogenes* in products equals 6.1 $\log_{10}$ cfu/g. This assumption is also underestimating exposure since others assume that *L. monocytogenes* can reach a maximal population density of 8 $\log_{10}$ in a food.[4,5] Altogether, these assumptions lead to an estimated lower exposure compared to other available data sets. In our study, it is estimated that only 120 servings include *L. monocytogenes* levels at or above 10^8 cells each year in the United States; by comparison, the FDA/FSIS[4] report, considering bacterial growth at the consumer step and 23 contaminated products, estimates 70,000,000+ servings at these levels. When tested with a maximum level of *L. monocytogenes* contamination of 8.1 $\log_{10}$ cfu/g, we confirmed the shift of the corresponding dose response to the right: with this maximum level, 0% of the cases would be predicted for a dose of 4 $\log_{10}$ cfu/g. Indeed, the maximum population density in a food has been shown to be an influential parameter for the predicted risk of invasive listeriosis.[54,55] Given the same dose response, the higher the maximum population density, the higher the predicted number of cases.[54,55] In addition, assumption on the maximum population density affects dose-response model parameters based on surveillance data.[5] The FAO/WHO risk assessment of *L. monocytogenes* in RTE foods[5] shows that a shift in the maximum population density by 2 $\log_{10}$ results in approximately one order of magnitude shift in the *r* value. The resulting dose-response presented here may be overestimating the probability of illness from a given dose.

As considered in previous risk assessments,[4,5] the assumption of equal exposure to contaminated food for all population subgroups does not consider the potential effectiveness of prevention campaigns to change behavior of susceptible populations, notably for pregnant women, people with cancer, transplant recipients, for older adults, or for people with diabetes. Reported consumption estimates for certain food types suggests differences do exist in food consumption across population subgroups.[25,56] Nevertheless, the model appeared relatively insensitive to this assumption when tested with an alternative assumption of a lower exposure for the more susceptible subgroups than for "healthy adults." Refinements accounting for differences in consumption habits across population subgroups would improve the current dose-response models. However, such data are currently not available for many of the 11 population subgroups analyzed here.

4.4. Dose-response Evaluation in Highly Susceptible Groups and in Outbreak Situations

For the most susceptible population subgroup (i.e., hematological cancer patients), the marginal probability (i.e., averaged across all strains) of illness following the ingestion of 1 *L. monocytogenes* cell is estimated at 9.5×10^{-9}. It is 9.3×10^{-7} following the ingestion of 100 cells and 7.2×10^{-5} for the ingestion of 10,000 cells (e.g., 100 g of product contaminated with 100 cfu/g). These estimates are considerably higher than the ones estimated by FAO/WHO,[5] averaged over all possible risk factors. The corresponding estimates, using their *r* parameter of 5.85×10^{-12}, would be 5.9×10^{-12}, 5.9×10^{-10}, and 5.9×10^{-8}, respectively, that is, 1,610, 1,576, and 1,220 times lower, respectively.

By characterizing specifically the most susceptible individuals and the most virulent strains in this study, the lognormal-Poisson dose-response analysis reconciles data observed in outbreaks with dose response derived from epidemiological studies, as illustrated Fig. 3. The high fat content of the food vehicle in the Finish butter outbreak (~80% fat) could potentially be partially responsible for this high probability of infection. High fat content in food may actually protect bacteria from gastric acid and, possibly, enhance uptake and survival in host cells *via* interaction with cell membrane lipids.[4,57]

4.5. The Need for Better Data

Assumptions were made in the derivation of this model that lead to higher risk predictions at low dose

(higher predicted marginal probability of illness) compared to previously published dose-response models.[4,5] The estimates presented here should generally be viewed as overestimating the probability of illness. The characterization of the range of the individual susceptibility within groups and of the range of the strain virulence variability should be refined for a better characterization of these dose-response relationships. A mix of illness data from France[26] and the United States,[1] and exposure data obtained in two states from the United States,[30,43] were used, with the underlying assumptions that characteristics of listeriosis would be comparable in these areas. More current and detailed exposure data and data on the relative risk of listeriosis among different population subgroups in the United States are needed to refine this model. The primary purpose of this study was to derive a framework and to test with currently available data; to provide a definitive dose-response model is a secondary goal that would likely require refinements.

5. CONCLUSIONS

The exponential model has the oversimplifying assumption of a constant probability of infection following the ingestion of *L. monocytogenes* in a given population. This study incorporates variability in strain virulence and host susceptibility into the dose-response relationships. Additional data are needed to better understand and model the process from the ingestion of *L. monocytogenes* cells to the development of invasive listeriosis. However, several general conclusions can be made based on the available data. Overall, our model predicts the expected number of cases linked to the consumption of 10,000 cfu or less in 55 out of 1,591 cases, i.e., 3.5% of cases. Notably, these servings are expected to represent 99.96% of all RTE servings, indicating that most cases are expected to be caused by highly contaminated food items. Importantly, however, most of these cases attributable to low contamination doses are predicted to occur in the most highly susceptible population subgroups, including, for example, pregnant women. Using the model and assumptions discussed above led to the conclusion that, while most of the cases are linked to a medium to high exposure doses to *L. monocytogenes*, those at greatest risk of developing listeriosis are also at a measurable risk of illness when consuming food contaminated with relatively low doses of *L. monocytogenes,* especially if highly virulent bacterial strains are involved.

ACKNOWLEDGMENTS

This work was supported, in part, by an appointment to the Research Participation Program at the Center for Food Safety and Applied Nutrition administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration. We thank Eric Ebel (USDA-FSIS) and the anonymous reviewers for their constructive comments on the first draft of the article.

APPENDIX: DERIVATIONS USING A BETA-POISSON MODEL

If a beta distribution $Be(\alpha, \beta)$ is chosen for f in Equation (2), this integrate leads to the "exact beta-Poisson,"[6]

$$P(\text{ill}; d, \alpha, \beta) = 1 - {}_1F_1(\alpha, \alpha + \beta, -d), \qquad \text{(A.1)}$$

in which ${}_1F_1$ is the Kummer confluent hypergeometric function. Equation (A.1) simplifies to the "beta-Poisson" dose-response model:

$$P(\text{ill}; d, \alpha, \beta) = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha}, \qquad \text{(A.2)}$$

when $\beta \gg \alpha$ and $\beta \gg 1$.[7,39] Note that these conditions are expected to be fulfilled for *L. monocytogenes*: the average probability of infection is very low,[30] thus $E[r] = \frac{\alpha}{\alpha+\beta} \ll 1$, leading to $\beta \gg \alpha$ and $\beta \gg 1$.

Assume $Be(\alpha_g, \beta_g)$ accounts for variability in r among *L. monocytogenes* strains and individuals within a given population subgroup g. Contrary to the lognormal distribution, the beta distribution does not easily allow for separation among interstrain and interindividual variability components of this distribution as in Equation (10). An overall (i.e., interindividual and interstrain) measure of the variability in r therefore needs to be estimated. Denote Q_{90}, the $\log_{10}$ of the combined 90% individual susceptibility and strain virulence variability. Q_{90} equals the range between the 5th and the 95th percentile of $Be(\alpha_g, \beta_g)$. Using and combining FDA

FDA/FSIS[4] strain-to-strain virulence variability distributions (Table IV-5 in Ref. 4) and host susceptibility variability (Table IV-7 in Ref. 4) lead to an overall $\log_{10}$ of the inter 5%–95% variability of $Q_{90} = 5.4$ $\log_{10}$.

Equivalently to Equation (9), the subroutine must find (α_g, β_g) solutions of:

$$\begin{aligned} E[c_g] &= \int_0^{\infty} M_{d,g} \left(1 - \left(1 + \frac{d}{\beta_g}\right)^{-\alpha_g}\right) \mathrm{d}d \\ Q_{90} &= \log_{10}(q_{0.95}) - \log_{10}(q_{0.05}) \end{aligned}, \quad \text{(A.3)}$$

with q_x the xth quantile of the $Be(\alpha_g, \beta_g)$ distribution.

The quantile function of beta distributions is not available in a closed form, and a numerical routine is required. Nevertheless, a solution exists for the parameters of a beta distribution given any combination of a lower and an upper quantile constraint.[58] The 11 pairs (α_g, β_g) were evaluated numerically using R optimization subroutines. As expected, the βs were extremely high. Similar αs were obtained for all populations. The parameters for the "healthy adult population" (i.e., the less susceptible subgroup) and the "hematological cancer population" (i.e., the more susceptible subgroup) were $(0.253, 3.86 \times 10^{10})$ and $(0.253, 9.9 \times 10^{7})$, respectively.

A $Be(0.253, 2.3 \times 10^{8})$ was estimated for the "solid organ transplant" population subgroup. With this set of parameters, the probability to obtain a r parameter equal or higher than 3.15×10^{-7}, estimated from the Finnish butter outbreak data,[5,31] equals 2.7×10^{-34}. This extremely low probability proves that the Finnish outbreak cannot be predicted using the beta-Poisson dose-response model, as parameterized here. Fig. A.1 illustrates the density of the underlying beta distribution of the beta-Poisson dose-response model and the underlying lognormal distribution of the lognormal-Poisson dose-response models. The graph clearly illustrates the contrast between the very sharp decrease in the density for the beta distribution compared to the smoother decrease for the lognormal distribution. With such parameters ($\beta \to \infty$), the beta distribution converge to a degenerate distribution with a single point mass at some $x \in [0, 1]$.[58] With parameters estimated from epidemiological data, the beta distribution is not flexible enough to predict r values high enough to explain the Finnish butter outbreak.

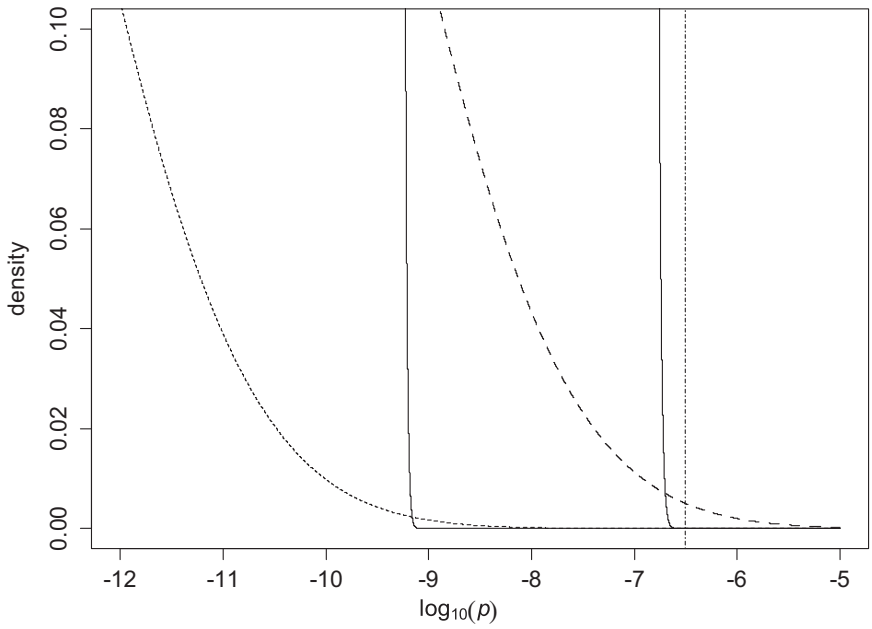

Fig. A.1. 1 Density of r according to the beta-Poisson dose response (plain) or the lognormal-Poisson dose response (dashed) for the healthy population (thin on the left) and the most susceptible population subgroup (hematological cancer population, thick on the right). The values estimated using the Finnish butter outbreak data by FAO/WHO[5] equals 3.15×10^{-7}, that is, $10^{-6.5}$ (dot-dashed vertical line).

REFERENCES

1. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. Foodborne illness acquired in the United States—Major pathogens. Emerging Infectious Diseases, 2011; 17(1):7–12.
2. Hoelzer K, Chen Y, Dennis S, Evans P, Pouillot R, Silk BJ, Walls I. New data, strategies, and insights for *Listeria monocytogenes* dose-response models: Summary of an interagency workshop, 2011. Risk Analysis, 2013; 33(9):1568–1581.
3. Hoelzer K, Pouillot R, Dennis S. Animal models of listeriosis: A comparative review of the current state of the art and lessons learned. Veterinary Research, 2012; 43(1):1–27.
4. FDA/FSIS. Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Food and Drug Administration, U.S. Department of Agriculture, Centers for Disease Control and Prevention, 2003. Available at: http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm183966.htm, Accessed March 20, 2014.
5. FAO/WHO. Risk assessment of *Listeria monocytogenes* in ready to eat foods—Technical report. Rome: Food and Agriculture Organization of the United Nations and World Health Organization Report No.: Microbiological Risk Assessment Series 5; 2004. Available at: http://www.fao.org/docrep/010/y5394e/y5394e00.htm, Accessed March 20, 2014.
6. Haas CN, Rose JB, Gerba CP. Quantitative Microbial Risk Assessment. New York: Wiley, 1999.
7. Teunis PF, Havelaar AH. The Beta Poisson dose-response model is not a single-hit model. Risk Analysis, 2000; 20(4):513–520.
8. 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 Analysis, 2013; 33(9):1677–1693.
9. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV. Food-related illness and death in the United States. Emerging Infectious Diseases, 1999; 5(5):607–625.
10. FDA/FSIS. Draft assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Food and Drug Administration, U.S. Department of Agriculture, Centers for Disease Control and Prevention, 2001.
11. Ross T, Rasmussen S, Fazil A, Paoli G, Summer J. Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat meats in Australia. International Journal of Food Microbiology, 2009; 131(2–3):128–137.
12. Sanaa M, Coroller L, Cerf O. Risk assessment of listeriosis linked to the consumption of two soft cheeses made from raw milk: Camembert of Normandy and Brie of Meaux. Risk Analysis, 2004; 24(2):389–399.
13. Pouillot R, Goulet V, Delignette-Muller ML, Mahe A, Cornu M. Quantitative risk assessment of *Listeria monocytogenes* in French cold-smoked salmon: II. Risk characterization. Risk Analysis, 2009; 29(6):806–819.
14. Gallagher D, Ebel ED, Gallagher O, Labarre D, Williams MS, Golden NJ, Pouillot R, Dearfield KL, Kause J. Characterizing uncertainty when evaluating risk management metrics: Risk assessment modeling of *Listeria monocytogenes* contamination in ready-to-eat deli meats. International Journal of Food Microbiology, 2013; 162(3):266–275.
15. Jacquet C, Doumith M, Gordon JI, Martin PM, Cossart P, Lecuit M. A molecular marker for evaluating the pathogenic potential of foodborne *Listeria monocytogenes*. Journal of Infectious Diseases, 2004; 189(11):2094–2100.
16. Nightingale KK, Ivy RA, Ho AJ, Fortes ED, Njaa BL, Peters RM, Wiedmann M. *inlA* premature stop codons are common among *Listeria monocytogenes* isolates from foods and yield virulence-attenuated strains that confer protection against fully virulent strains. Applied and Environmental Microbiology, 2008; 74(21):6570–6583.
17. Nightingale KK, Windham K, Martin KE, Yeung M, Wiedmann M. Select *Listeria monocytogenes* subtypes commonly found in foods carry distinct nonsense mutations in *inlA*, leading to expression of truncated and secreted internalin A, and are associated with a reduced invasion phenotype for human intestinal epithelial cells. Applied and Environmental Microbiology, 2005; 71(12):8764–8772.
18. Ward TJ, Evans P, Wiedmann M, Usgaard T, Roof SE, Stroika SG, Hise K. Molecular and phenotypic characterization of *Listeria monocytogenes* from U.S. Department of Agriculture Food Safety and Inspection Service surveillance of ready-to-eat foods and processing facilities. Journal of Food Protection, 2010; 73:861–869.
19. Bonazzi M, Lecuit M, Cossart P. *Listeria monocytogenes* internalin and E-cadherin: From bench to bedside. Cold Spring Harbor Perspectives in Biology, 2009; 1(4):1–5.
20. Bonazzi M, Lecuit M, Cossart P. *Listeria monocytogenes* internalin and E-cadherin: From structure to pathogenesis. Cellular Microbiology, 2009; 11(5):693–702.
21. Lecuit M. Understanding how *Listeria monocytogenes* targets and crosses host barriers. Clinical Microbiology and Infection, 2005; 11(6):430–436.
22. Lecuit M, Vandormael-Pournin S, Lefort J, Huerre M, Gounon P, Dupuy C, Babinet C, Cossart P. A transgenic model for listeriosis: Role of internalin in crossing the intestinal barrier. Science, 2001; 292(5522):1722–1725.
23. Van Stelten A, Simpson JM, Chen Y, Scott VN, Whiting RC, Ross WH, Nightingale KK. Significant shift in median guinea pig infectious dose shown by an outbreak-associated *Listeria monocytogenes* epidemic clone strain and a strain carrying a premature stop codon mutation in *inlA*. Applied and Environmental Microbiology, 2011; 77(7):2479–2487.
24. Chen Y, Ross WH, Whiting RC, Van Stelten A, Nightingale KK, Wiedmann M, Scott VN. Variation in *Listeria monocytogenes* dose responses in relation to subtypes encoding a full-length or truncated internalin A. Applied and Environmental Microbiology, 2011; 77(4):1171–1180.
25. Silk BJ, Date KA, Jackson KA, Pouillot R, Holt KG, Graves LM, Ong KL, Hurd S, Meyer R, Marcus R, Shiferaw B, Norton DM, Medus C, Zansky SM, Cronquist AB, Henao OL, Jones TF, Vugia DJ, Farley MM, Mahon BE. Invasive listeriosis in the Foodborne Diseases Active Surveillance Network (FoodNet), 2004–2009: Further targeted prevention needed for higher-risk groups. Clinical Infectious Diseases, 2012; 54 (Suppl 5):S396–404.
26. Goulet V, Hebert M, Hedberg C, Laurent E, Vaillant V, De Valk H, Desenclos JC. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. Clinical Infectious Diseases, 2012; 54(5):652–660.
27. Pouillot R, Hoelzer K, Jackson KA, Henao OL, Silk BJ. Relative risk of listeriosis in Foodborne Diseases Active Surveillance Network (FoodNet) sites according to age, pregnancy, and ethnicity. Clinical Infectious Diseases, 2012; 54(Suppl 5):S405–410.
28. Mook P, O'Brien SJ, Gillespie IA. Concurrent conditions and human listeriosis, England, 1999–2009. Emerging Infectious Diseases, 2011; 17(1):38–43.
29. Buchanan RL, Havelaar AH, Smith MA, Whiting RC, Julien E. The key events dose-response framework: Its potential for application to foodborne pathogenic microorganisms. Critical Reviews in Food Science and Nutrition, 2009; 49(8):718–728.

30. Chen Y, Ross WH, Scott VN, Gombas DE. *Listeria monocytogenes:* Low levels equal low risk. Journal of Food Protection, 2003; 66(4):570–577.
31. Lyytikainen O, Autio T, Maijala R, Ruutu P, Honkanen-Buzalski T, Miettinen M, Hatakka M, Mikkola J, Anttila VJ, Johansson T, Rantala L, Aalto T, Korkeala H, Siitonen A. An outbreak of *Listeria monocytogenes* serotype 3a infections from butter in Finland. Journal of infectious Diseases, 2000; 181(5):1838–1841.
32. Bollaerts K, Aerts M, Faes C, Grijspeerdt K, Dewulf J, Mintiens K. Human salmonellosis: Estimation of dose-illness from outbreak data. Risk Analysis, 2008; 28(2):427–440.
33. FAO/WHO. Risk Assessment of *Salmonella* in eggs and broiler chickens. Technical report. Rome: Food and Agriculture Organization of the United Nations and World Health Organization Report No.: Microbiological Risk Assessment Series 3; 2002. Available at: http://www.fao.org/DOCREP/005/Y4392E/Y4392E00.HTM, Accessed March 10, 2014.
34. Teunis PFM, Kasuga F, Fazil A, Ogden ID, Rotariu O, Strachan NJC. Dose response modeling of *Salmonella* using outbreak data. International Journal of Food Microbiology, 2010; 144(2):243–249.
35. Teunis P, Van den Brandhof W, Nauta M, Wagenaar J, Van den Kerkhof H, Van Pelt W. A reconsideration of the *Campylobacter* dose-response relation. Epidemiology and Infection, 2005; 133(4):583–592.
36. Teunis PF, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, Le Pendu J, Calderon RL. Norwalk virus: How infectious is it? Journal of Medical Virology, 2008; 80(8):1468–1476.
37. Thebault A, Teunis PF, Le Pendu J, Le Guyader FS, Denis JB. Infectivity of GI and GII noroviruses established from oyster related outbreaks. Epidemics, 2013; 5(2):98–110.
38. Rocourt J. Risk factors for listeriosis. Food Control, 1996; 7(4–5):195–202.
39. Furumoto WA, Mickey R. A mathematical model for the infectivity-dilution curve of tobacco mosaic virus: Theoretical considerations. Virology, 1967; 32(2):216–223.
40. Teunis PF, Nagelkerke NJ, Haas CN. Dose response models for infectious gastroenteritis. Risk Analysis, 1999; 19(6):1251–1260.
41. Haas CN, Thayyar- Madabusi A, Rose JB, Gerba CP. Development and validation of dose-response relationship for *Listeria monocytogenes*. Quantitative Microbiology, 1999; 1(1):89–102.
42. Goulet V, Marchetti P. Listeriosis in 225 non-pregnant patients in 1992: Clinical aspects and outcome in relation to predisposing conditions. Scandinavian Journal of Infectious Diseases, 1996; 28(4):367–374.
43. Gombas DE, Chen Y, Clavero RS, Scott VN. Survey of *Listeria monocytogenes* in ready-to-eat foods. Journal of Food Protection, 2003; 66(4):559–569.
44. R Development Core Team. R: A language and environment for statistical computing 2008 [cited March 20, 2012]. Available at: http://www.R-project.org, Accessed March 20, 2014.
45. Cossart P, Pizarro-Cerda J, Lecuit M. Invasion of mammalian cells by *Listeria monocytogenes*: Functional mimicry to subvert cellular functions. Trends in Cell Biology, 2003; 13(1): 23–31.
46. Moran PA. The dilution assay of viruses. Journal of Hygiene, 1954; 52(2):189–193.
47. Painter J, Slutsker L. Listeriosis in humans. Pp. 85–109 in Ryser ET, Marth EH (eds). *Listeria*, Listeriosis and Food Safety. 3rd ed. Boca Raton: CRC Press, 2007.
48. CDC. 2011 National Diabetes Fact Sheet 2011 [cited January 17, 2014]. Available at: http://www.cdc.gov/diabetes/pubs/pdf/ndfs˙2011.pdf, Accessed March 20, 2014.
49. American Cancer Society. Cancer Facts & Figures 2012. Atlanta: American Cancer Society, 2012. Available at: http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-031941.pdf, Accessed March 20, 2014.
50. World Bank. Indicators 2014 [cited January 17, 2014]. Available at: http://data.worldbank.org/indicator, Accessed March 20, 2014.
51. Anonymous. Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe—United States, August–September 2011. Morbidity and Mortality Weekly Report, 2011; 60(39):1357–1358.
52. Varma JK, Samuel MC, Marcus R, Hoekstra RM, Medus C, Segler S, Anderson BJ, Jones TF, Shiferaw B, Haubert N, Megginson M, McCarthy PV, Graves L, Gilder TV, Angulo FJ. *Listeria monocytogenes* infection from foods prepared in a commercial establishment: A case-control study of potential sources of sporadic illness in the United States. Clinical Infectious Diseases, 2007; 44(4):521–528.
53. Gaul LK, Farag NH, Shim T, Kingsley MA, Silk BJ, Hyytia-Trees E. Hospital-acquired listeriosis outbreak caused by contaminated diced celery—Texas, 2010. Clinical Infectious Diseases, 2013; 56(1):20–26.
54. Ellouze M, Gauchi JP, Augustin JC. Global sensitivity analysis applied to a contamination assessment model of *Listeria monocytogenes* in cold smoked salmon at consumption. Risk Analysis, 2010; 30(5):841–852.
55. Pouillot R, Lubran MB. Predictive microbiology models *vs*. modeling microbial growth within *Listeria monocytogenes* risk assessment: What parameters matter and why. Food Microbiology, 2011; 28(4):720–726.
56. Hoelzer K, Pouillot R, Egan K, Dennis S. Produce consumption in the United States: An analysis of consumption frequencies, serving sizes, processing forms, and high-consuming population subgroups for microbial risk assessments. Journal of Food Protection, 2012; 75(2):328–340.
57. Coleman M, Marks H. Topics in dose-response modeling. Journal of Food Protection, 1998; 61(11):1550–1559.
58. van Dorp JR, Mazzuchi TA. Solving for the parameters of a beta distribution under two quantile constraints. Journal of Statistical Computation and Simulation, 2000; 67(2): 189–201.