Journal of Food Protection, Vol. 70, No. 2, 2007, Pages 363–372

Second-Order Modeling of Variability and Uncertainty in Microbial Hazard Characterization

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MS 06-163: Received 20 March 2006/Accepted 5 July 2006

ABSTRACT

This study describes an analytical framework that permits quantitative consideration of variability and uncertainty in microbial hazard characterization. Second-order modeling that used two-dimensional Monte Carlo simulation and stratification into homogeneous population subgroups was applied to integrate uncertainty and variability. Specifically, the bootstrap method was used to simulate sampling error due to the limited sample size in microbial dose-response modeling. A data set from human feeding trials with *Campylobacter jejuni* was fitted to the log-logistic dose-response model, and results from the analysis of FoodNet surveillance data provided further information on variability and uncertainty in *Campylobacter* susceptibility due to the effect of age. Results of our analyses indicate that uncertainty associated with dose-response modeling has a dominating influence on the analytical outcome. In contrast, inclusion of the age factor has a limited impact. While the advocacy of more closely modeling variability in hazard characterization is warranted, the characterization of key sources of uncertainties and their consistent propagation throughout a microbial risk assessment actually appear of greater importance.

Over the past decade, heightened awareness about the consequences of foodborne illnesses has stimulated the application of quantitative risk assessment to food safety problems *(5, 21, 24).* A four-step paradigm—hazard identification, exposure assessment, hazard characterization, and risk characterization—has been commonly followed *(9).* Practically, hazard characterization has taken the form of dose-response assessment, in which a mathematical function links an exposure dose to an infection or illness probability. In some instances, a constant has been used to adjust for increased susceptibility in a population subgroup or for animal-to-human extrapolation *(7, 31).* A more thorough consideration of the multifaceted interaction between pathogen, host, and food medium is commonly advocated *(9).* For example, the development of methods that can incorporate the impact of host factors, such as age and immune status, on susceptibility is deemed necessary *(20).*

Quantitative risk assessment models are commonly the objects of a probabilistic analysis *(23).* Within this context, two distinct sources of variation—uncertainty and variability—are often concurrent. Variability relates to the interindividual, temporal, or spatial heterogeneity of the population under study, while uncertainty refers to the lack of knowledge about specific factors, parameters, or models *(4, 14, 29).* Environmental risk assessors have pointed out that, when interpreting the results of probabilistic risk assessment, variability and uncertainty have different implications *(2, 4, 17, 18).* The distinction is particularly relevant for policy reasons. An understanding of sources of variability permits a characterization of risks that are specific to those population subgroups of potential concern to a decision maker (e.g., children, pregnant women, immunocompromised individuals) or a targeting of risk management strategies to controllable sources of variation. In contrast, knowledge of sources of uncertainty can both qualify the certitude of the conclusions reached and aid in determining where additional research can be focused in order to reduce uncertainty *(14).* Separation of uncertainty and variability throughout a risk assessment model is consequently advocated *(10, 20, 30).*

Conceptualization of uncertainty and variability in microbial risk assessment has largely mimicked research done in the field of environmental risk assessment. The utility of probabilistic analysis has long been recognized *(21),* and this approach nowadays prevails *(23).* Within this context, the different implications of uncertainty and variability have likewise been noted *(27).* Separation of variability and uncertainty is increasingly being carried out. For example, separation of variability and uncertainty was extensively employed in the risk assessment for *Listeria monocytogenes* in selected ready-to-eat foods by means of a two-dimensional Monte Carlo simulation *(7).* However, the two-dimensional framework was not maintained throughout the model. The two-dimensional exposure assessment was actually converted into a one-dimensional simulation (viewed to represent uncertainty only) to make dose-response modeling possible. Another example includes the draft risk assessment for *Escherichia coli* O157:H7 in ground beef for which separation of variability and uncertainty was selec-

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TABLE 1. *Data from* Campylobacter jejuni *human feeding trial*

Dose (CFU)	Total no. of exposed subjects	No. of subjects infected[a]	No. of subjects with fever or diarrhea
8×10^2	10	5	2
8×10^3	10	6	1
8×10^4	13	11	8
8×10^5	11	8	1
1×10^6	19	15	3
1×10^8	5	5	0

[a] Infected subjects are defined as those volunteers with positive stool cultures.

tively used for most parts of the model, specifically in the exposure assessment module. However, the hazard characterization module of the model did not maintain the two-dimensional framework *(37).*

It is perhaps not coincidental that dose-response assessment represents such an impediment to the realization of second-order modeling. While microbial risk assessors have often modeled exposure in great detail, hazard characterization has essentially relied on the predictive capability of dose-response functions. Quantitative approaches have been proposed to deal with model uncertainty *(22, 25).* However, although confidence bands of specific microbial dose-response functions have been examined *(33),* there has never been an attempt to incorporate parameter uncertainty associated with the dose-response relationship into a risk assessment, to our knowledge.

This study develops an analytical framework that permits a quantitative consideration of variability and uncertainty in microbial hazard characterization. Second-order modeling that employs two-dimensional Monte Carlo simulation and stratification into homogeneous population subgroups was applied to integrate uncertainty and variability. Specifically, the bootstrap method was used to simulate sampling error due to the limited sample size in microbial dose-response modeling *(16).* To demonstrate the process, a data set from human feeding trials with *Campylobacter jejuni* was considered, with a log-logistic dose-response model used as a test bed *(3).* Results from the analysis of FoodNet surveillance data were further used to reflect both variability and uncertainty in *Campylobacter* susceptibility due to the effect of age. The relevance of the findings in the context of microbial risk assessment and policy making is discussed.

MATERIALS AND METHODS

Data. *C. jejuni* infects the gastrointestinal tract and is usually transmitted by the consumption of contaminated food or water. This includes meats (especially chicken), water taken from contaminated sources, and milk and milk products that have not been pasteurized. There are an estimated 2.4 million *Campylobacter* infections in the United States annually, and over 95% of diagnosed *Campylobacter* infections have been attributed to *C. jejuni (1, 28).* In an effort to model exposure doses from the consumption of poultry-related products, we used the available data along with our collective expertise. Mean exposure doses are modeled by means of a Monte Carlo simulation, assuming input distributions for serving size and microbial concentration of *C. jejuni* in meat, skin, and exudates. The mean serving size for poultry was obtained from the Continuing Survey of Food Intakes by Individuals *(35).* The microbial concentration was obtained from the 1996 Food Safety Inspection Service Nationwide Broiler Chicken Microbiological Baseline Data Collection Program *(36).* Specifically, we used a lognormal distribution (mean = 1.87; standard deviation = 1.68) to represent the exposure dose in CFU per serving. This distribution is assumed to represent the average dose to which the population is exposed at different occasions. The actual dose contained in a serving is expected to follow a Poisson distribution, whose parameter is the mean dose given by the lognormal distribution model.

TABLE 2. *Incidence of* Campylobacter *infection, by year and demographic factor, from original FoodNet sites (1996 to 1999)*

Variable	Annual incidence, cases per 100,000 population				
	1996	1997	1998	1999	Average, 1996–1998
Overall	23.6	25.2	21.4	17.5	21.9
Site					
California	57.6	48.8	36.8	32.2	43.8
Connecticut	16.2	18.9	17.0	14.3	16.6
Georgia	12.0	14.1	12.9	9.7	12.2
Minnesota	19.1	25.0	21.2	16.4	20.4
Oregon	21.7	22.6	21.1	17.8	20.8
Gender					
Female	21.6	22.1	18.5	15.2	19.4
Male	25.4	28.2	24.3	19.7	24.4
Age (yr)					
<1	69.2	57.9	52.2	45.4	56.2
1–4	42.0	49.2	40.1	33.6	41.2
5–9	18.7	18.6	14.6	12.7	16.2
10–14	12.0	11.2	9.5	7.3	10.0
15–19	19.6	16.6	14.4	13.6	16.0
20–29	34.3	32.8	29.9	24.0	30.3
30–39	24.9	29.9	23.8	20.5	24.8
40–49	21.9	23.4	20.0	16.3	20.4
50–59	17.7	23.8	20.2	15.8	19.4
≥60	16.3	17.5	15.9	11.3	15.3

Data from human challenge studies with *C. jejuni* strain A3249, in which 68 individuals were stratified into six dosage groups, were considered the basis for developing the dose-response relationship (Table 1) *(3).* These data are part of a larger study that involved 111 adult volunteers who were given two strains of *C. jejuni* in order to establish certain basic features of the infection and illness. These features included the quantity of organisms needed to cause illness, the pathology of the illness, the development of an immune response to infection, the development of illness, and the extent of homologous immunity after illness. Infection was defined by positive cultures of stool specimens. The data in Table 1 demonstrate that the rate of infection increased from 50 to 100% as the dose was raised from 800 to 10^8 CFU per subject.

The number of *Campylobacter* infections corresponding to the years 1996, 1997, 1998, and 1999 was extracted from annual FoodNet surveillance reports *(8).* The data included the frequency tables concerning the three factors of age, gender, and location,

stratified by year (Table 2). These data were used to estimate measures of relative risk in order to characterize interindividual variability in susceptibility within the framework of microbial risk assessment.

Poisson regression analysis. A common analytical goal of surveillance data is to estimate incidences (rates of illness per infection per population at risk) and to establish the potential relationship to a set of available explanatory variables (e.g., site, age). Surveillance data often come in the form of discrete counts of events. When frequencies are contained, an adequate assumption is that the counts follow a Poisson distribution *(32).* The Poisson regression is an analytical method that can achieve the goal of estimating the incidences and establishing the potential relationships while preserving and exploiting as much as possible the discreteness of the count variable.

Computational implementation of the Poisson regression was carried out within the framework of Generalized Linear Models *(32).* We assumed that the dependent variable Y was Poisson distributed with mean and variance μ. If only a single explanatory model is considered, the base Generalized Linear Models regression model for μ is written as follows:

$$\log(\mu) = \alpha + x\beta \tag{1}$$

When the interest lies in modeling rates, an exposure variable N (e.g., population at risk) needs to be defined. The rate is then Y/N. The expected value becomes μ/N, which is modeled as follows:

$$\log(\mu) = \alpha + x\beta + \log(N) \tag{2}$$

For multiple explanatory variables, the model is written as follows:

$$\log(\mu_i) = \log(N_i) + X_i' \times \beta \tag{3}$$

where X_i is a matrix that holds the explanatory variables. A useful characteristic of the Poisson log-linear model is that the exponentiation of parameter coefficients leads to measures of relative risk, i.e., the incidence rate ratio. The risk of *Campylobacter* infection in seven distinct age groups (younger than 1 year of age, 1 to 9, 10 to 19, 30 to 39, 40 to 49, 50 to 59, and older than 60) relative to a reference (individuals 20 to 29 years of age) was calculated with Poisson regression, considering explanatory variables such as age, gender, and year and possible interactions between these variables. The human feeding trial with *C. jejuni* was conducted with young adults *(3),* and hence, individuals between 20 and 29 years old were assumed to represent the reference group.

Reported counts of *Campylobacter* infections for the 4-year period between 1996 and 1999 were extracted from annual FoodNet reports *(8),* while denominators for the population at risk were derived from online data (year 2000) of the U.S. Census Bureau *(34).* The coefficients of seven age groups are thought to follow a normal distribution in which the mean is the estimate of the age parameter from the Poisson regression, and the standard deviation is the standard error of such a parameter *(12).*

The goodness of fit for the Poisson regression analysis was investigated through residual analysis. The transformation of the dependent variable that is closest to normality and is standardized to mean 0 and variance 1 defines the Anscombe residual *(6).* For a Poisson-distributed variable, the transformation $y^{2/3}$ is closest to normality, and the Anscombe residual is calculated as follows:

$$a_i = \frac{1.5*(y_i^{2/3} - \hat{y}_i^{2/3})}{\hat{y}_i^{1/6}} \tag{4}$$

where y_i is the observed value for a specific combination of levels of the explanatory variables, and $\hat{y}_i$ is the relative predicted value. Plots of the Anscombe residuals against the levels of the explanatory variables were used to assess the goodness of fit of the final models. The normality of the residual was checked through the Kolmogorov-Smirnov test and normal probability plots.

Dose-response model. The log-logistic equation was used to model the dose-response relationship and hence estimate the infection probability for an age group (P_i) *(11).* When a log-logistic model is applied, the P_i for the reference age group (i.e., individuals between 20 and 29 years old) can be expressed as follows:

$$P_{20\text{ to }29} = f(\beta_0, \beta_1; d) = \frac{1}{1 + e^{-[\beta_0 + \beta_1 \times \log(d)]}} \tag{5}$$

where β_0 and β_1 are the parameters of the log-logistic dose-response model, and d is the dose. Equation 5 was the baseline model used in our analyses.

Equation 5 can be extended to model dose response for the remaining seven age groups. Specifically, the Poisson regression coefficients are defined as β_{0i}' and are summed to the parameter β_0. The age-specific infection probability P_i is thus as follows:

$$P_i = f(\beta_0, \beta_{0i}', \beta_1; d) = \frac{1}{1 + e^{-[(\beta_0 + \beta_{0i}') + \beta_1 \times \log(d)]}} \tag{6}$$

This implies two assumptions. First, risk differences among age groups that result from the FoodNet surveillance data are viewed to arise solely on the basis of differences in susceptibility. In other words, exposure is assumed to be comparable for all age groups. Second, susceptibility distributions differ from one age group to another only in terms of the median, while the dispersion is similar for all age groups (i.e., the parameter β_1 is correct independently of age). To account for varying serving size, and thus dose size in different age groups, a factor, γ_i, that proportionally adjusts dose can be introduced. P_i is then as follows:

$$P_i = f(\beta_0, \beta_{0i}', \beta_1, \gamma_i; d) = \frac{1}{1 + e^{-[(\beta_0 + \beta_{0i}') + \beta_1 \times \log(\gamma_i \times d)]}} \tag{7}$$

As calculated from the Continuing Survey of Food Intakes by Individuals *(35),* the mean serving size for poultry in select age groups is 32.8 g for individuals younger than 1 year of age; 103.5 g for the 20 to 29 age group; and 85.3 g for people older than 60 years *(35).* When compared to young adults, the dose adjustment factor γ_i was thus assumed to be 0.3 and 0.8 for infants and the elderly, respectively.

For any given dose, the infection probability P in the heterogeneous population is the sum of age-specific infection probabilities P_i, each weighted for the fraction of the relative age group δ_i in the population, i.e.,

$$P = \sum_i \delta_i P_i \tag{8}$$

The age-specific fraction δ_i for the estimated population of the United States in July 2000 was 0.014 (younger than 1 year of age); 0.123 (1 to 9 years of age); 0.145 (10 to 19 years of age); 0.131 (20 to 29 years of age); 0.145 (30 to 39 years of age); 0.158 (40 to 49 years of age); 0.118 (50 to 59 years of age); and 0.166 (older than 60 years of age) *(34).*

Parameter estimation for the dose-response model. Given the nonlinear character of the log-logistic dose-response model, the maximum likelihood estimation was selected as the method of choice for calculating the values of model parameters by the following log-likelihood function for binary data (i.e., β_0^*, β_1^*) *(26)*:

$$l(\hat{\pi}; y) = \sum_i [y_i \log \hat{\pi}_i + (m_i - y_i)\log(1 - \hat{\pi}_i)] \tag{9}$$

where m_i is the number of trials in group i, y_i is the number of successes, and π_i is the fitted probability. The maximum likelihood estimates are those parameter values that maximize this equation.

The deviance as a measure of the goodness of fit is defined as twice the difference between the maximum achievable log likelihood and that attained under the fitted model and can be written as follows:

$$D(y; \hat{\pi}) = 2 \sum_i \left[y_i \log\left(\frac{y_i}{\hat{\mu}_i}\right) + (m_i - y_i)\log\left(\frac{m_i - y_i}{m_i - \hat{\mu}_i}\right)\right] \quad (10)$$

where $\hat{\mu}_i$ is the expected value of the number of successes in group i on the basis of the fitted model. The deviance is commonly regarded as being asymptotically χ^2_{n-p} distributed, where n is the number of groups, and p is the number of parameters fitted.

Bootstrap simulation applied to dose-response modeling. The bootstrap method extends the standard approach of obtaining a single ''best'' estimate (e.g., maximum likelihood estimate) for parameters of a microbial dose-response model by generating a distribution of the parameter values. Bootstrap simulation can provide estimates of confidence intervals in situations for which analytical mathematical solutions may not exist. Bootstrap simulation is based on drawing multiple random samples, each of size n, with replacement from the data set *(16).* The sought statistic s is then calculated for each bootstrap sample, and a series of bootstrap replications is the result. As the number of bootstrap samples increases, the distribution of the bootstrap replications becomes a reliable estimate of the population statistic.

Data for human feeding trials are typically sparse (i.e., few dose-response pairs), the dose has to be viewed as a fixed factor, and the response is binomial (i.e., has a domain between 0 and 1). A bootstrap procedure for such data has been proposed *(13, 15).* The crucial step of the bootstrap method is the process by which the observed data set is resampled, i.e., how bootstrap samples are generated. We considered a binomial distribution for resampling from the data for human feeding trials. The outcome of the resampling scheme is essentially a resampled number of infected individuals (r_i^*) for each of i dosage groups as follows:

$$r_i^* = \text{binomial}(n_i, r_i/n_i) \quad (11)$$

where n_i is the total number of exposed individuals in the ith dosage group, and r_i is the number of infected individuals in the same group. The ratio of r_i:n_i is also known as the observed infection.

Model application. Both ''first-order analysis'' and ''second-order analysis'' were conducted on the proposed dose-response model. Specifically, the term first-order is used for an analysis that, while probabilistic, confounds uncertainty and variability. In contrast, the two elements are kept separated in a second-order analysis. The effect of age was separated by stratifying the analysis, while the other sources of uncertainty and variability, such as the sampling error due to the limited sample size for the human trial data and the dose distribution, respectively, were propagated by Monte Carlo simulation.

First-order analysis drew 500 samples from the lognormal distribution of doses described earlier. The other parameters were the point estimates of the dose-response function or the Poisson regression. For the second-order analysis, dose was generated through a two-dimensional simulation that nested one iterative loop into another. The first loop simulated variability by creating 500 samples of the variable dose from the mentioned lognormal distribution. Five hundred Poisson random samples were generated, starting from those first 500 samples. This second loop represented uncertainty. Parameter uncertainty of the dose-response function was simulated by the bootstrap method. Uncertainty in the age effect was simulated by drawing 500 samples from seven normal distributions (one for each age group, excluding the 20- to 29-year-old baseline) whose parameters were defined on the basis of the results obtained in the Poisson regression. The three data sets, including dose values, uncertainty associated with the dose-response function, and uncertainty associated with the age effect, were merged, and equation 7 was resolved. Each one of 250,000 observations (i.e., 500 × 500) thus contained a value for dose and the corresponding infection probability for each of the eight age groups.

TABLE 3. *Incidence rate ratio for* Campylobacter *for different age categories based on the Poisson log-linear regression model*

Age categories	Incidence rate ratio (β_0')	SE	Lower confidence limit[a]	Upper confidence limit[a]
<1	1.87	0.044	1.72	2.04
1–9	0.85	0.050	0.77	0.94
10–19	0.43	0.040	0.39	0.46
20–29[b]	1.00	—	—	—
30–39	0.86	0.046	0.79	0.94
40–49	0.72	0.041	0.66	0.78
50–59	0.65	0.057	0.58	0.73
≥60	0.51	0.042	0.47	0.56

[a] Lower and upper limits based on 95% confidence intervals.
[b] Reference level.

RESULTS

Incidence rate ratio for *Campylobacter.* The risk of *Campylobacter* infection in seven distinct age groups (younger than 1 year of age, 1 to 9, 10 to 19, 30 to 39, 40 to 49, 50 to 59, and older than 60) relative to a reference (individuals 20 to 29 years of age) was calculated with Poisson regression, considering explanatory variables such as age, gender, and year and the possible interaction between these variables. For the seven age categories, the mean coefficients, along with standard errors and the lower and upper bounds of the 95% confidence intervals for incidence rates, are given in Table 3. Rates of *Campylobacter* infection were higher in infants (children younger than 1 year of age) and in adults (20 to 29 years of age) and appeared fairly similar for the other age groups. Although more detailed discrimination would require specific contrasts between the different age groups, the age group 10 to 19 had the lowest risk, and people older than 60 years had the second lowest risk. On the basis of the confidence intervals, these results are statistically significant. Age groups 1 to 9 and 30 to 39 had a similar risk, as did people between 40 and 59 years of age.

The goodness of fit of the final multivariate model for the *Campylobacter* data was satisfactory. The residuals appeared normally distributed, with a mean of 0.044 and a standard deviation of 1.265 (Fig. 1a, Kolmogorov-Smirnov statistics $P > 0.15$). Also, no particular pattern was evident from the graphical representations of the Anscombe residuals (Fig. 1b and 1c).

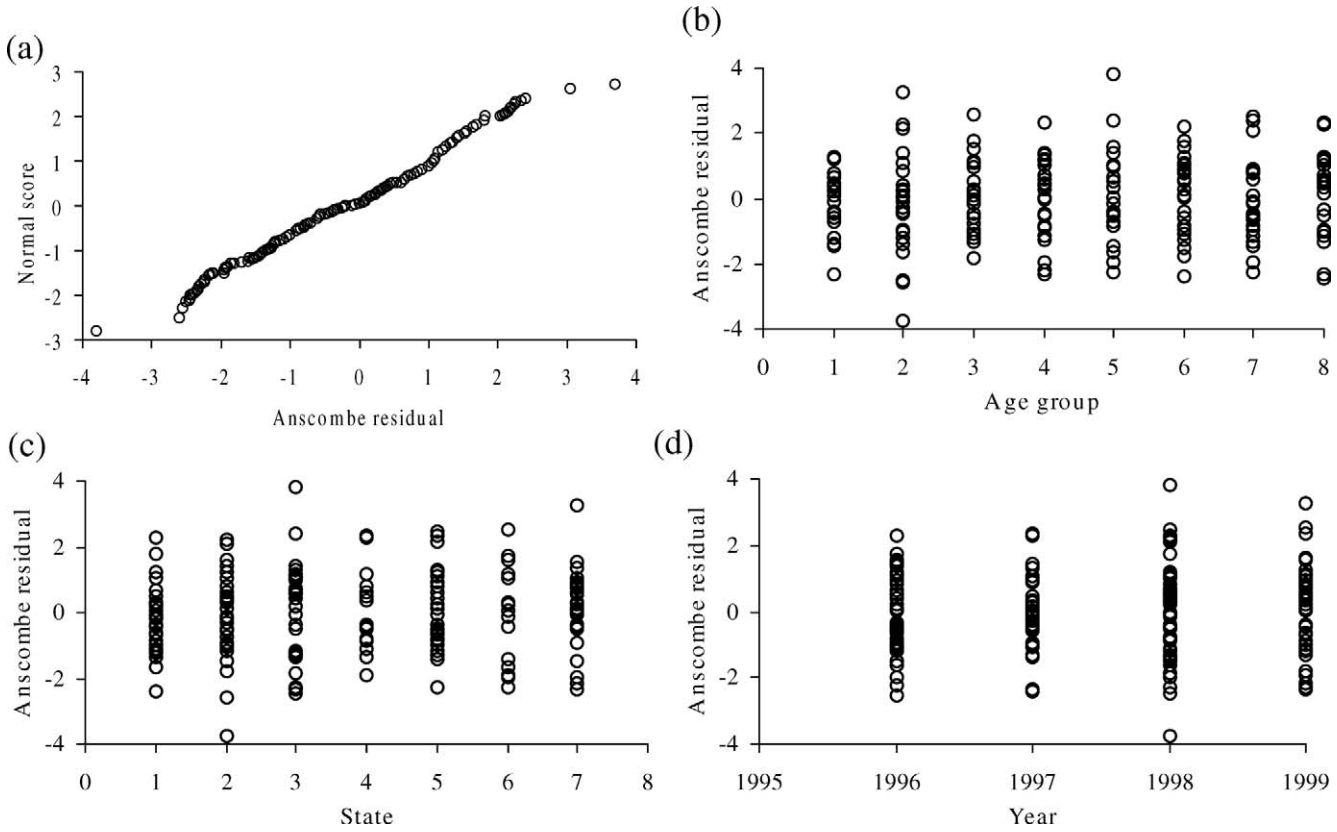

FIGURE 1. *Goodness-of-fit results for the Poisson regression analysis: normal probability plot of Anscombe residuals (a); Anscombe residuals of final model versus age groups (b), states (c), and different years of FoodNet data (d).*

First-order analysis of variability and uncertainty. The first set of results focuses on the combined effect of variability in dose and parameter uncertainty of the dose-response function. Figure 2 displays the results of the first-order analysis for the dose-response relationship and the corresponding joint probability when age was not considered. Confidence intervals represent the uncertainty associated with each value of the response (i.e., the probability of infection). Different values of probability of infection represent variability.

From these graphs, it is apparent that the dose-response function creates a perfect correlation between dose and response, i.e., given a dose, the response is defined with certitude. The maximum likelihood estimations for parameters β_0 and β_1 of the log-logistic dose-response model were -1.385 and 0.484, respectively, with a deviance of 2.37. While the parameter values are mainly of interest as a reference to the further results, the goodness of fit of the dose-response model is worth considering. On the basis of the significance level of the observed deviance (Pr(deviance) $= 0.67$), the log-logistic model resulted in an adequate fit for the *C. jejuni* data.

Figure 3 illustrates the influence of age on the dose-response relationship based on the first-order analysis of variability and uncertainty. The ''combined'' curve represents the weighted sum of the age-specific responses, which is meant to reflect the dose-response relation for the heterogeneous population based on equation 8. As shown in Table 3, the epidemiological analysis suggests that infants (younger than 1 year of age) have about a twofold greater risk than the reference group of young adults (i.e., age between 20 and 29). At the other extreme, an age between 10 and 19 years or older than 60 years is associated with a nearly halved relative risk. The ordering of the curves in Figure 3 closely reflects the relative risks in Table 3. The distances between curves remain proportionally constant throughout dose levels. As is especially evident from the 95% confidence interval graphs, the line for the combined population is somewhat less steep than are the age-specific ones.

Second-order analysis of variability and uncertainty. In contrast to the results for the combined analysis of variability and uncertainty, second-order analysis accommodates the sampling error arising from dose-response modeling, and the dose-response relation becomes a surface. Figure 4 directly contrasts the results from three analytical approaches that represent an increasing level of sophistication. The deterministic analysis considers only the median of the dose distribution (i.e., the mean in a log scale, 1.87), and the response (0.382) directly results from the dose-response function. Stochastic simulation in a single dimension generates a similar pair of most likely values (log dose, 1.97; infection probability, 0.394). However, it is now possible to make a statement of confidence regarding the range of these estimates. Specifically, the 90% confidence interval for dose is -0.74 to 4.49, and that for response ranges from 0.149 to 0.687. The distinct advan-

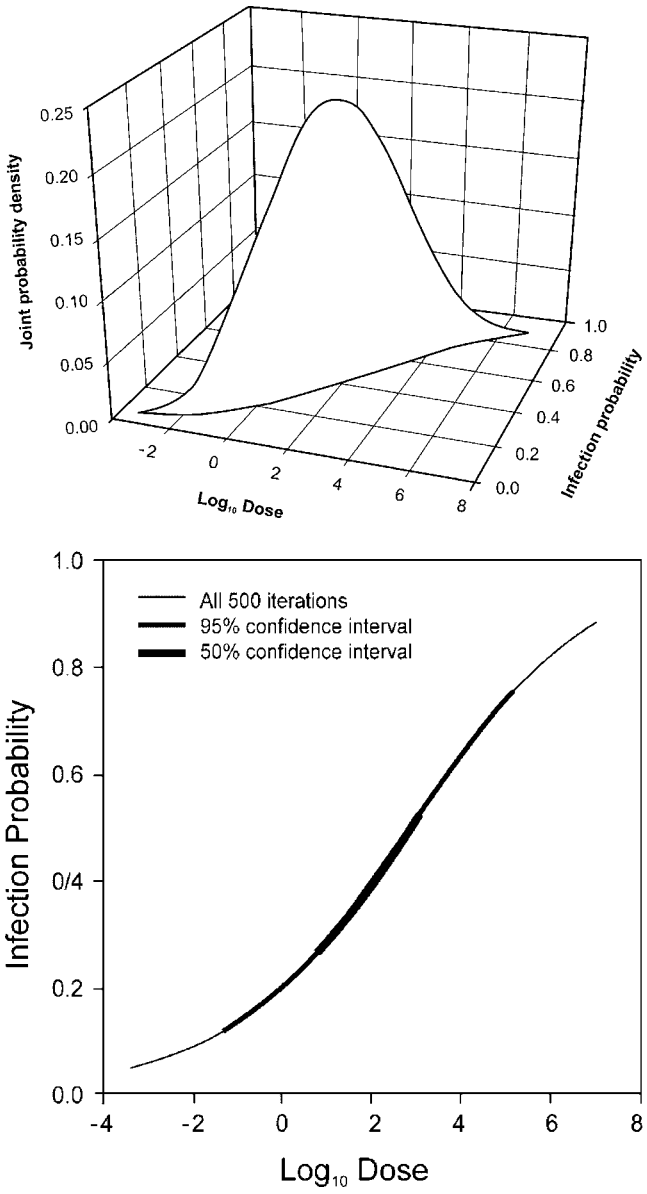

FIGURE 2. *First-order analysis of the dose-response relationship.*

tage of the two-dimensional probabilistic simulation is that it allows confidence statements for any given value of dose and response. The most likely pairs are notably shifted toward higher values of both dose and response.

Figure 5 shows the results for different age groups. Fractiles of the empirical cumulative distribution of the joint probability are drawn as contour plots. These contours can be interpreted as confidence regions such that the confidence level is equal to 1 minus the fractile, multiplied by 100. In particular, the 0.05 fractile describes the 95% confidence region, the 0.25 fractile describes the 75% confidence region, and so on. Similar to the first-order analysis, the vertical shift between age groups is maintained in the second-order analysis, and the shape of the confidence regions appears very similar. When the results for infants are compared with those of the 20 to 29 age group, it would appear that, for any given dose, a relative risk of 2 roughly translates into a 0.10 to 0.15 increase in infection probability independently of the considered dose or confidence level. Conversely, a 0.10 to 0.20 decrease in infection probability seems to be associated with a relative risk of 0.5, as judged from the 10 to 19 age group. In contrast, a similar pattern cannot be inferred starting from the *y*-axis (infection probability), because the vertical shift of the confidence regions is not linked to a proportional horizontal change. For any given infection probability, the associated confidence interval of dose varies from one age group to another.

DISCUSSION

The methodology presented in this study achieves two advancements in microbial risk assessment. First, the integration of an important source of uncertainty (sampling error) in dose-response modeling more openly reflects the degree of reliability inherent in the model and its outcomes. This has relevant implications in terms of risk management. Second, the potential role of a specific host factor and, by reflection, the need for a more detailed characterization of the multifaceted process of foodborne infection are evaluated.

Parameter estimates of the log-logistic dose-response model were conducted by the human feeding trial with *C. jejuni (3).* Other researchers have reported estimates for this data set by alternative dose-response models. For instance, Teunis et al. *(33)* and Holcomb et al. *(19)* both considered the beta-Poisson model, while Teunis et al. also considered the exponential model. Although we also evaluated the beta-Poisson model in our analyses (data not shown), the estimation process for the parameters of the log-logistic model by the maximum likelihood estimation approach offered better warranties of stability. In comparison to the beta-Poisson model, the parameter estimates of the log-logistic model thus incurred a lesser chance of being biased. Another key consideration with regard to the beta-Poisson model was that relevant portions of the confidence regions for the parameter estimates intersected an implausible region (i.e., parameter $\alpha >$ parameter β). Thus, we were not able to reject the possibility that the theoretical assumptions governing the derivation of the beta-Poisson model were violated. Thus, for our case, the beta-Poisson model was devoid of its claimed biological fundaments and reverted to an empirical model very much as in the log-logistic model.

Our analysis showed that a high degree of uncertainty was revealed by the second-order analysis. While at first glance disconcerting to the risk manager, the increasingly sophisticated methodology does not affect the ability to identify the most likely outcomes. As shown in Figure 4, when moving from a deterministic to a stochastic analysis and from first-order to second-order modeling, the most likely values of dose and response can always be identified. The location of these pairs varies because of the random nature of the Monte Carlo simulation (deterministic versus first-order stochastic analysis) and an increased complexity of the applied models (second-order stochastic analysis). The notable improvement is in our ability to make statements on the degree of confidence and belief in the results. While the first-order analysis characterizes the confidence of the most likely estimates, a similar statement is possible

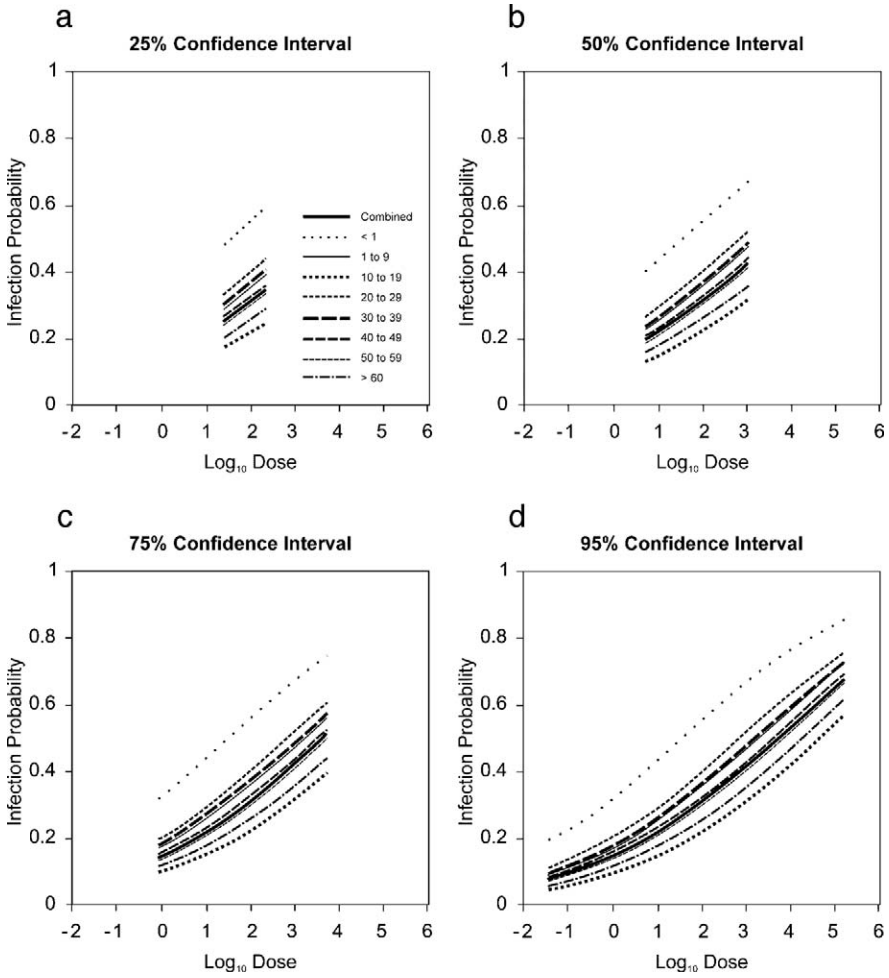

FIGURE 3. *First-order analysis of the dose-response relationship for different age groups: (a) 25% confidence intervals; (b) 50% confidence intervals; (c) 75% confidence intervals; and (d) 95% confidence intervals.*

for any point in the dose-response plan (surface) when the second-order analysis is applied.

The capability of making confidence statements has far-reaching implications in terms of risk management. This was originally pointed out in environmental risk assessment *(2, 4, 17, 18)* but has since been recognized in the food safety arena *(23).* Figure 6 shows the possible interpretation of second-order analysis for policy making. Confidence statements are possible irrespective of whether a risk manager is interested in establishing the infection probability for a given dose or the dose for a given infection probability. As a strategy based on postexposure mitigation is obviously untenable in food safety, the exposure process (whether in terms of dose modulation or avoiding the exposure altogether) clearly is pivotal to risk management options. Thus, a representation of the final risk that still conveys the level of exposure offers an unparalleled insight for a decision-maker. For the sake of illustration, the exposure scenario considered in this study is simple. At best, it represents a single exposure to a food item contaminated with *C. jejuni.* However, replacing dose and infection probability with other relevant variables from the steps of exposure assessment or hazard characterization, respectively, can be envisioned. For instance, the *x* axis could represent the dose before preparation, and the *y* axis could represent the number of exposed individuals. The gained insight would be equivalent.

In this study, a relatively small sample size of 500 iterations was chosen. Morgan and Henrion *(29)* discuss formal ways of determining the number of iterations that would fulfill a desired level of accuracy. Given the two-dimensional structure of a second-order analysis, an increase in sample size implies exponentiation of the observation number. Theoretically, computation capability is the limiting factor in achieving better accuracy through an increase in iterations. For the baseline model, we contrasted a simulation with 500 iterations to one with 1,000 simulations (250,000 and 1,000,000 observations, respectively). Graphical appearance of the results was similar for the two cases (data not shown). The larger simulation required a longer, but still manageable computation time. However, it was at the level of outcome analysis that the added burden became manifest. Imposing a more limited number of iterations for a comparable level of accuracy, Latin Hypercube Sampling should be considered an alternative to the Monte Carlo method *(29).*

As was apparent for our analysis of *Campylobacter* infection, a consideration of the age effect was associated with changes in the estimated risk (Figs. 3 and 5). While not as dramatic as those linked to the sampling error of the dose-response function, the differences are nonetheless perceptible. A relative risk of 1.87 for *Campylobacter* infection was established for infants (younger than 1 year of age) in comparison to young adults (20 to 29 years of age, refer-

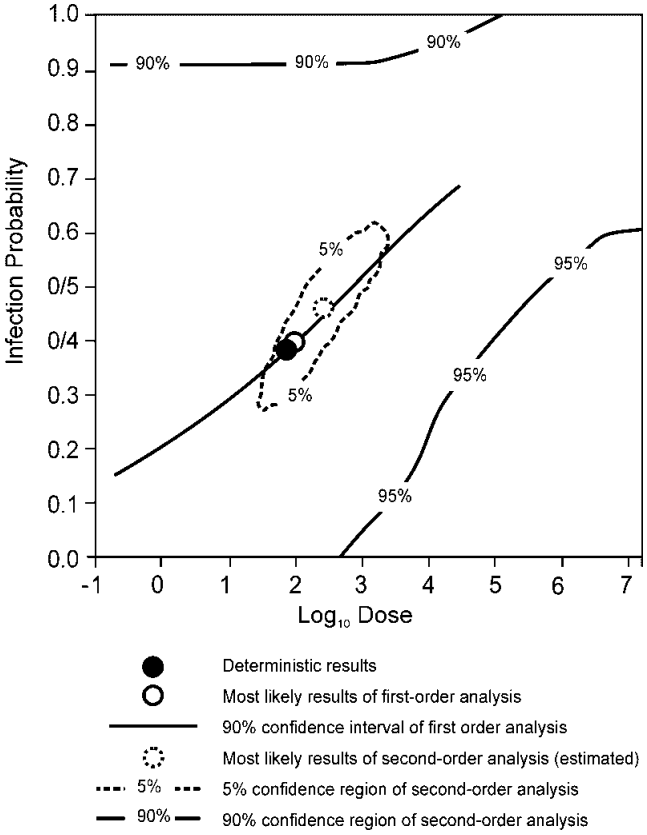

FIGURE 4. *Relationship between deterministic and probabilistic results.*

ence group) (Table 3). Such a relative risk is associated with a 0.1 to 0.15 increase in infection probability. Similarly, the 0.43 relative risk for the 10 to 19 age group translates into a 0.1 to 0.2 decrease in response. That relative risks are ratios explains why the differences for the other age groups are more limited than are those at extreme relative risks. Also, since differences in response between age groups remain constant over the observed dose range, their relevance diminishes with increasing dose. From the graphical results, one wonders whether consideration of the age effect matters in terms of the risk assessment. Overall, the incertitude is perhaps indicative that relative risks of 0.5 and 2 represent pragmatic thresholds and that only host factors associated with a relative risk lower than 0.5 or greater than 2.0 ought to be pondered for inclusion in a microbial hazard characterization. Finally, it is noteworthy that higher probabilities for a *Campylobacter* infection are estimated for the reference group (20 to 29 years of age) than for the whole population. As pointed out, the reference group values arise from simply fitting the dose-response function to the data from the human feeding trial. Because the age effect was not considered, it would appear that there has been an implicit tendency to overestimate the risk when considering campylobacteriosis.

The conclusions regarding the influence of age rely on the soundness of the assumption made in formulating equation 2. Specifically, it was assumed that only the mean of susceptibility changes across population subgroups. The concept of susceptibility is already so challenging in qualitative terms that creating speculative quantitative statements is hardly warranted. However, while we cannot justify the assumption, there appears to be no evidence to refute it. Also, short of ignoring the age effect, the proposed approach is the simplest that we can advance and is thus the one that carries the least assumptions.

In conclusion, the present study proposes an analytical framework that integrates uncertainty associated with dose-

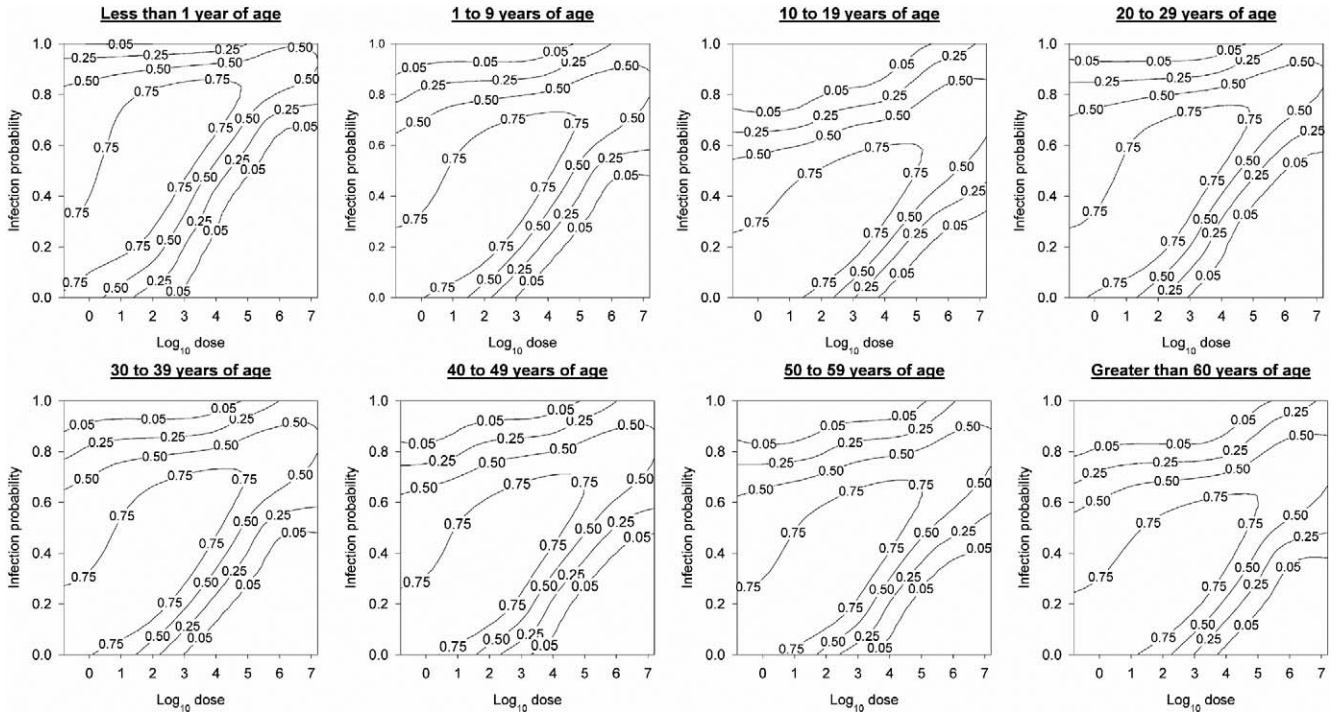

FIGURE 5. *Second-order analysis of the dose-response relationship for different age groups.*

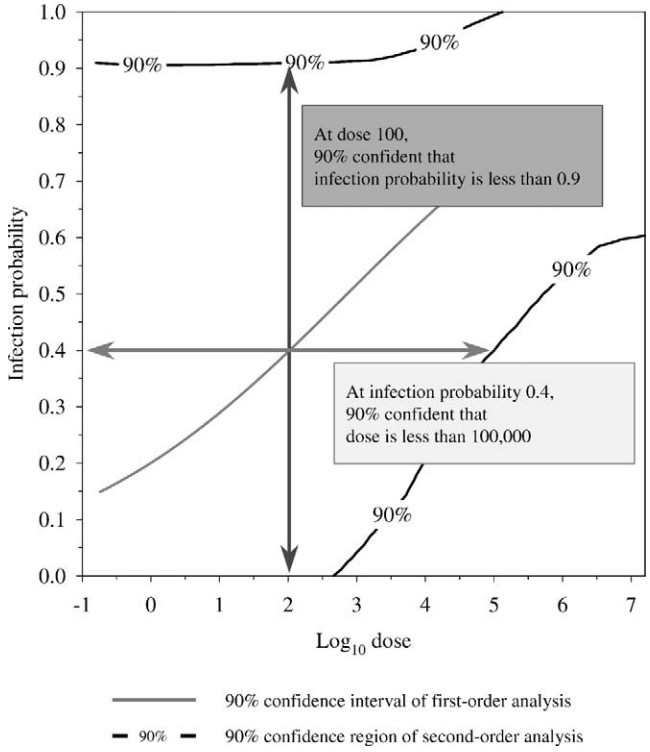

FIGURE 6. *Interpretation of second-order analysis in policy making.*

response modeling as well as variability due to age, as applied to *Campylobacter* infection. There are, of course, many other sources of uncertainty and variability that could be considered in future analyses. Nonetheless, in the present study, we can conclude that the former has a dominating influence on the analytical outcome. In contrast, inclusion of the age factor has a limited impact. Although this is only one example, the process suggests that biological plausibility and epidemiological evidence do not necessarily translate into risk assessment relevance. While the advocacy of more closely modeling variability in microbial risk assessment is warranted, for hazard characterization, this may reflect a misplaced emphasis. Indeed, in the case illustrated, the characterization of key sources of uncertainties and their consistent propagation throughout a microbial risk assessment actually appear to be of greater importance.

ACKNOWLEDGMENTS

This study was supported by funding from the Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture (CSREES, USDA grants 96-354003606 and 2001-51110-11365).

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