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# Dose-Response Model for 13 Strains of *Salmonella*

**Thomas Oscar***

Data from a human feeding trial with healthy men were used to develop a dose-response model for 13 strains of *Salmonella* and to determine the effects of strain variation on the shape of the dose-response curve. Dose-response data for individual strains were fit to a three-phase linear model to determine minimum, median, and maximum illness doses, which were used to define Pert distributions in a computer simulation model. Pert distributions for illness dose of individual strains were combined in an Excel spreadsheet using a discrete distribution to model strain prevalence. In addition, a discrete distribution was used to model dose groups and thus create a model that simulated human feeding trials. During simulation of the model with @Risk, an illness dose and a dose consumed were randomly assigned to each consumption event in the simulated feeding trial and if the illness dose was greater than the dose consumed then the model predicted no illness, otherwise the model predicted that an illness would occur. To verify the dose-response model predictions, the original feeding trial was simulated. The dose-response model predicted a median of 69 (range of 43–101) illnesses compared to 74 in the original trial. Thus, its predictions were in agreement with the data used to develop it. However, predictions of the model are only valid for eggnog, healthy men, and the strains and doses of *Salmonella* used to develop it. When multiple strains of *Salmonella* were simulated together, the predicted dose-response curves were irregular in shape. Thus, the sigmoid shape of dose-response curves in feeding trials with one strain of *Salmonella* may not accurately reflect dose response in naturally contaminated food where multiple strains may be present.

**KEY WORDS:** Dose-response modeling; human feeding trial; *Salmonella*; simulation; strain variation

## 1. INTRODUCTION

*Salmonella* are gram-negative, rod-shaped bacteria that inhabit the intestinal tracts of humans and animals and are classified as either typhoid or nontyphoid.[1] Typhoid strains, such as *S.* Typhi and *S.* Paratyphi, cause typhoid fever, which is transmitted from person to person, whereas nontyphoid strains, such as *S.* Typhimurium and *S.* Enteritidis, cause gastroenteritis and are usually acquired from animal food products.[2] Some nontyphoid strains, such as *S.* Pullorum and *S.* Gallinarum in poultry, are highly pathogenic in the animal host but weakly pathogenic in humans.[3] On the other hand, most nontyphoid strains are nonpathogenic in the animal host but highly pathogenic in humans.[2] Consequently, they are difficult to detect in slaughter animals and thus account for most of the cases of human salmonellosis.[3]

In developing countries, typhoid fever is declining, whereas nontyphoid salmonellosis is increasing.[4] These epidemiological changes are attributed to prophylactic treatment of typhoid fever and changes in animal production and processing practices that facilitate the spread of nontyphoid *Salmonella* among food animals.[4] The U.S. Centers for Disease Control and Prevention estimate that nontyphoid *Salmonella* of food origin causes 1.3 million illnesses and 553 deaths per year.[5]

* U.S. Department of Agriculture, Agriculture Research Service, Microbial Food Safety Research Unit, Room 211, Center for Food Science and Technology, University of Maryland Eastern Shore, Princess Anne, MD 21853; toscar@umes.edu.



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The number of nontyphoid *Salmonella* that must be ingested to cause gastroenteritis ranges from less than 100 to greater than $10^9$.[6] This large variation in illness dose most likely reflects differences in virulence among strains of *Salmonella*, differences in resistance among humans, and the interacting effect of the food matrix on pathogen virulence and host resistance.[6] Thus, predicting responses of consumers to ingestion of food contaminated with *Salmonella* requires knowledge of how the food matrix, pathogen virulence, and host resistance interact to determine the dose of pathogen ingested that causes illness.

The human feeding trial is a useful black box method for establishing a mathematical relationship between the dose of pathogen ingested and the response of the host population. In a typical feeding trial, groups of human volunteers are fed known doses of a pathogen (i.e., dose groups) in a common food vehicle, such as milk[7] or eggnog,[8] and then the subjects are monitored for signs of a response, such as fecal shedding, antibody production, fever, abdominal cramping, vomiting, headache, and diarrhea. Responses of individual subjects in such trials fall within a continuum from no response to severe illness. Nonetheless, to model the data, criteria are established to classify responses as either positive or negative for infection or illness. Using the latter approach, dose-response models have been developed for a number of pathogens, such as *Campylobacter jejuni*,[9] *Cryptosporidium parvum*,[10] *Escherichia coli* O157:H7,[11] *Giardia lambia*,[12] *Salmonella* spp.,[13] *Shigella* spp.,[14] and viruses.[15] The two most popular dose-response models for microbial pathogens are the exponential model and the beta-Poisson model,[15,16] although a number of others, such as the Gompertz, log-logistic, and Weibull gamma have also been used.[13,17]

The aforementioned dose-response models are analogous to primary models, such as the Gompertz[18] and Baranyi[19] models for microbial growth, in that they model a response as a function of one variable while other variables are held constant. For example, growth of a pathogen population in laboratory media is modeled as a function of time at a given temperature, pH, and water activity. In an analogous manner, in dose-response modeling, the response (i.e., infection or illness) of a host population is modeled as a function of dose for a given food matrix, pathogen strain, and host population, usually healthy adults.

The instances in which dose-response modeling and growth modeling differ are that in growth modeling data are collected for a matrix of growth conditions and then secondary[18] and tertiary[20] models that predict the primary response (i.e., growth) as a function of time and the growth condition variables (i.e., temperature, pH, and water activity) are generated. In an analogous manner, it should be possible to collect data for a matrix of dose-response conditions and then construct secondary and tertiary models that predict the primary response (i.e., infection or illness) as a function of dose ingested and the dose-response variables (e.g., food pH, food fat%, pathogen strain, pathogen prevalence, host age, and host health status).

A difficulty in applying a growth modeling approach to dose-response modeling is that most data sets from human feeding trials are limited in scope because they include one or a limited number of dose-response conditions. One exception is a large feeding trial in which 13 strains of *Salmonella* were fed to healthy men.[8,21,22] Using the illness data from this feeding trial,[8,21,22] the objectives of the current study were to develop a dose-response model for predicting salmonellosis as a function of dose consumed and strain variation and to use the model to investigate the effect of strain variation on the shape of the dose-response curve.

## 2. METHODS

### 2.1. Human Feeding Trial Data

Illness data from a feeding trial that was conducted over 50 years ago with healthy men confined in an institutional setting [8,21,22] were used for model development (Fig. 1). Subjects in the trial were fed one strain of nontyphoid *Salmonella* in a glass of eggnog after the noon meal. Typically there were six (range of five to eight) men per dose group (i.e., a group of subjects that were fed the same dose and strain of *Salmonella*). Strains were isolated from spray-dried whole egg and included Anatum (three strains), Bareilly (one strain), Derby (one strain), Meleagridis (three strains), Newport (one strain), and Pullorum (four strains). Severity of illness ranged from mild to severe (i.e., hospitalization) but was not considered in this study.

### 2.2. Dose-Response Modeling

Incidence of salmonellosis ($Y$; %) in the test population for individual strains of *Salmonella* was graphed as a function of dose consumed ($X$; $\log_{10}$) and then the data were fit (version 3.0, Prism, GraphPad Software, Inc., San Diego, CA) to a three-phase

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Serotype | Dose ($\log_{10}$) | Ill | Feedings | Incidence |
| 2 | Anatum I | 4.08 | 0 | 5 | 0% |
| 3 | Anatum I | 4.38 | 0 | 6 | 0% |
| 4 | Anatum I | 4.82 | 0 | 6 | 0% |
| 5 | Anatum I | 4.97 | 0 | 6 | 0% |
| 6 | Anatum I | 5.15 | 0 | 6 | 0% |
| 7 | Anatum I | 5.41 | 0 | 6 | 0% |
| 8 | Anatum I | 5.77 | 2 | 6 | 33% |
| 9 | Anatum I | 5.93 | 3 | 6 | 50% |
| 10 | | | | | |
| 11 | Anatum II | 4.95 | 0 | 6 | 0% |
| 12 | Anatum II | 5.65 | 0 | 6 | 0% |
| 13 | Anatum II | 6.02 | 0 | 6 | 0% |
| 14 | Anatum II | 6.59 | 0 | 6 | 0% |
| 15 | Anatum II | 7.00 | 0 | 6 | 0% |
| 16 | Anatum II | 7.38 | 0 | 6 | 0% |
| 17 | Anatum II | 7.65 | 1 | 6 | 17% |
| 18 | Anatum II | 7.83 | 4 | 8 | 50% |
| 19 | | | | | |
| 20 | Anatum III | 5.20 | 0 | 6 | 0% |
| 21 | Anatum III | 6.10 | 2 | 6 | 33% |
| 22 | Anatum III | 6.67 | 4 | 6 | 67% |
| 23 | | | | | |
| 24 | Bareilly | 5.10 | 1 | 6 | 0.17 |
| 25 | Bareilly | 5.84 | 2 | 6 | 0.33 |
| 26 | Bareilly | 6.23 | 4 | 6 | 0.67 |
| 27 | | | | | |
| 28 | Derby | 5.14 | 0 | 6 | 0.00 |
| 29 | Derby | 5.85 | 0 | 6 | 0.00 |
| 30 | Derby | 6.22 | 0 | 6 | 0.00 |
| 31 | Derby | 6.81 | 0 | 6 | 0.00 |
| 32 | Derby | 7.18 | 3 | 6 | 0.50 |
| 33 | | | | | |
| 34 | Meleagridis I | 4.08 | 0 | 6 | 0.00 |
| 35 | Meleagridis I | 4.38 | 0 | 6 | 0.00 |
| 36 | Meleagridis I | 4.72 | 0 | 6 | 0.00 |
| 37 | Meleagridis I | 4.98 | 0 | 6 | 0.00 |
| 38 | Meleagridis I | 5.19 | 0 | 6 | 0.00 |
| 39 | Meleagridis I | 5.48 | 0 | 6 | 0.00 |
| 40 | Meleagridis I | 5.86 | 0 | 5 | 0.00 |
| 41 | Meleagridis I | 6.06 | 0 | 6 | 0.00 |
| 42 | Meleagridis I | 6.74 | 0 | 6 | 0.00 |
| 43 | Meleagridis I | 7.38 | 1 | 5 | 0.20 |
| 44 | Meleagridis I | 7.70 | 4 | 6 | 0.67 |
| 45 | | | | | |
| 46 | Meleagridis II | 6.00 | 0 | 6 | 0.00 |
| 47 | Meleagridis II | 6.74 | 0 | 6 | 0.00 |
| 48 | Meleagridis II | 7.00 | 1 | 6 | 0.17 |
| 49 | Meleagridis II | 7.30 | 2 | 6 | 0.33 |
| 50 | Meleagridis II | 7.61 | 5 | 6 | 0.83 |
| 51 | | | | | |
| 52 | Meleagridis III | 5.20 | 0 | 6 | 0.00 |
| 53 | Meleagridis III | 6.18 | 0 | 6 | 0.00 |
| 54 | Meleagridis III | 6.89 | 1 | 6 | 0.17 |
| 55 | Meleagridis III | 7.00 | 2 | 6 | 0.33 |
| 56 | | | | | |
| 57 | Newport | 5.18 | 1 | 6 | 0.17 |
| 58 | Newport | 5.59 | 1 | 8 | 0.13 |
| 59 | Newport | 6.13 | 3 | 6 | 0.50 |
| 60 | | | | | |
| 61 | Pullorum I | 4.00 | 0 | 6 | 0.00 |
| 62 | Pullorum I | 9.25 | 0 | 6 | 0.00 |
| 63 | Pullorum I | 10.00 | 6 | 6 | 1.00 |
| 64 | Pullorum I | 10.20 | 6 | 6 | 1.00 |
| 65 | | | | | |
| 66 | Pullorum II | 6.14 | 0 | 6 | 0.00 |
| 67 | Pullorum II | 8.21 | 0 | 6 | 0.00 |
| 68 | Pullorum II | 9.83 | 4 | 5 | 0.80 |
| 69 | | | | | |
| 70 | Pullorum III | 6.36 | 0 | 6 | 0.00 |
| 71 | Pullorum III | 7.97 | 0 | 6 | 0.00 |
| 72 | Pullorum III | 9.11 | 0 | 6 | 0.00 |
| 73 | Pullorum III | 9.88 | 6 | 6 | 1.00 |
| 74 | | | | | |
| 75 | Pullorum IV | 6.27 | 0 | 6 | 0.00 |
| 76 | Pullorum IV | 8.04 | 0 | 6 | 0.00 |
| 77 | Pullorum IV | 8.14 | 0 | 6 | 0.00 |

linear model:[23]

$$Y = 0 \qquad \text{if } X \le X_{\min}$$
$$Y = \alpha(X - X_{\min}) \qquad \text{if } X_{\min} < X < X_{\max}$$
$$Y = 100 \qquad \text{if } X \ge X_{\max}$$

where $X_{\min}$ was the minimum illness dose ($\log_{10}$), $X_{\max}$ was the maximum illness dose ($\log_{10}$), and $\alpha$ was the slope of the linear portion of the dose-response curve. Median illness dose was calculated from the dose-response curve.

The three-phase linear model is capable of predicting a minimum illness dose of one pathogen; however, data at low doses would be required. Thus, the author does not reject current thinking that one pathogen can cause illness. In the current study, the lowest minimum illness dose obtained was 4.78 $\log_{10}$ for *S.* Bareilly. As discussed later, the use of healthy men in the feeding trial or the feeding of only high doses may explain the high minimum illness doses observed when modeling the data. Had high-risk individuals and lower doses been included in the feeding trial, the dose-response curves for individual strains of *Salmonella* in the present study may have produced curve-fits with a minimum illness dose of one.

The minimum, median, and maximum illness doses from the three-phase linear model fits were used to define Pert distributions for illness dose of individual strains. This was done so that a dose-response model could be created that simulated human feeding trials and predicted dose response as a function of dose consumed and strain variation, where strain variation refers to variation in strain virulence and prevalence. The model (Fig. 2) was created in an Excel 2000 spreadsheet (Microsoft Corporation, Redmond, WA) and was simulated using @Risk (version 4.0, @Risk, Palisade Corporation, Newfield, NY). Discrete distributions were used to model the dose groups and strain prevalence. During simulation, an illness dose (ID; $\log_{10}$) from the discrete distribution for strain prevalence and a dose consumed ($X$; $\log_{10}$) from the discrete distribution for dose consumed were randomly

**Fig. 1.** Data set used to develop the dose-response model in Fig. 2. Results from the original feeding trial were recorded in an Excel spreadsheet by strain and dose group, where each row of results in the spreadsheet represents a dose group. Entrance of the results in a spreadsheet facilitated their use in the dose-response model. As explained further in the legend to Fig. 2, the original feeding trial was simulated to verify the dose-response model predictions by using the cell addresses in this spreadsheet to define discrete distributions for the frequencies of dose groups for the individual strains in the dose-response model.

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Strain | Frequency | Illness Dose ($\log_{10}$) | Formula |
| 2 | Anatum I | 100 | 5.93 | =RiskPert(5.45,5.93,6.41) |
| 3 | Anatum II | 0 | 7.83 | =RiskPert(7.56,7.83,8.1) |
| 4 | Anatum III | 0 | 6.39 | =RiskPert(5.53,6.39,7.24) |
| 5 | Bareilly | 0 | 5.99 | =RiskPert(4.78,5.99,7.2) |
| 6 | Derby | 0 | 7.18 | =RiskPert(6.88,7.18,7.49) |
| 7 | Meleagridis I | 0 | 7.59 | =RiskPert(7.24,7.59,7.93) |
| 8 | Meleagridis II | 0 | 7.35 | =RiskPert(6.9,7.35,7.81) |
| 9 | Meleagridis III | 0 | 7.11 | =RiskPert(6.78,7.11,7.44) |
| 10 | Newport | 0 | 6.27 | =RiskPert(4.92,6.27,7.63) |
| 11 | Pullorum I | 0 | 9.67 | =RiskPert(9.33,9.67,10) |
| 12 | Pullorum II | 0 | 9.43 | =RiskPert(8.75,9.43,10.1) |
| 13 | Pullorum III | 0 | 9.46 | =RiskPert(9.11,9.46,9.8) |
| 14 | Pullorum IV | 0 | 9.32 | =RiskPert(8.07,9.32,10.57) |
| 15 | | | | |
| 16 | Dose Consumed | 8.14 | | =RiskDiscrete(Data!B2:B9,Data!D2:D9) |
| 17 | Illness Dose | 5.93 | | =RiskDiscrete(C2:C14,B2:B14) |
| 18 | Illness (0=no, 1=yes) | 1 | | =RiskOutput() + IF(B16<B17,0,1) |

**Fig. 2.** A simulation dose-response model for predicting the incidence of salmonellosis as a function of dose consumed and strain variation. The model was constructed in an Excel spreadsheet and was simulated using @Risk. The $\log_{10}$ minimum, median, and maximum illness doses from the three-phase linear model fits of the dose-response data for the individual strains of *Salmonella* (Table I) were used to define Pert distributions for illness dose of the individual strains (see Fig. 7 for an example). The frequencies of occurrence of the individual strains in the simulation scenario were entered in cells B2 to B14 of the model. A discrete distribution was used to model illness dose of individual consumption events in cell B17 of the model. Similarly, a discrete distribution was used to model the frequencies of occurrence of the dose groups for individual strains, whereas the iterations for the simulation of the scenario were used to model the number of subjects in the feeding trial. During simulation of the model, @Risk randomly selected an illness dose from the Pert distributions for the individual strains. The randomly selected illness doses for an iteration of the model are shown in cells C2 to C14 of the model. In addition, @Risk randomly selected a dose from the discrete distribution for the dose groups. The dose for an iteration of the model is shown in cell B16 of the model. To determine which of the 13 illness doses was used to calculate the response for the iteration, @Risk used the discrete distribution for illness dose in cell B17 to randomly select one of the 13 possible illness doses for the iteration. The selection of the illness dose was based on the frequencies of occurrence of the strains in the dose groups of the feeding trial. Finally, the dose response was calculated. If the dose consumed (cell B16) was less than the illness dose (cell B17) the model returned a zero indicating that an illness did not occur, otherwise the model returned a one indicating that an illness occurred. The sample scenario shown here was for simulating the original feeding trial for *Salmonella* Anatum I and therefore the frequency of occurrence of *S.* Anatum I was set to 100 and the frequencies of occurrence of all other *Salmonella* were set to zero. Note that the input settings for the discrete distribution for the dose groups are the cell addresses that corresponded to the location of the *S.* Anatum I data in the Data! spreadsheet shown in Fig. 1. The model scenario was simulated for 47 iterations, which was the number of feedings in the original trial. The output of the model was a discrete distribution for the 47 consumption events where there were two possible outcomes: no illness (i.e., output = 0) or illness (i.e., output = 1).

assigned to each consumption event in the simulated human feeding trial and individual dose response was calculated:

$$\text{Illness}\,(0 = \text{no}, 1 = \text{yes}) = \text{IF}(X < \text{ID}, 0, 1)$$

where no illness occurred (i.e., output = 0) when the dose was less than the illness dose, otherwise an illness occurred (i.e., output = 1). Note that the discrete distribution for dose consumed in Fig. 2 is linked to the database in Fig. 1 with cell addresses that correspond to the dose groups of *S.* Anatum I.

### 2.3. Dose-Response Model Verification

To verify the dose-response model predictions, the original feeding trials for individual strains were simulated using @Risk settings of 100 simulations (i.e., 100 replicates of the feeding trial), random selection of different random number generator seeds, where each seed generated a unique outcome of the model, and Latin Hypercube sampling (results were similar using Monte Carlo sampling). The number of iterations per simulation was equal to the number of feedings in the original trial. Illnesses per trial were calculated (output mean *x* iterations) and range and central tendency (median) of illnesses among the 100 trials were compared to observed illnesses in the original trial.

### 2.4. Effect of Strain Variation

Four "what if" feeding trials were simulated to investigate effects of strain variation (i.e., strain virulence and strain prevalence) on the shape of the dose-response curve. Dose groups from $10^4$ to $10^{10}$ in increments of $10^{0.1}$ *Salmonella* per glass of eggnog and 10,000 subjects per dose group were simulated for each trial. Simulation settings of 10,000 iterations (i.e., number of subjects per dose group), one simulation per trial, a seed of one (arbitrary selection), and Latin Hypercube sampling were used. Dose-response curves of the incidence of salmonellosis versus dose consumed were generated and visually compared.

## 3. RESULTS

### 3.1. Dose-Response Modeling

None of the data for individual strains completely defined the dose-response curve. Some strains (Bareilly and Newport in Fig. 4) lacked data at low and high dose responses, some strains (Anatum I, II, and III in Fig. 3, Derby in Fig. 4, Meleagridis I, II, and

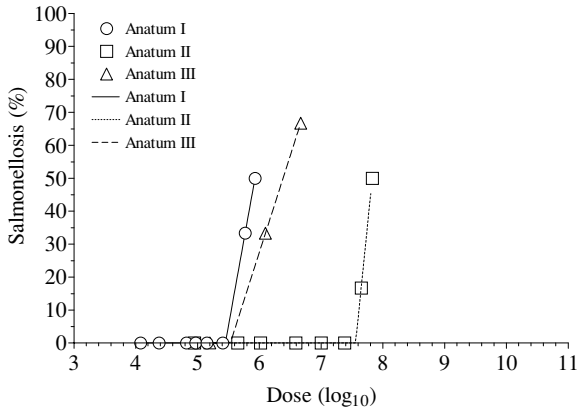


**Fig. 3.** Three-phase linear model fits for the three strains of *Salmonella* Anatum. Despite incomplete data at high dose responses, the model fit converged in all cases.

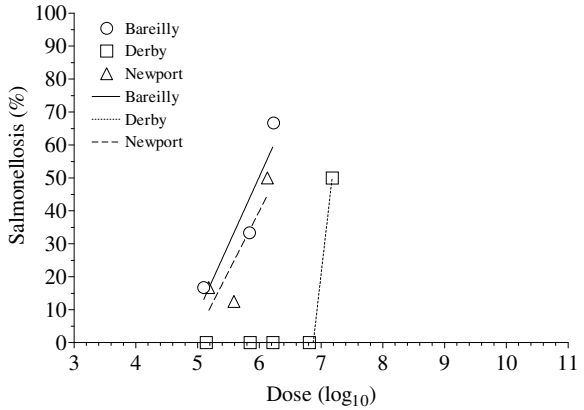


**Fig. 4.** Three-phase linear model fits for *Salmonella* Bareilly, Derby, and Newport. Despite incomplete data at low and high dose responses, the model fit converged in all cases.

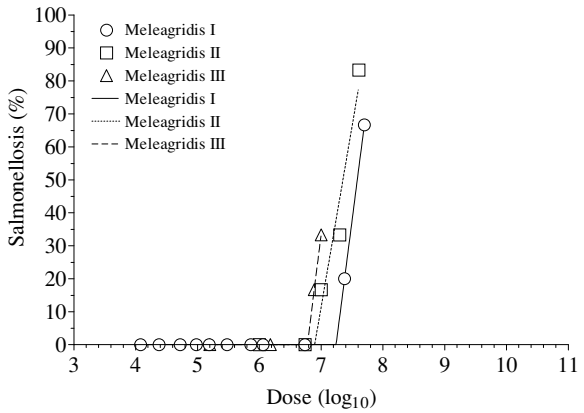


**Fig. 5.** Three-phase linear model fits for the three strains of *Salmonella* Meleagridis. Despite incomplete data at high dose responses, the model fit converged in all cases.

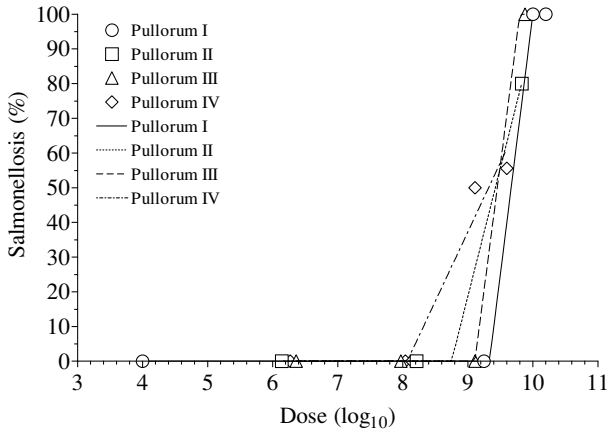


**Fig. 6.** Three-phase linear model fits for the four strains of *Salmonella* Pullorum. Despite missing data at low, intermediate, and high dose responses, the model fit converged in all cases.

III in Fig. 5, and Pullorum II and IV in Fig. 6) lacked data at high dose responses and some strains (Pullorum I and III in Fig. 6) lacked data at intermediate dose responses. The three-phase linear model was selected because it was capable of fitting dose-response curves with missing data by extrapolating beyond the data. The advantages and disadvantages of such extrapolation are addressed later. In fact, the model fit converged in all cases with $R^2$ that ranged from 0.73 to 1.00 (Table I). Variation in minimum, median, and maximum illness doses was observed among strains and thus justified the need for a dose-response model that considered differences in strain virulence.

**Table I.** Parameters and Goodness-of-Fit of the Three-Phase Linear Model to the Dose-Response Data for the Individual Strains of *Salmonella*

| Strains | $X_{min}$ | $X_{med}$ | $X_{max}$ | $df$ | $R^2$ |
|---|---|---|---|---|---|
| Anatum I | 5.45 | 5.93 | 6.41 | 6 | 1.000 |
| Anatum II | 7.56 | 7.83 | 8.10 | 6 | 1.000 |
| Anatum III | 5.53 | 6.39 | 7.24 | 1 | 1.000 |
| Bareilly | 4.78 | 5.99 | 7.20 | 1 | 0.870 |
| Derby | 6.88 | 7.18 | 7.49 | 3 | 1.000 |
| Newport | 7.24 | 7.59 | 7.93 | 1 | 0.729 |
| Meleagridis I | 6.90 | 7.35 | 7.81 | 9 | 1.000 |
| Meleagridis II | 6.78 | 7.11 | 7.44 | 3 | 0.964 |
| Meleagridis III | 4.92 | 6.27 | 7.63 | 2 | 1.000 |
| Pullorum I | 9.33 | 9.67 | 10.00 | 2 | 1.000 |
| Pullorum II | 8.75 | 9.43 | 10.10 | 1 | 1.000 |
| Pullorum III | 9.11 | 9.46 | 9.80 | 2 | 1.000 |
| Pullorum IV | 8.07 | 9.32 | 10.57 | 3 | 0.967 |

Abbreviations: $X_{min} = \log_{10}$ minimum illness dose; $X_{med} = \log_{10}$ median illness dose; $X_{max} = \log_{10}$ maximum illness dose; $df$ = degrees of freedom; and $R^2$ = coefficient of determination.

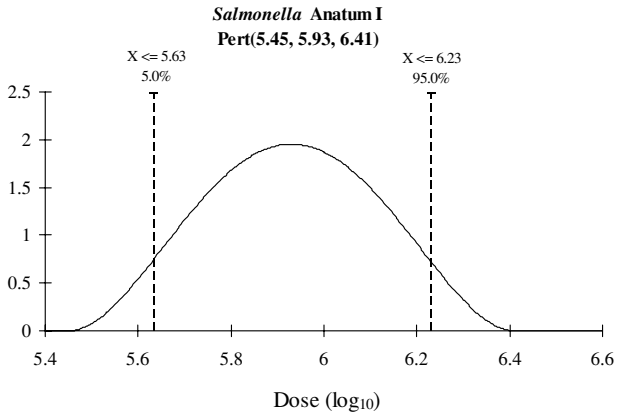


**Fig. 7.** An example of a Pert distribution for illness dose of *Salmonella* (i.e., Anatum I) that was obtained by using the $log_{10}$ minimum, median, and maximum illness doses from the three-phase linear model fit (Fig. 3 and Table I) of the dose-response data.

**Table III.** "What If" Scenarios for Determining the Effect of Strain Variation on the Shape of the Dose-Response Curve for Salmonellosis

| | Scenarios | | | |
|---|---|---|---|---|
| Strains | A | B | C | D |
| Anatum I | 100[a] | 25 | 0 | 10 |
| Anatum II | 0 | 75 | 0 | 10 |
| Anatum III | 0 | 0 | 10 | 0 |
| Bareilly | 0 | 0 | 0 | 0 |
| Derby | 0 | 0 | 20 | 10 |
| Newport | 0 | 0 | 0 | 10 |
| Meleagridis I | 0 | 0 | 0 | 0 |
| Meleagridis II | 0 | 0 | 30 | 0 |
| Meleagridis III | 0 | 0 | 0 | 10 |
| Pullorum I | 0 | 0 | 0 | 10 |
| Pullorum II | 0 | 0 | 40 | 0 |
| Pullorum III | 0 | 0 | 0 | 10 |
| Pullorum IV | 0 | 0 | 0 | 10 |

[a]Frequency.

### 3.2. Dose-Response Model Verification

Dose-response curves for individual strains were converted into Pert distributions for illness dose using minimum, median, and maximum values from the three-phase linear model fits. A representative example of a Pert distribution for illness dose is shown in Fig. 7. Pert distributions for illness dose were then combined in a computer spreadsheet to form a simulation model for predicting the incidence of salmonellosis as a function of dose consumed and strain prevalence (Fig. 2). To verify this approach to dose-response modeling, the original feeding trial was simulated and the results obtained are shown in Table II. In all cases the number of observed illnesses was within the range of illnesses predicted by the model and overall, the model predicted a median of 69 (range of 43 to 101) illnesses compared to 74 illnesses in the original trial. Thus, the dose-response model predictions were in agreement with the data used to develop it.

### 3.3. Effects of Strain Variation on Dose Response

Four "what if" scenarios with different combinations of the 13 strains (Table III) were simulated to determine effects of strain variation on the shape of

**Table II.** Illnesses Observed in the Original Feeding Trial and Predicted by the Dose-Response Model for *Salmonella*

| Strains | Feedings per Trial | Trials | Observed Illnesses | Predicted Illnesses | | |
|---|---|---|---|---|---|---|
| | | | | Minimum | Median | Maximum |
| Anatum I | 47 | 100 | 5 | 1 | 4 | 9 |
| Anatum II | 50 | 100 | 5 | 1 | 4 | 7 |
| Anatum III | 18 | 100 | 6 | 3 | 6 | 8 |
| Bareilly | 18 | 100 | 7 | 4 | 6 | 9 |
| Derby | 30 | 100 | 3 | 0 | 3 | 5 |
| Meleagridis I | 64 | 100 | 5 | 2 | 5 | 8 |
| Meleagridis II | 30 | 100 | 8 | 6 | 8 | 12 |
| Meleagridis III | 24 | 100 | 3 | 0 | 1 | 4 |
| Newport | 20 | 100 | 5 | 1 | 3 | 6 |
| Pullorum I | 24 | 100 | 12 | 12 | 12 | 12 |
| Pullorum II | 17 | 100 | 4 | 4 | 5 | 5 |
| Pullorum III | 24 | 100 | 6 | 6 | 6 | 6 |
| Pullorum IV | 30 | 100 | 5 | 3 | 6 | 10 |
| Total | | | 74 | 43 | 69 | 101 |

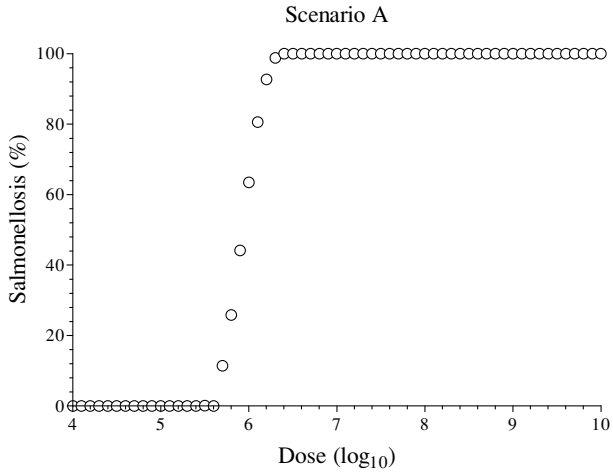


**Fig. 8.** Dose-response curve for salmonellosis that was obtained by simulating scenario A in Table III, which was for one strain of *Salmonella*.

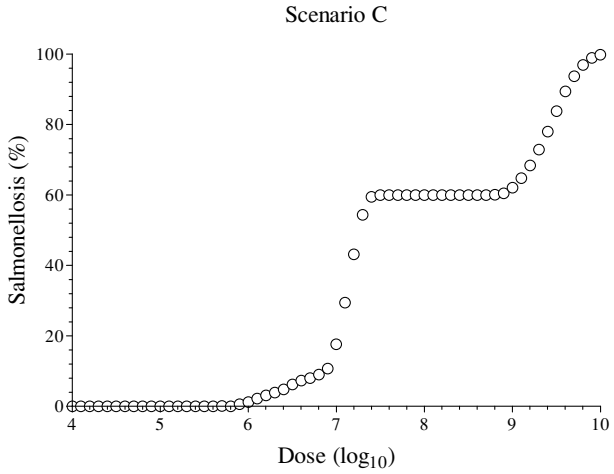


**Fig. 10.** Dose-response curve for salmonellosis that was obtained by simulating scenario C in Table III, which was for four strains of *Salmonella* with different virulence.

the dose-response curve. When one strain (Anatum I) was simulated, the dose-response curve had three phases (Fig. 8). When two strains of different virulence (Anatum I and II) were simulated, a five-phase dose-response curve was obtained with a bottom, middle, and top asymptote (Fig. 9). The middle asymptote occurred in the region where the dose consumed was greater than the maximum illness dose of the more virulent strain (Anatum I) and less than the minimum illness dose of the less virulent strain (Anatum II). When four (Fig. 10) or eight (Fig. 11) strains were simulated, the shape of the dose-response curve became less sigmoid and more complex. These results demonstrated that the predicted dose-response curve does not have a sigmoid shape when multiple strains with different virulence are present.

## 4. DISCUSSION

Infection and illness data from a human feeding trial in Chicago[8,21,22] have been used to develop different types of dose-response models for *Salmonella*. Teunis *et al.*[10] used data for Meleagridis III to model probability of illness as a function of infection

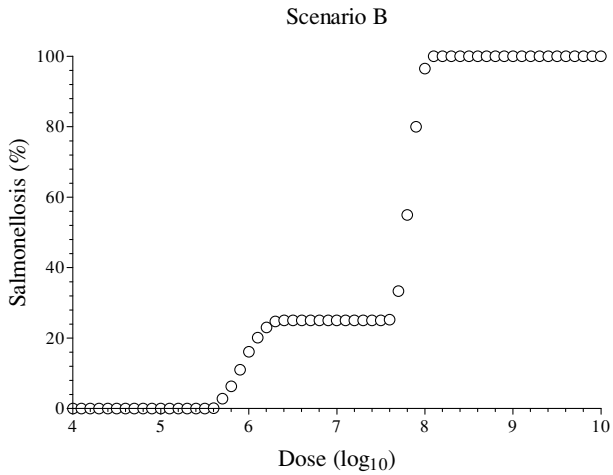


**Fig. 9.** Dose-response curve for salmonellosis that was obtained by simulating scenario B in Table III, which was for two strains of *Salmonella* with different virulence.

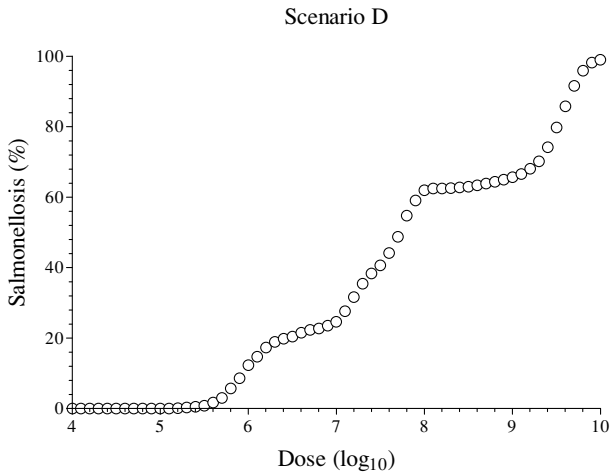


**Fig. 11.** Dose-response curve for salmonellosis that was obtained by simulating scenario D in Table III, which was for eight strains of *Salmonella* with different virulence.

using a beta-Poisson dose-response model. Coleman and Marks[13] modeled combined illness data for 9 of 13 strains using logistic and Gompertz dose-response models. Latimer *et al.*[24] categorized selected strains into low (Anatum II and Meleagridis I) and moderate (Anatum I, Bareilly, and Newport) virulence groups and developed a composite model for predicting probability of illness as a function of group prevalence. In the current study, a dose-response model for predicting salmonellosis as a function of dose consumed for all 13 strains of *Salmonella* in the Chicago feeding trial was developed. The current model differs from previous models in that it can predict dose response as a function of the prevalence of all 13 strains in the original study.

Dose-response experiments, whether conducted with animals or humans, are expensive and time consuming. Consequently, it is difficult to obtain sufficient data to properly define dose-response curves for even one dose-response condition. In fact, most dose-response experiments have insufficient numbers of dose groups and insufficient numbers of subjects per dose group to accurately define complete dose-response curves. An important characteristic of the three-phase linear model, which was used in the current study, was the ability to fit incomplete dose-response curves. In fact, the three-phase linear model was able to model dose response even when data were missing at low, intermediate, and/or high dose responses. The ability of the three-phase linear model to fit incomplete dose-response data can lower the cost of future feeding trials by reducing the number of dose groups needed to estimate the dose-response relationship.

When an attempt was made to fit the incomplete dose-response data to the more commonly used exponential and beta-Poisson dose-response models, the curve-fitting routine, which used a Levenberg-Marquardt method of iteration, failed to converge. However, other modelers have successfully fit exponential and beta-Poisson models to similar dose-response data by using a maximum likelihood method.[11,12] Thus, the method of curve fitting may explain the lack of fit in the present study. Nonetheless, it should be mentioned that like the exponential and beta-Poisson models, the three-phase linear model is capable of fitting dose-response curves with a minimum illness dose of one. However, this would require data at low doses, as the three-phase linear model does not automatically assume a minimum illness dose of one.

In agreement with Coleman and Marks[13] and Latimer *et al.*,[24] variation in virulence among the 13 strains of *Salmonella* was observed. In the current study, the minimum illness dose ranged from $10^{4.8}$ for Bareilly to $10^{9.3}$ for Pullorum I. High minimum illness doses in this study reflect the highly resistant host population (healthy men) as well as strains with low virulence, such as Pullorum, which is highly pathogenic in chickens but only weakly pathogenic in humans.[3] To model variation in virulence as well as prevalence of the strains, a simulation approach was used in the present study. To verify this approach, the ability of the dose-response model to simulate the original feeding trial was tested and the predicted cases of salmonellosis were in close agreement with those observed in the original trial. Thus, the dose-response approach was verified.

Although the model was verified for predicting dose response for the Chicago trial, which was conducted in the late 1940s, the use of the model to predict dose response in the current marketplace is not recommended for the following reasons. First, of the 13 strains of *Salmonella* tested only Newport is listed in the top 15 serotypes isolated from human clinical samples in the United States between 1987 and 1997.[25] Second, selection of only healthy men in the Chicago trial and repeat feeding of some subjects, which was shown to increase resistance to salmonellosis,[26] resulted in a host population with high resistance to salmonellosis and may account for the high illness doses observed among the 13 strains of *Salmonella*. Thus, the model predictions are only applicable to the very resistant portion of the consumer population and would grossly underpredict the public health risk of salmonellosis if the model were applied to the general consumer population, which contains many individuals with considerably lower resistance to *Salmonella* due to such factors as young or old age, chemotherapy, AIDS, malnutrition, and pregnancy, all of which compromise the immune system and result in low resistance to infectious disease, such as salmonellosis.

Clearly, there is a need for more feeding trials with foods, strains of *Salmonella*, and consumers that are more typical of those found in the current marketplace. A key to being able to conduct such studies in an ethical manner is to greatly lower the risk of adverse health effects among volunteers. This could be accomplished by feeding low doses of *Salmonella*, by using infection rather than illness as the response endpoint, and by using the three-phase linear model to minimize the number of subjects needed to

estimate the dose-response curve. Although infection is a less desirable endpoint than illness, having infection as the dose-response endpoint may increase the feasibility of conducting human feeding trials, may increase the feasibility of including high-risk individuals in the dose groups, and would result in more conservative dose-response models than illness-based dose-response models.

In feeding trials where one combination of food, pathogen, and host factors is investigated, the dose-response curve follows a sigmoid pattern that can be fit to a number of dose-response models.[17] In contrast, in the current study, when a model capable of generating dose-response curves as a function of strain virulence and prevalence was simulated, the dose-response curves obtained were irregular in shape. Similarly, Latimer *et al*.[24] found that the predicted dose-response curves for *Salmonella* are irregular in shape when strain virulence and prevalence are included in the model. By analogy, inclusion of multiple food and host factors in the feeding trial and model may also add further irregularity to the shape of the dose-response curve. Thus, an approach such as that used in the current study may be needed to develop models that are capable of predicting dose response as a function of a matrix of food, pathogen, and host factors.

## ACKNOWLEDGMENTS

Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

## REFERENCES

1. D'Aoust, J.-Y. (1991). Pathogenicity of foodborne *Salmonella*. *International Journal of Food Microbiology*, *12*, 17–40.
2. Prost, E., & Reimann, H. (1967). Food-borne salmonellosis. *Annual Review of Microbiology*, *21*, 495–528.
3. Baird-Parker, A. C. (1990). Foodborne salmonellosis. *Lancet*, *336*, 1231–1235.
4. Cohen, M. L., & Tauxe, R. V. (1986). Drug-resistant *Salmonella* in the United States: An epidemiologic perspective. *Science*, *234*, 964–969.
5. Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M., & Tauxe, R. V. (1999). *Food-Related Illness and Death in the United States*. http://www.cdc.gov/ncidod/eid/vol5no5/mead.htm.
6. Blaser, M. J., & Newman, L. S. (1982). A review of human salmonellosis: I. Infective dose. *Review of Infectious Disease*, *4*, 1096–1106.
7. Dupont, H. L., Hornick, R. B., Dawkins, A. T., Snyder, M. J., & Formal, S. B. (1969). The response of man to virulent *Shigella flexneri* 2a. *Journal of Infectious Disease*, *119*, 296–299.
8. McCullough, N. B., & Eisele, C. W. (1951). Experimental human salmonellosis. I. Pathogenicity of strains of *Salmonella meleagridis* and *Salmonella anatum* obtained from spray-dried whole egg. *Journal of Infectious Disease*, *88*, 278–279.
9. Medema, G. J., Teunis, P. F. M., Havelaar, A. H., & Haas, C. N. (1996). Assessment of the dose-response relationship of *Campylobacter jejuni*. *International Journal of Food Microbiology*, *30*, 101–111.
10. Teunis, P. F. M., Nagelkerke, N. J. D., & Haas, C. N. (1999). Dose response models for infectious gastroenteritis. *Risk Analysis*, *19*, 1251–1260.
11. Haas, C. N., Thayyar-Madabusi, A., Rose, J. B., & Gerba, C. P. (2000). Development of a dose-response relationship for *Escherichia coli* O157:H7. *International Journal of Food Microbiology*, *56*, 153–159.
12. Regli, S., Rose, J. B., Haas, C. N., & Gerba, C. P. (1991). Modeling the risk from *Giardia* and viruses in drinking water. *Journal of American Water Works Association*, *83*, 76–84.
13. Coleman, M. E., & Marks, H. M. (1998). Topics in dose-response modeling. *Journal of Food Protection*, *61*, 1550–1559.
14. Crockett, C. S., Haas, C. N., Fazil, A., Rose, J. B., & Gerba, C. P. (1996). Prevalence of shigellosis in the U.S.: Consistency with dose-response information. *International Journal of Food Microbiology*, *30*, 87–99.
15. Haas, C. N. (1983). Estimation of risk due to low doses of microorganisms: A comparison of alternative methodologies. *American Journal of Epidemiology*, *118*, 573–582.
16. Rose, J. B., Haas, C. N., & Gerba, C. P. (1995). Linking microbiological criteria for foods with quantitative risk assessment. *Journal of Food Safety*, *15*, 121–132.
17. Holcomb, D. L., Smith, M. A., Ware, G. O., Hung, Y. C., Brackett, R. E., & Doyle, M. P. (1999). Comparison of six dose-response models for use with food-borne pathogens. *Risk Analysis*, *19*, 1091–1100.
18. Gibson, A. M., Bratchell, N., & Roberts, T. A. (1988). Predicting microbial growth: Growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. *International Journal of Food Microbiology*, *6*, 155–178.
19. Baranyi, J., Roberts, T. A., & McClure, P. (1993). A non-autonomous differential equation to model bacterial growth. *Food Microbiology*, *10*, 43–59.
20. Buchanan, R. L. (1993). Developing and distributing user-friendly application software. *Journal of Industrial Microbiology*, *12*, 251–255.
21. McCullough, N. B., & Eisele, C. W. (1951). Experimental human salmonellosis. III. Pathogenicity of strains of *Salmonella newport*, *Salmonella derby*, and *Salmonella Bareilly* obtained from spray-dried whole egg. *Journal of Infectious Disease*, *89*, 209–213.
22. McCullough, N. B., & Eisele, C. W. (1951). Experimental human salmonellosis. IV. Pathogenicity of strains of *Salmonella pullorum* obtained from spray-dried whole egg. *Journal of Infectious Disease*, *89*, 259–265.
23. Buchanan, R. L., Whiting, R. C., & Damert, W. C. (1997). When is simple good enough: A comparison of the Gompertz, Baranyi and three-phase linear models for fitting bacterial growth curves. *Food Microbiology*, *14*, 313–326.
24. Latimer, H. K., Jaykus, L. A., Morales, R. A., Cowen, P., & Crawford-Brown, D. (2001). A weighted composite dose-response model for human salmonellosis. *Risk Analysis*, *21*, 295–305.
25. Olsen, S. J., Bishop, R., Brenner, F. W., Roels, T. H., Bean, N., Tauxe, R. V., & Slutsker, L. (2001). The changing epidemiology of *Salmonella*: Trends in serotypes isolated from humans in the United States, 1987–1997. *Journal of Infectious Disease*, *183*, 753–761.
26. McCullough, N. B., & Eisele, C. W. (1951). Experimental human salmonellosis: II. Immunity studies following experimental illness with *Salmonella meleagridis* and *Salmonella anatum*. *Journal of Immunology*, *66*, 595–608.