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Research Paper

Monte Carlo Simulation Model for Predicting *Salmonella* Contamination of Chicken Liver as a Function of Serving Size for Use in Quantitative Microbial Risk Assessment

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ABSTRACT

The first step in quantitative microbial risk assessment (QMRA) is to determine the distribution of pathogen contamination among servings of the food in question at some point in the farm-to-table chain. In the present study, the distribution of *Salmonella* contamination among servings of chicken liver for use in the QMRA was determined at meal preparation. *Salmonella* prevalence (*P*), most probable number (MPN, *N*), and serotype for different serving sizes were determined by use of a combination of five methods: (i) whole sample enrichment; (ii) quantitative PCR; (iii) culture isolation; (iv) serotyping; and (v) Monte Carlo simulation. Epidemiological data also were used to convert serotype data to virulence (*V*) values for use in the QMRA. A Monte Carlo simulation model based in Excel and simulated with @Risk predicted *Salmonella P, N,* serotype, and *V* as a function of a serving size of one (58 g) to eight (464 g) chicken livers. *Salmonella P* of chicken livers was 72.5% (58 of 80) per 58 g. Four *Salmonella* serotypes were isolated from chicken livers: (i) Infantis ($P = 28\%$, $V = 4.5$); (ii) Enteritidis ($P = 15\%$, $V = 5$); (iii) Typhimurium ($P = 15\%$, $V = 4.8$); and (iv) Kentucky ($P = 15\%$, $V = 0.8$). *Salmonella N* was 1.76 log MPN/58 g (median) with a range of 0 to 4.67 log MPN/58 g, and the median *Salmonella N* was not affected ($P > 0.05$) by serotype. The model predicted a nonlinear increase ($P \leq 0.05$) of *Salmonella P* from 72.5%/58 g to 100%/464 g, a minimum *N* of 0 log MPN/58 g to 1.28 log MPN/464 g, and a median *N* from 1.76 log MPN/58 g to 3.22 log MPN/464 g. Regardless of serving size, predicted maximum *N* was 4.74 log MPN per serving, mean *V* was 3.9 per serving, and total *N* was 6.65 log MPN per lot (10,000 chicken livers). The data acquired and modeled in this study address an important data gap in the QMRA for *Salmonella* and whole chicken liver.

HIGHLIGHTS

- Quantitative data for *Salmonella* contamination of chicken liver were collected.
- A model for *Salmonella* contamination of chicken liver servings was developed.
- *Salmonella* prevalence should be expressed as a function of sample size.
- *Salmonella* serotype data should be collected for risk assessment.
- A dap gap in risk assessment for *Salmonella* and chicken liver was addressed.

Key words: Chicken liver; Contamination; Predictive microbiology; Predictive model; Quantitative microbial risk assessment; *Salmonella*

Quantitative microbial risk assessment (QMRA) is a holistic approach to food safety that uses process risk models to simulate consumer exposure and response to pathogens that contaminate food produced by specific farm-to-table scenarios *(3, 17, 31, 33)*. A process risk model could be used at the processing plant exit to identify unsafe lots of food before they are shipped to consumers and cause foodborne illness *(15, 25, 38)*. In addition to pathogen prevalence, a QMRA considers pathogen level, type, virulence, and postprocessing risk factors such as temperature abuse, cross-contamination, undercooking, host resistance, and food consumption behavior in its assessment of foodborne illness risk *(1, 23, 24, 37)*.

The first step of QMRA is to determine distribution of pathogen contamination (prevalence, level, and type) among servings of food at some point in the production chain *(19, 20)*. Obtaining these data is time-consuming and expensive and realistically can be done only at one point in the food production chain and for one sample size. However, data collected with one sample size can be used in a Monte Carlo simulation model to predict pathogen contamination as a function of serving size *(18, 24, 25)*.

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Thus, it is possible to collect pathogen contamination data for QMRA in a cost-efficient and timely manner.

Chicken liver is a high-risk food because it is often contaminated with *Salmonella (10, 12)* and *Campylobacter (34)* bacteria, which are leading causes of foodborne illness *(32)*. Blended chicken liver (e.g., paté) and whole chicken livers are often undercooked to maintain eating quality *(28),* resulting in sporadic cases and outbreaks of foodborne illness *(13)*. An important need in QMRA for chicken liver is quantitative data for *Salmonella* contamination. Consequently, in the present study, data for *Salmonella* contamination of whole chicken liver were collected at meal preparation and used in a Monte Carlo simulation model *(24, 25)* to predict *Salmonella* prevalence, level, serotype, and virulence as a function of serving size for use in a QMRA that predicts risk of salmonellosis from individual lots of whole chicken liver. Consequently, data for *Salmonella* contamination were collected with one brand and over a limited time to simulate a lot of whole chicken livers. Although blended chicken liver (e.g., paté) is responsible for more cases of foodborne illness *(13),* whole chicken livers were examined in the present study because this is the primary type of chicken liver preparation sold, prepared, and consumed in the study area (Delmarva Peninsula, eastern United States), which is a major area of chicken production.

MATERIALS AND METHODS

Materials. Chicken livers were purchased at a local retail store (Salisbury, MD) from 30 April to 9 October 2018. They were sold in sealed plastic containers (473.2 mL) and were of a single brand. Weight and pH (pH Spear, Oaktron Instruments, Vernon Hills, IL) of chicken livers were determined at the time of analysis.

Buffered peptone water (BPW) was from Microbiology International (Frederick, MD). Rappaport-Vassiliadis broth (RVB) and xylose lysine Tergitol 4 (XLT4) agar were from BD (Sparks, MD). iQ-Check for *Salmonella* test kits were from Bio-Rad (Hercules, CA). Reveal 2.0 test kits for *Salmonella* were from Neogen (East Lansing, MI). Excel Office 365 was from Microsoft Corporation (Redmond, WA). @Risk version 7.6 was from Palisade Corporation (Ithaca, NY). Prism version 9.1 was from GraphPad Software (San Diego, CA).

***Salmonella* levels.** Whole sample enrichment followed by quantitative PCR (WSE-qPCR) was used to determine *Salmonella* prevalence and level on or in whole chicken livers *(24, 25)*. For standard curve development, cultures of *Salmonella* Typhimurium and *Salmonella* Infantis, which were isolated from chicken livers in the present study, were incubated for 96 h at 22°C in 1 mL of BPW to obtain stationary-phase cells for inoculation of chicken livers. The 96-h cultures were serially diluted (1:10) in BPW to 10^{-7} most probable number (MPN), and then 5 μL of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, or 10^{-7} dilutions were inoculated onto chicken livers ($n = 72$) to achieve initial levels of 0 to 5 log MPN per whole chicken liver. The level of *Salmonella* in the undiluted cultures used for inoculation was determined using an automated miniature MPN method *(21)*. The MPN was calculated using an Excel spreadsheet calculator *(11)*.

Inoculated samples (one whole chicken liver) were incubated in 400 mL of BPW in stomacher bags for 6 h at 40°C and 80 rpm *(24, 25)*. After 6 h of WSE, 1 mL of the BPW culture was removed and used for qPCR with iQ-Check for *Salmonella*. The PCR cycle that detected *Salmonella* or the cycle threshold (C_T) value was graphed as a function of inoculated log MPN of *Salmonella*. Data were then fitted to the Weibull model using Prism software:

$$C_T = C_{T0} - \left(\frac{N}{a}\right)^b$$

where C_{T0} is the C_T value at 0 log MPN *Salmonella* per liver, N is the level of *Salmonella* (log MPN) per liver, a is a regression coefficient, and b is a shape parameter.

Some inoculated food samples will have indigenous *Salmonella* on or in them, which will lower the C_T *(20, 22)*. Inclusion of these samples in curve fitting would result in a standard curve that underestimates the *Salmonella* MPN. Therefore, to develop a more accurate and fail-safe standard curve for enumeration of *Salmonella,* samples with low C_T within a dose of inoculated *Salmonella* were excluded during curve fitting. In addition, C_{T0} was fixed to the maximum observed C_T value in the study during curve fitting to ensure that the minimum MPN of *Salmonella* interpolated from the standard curve for naturally contaminated samples was $\geq$0 log MPN per liver.

To determine *Salmonella* prevalence and level on naturally contaminated chicken livers, uninoculated chicken livers ($n = 80$) were subjected to the same WSE-qPCR procedure as described for inoculated chicken livers used in standard curve development. The number of uninoculated livers analyzed ($n = 80$) was based on time, cost, and *Salmonella* prevalence. The goal was to obtain enough data to define a pert distribution for simulating *Salmonella* level in the model.

***Salmonella* isolation and serotyping.** At 6 h of WSE for uninoculated chicken livers that tested positive for *Salmonella* in the qPCR assay, a second 1-mL BPW enrichment culture sample was collected and used for culture isolation of *Salmonella* and confirmation of qPCR results *(24, 25)*. *Salmonella* was isolated in three steps: (i) incubation of the BPW enrichment sample (1 mL) for an additional 24 h at 40°C; (ii) selective enrichment of 10 μL of BPW enrichment culture in 1 mL of RVB for 24 h at 42°C; and (iii) selective growth of 1 μL of RVB enrichment culture streaked onto XLT4 agar and incubated for 24 h at 40°C. One presumptive colony of *Salmonella* was picked per XLT4 plate or sample, regrown in BPW for 24 h at 40°C, confirmed by the Reveal 2.0 antigen-antibody test for *Salmonella,* and then serotyped by a *Salmonella* Reference Center (U.S. Department of Agriculture [USDA], National Veterinary Services Laboratory, Ames, IA).

Contamination model. Data for *Salmonella* contamination of individual chicken livers (Table 1) were used in a published Monte Carlo simulation model *(24, 25)* to predict *Salmonella* prevalence, level, serotype, and virulence as a function of serving sizes of one to eight chicken livers. The model was developed in Excel and simulated with @Risk, a spreadsheet add-in program. A rare events modeling method was used in which a discrete distribution was used to simulate the *Salmonella* serotype prevalence data (Table 1) and a pert (minimum, mode, and maximum) distribution was used to simulate the *Salmonella* level data (Table 1).

Outputs from the pert distributions for *Salmonella* level in the model were used to calculate model outputs only when the output of the corresponding discrete distribution for *Salmonella* serotype prevalence indicated that *Salmonella* was present. Because not all outputs from the pert distributions were used to calculate model outputs, the sensitivity analysis provided by @Risk was not

TABLE 1. Salmonella *isolation date, cycle threshold (C_T), serotype, and level on and in contaminated chicken liver*

Date (mo/day/yr)	C_T	Serotype	Level (log MPN/liver)
4/30/2018	25.52	Infantis	2.64
4/30/2018	26.15	Infantis	2.45
4/30/2018	27.13	Typhimurium	2.17
4/30/2018	27.44	Typhimurium	2.09
4/30/2018	27.73	Infantis	2.01
4/30/2018	28.43	Typhimurium	1.81
4/30/2018	30.86	Typhimurium	1.17
4/30/2018	31.05	Typhimurium	1.12
4/30/2018	31.10	Typhimurium	1.11
4/30/2018	32.55	Typhimurium	0.75
5/7/2018	31.13	Infantis	1.10
5/7/2018	31.34	Infantis	1.05
5/7/2018	31.40	Kentucky	1.03
5/7/2018	31.75	Infantis	0.95
5/7/2018	32.00	Infantis	0.89
5/7/2018	32.22	Infantis	0.83
5/7/2018	32.35	Infantis	0.80
5/14/2018	24.31	Kentucky	2.99
5/14/2018	24.92	Infantis	2.81
5/14/2018	27.09	Infantis	2.19
5/14/2018	27.49	Enteritidis	2.07
5/14/2018	27.57	Typhimurium	2.05
5/14/2018	27.71	Enteritidis	2.01
5/14/2018	29.03	Enteritidis	1.65
5/14/2018	29.67	Enteritidis	1.48
5/21/2018	26.66	Typhimurium	2.31
5/21/2018	26.70	Infantis	2.30
5/21/2018	29.89	Typhimurium	1.42
5/21/2018	30.27	Typhimurium	1.32
5/21/2018	34.65	Infantis	0.29
5/21/2018	35.07	Infantis	0.21
5/21/2018	36.21	Typhimurium	0.02
6/11/2018	25.28	Enteritidis	2.71
6/11/2018	30.15	Enteritidis	1.35
6/11/2018	35.44	Enteritidis	0.14
6/24/2018	27.65	Kentucky	2.03
6/24/2018	28.79	Kentucky	1.71
6/24/2018	29.90	Kentucky	1.42
6/24/2018	30.55	Kentucky	1.25
6/24/2018	31.15	Kentucky	1.10
6/24/2018	32.40	Infantis	0.79
6/24/2018	34.47	Kentucky	0.33
6/24/2018	35.11	Kentucky	0.20
8/13/2018	24.03	Infantis	3.08
8/13/2018	24.05	Infantis	3.07
8/13/2018	25.45	Infantis	2.66
8/13/2018	26.20	Infantis	2.44
8/13/2018	26.53	Infantis	2.34
8/13/2018	26.54	Kentucky	2.34
8/13/2018	26.58	Infantis	2.33
8/13/2018	27.04	Kentucky	2.20
8/13/2018	27.19	Infantis	2.16
8/13/2018	28.03	Kentucky	1.92
8/27/2018	18.82	Enteritidis	4.67
8/27/2018	28.1	Enteritidis	1.96
8/27/2018	28.54	Enteritidis	1.85
8/27/2018	29.67	Enteritidis	1.58
8/27/2018	32.41	Enteritidis	0.98

accurate and thus was not used. Nonetheless, both *Salmonella*-contaminated and noncontaminated servings were simulated together to provide a more accurate simulation and prediction of *Salmonella* contamination as a function of serving size.

The pert distribution was used in the model to simulate variability of *Salmonella* level among chicken livers because it is an easy distribution to define, is flexible, and provides good generalization for small sets of data. This distribution is flexible because it can vary in shape from a normal distribution to a lognormal distribution that is skewed to the right or left, which gives it the ability to simulate a wide range of distribution types.

To further justify use of the pert distribution to simulate *Salmonella* level per chicken liver, the BestFit option of @Risk was used to fit probability distributions to the *Salmonella* level data (Table 1). Before fitting of probability distributions, a lower bound of 0 log MPN per chicken liver and an upper bound of 5 log MPN per chicken liver were set. This approach resulted in only five possible distributions for fitting: (i) BetaGeneral; (ii) Kumaraswamy; (iii) pert; (iv) triangle; and (v) uniform. The Akaike information criterion (AIC) was used to identify the best-fitting distribution. Lower and upper bounds were set to avoid identification and use of an unbounded probability distribution that could make inaccurate (<0 or >5 log MPN) predictions of *Salmonella* level on or in chicken liver.

Epidemiological data were used to assign a virulence (V) value from low (0.1) to high (5.0) in 0.1-unit increments for each *Salmonella* serotype *(25)*. For serotypes ranked in the top 20 of human clinical isolates in 2016,

$$V = 5.1 - 0.1(r)$$

where r is the epidemiological rank from 1 to 20. For *Salmonella* serotypes ranked outside the top 20 human clinical isolates,

$$V = 3.1 \times (c/257)$$

where c is the number of illness cases for that serotype and 257 is the number of illness cases for the 20th ranked serotype, which was *Salmonella* Anatum.

The virulence value is used in a disease triangle dose-response model to identify illness dose for individual servings of food *(25)*. Although the disease triangle dose-response model was not used in the present study, it is described briefly here so that the importance of obtaining data for *Salmonella* serotype and predicting *Salmonella* virulence can be understood. The virulence value predicted by the model is added to similar values for food consumption behavior and host resistance in the dose-response model to obtain a disease triangle score for the simulated serving. The disease triangle score is used to calculate the minimum, mode, and maximum values of a pert distribution for illness dose. The dose-response model has 115 possible pert distributions for illness dose. Thus, the disease triangle score identifies which pert distribution to use for the simulated serving.

Once the pert distribution for illness dose is identified, it is randomly sampled to provide an illness dose for the serving simulated. The illness dose is used to calculate the severity of salmonellosis as follows: when the ratio of the level of *Salmonella* consumed to illness dose is >0 but <1 an infection occurs, whereas when the ratio is ≥ 1 an illness of increasing severity occurs. Thus, *Salmonella* serotype and predicted virulence are important information for a QMRA.

Model simulation. Eight scenarios were simulated, one for each serving size of one to eight chicken livers. Model scenarios were simulated with @Risk settings of Latin Hypercube sampling, Mersenne Twister, four simulations per scenario to assess

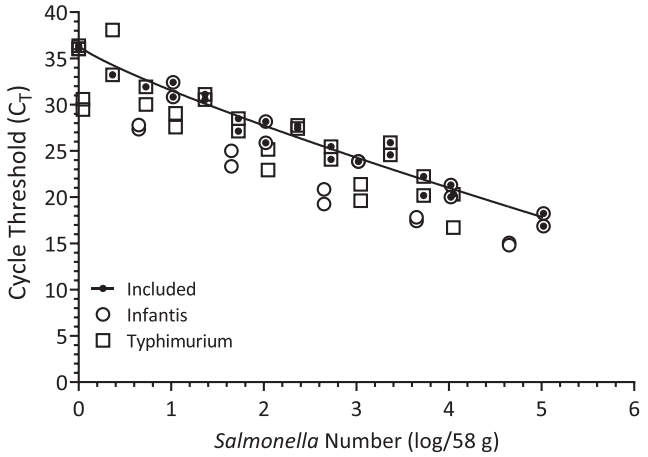

FIGURE 1. *Standard curve for enumeration (log MPN) of* Salmonella *on and in chicken liver by whole sample enrichment and quantitative PCR assay. "Included" refers to the data used to develop the standard curve.*

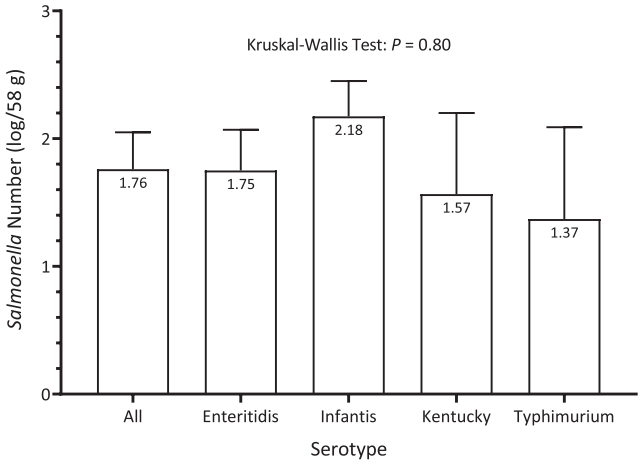

FIGURE 2. Salmonella *level in contaminated chicken liver as a function of serotype. Bars are median* Salmonella *MPN per contaminated chicken liver with 95% confidence intervals.*

variability and uncertainty of model outputs, and random selection of a different random number generator seed to initiate each simulation of a scenario. Number of servings per simulation depended on serving size (1,250 servings of eight chicken livers to 10,000 servings of one chicken liver) and lot size (which was fixed at 10,000 chicken livers).

Data, statistical analysis, and regression modeling. Simulation results were exported to Excel, and *Salmonella* prevalence (percent positive per lot), virulence (average per contaminated serving), and level (minimum, median, and maximum MPN per contaminated serving and total MPN per lot) were recorded or calculated. Total level of *Salmonella* per lot was calculated by multiplying the mean MPN of *Salmonella* per serving by the number of servings in the lot.

Linear and nonlinear regressions were used in Prism to model *Salmonella* prevalence, virulence, and level as a function of serving size. A nonparametric Kruskal-Wallis test in Prism was used to evaluate the effect of serotype on median MPN of *Salmonella* per chicken liver. Lastly, correlation analysis in Prism was used to evaluate the relationship between *Salmonella* level and weight of chicken livers. For this analysis, a value of –0.01 log MPN was assigned to chicken livers ($n = 22$) that were not contaminated with *Salmonella*.

Literature search. Results of the present study were compared with those of relevant published studies. The relevant studies were identified through a literature search in multiple databases using the USDA Digitop Navigator platform with a string of '*Salmonella*' and 'chicken' and 'liver' and 'prevalence' and 'retail' and a second search with the same string except that 'prevalence' was replaced with 'number.' The first search netted 17 hits, of which 3 were duplicates, 4 were not *Salmonella,* 1 was not retail, and 3 were not liver. The second search netted five hits of which two were duplicates and two were not number. Thus, six relevant studies were identified for *Salmonella* prevalence, and one relevant study was identified for *Salmonella* level. Within the *Salmonella* prevalence studies, only results obtained by WSE followed by culture isolation were reviewed because this was most similar to the method used in the present study and thus provided the most relevant comparisons.

RESULTS

Chicken liver. Whole chicken livers ($n = 80$) analyzed for natural *Salmonella* contamination (Table 1) weighed 58.3 ± 8.4 g (mean ± standard deviation). The pH at the time of analysis was 6.38 ± 0.16.

***Salmonella* enumeration.** The standard curve for enumeration of *Salmonella* by WSE-qPCR (Fig. 1) was

$$C_T = 36.38 - \left(\frac{N}{0.1506}\right)^{0.8332}$$

with a standard error of 0.0292 for the regression coefficient (0.1506) and 0.0517 for the shape parameter (0.8332) and a coefficient of determination (R^2) of 0.962 for replicate values as opposed to mean values. This standard curve and the interpolation function of Prism were used to convert C_T values obtained for chicken livers that were naturally contaminated with *Salmonella* into log MPN (Table 1).

***Salmonella* contamination.** *Salmonella* prevalence for naturally contaminated chicken livers was 72.5% (58 of 80 samples) per 58 g (mean weight of chicken livers examined), whereas *Salmonella* level was 1.76 log MPN (median) with a range of 0.02 to 4.67 log MPN per 58 g or per one chicken liver (Table 1). Four *Salmonella* serotypes were isolated. Prevalence of serotypes among contaminated chicken livers was 37.9% (22 of 58 samples) for *Salmonella* Infantis ($r = 6$, $V = 4.5$) and 20.7% (12 of 58 samples) for *Salmonella* Enteritidis ($r = 1$, $V = 5$), *Salmonella* Typhimurium ($r = 3$, $V = 4.8$), and *Salmonella* Kentucky ($c = 63$, $V = 0.8$) per 58 g or one chicken liver. Composite *Salmonella* virulence per contaminated chicken liver was $3.9 = (0.379 \times 4.5) + (0.207 \times 5) + (0.207 \times 4.8) + (0.207 \times 0.8)$ per 58 g.

Median level of *Salmonella* per 58 g of contaminated chicken liver was not affected ($P > 0.05$) by serotype (Fig. 2). Thus, a single pert distribution with values of 0 (minimum), 1.5 (mode), and 5 (maximum) log MPN/58 g

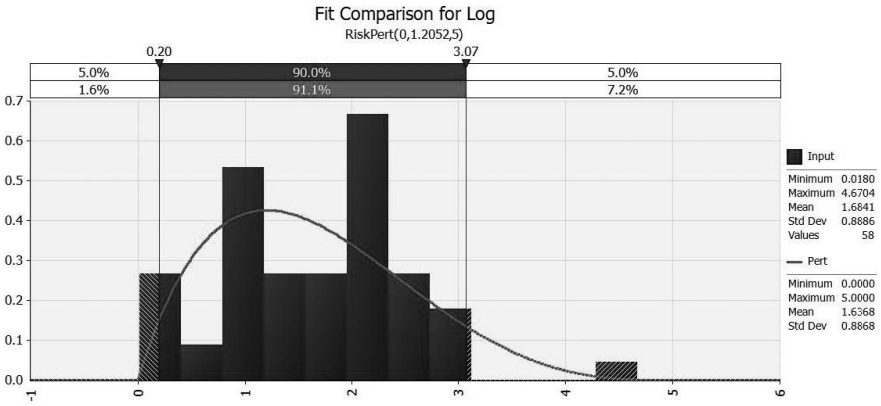

FIGURE 3. *Pert distribution for simulation of* Salmonella *level on or in chicken livers. Values are log transformed.*

was used to simulate *Salmonella* level per contaminated chicken liver in the model.

Use of a pert distribution to simulate *Salmonella* level (Table 1 and Fig. 3) was verified with the BestFit option of @Risk and the AIC (lower AIC indicates better fit). The AIC was 156.7 for the pert distribution, 157.1 for the BetaGeneral distribution, 161.0 for the triangle distribution, 161.7 for the Kumaraswamy distribution, and 186.7 for the uniform distribution. Thus, the pert distribution was the best-fitting distribution for the data on *Salmonella* level per chicken liver (Table 1 and Fig. 3), and the uniform distribution was the worst-fitting distribution for these data.

Single serving. Figure 4 shows simulation results for a single serving of four chicken livers. This example is provided to demonstrate how the model works. In this simulation, three of the four chicken livers in the serving were contaminated with one of three *Salmonella* serotypes: Kentucky, Typhimurium, and Enteritidis. The cumulative level of *Salmonella* among the four chicken livers was 135 MPN (2.13 log MPN), and the composite *V* score was 4.9.

The current version of the model can simulate a serving size up to eight chicken livers but can be expanded to include larger serving sizes. In this example (Fig. 4), chicken liver 5 was not contaminated with *Salmonella,* whereas chicken livers 6, 7, and 8 were contaminated with *Salmonella* serotypes Infantis, Typhimurium, and Infantis, respectively. However, the model ignored chicken livers 5 to 8 in its prediction of *Salmonella* contamination because the simulated serving size was only four chicken livers.

Model performance. *Salmonella* contamination data (Table 1) were obtained with a sample size of one chicken liver, with a mean weight of 58 g. These data were used in a Monte Carlo simulation model (Fig. 4) to predict *Salmonella* prevalence (*P*), *N,* serotype, and *V* as a function of serving size from 58 g (one chicken liver) to 464 g (eight chicken livers) in 58-g increments. Model performance was evaluated by simulating *Salmonella* contamination for a serving size of one chicken liver.

The model predicted that for one chicken liver or 58 g, *Salmonella P* was 72.5% (Fig. 5), median *N* was 1.76 log MPN (Fig. 6), minimum *N* was 0 log MPN (Fig. 7), maximum *N* was 4.74 log MPN (Fig. 8), and *V* was 3.9 (Fig. 9). These predictions were identical to observed data, except for maximum *Salmonella N* (Fig. 8), which was observed as 4.67 log MPN per chicken liver (58 g).

Salmonella		Serving Size Distribution		*Salmonella* Number			Virulence	Outputs	Settings
Serotype	Number (log)	Frequency	Liver	per Liver	Cumulative	Serotype	Contribution	Liver per Serving	Avg Livers per Serving
4	0.123	0	1	1	1	Kentucky	0.006		
0	1.123	0	2	0	1			4	4
2	1.719	0	3	52	53	Typhimurium	1.849	Number	Livers per lot
3	1.911	1	4	82	135	Enteritidis	3.037	135	10,000
0	1.615	0	5					Virulence	Servings
1	0.569	0	6					4.9	2,500
2	3.171	0	7					Prevalence	
1	2.319	0	8					1	

Code	Serotype	Virulence
0	None	0.0
1	Infantis	4.5
2	Typhimurium	4.8
3	Enteritidis	5.0
4	Kentucky	0.8

FIGURE 4. *Monte Carlo simulation model for predicting* Salmonella *contamination (prevalence, level, serotype, and virulence) as a function of serving size from one (58 g) to eight (464 g) chicken livers. Results are for a serving size of four chicken livers (232 g).*

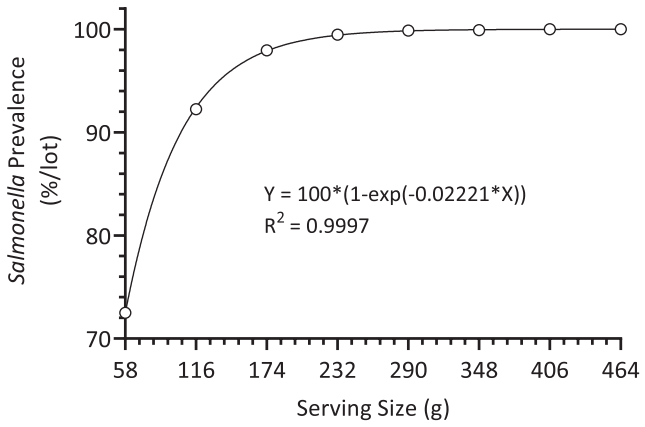

FIGURE 5. Salmonella *prevalence (*P*) on and in chicken liver as a function of serving size. The nonlinear regression model can be used to predict* Salmonella P *from 58 g (one chicken liver) to 464 g (eight chicken livers). Symbols are means ± standard deviations for four simulations.*

Serving size. The model (Fig. 4) was used to predict *Salmonella* contamination as a function of serving size from one to eight chicken livers in increments of one chicken liver (58 g). The model predicted that *Salmonella P* (Fig. 5) would increase significantly ($P \leq 0.05$) in a nonlinear manner as a function of serving size until it reached 100%. Likewise, the model predicted that minimum (Fig. 7) and median (Fig. 6) *Salmonella N* per contaminated serving would increase significantly ($P \leq 0.05$) in a nonlinear manner as a function of serving size. In contrast, maximum *Salmonella N* per contaminated serving (Fig. 8), mean *Salmonella V* per contaminated serving (Fig. 9), and total *Salmonella N* per lot (6.65 log MPN) were not affected ($P > 0.05$) by serving size. The lack of change of total *Salmonella N* per lot was expected because no matter how a lot of food is partitioned, total *Salmonella N* should not change. This result is important because it shows that the model is properly simulating the pathogen contamination data.

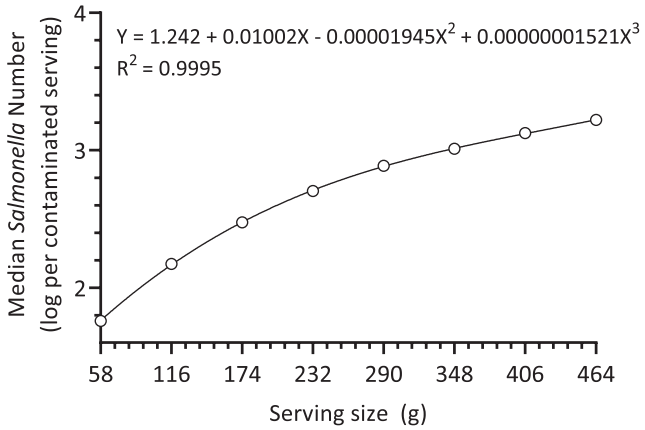

FIGURE 6. *Median* Salmonella *level (*N*) on and in chicken liver as a function of serving size. The nonlinear regression model can be used to predict median* Salmonella N *from 58 g (one chicken liver) to 464 g (eight chicken livers). Symbols are means ± standard deviations for four simulations.*

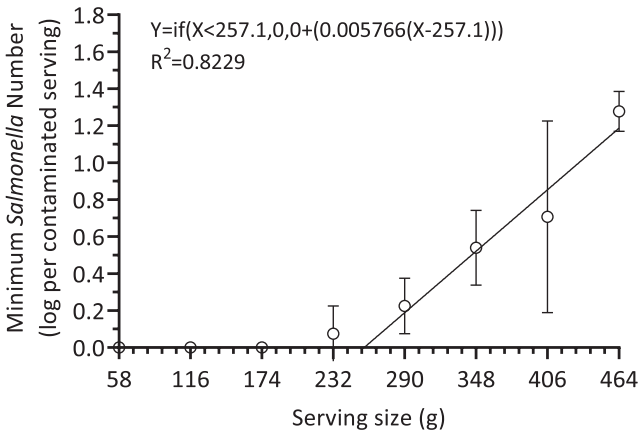

FIGURE 7. *Minimum* Salmonella *level (*N*) on and in chicken liver as a function of serving size. The nonlinear regression model can be used to predict minimum* Salmonella N *from 58 g (one chicken liver) to 464 g (eight chicken livers). Symbols are means ± standard deviations for four simulations.*

The nonlinear equations in Figure 5 for *Salmonella P,* in Figure 7 for minimum *Salmonella N,* and in Figure 6 for median *Salmonella N* can be used to predict these *Salmonella* contamination variables as a function of any serving size (e.g., 75, 111, and 163 g) from 58 g (one chicken liver) to 464 g (eight chicken livers).

Correlation analysis. *Salmonella* level was not correlated ($r = 0.088$; $P = 0.44$) with the weight of chicken livers used in the WSE-qPCR assay (Fig. 10). The weight of each chicken liver ranged from 38.6 to 75 g, with a mean of 58 g.

DISCUSSION

Important contributions of the present study were collection of new data for *Salmonella* contamination (prevalence, level, and serotype) of chicken liver and

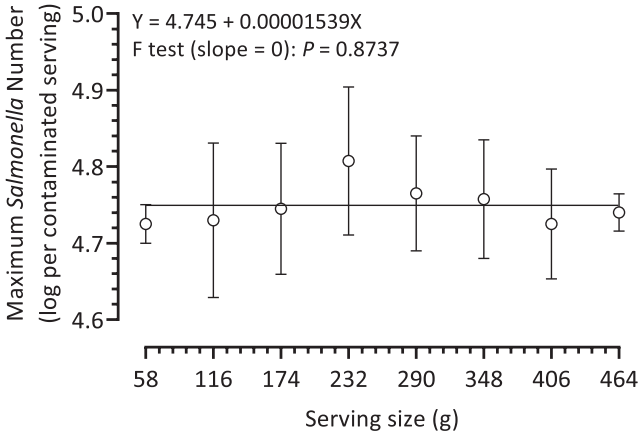

FIGURE 8. *Maximum* Salmonella *level (*N*) on and in chicken liver as a function of serving size. The slope of the linear regression line was not different (*P *> 0.05) from zero. Therefore, the* y *intercept can be used to predict maximum* Salmonella N *as a function of serving size of 58 g (one chicken liver) to 464 g (eight chicken livers). Symbols are means ± standard deviations for four simulations.*

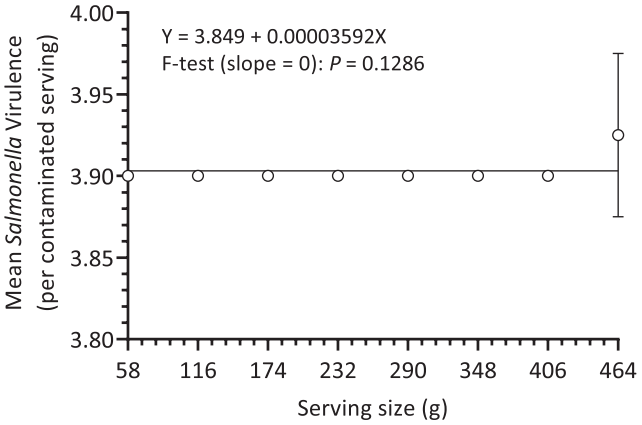

FIGURE 9. *Composite virulence (*V*) of* Salmonella *on and in chicken liver as a function of serving size. The slope of the linear regression line was not different (*P *> 0.05) from zero. Therefore, the* y *intercept can be used to predict composite* Salmonella V *as a function of serving size of 58 g (one chicken liver) to 464 g (eight chicken livers). Symbols are means ± standard deviations for four simulations.*

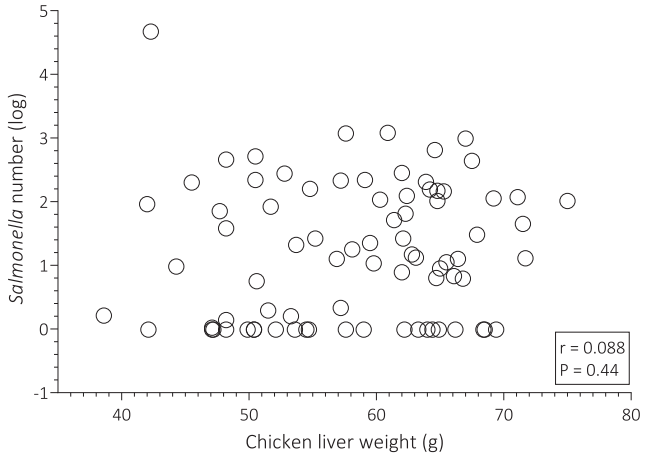

FIGURE 10. *Correlation analysis of* Salmonella *level versus weight of chicken livers. A value of −0.01 log MPN was assigned to chicken livers that were not contaminated with* Salmonella. r, *correlation coefficient;* P, *probability.*

development of a model to predict how *Salmonella* contamination of chicken liver changes as a function of serving size. These data are needed for a QMRA that predicts risk of salmonellosis from individual lots of food *(24, 25)*. In this discussion, each type of contamination data will be examined by comparison with similar data from studies published from 2010 to the present. Earlier studies were excluded because they may not adequately represent conditions in the current chicken industry.

Sample size. In the present study, data for *Salmonella* contamination were collected with a sample size of one whole chicken liver. The distribution of *Salmonella* within a whole chicken liver (i.e., mapping) was not determined and has not been reported for chicken liver. However, the distribution of *Salmonella* on and in chicken liver could depend on how the contamination occurred. For example, if contamination occurred during grow-out, *Salmonella* could be concentrated in biliary ducts or other internal regions in proximity to the route of infection from the gastrointestinal tract *(8, 27)*. However, if contamination occurred during processing by cross-contamination, *Salmonella* could be predominately on the outer surface of the chicken liver *(5, 7)*. If contamination occurred from cross-contamination during storage in plastic containers and submergence in a blood-like liquid with other *Salmonella*-contaminated livers, *Salmonella* could be located throughout the liver and levels could be high if the container were temperature abused during storage *(23)*. Without knowledge of the distribution of *Salmonella* on or in the chicken liver matrix, the best analytical unit to obtain accurate and unbiased *Salmonella* contamination data for QMRA would be the whole chicken liver.

A novel aspect of the current study is that data for *Salmonella* prevalence, level, and serotype were collected with one sample and one sample size (one whole chicken liver) and used in a Monte Carlo simulation model to predict *Salmonella* prevalence, level, serotype, and virulence as a function of a serving size of one to eight chicken livers. This modeling approach saves time and money by acquiring data for multiple servings sizes (one to eight chicken livers) based on a single sample size (one chicken liver).

In the present study, the weight of individual chicken livers used to obtain data for *Salmonella* contamination was not correlated with data for *Salmonella* level (Fig. 10). This outcome is not surprising because each liver came from a different chicken and whether they were contaminated with *Salmonella* and the level and serotypes of *Salmonella* present would depend on a number of rare, random, variable, uncertain, and independent events such as *Salmonella* infection of the chicken during grow-out, cross-contamination of the chicken liver during processing, and cross-contamination and temperature abuse of the chicken liver during storage in a plastic container with other chicken livers that could be contaminated with *Salmonella*. Thus, the lack of correlation between *Salmonella* level and weight of whole chicken livers further justifies use of the whole chicken liver as the analytical unit for data collection and does not contradict, as explained below, the conclusion that *Salmonella* level increases in a nonlinear manner as a function of serving size.

***Salmonella* prevalence.** In the present study, whole chicken livers were obtained at retail from one location in the United States. Individual chicken livers (mean weight = 58 g) were enriched in BPW before detection of *Salmonella* by qPCR and culture isolation and confirmation by antigen-antibody tests including serotyping. Prevalence of *Salmonella* was 72.5% (58 of 80 samples) per chicken liver (58 g). These data were used in a Monte Carlo simulation model (Fig. 4) and in a nonlinear regression model (Fig. 5) to predict *Salmonella* prevalence as a function of serving size from one (58 g) to eight (464 g) chicken livers. The models predicted that *Salmonella P* would increase in a nonlinear

manner from 72.5% per one chicken liver to 100% per eight chicken livers.

Procura et al. *(29)* obtained chicken livers from nine processing plants located in three regions of Argentina. One- to 2-g samples (assumed mean = 1.5 g) of chicken liver were enriched in BPW followed by culture isolation for *Salmonella*. Although results were reported per chicken liver, actual *Salmonella P* was 4.8% (32 of 666 samples) per 1.5 g of chicken liver because that was the size of sample analyzed.

Jung et al. *(12)* obtained chicken livers from 81 retail outlets in three mid-Atlantic U.S. states. Samples (50 g) of chicken liver were homogenized by stomaching in 450 mL of recovery medium, resulting in 500 mL of homogenate containing chicken liver at 0.1 g/mL. Next, 450 mL of this homogenate was enriched followed by PCR assay and culture isolation to detect *Salmonella*. The other 50 mL of homogenate was set aside for possible enumeration of *Salmonella* as explained below. *Salmonella P* was reported as 59.4% (148 of 249 samples) per chicken liver. However, because only 450 of the 500 mL of chicken liver homogenate was analyzed, the actual *Salmonella P* was 59.4% per 45 g (450 mL × 0.1 g/mL) of chicken liver.

In four studies, 25-g samples of chicken liver were used to determine *Salmonella P* by WSE and culture isolation. Goncuoglu et al. *(10)* obtained chicken livers from retail markets in Ankara, Turkey, and reported a *Salmonella P* of 33.6% (37 of 110 samples). Rahimi *(30)* obtained chicken livers from retail markets in two locations in Iran and obtained a *Salmonella P* of 18% (9 of 50 samples). Zdragas et al. *(36)* obtained chicken livers from 22 brands in retail markets in Greece and found a *Salmonella P* of 33.3% (10 of 30 samples). Liu et al. *(14)* obtained chicken livers from three retail locations in one city in the southern United States and reported a *Salmonella P* of 9.7% (7 of 72 samples).

Because the size of sample (25 g) used to determine *Salmonella P* in these four studies was the same, differences in *Salmonella P* among these studies cannot be explained by differences in sample size. Differences were likely due to other factors such as geographic location, chicken production system, and experimental methods used to determine *Salmonella P.* Nonetheless, results of the present study indicate that *Salmonella P* in chicken liver increases in a nonlinear manner as a function of serving size, which agrees with results of previous studies with similar data collection and modeling methods *(18, 24, 25)*. The results of the present study also indicate that *Salmonella P* should be expressed as a function of the size of the sample used to determine it rather than just expressing it as a percentage.

Expression of *Salmonella P* as a percentage without indicating or considering the size of sample used to determine it could result in inappropriate food safety decisions. For example, in the United States, chicken carcasses are regularly sampled for *Salmonella* using the whole carcass rinse and aliquot method *(6, 35)*. With this method, a whole chicken carcass is placed in a plastic bag with 400 mL of recovery medium and shaken for 1 min. A 30-mL aliquot of the recovery medium is then enriched followed by PCR assay and culture isolation to detect *Salmonella*. Prevalence results from this method are expressed as a percentage and interpreted as if they represented the prevalence of *Salmonella* among whole chicken carcasses. However, the relationship between *Salmonella P* in the 30 mL of carcass rinse and actual *Salmonella P* for the whole carcass is not known. When the whole sample enrichment method is used to determine *Salmonella P* for whole chicken carcasses that have low (<7.5%) prevalence of *Salmonella* by the carcass rinse and aliquot method, much higher rates of *Salmonella P* are observed *(2, 26)*. Thus, by not expressing *Salmonella P* as a function of the size of sample used to determine it, inappropriate food safety decisions can be made. In this case, that decision might be that the particular lot of chicken poses low risk of salmonellosis when in fact the risk may be higher.

The conclusion that *Salmonella P* should be expressed as a function of sample size can be further examined using the following generic example. If a 250-g sample of food were contaminated with one pathogen cell and the sample were divided into 10 25-g samples and all 25-g samples were analyzed separately, pathogen levels would be 0, 0, 0, 0, 1, 0, 0, 0, 0, and 0 cells per 25-g sample, and pathogen prevalence would be 10% per 25 g. However, if pairs of 25-g samples were analyzed together, pathogen levels would be 0, 0, 1, 0, and 0 cells per 50-g sample, and pathogen prevalence would be 20% per 50 g, whereas if half of the 25-g samples were analyzed together, pathogen levels would be 0 and 1 cell per 125-g sample, and pathogen prevalence would be 50% per 125-g sample. If all 25-g samples were analyzed together, pathogen levels would be 1 cell per 250-g sample, and pathogen prevalence would be 100% per 250 g. Thus, the conclusion that *Salmonella P* should be expressed as a function of sample size is supported by the one pathogen cell test.

***Salmonella* serotype and virulence.** In the present study, chicken livers were contaminated with four *Salmonella* serotypes: Infantis ($P = 37.9\%$, $r = 6$, $V = 4.5$), Enteritidis ($P = 20.7\%$, $r = 1$, $V = 5$), Typhimurium ($P = 20.7\%$, $r = 3$, $V = 4.8$), and Kentucky ($P = 20.7\%$, $c = 63$, $V = 0.8$). The calculated and simulated composite *Salmonella V* was 3.9 per contaminated serving, which is considered high risk. This V value can be used in a disease triangle dose-response model to predict consumer response to *Salmonella* exposure *(25)*. Thus, food safety risk assessments must include data for *Salmonella* serotype and predictions of *Salmonella* virulence. However, only three of six studies reviewed for *Salmonella* prevalence reported serotype data.

Procura et al. *(29)* found that chicken livers in Argentina were contaminated with *Salmonella* serotypes Schwarzengrund ($P = 78\%$, $c = 208$, $V = 2.5$), Enteritidis ($P = 18\%$; $r = 1$, $V = 5$), and Typhimurium ($P = 4\%$, $r = 3$, $V = 4.8$), with a composite *Salmonella V* of 3.0 per contaminated serving, which was lower than that observed in the present study ($V = 3.9$ per contaminated serving).

Although Zdragas et al. *(36)* did not report *Salmonella P* of serotypes isolated from chicken liver, they did report that serotypes Enteritidis ($r = 1$, $V = 5$), Hadar ($c = 203$, $V = 2.5$), and Typhimurium ($r = 3$, $V = 4.8$) were recovered from chicken liver and that the *P* values of these serotypes were 22.9, 29.2, and 10.4%, respectively, for all poultry products examined. Assuming similar *P* in chicken liver, composite *Salmonella V* was 3.8 per contaminated serving, which was similar to that in the present study ($V = 3.9$ per contaminated serving).

Rahimi *(30)* reported that chicken livers in Iran were contaminated with *Salmonella* serotypes Typhimurium ($P = 33.3\%$, $r = 3$, $V = 4.8$), Enteritidis ($P = 55.6\%$, $r = 1$, $V = 5$), and others ($P = 11.1\%$, assumed $V = 2.5$), with a composite *Salmonella V* of 4.7 per contaminated serving, which was higher than that in the present study.

Thus, serotypes and virulence of *Salmonella* strains that contaminate chicken livers differ among studies, as expected, likely due to differences in geographic location, chicken production systems, and experimental methods. Nonetheless, *Salmonella* serotype data are important for QMRA because virulence differs among serotypes *(16)*.

***Salmonella* levels.** In the present study, *Salmonella N* was determined by WSE-qPCR assay, which had a range of enumeration from 0 to 5 log MPN per one chicken liver (58 g). To obtain accurate data for QMRA, the enumeration method must be able to detect a single pathogen cell in the size of sample analyzed. This requirement was met in the present study.

The *Salmonella N* of naturally contaminated chicken livers ranged from 0 to 4.67 log MPN, with a median of 1.76 log MPN per chicken liver (58 g) (Table 1). These data, which were obtained with a sample size of one chicken liver, were used in a Monte Carlo simulation model to predict *Salmonella N* as a function of serving size of one (58 g) to eight (464 g) chicken livers.

The model predicted that minimum and median *Salmonella N* increased in a nonlinear manner as a function of serving size. In contrast, maximum *Salmonella N* was not affected by serving size and was predicted to be 4.74 log MPN for all serving sizes. This result can be explained by looking at results for the most highly contaminated serving ($N = 4.71$ log MPN) in a simulation for a serving size of four chicken livers. In this case, the *Salmonella* levels on the four chicken livers that by random chance made up the serving were 1.48, 2.62, 4.65, and 3.75 log MPN. Thus, maximum *Salmonella N* (4.71 log MPN) for this serving was mainly determined by the most highly contaminated chicken liver in the serving (4.65 log MPN), and by random chance the probability of having two or more highly contaminated chicken livers (>4.5 log MPN) in the same serving was very low (<0.01; see Fig. 3) and did not increase significantly as a function of serving size, which explains why maximum *Salmonella N* did not increase as a function of serving size.

The data collected in the present study for *Salmonella* contamination of chicken livers (Table 1) indicated that *Salmonella* was not uniformly distributed among and within chicken livers. For *Salmonella* to be uniformly distributed among chicken livers, each chicken liver would have the same level and serotypes of *Salmonella,* whereas for *Salmonella* to be uniformly distributed within a chicken liver, each gram of chicken liver would have the same level and serotypes of *Salmonella*. However, neither of these situations was observed in the present study or by Jung et al. *(12)*.

When data for *Salmonella N* on and in chicken livers (Table 1) were fitted to different probability distributions, the worst-fitting distribution was the uniform distribution. The model (Fig. 4), which used the data in Table 1 to simulate *Salmonella* contamination of chicken liver as a function of serving size, predicted that *Salmonella N* would increase in a nonlinear (Figs. 5 and 6) rather than a linear manner as a function of serving size, which was further evidence of a nonuniform distribution of *Salmonella* among and within chicken livers. Thus, *Salmonella N* should be expressed and simulated as a function of sample size instead of per gram. Expression of *Salmonella N* per gram is based on the incorrect assumption that *Salmonella* is uniformly distributed among and within chicken livers.

To further examine these conclusions, an example was evaluated in which the observed levels of *Salmonella* on four chicken livers were 1, 0, 52, and 82 MPN (Fig. 4). To keep this example simple for clarity, the assumption was that all four chicken livers weighed 58 g. Based on these data, two assumptions for predicting *Salmonella N* per serving were compared: a uniform distribution (method A) and a nonuniform distribution (method B).

Assuming a uniform distribution among and within chicken livers, *Salmonella N* per gram was calculated as 0.58 cells (135 cells per 232 g). Assuming a nonuniform distribution among and within chicken livers, *Salmonella N* per 58 g or one chicken liver was 1, 0, 52, and 82 cells for the four chicken livers, which match the observed data. With method A, *Salmonella N* per serving was calculated by multiplying the level per gram (0.58 cells per g) by the serving size in grams (58, 116, 174, and 232 g). With method B, *Salmonella* level per serving was calculated by summing the *Salmonella* levels on or in each chicken liver in the serving. Method A was used by Jung et al. *(12),* whereas method B was used in the present study (Fig. 4).

In this example, the serving size is one, two, three, or four chicken livers. When the serving size is one, two, or three chicken livers, random chance determines which chicken livers make up the serving. However, to keep this example simple, the assumption was that the chicken livers are selected in order. Thus, there is only one possible outcome for each serving size. The observed *Salmonella* level is 1 cell for the first chicken liver or for a serving size of one chicken liver, 1 cell for the first two chicken livers ($1 + 0$) or for a serving size of two chicken livers, 53 cells for the first three chicken livers ($1 + 0 + 52$) or for a serving size of three chicken livers, and 135 cells for all four chicken livers ($1 + 0 + 52 + 82$) or for a serving size of four chicken livers. These are the same results obtained with method B (Fig. 4) because this method simulates how chicken livers are contaminated and consumed in the real

world. In this example, method B correctly predicted *Salmonella* level for each serving size. Method B also correctly predicted that *Salmonella* level increases in a nonlinear manner as a function of serving size because the method correctly assumed and simulated a nonuniform distribution of *Salmonella* among and within the chicken livers.

In contrast, with method A *Salmonella N* per serving was predicted to be 33.6 cells (0.58 cells per g × 58 g) for the first chicken liver or for a serving size of one chicken liver, 67.3 cells (0.58 cells per g × 116 g) for the first two chicken livers or for a serving size of two chicken livers, 100.9 cells (0.58 cells per g × 174 g) for the first three chicken livers or for a serving size of three chicken livers, and 135 cells (0.58 cells per g × 232 g) for all four chicken livers or for a serving size of four chicken livers. Thus, method A incorrectly predicted *Salmonella N* for three of the four serving sizes.

Method A also incorrectly predicted that *Salmonella N* increases in a linear manner as a function of serving size because this method incorrectly assumed and simulated a uniform distribution of *Salmonella* among and within the chicken livers. The only correct prediction made by method A was for a serving size of four chicken livers, which was the size of sample (232 g) used to determine *Salmonella N* per gram. This result supports the conclusion that to obtain accurate data for QMRA, *Salmonella N* should be expressed as a function of the size of the sample used to determine *N* instead of per gram. The failure of method A to accurately predict *Salmonella N* per serving is due to inaccurate simulation of how chicken liver is contaminated and consumed in the real world.

Of the six studies reviewed, only one provided data for *Salmonella* level *(12)*. Jung et al. *(12)* reported the distribution of *Salmonella N* among five categories: (i) ≤0.3 MPN/g (68 of 148 samples, 46%); (ii) 0.31 to 3 MPN/g (56 of 148 samples, 38%); (iii) 3.01 to 30 MPN/g (16 of 148 samples, 11%); (iv) 30.01 to 110 MPN/g (6 of 148 samples, 4%); and (v) >110 MPN/g (2 of 148 samples, 1%). These enumeration data were obtained with three replicates and three sample sizes (10, 1, or 0.1 mL of original homogenate) for the MPN assay. Thus, the total mass of chicken liver used in the MPN assay was 3.33 g because the concentration of chicken liver in the original homogenate was 0.1 g/mL (50 g in 500 mL of homogenate).

Based on an MPN calculator *(11),* the enumeration range of the MPN assay used by Jung et al. *(12)* was 1 MPN/3.33 g (0, 0, 1) to 363 MPN/3.33 g (3, 3, 2). Thus, this approach satisfied the requirement of a minimum level of enumeration of one cell of the pathogen in the size of sample analyzed. However, the range of enumeration was too narrow to obtain complete and accurate data for QMRA. In comparison, the WSE-qPCR method used in the present study had an enumeration range of 1 (0 log MPN) to 100,000 (5 log MPN) *Salmonella* cells per chicken liver (58 g).

Although only *Salmonella*-positive samples from the enrichment culture assay for *Salmonella P* were enumerated by Jung et al. *(12)* for *Salmonella,* 46% (68 of 148 samples) had an MPN of 0 MPN/3.33 g (0, 0, 0), which indicated that *Salmonella* was not detected in the MPN assay. This result was obtained because a smaller sample (3.33 g) was used to determine *Salmonella N* than was used for *Salmonella P* (45 g) and because *Salmonella* is not uniformly distributed among and within chicken livers. Thus, when the same sample and same size sample are not used, results for *Salmonella P* and *N* are confounded and should not be used together in a QMRA because they will not provide accurate results.

The results of the MPN assay of Jung et al. *(12)* can be corrected. The corrected results for *Salmonella N* are (i) 0 MPN/3.33 g (46%); (ii) 1 to 10 MPN/3.33 g (38%); (iii) >10 to 100 MPN/3.33 g (11%); (iv) >100 to 363 MPN/3.33 g (4%); and (v) >363 MPN/3.33 g (1%). However, because 68 samples were below the lower limit of enumeration and two samples were above the upper limit of enumeration, it was not possible to accurately determine minimum, median, and maximum *N* for defining a pert distribution for use in a QMRA. Thus, use of results such as those of Jung et al., which are incomplete, inaccurate, and biased, would result in a QMRA that provides an inaccurate assessment of consumer exposure to *Salmonella* and risk of salmonellosis.

Recognizing that these conclusions may be controversial because the method used by Jung et al. *(12)* is widely used and accepted in QMRAs, a further examination of these conclusions is warranted. In the method used by Jung et al. *(12),* 50 g of chicken liver was added to 450 mL of recovery medium in a plastic bag and homogenized by stomaching. This resulted in creation of 500 mL of homogenate containing chicken liver at 0.1 g/mL. To determine *P,* 450 mL of the homogenate (45 g of chicken liver) was enriched followed by PCR detection and culture confirmation of *Salmonella*. The other 50 mL of the homogenate (containing 5 g of chicken liver) was set aside for possible enumeration of *Salmonella* with an MPN assay. However, the MPN assay was conducted for only the samples that tested positive for *Salmonella* in the enrichment assay used to determine *Salmonella P.*

Of 249 samples of homogenate (450 mL) used in the enrichment assay for *Salmonella P,* only 148 were positive for *Salmonella*. Thus, only 148 of 249 samples set aside were used in a three-replicate, three-sample-size (1, 0.1, and 0.01 g) MPN assay to determine *Salmonella N*. However, 46% (68 of 148 samples) of the samples set aside had an MPN result of zero positive tubes, and 1% (2 of 148 samples) of the samples set aside had an MPN result of all positive tubes. Thus, for 47% (70 of 148 samples) of the samples set aside that were examined, no MPN data were obtained, which resulted in incomplete, inaccurate, and biased results for *Salmonella N.*

Why did this occur? There are at least three possible reasons. First, a different sample was used to determine *Salmonella P* and *N*. Second, a different size sample was used to determine *Salmonella P* (45 g) and *N* (3.33 g). Third, the upper limit of enumeration of the MPN assay was below the level of *Salmonella* in the two most highly

contaminated samples. Thus, the method did not provide complete, accurate, or unbiased data for the QMRA.

To better understand these potentially controversial statements, the one pathogen cell test can be used.

When a 250-g sample of food is contaminated with one pathogen cell and is divided into five 50-g samples that are further divided into five 45-g samples for determination of pathogen prevalence and five 5-g samples that are set aside for pathogen enumeration, there is a 90% chance that the one pathogen cell will be in a sample used for determining pathogen prevalence, a 10% chance that the one pathogen cell will be in a set aside sample, and a 6.67% chance that the one pathogen cell will be in the set aside sample for the MPN assay used to determine pathogen level.

When the pathogen cell is in a 45-g sample used to determine pathogen prevalence, pathogen level will be 0, 0, 1, 0, and 0 cells per 45 g and pathogen prevalence will be 20% per 45 g. However, when the set aside sample from the one sample that tested positive for the pathogen in the prevalence assay is tested in the MPN assay, all of the tubes will test negative for the pathogen because the one pathogen cell was in the 45-g portion of the original 50-g sample that was used to determine pathogen prevalence.

However, when the pathogen cell is in a set aside sample, it will not be detected and enumerated because the MPN assay will not be conducted for any of the set aside samples because all of the samples used in the enrichment assay for pathogen prevalence have tested negative for the pathogen. Thus, the one pathogen cell test can be used to demonstrate that the design of the method used by Jung et al. *(12)* does not provide accurate data for a QMRA because the same sample and same size of sample are not used to determine pathogen prevalence and level. In contrast, the data collection methods used in the present study (WSE, qPCR, culture isolation, serotyping, and Monte Carlo simulation) use the same sample and therefore same sample size to obtain data for *Salmonella P, N,* and serotype. Thus, these methods pass the one pathogen cell test and therefore provide complete, accurate, and unbiased data for a QMRA.

The definition of what "same sample" means and the importance of using the same sample to collect data for foodborne pathogen contamination (*P, N,* and serotype) for a QMRA may not be obvious and may require further explanation using a simple example. In this example, when a 250-g sample of food is contaminated with one pathogen cell and this sample is divided into two 125-g samples, one sample will test positive for the pathogen and the other will test negative for the pathogen. If the 125-g samples were still part of the same sample, they would provide the same result, but they do not provide the same result when they are separate samples. The two 125-g samples are from the same sample but they are not the same sample. Thus, to obtain accurate data for pathogen *P, N,* and serotype for use in a QMRA, the same sample and same sample size must be used, as was done in the present study.

What is the consequence of providing inaccurate data for a QMRA? As in any endeavor, inaccurate data and information will result in an incorrect decision. The method used by Jung et al. *(12)* has been the method of choice to obtain pathogen *P* and *N* data for QMRAs *(4, 9)* that provide the scientific basis for food safety decisions directed at protecting public health. However, rates of foodborne illness from human bacterial pathogens in the United States have stayed the same or increased instead of decreasing *(32)*. Thus, the current approach to food safety and QMRA does not seem to be working. Perhaps it is time to try a something new *(25)*. The data collection and modeling methods used in the present study provide accurate data for a QMRA and can address an important data gap in QMRAs for *Salmonella* in whole chicken livers.

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