Journal of Food Protection, Vol. 67, No. 6, 2004, Pages 1201–1208

Simulation Model for Enumeration of *Salmonella* on Chicken as a Function of PCR Detection Time Score and Sample Size: Implications for Risk Assessment†

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MS 03-354: Received 21 August 2003/Accepted 31 December 2003

ABSTRACT

A data gap commonly identified in risk assessments is the lack of quantitative information on the contamination of food with pathogens. A simulation model that predicts the incidence and distribution of *Salmonella* contamination on chicken as a function of PCR detection time score and sample size was developed with data from challenge studies with preenrichment samples that were composed of 25 g of chicken and 225 ml of buffered peptone water inoculated with $10^{0.7}$ to 10^6 *Salmonella* and incubated at 37°C. At 0, 2, 4, 6, 8, 10, 12, and 24 h of incubation, subsamples were collected and tested for *Salmonella* by PCR, and a PCR detection time score based on the widths of the bands in the electrophoresis gel was obtained for each preenrichment sample. Standard curves relating PCR detection time score to initial density of *Salmonella* inoculated were developed for sterile and nonsterile preenrichment samples. Presence of other microorganisms in the preenrichment sample decreased the PCR detection time score at low ($<10^2$ per 25 g) but not at high ($>10^2$ per 25 g) initial densities of *Salmonella* and resulted in a nonlinear standard curve rather than the linear standard curve obtained for sterile samples. The predicted incidence and distribution of *Salmonella* contamination on chicken increased in a nonlinear manner as sample size increased from 25 to 500 g. The new method reduced the time and cost of *Salmonella* enumeration by eliminating the selective enrichment, selective plating, and confirmation steps of the traditional most-probable-number method. Results are useful for risk assessment because they consider the uncertainty of the standard curve predictions and because they provide distributions of *Salmonella* contamination for different size samples of chicken that can be directly used in risk assessment.

A data gap that is routinely identified in risk assessments is the lack of quantitative information on the contamination of food with pathogens *(7).* Most data are qualitative rather than quantitative because enumeration of pathogens in food requires more labor and time than does determining pathogen incidence, especially since the advent of rapid detection methods *(25).* Because the incidence of pathogen contamination of food samples may be low (e.g., <10%), a large number of samples must be evaluated to define a probability distribution with low uncertainty for risk assessment. Moreover, pathogens are usually present in low numbers relative to other microorganisms in food, which makes enumeration difficult. For example, the number of *Salmonella* cells on contaminated chickens is usually <30 *(13, 23, 26, 28),* whereas the total number of bacteria usually exceeds 10^5.

Although low numbers of *Salmonella* cells on chicken can be enumerated using the traditional most-probable-number (MPN) method *(11, 12),* this method is labor and materials intensive and time-consuming because it involves preenrichment, selective enrichment, selective plating, and confirmation steps. However, with the advent of molecular methods such as PCR that have high specificity for *Salmonella (15)* it is now possible to develop enumeration methods that require only preenrichment *(1),* thus saving labor and time by eliminating the selective enrichment, selective plating, and confirmation steps of the traditional method.

Recently, Bailey *(1)* evaluated a commercial PCR method (BAX, Qualicon, Wilmington, Del.) for its ability relative to that of the conventional culture method to detect *Salmonella* in poultry samples. Using serial dilutions, Bailey demonstrated that the size of the PCR band in the electrophoresis gel was related to the density of *Salmonella* in the preenrichment sample, with an increase from a faint band at 10^2 cells per ml to a full band at 10^7 cells per ml. A visual scoring system based on PCR band size was developed for semiquantitative enumeration of *Salmonella* in preenrichment samples *(1).* In the current study, a modified version of the method of Bailey *(1)* was used to develop a quantitative method for enumeration of *Salmonella* in preenrichment samples of chicken.

The incidence of *Salmonella* contamination of chicken is dependent on the size of sample analyzed. For example, Cox et al. *(8)* found that the incidence of *Salmonella* contamination of chicken was 45% for a whole carcass rinse sample but was only 11% for a partial carcass rinse sample (i.e., neck skin). Likewise, Surkiewicz et al. *(23)* reported that the incidence of *Salmonella* contamination of chicken

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was 4.9% when they used 10 ml of carcass rinse but was 20.5% when they used 270 ml of carcass rinse. The number of cells and the distribution of *Salmonella* contamination on chicken probably is also dependent on the size of sample analyzed and, like the prevalence, may increase in a nonlinear manner as a function of sample size. The implication for risk assessment is that enumeration data would be needed for different size samples of chicken because it would not be possible to linearly extrapolate data obtained with one size of sample to samples of other sizes. However, it is not practical to collect enumeration data for multiple sizes of samples because of the time and cost involved. Consequently, in the present study, a simulation model was developed to allow extrapolation of enumeration data obtained with one size of sample to samples of other sizes for use in risk assessment.

MATERIALS AND METHODS

Organisms. *Salmonella* Typhimurium (14028, American Type Culture Collection, Manassas, Va.) and *Salmonella* Worthington isolated from a broiler chicken *(18)* were used to develop the enumeration method. Stock cultures of the organisms were maintained at −70°C in brain heart infusion (BHI) broth (Difco, Becton Dickinson, Sparks, Md.) that contained 15% glycerol (Sigma, St. Louis, Mo.).

Starter cultures. Five microliters of the stock culture (10^9 cells per ml) was added to 5 ml of BHI broth in a 25-ml Erlenmeyer flask and sealed with a foam plug. Starter cultures were incubated for 23 h at 37°C and 150 orbits per min to obtain stationary-phase cells for inoculation. The number of *Salmonella* cells in the starter cultures was determined by viable cell counts on BHI agar *(17)* and was used to calculate the number of *Salmonella* cells inoculated.

Challenge studies. Stationary-phase cultures (10^{10} cells per ml) were serially diluted (10^{-4} to 10^{-9}) in sterile buffered peptone water (Difco, Becton Dickinson) and then 0.5 or 1 ml was added to a stomacher bag that contained 225 ml of sterile buffered peptone water and 25 g of chicken. The initial density of *Salmonella* cells was $10^{0.7}$ (i.e., 10^{10} cells per ml $\times$ 10^{-9} $\times$ 0.5 ml) to 10^6 (i.e., 10^{10} cells per ml $\times$ 10^{-4} $\times$ 1 ml) per 25 g of chicken. In challenge studies with sterile chicken, breast and thigh meat were cooked for 15 min at 121°C in an autoclave to remove all microorganisms before being inoculated with *Salmonella.*

After inoculation, preenrichment samples were homogenized for 2 min at normal speed in a model 400 Stomacher blender (Seward, London, UK) followed by incubation at 37°C without shaking. At 0, 2, 4, 6, 8, 10, 12, and 24 h of incubation, the bag contents were mixed by hand and then a 1-ml subsample was removed and added to 9 ml of cold BHI broth to stop the growth of *Salmonella.* Subsamples were held at refrigerated temperatures until analyzed for *Salmonella* within 30 h using the BAX system.

PCR analysis. Detection of *Salmonella* with the BAX system was done as described by Bailey *(1)* except that subsamples were not preincubated before PCR analysis. One gel consisting of 18 lanes was run per preenrichment sample, which was composed of 25 g of chicken and 225 ml of buffered peptone water incubated for 24 h at 37°C. Lanes 1 to 8 contained the subsamples (0, 2, 4, 6, 8, 10, 12, and 24 h, respectively), lane 9 contained molecular weight markers, lanes 10 to 17 were positive controls (subsamples from 0, 2, 4, 6, 8, 10, 12, and 24 h, respectively, that were inoculated into control tubes that contained reagents for confirming that the PCR was not inhibited by substances in the preenrichment sample), and lane 18 contained molecular weight markers. In all gels, the positive controls had the proper size band for the PCR product, indicating the absence of PCR inhibitors in the subsamples. The scoring system, which was modified from that of Bailey *(1),* for lanes 1 to 8 of the gel was 0 = no band; 1 = faint band; 2 = less than full band; and 3 = full band. Thus, preenrichment samples received a PCR detection time score of 0 to 24, representing the sum of the PCR bandwidth scores for the eight subsamples.

Standard curve. The PCR detection time scores for the preenrichment samples were graphed as a function of the log number of inoculated *Salmonella* cells, which ranged from $10^{0.7}$ to 10^6 cells per 25 g of chicken, and the resulting curves were fit to a first or second order polynomial using Prism 4.0 (GraphPad, San Diego, Calif.).

Simulation modeling. A model (Table 1) for predicting the incidence and distribution of *Salmonella* contamination on chicken as a function of PCR detection time score and sample size was created in an Excel spreadsheet (Microsoft, Redmond, Wash.) and was simulated using @Risk standard version 4.0 (Palisade, Newbury, N.Y.). Formulas used in the model are presented in Table 2. The PCR detection time scores of 12 naturally contaminated (i.e., with spoilage and perhaps pathogenic organisms) 25-g samples of chicken, which were purchased from local retail outlets and were not inoculated with *Salmonella* (Table 3), were determined as described above and were used to define the frequency of the PCR detection time scores in the model (Table 1).

The uncertainty of the predicted number of *Salmonella* cells for the individual PCR detection time scores was modeled with a triangle or pert distribution that required three input values (i.e., minimum, most likely, and maximum), which were derived from the final standard curve and its 95% prediction interval as shown in Figure 1 and as described in Tables 2 and 4. The model (Table 1) was designed to simulate samples from 25 to 500 g in 25-g increments. During simulation, @Risk randomly assigned a level of *Salmonella* contamination to each 25-g sector in the model, and then the total predicted number of *Salmonella* cells per sample or per iteration of the model was determined by summing the sectors for the size of sample simulated.

Simulation settings. The scenario depicted in Table 1 was simulated for chicken samples of 25, 50, 100, 200, 400, and 500 g to determine the effect of size of sample on the incidence and distribution of *Salmonella* contamination. @Risk settings of Latin hypercube sampling (similar results were obtained with Monte Carlo sampling), 10^4 iterations, and a random number generator seed of 1 were used to simulate the scenarios. Results of the simulations were filtered to remove the samples that were not contaminated with *Salmonella* (i.e., random iterations or samples with a predicted value of zero for the level of *Salmonella* contamination), and then the incidence of contaminated samples and the number of cells and distribution of *Salmonella* among contaminated samples were determined.

RESULTS AND DISCUSSION

The use of a standard curve to predict the quantity of a substance in a test sample is a common scientific practice. In the current study, this approach was used to develop an enumeration method for *Salmonella* cells that eliminated the selective enrichment, selective plating, and confirmation steps of the traditional MPN method. The new method was

TABLE 1. *Simulation model for enumeration of* Salmonella *cells on chicken as a function of PCR detection time score and sample size*

Model cell no.	Model cell letters[a]						
1	PCR detection time score		*Salmonella*		Chicken sample		
2	Score (A)	Frequency (B)	Log number per 25 g (C)	Number per 25 g (D)	Sector (E)	*Salmonella* per 25 g (F)	Size, g (G)
3	0	10		0	1	0	25
4	1	1	0.18	2	2	0	50
5	2	0	0.26	2	3	0	75
6	3	0	0.43	3	4	0	100
7	4	1	0.66	5	5	0	125
8	5	0	0.89	8	6	0	150
9	6	0	1.13	13	7	0	175
10	7	0	1.36	23	8	0	200
11	8	0	1.62	41	9	0	225
12	9	0	1.87	74	10	0	250
13	10	0	2.14	138	11	0	275
14	11	0	2.43	266	12	0	300
15	12	0	2.74	554	13	0	325
16	13	0	3.07	1,166	14	0	350
17	14	0	3.41	2,551	15	0	375
18	15	0	3.81	6,407	16	0	400
19	16	0	4.27	18,478	17	0	425
20	17	0	4.65	44,668	18	0	450
21	18	0	5.12	131,826	19	0	475
22	19	0	5.59	390,541	20	0	500
23					Total	0	

[a] Cell letters (and numbers) refer to model formulas presented in Table 2.

based on the concept that the time to detection by PCR in a preenrichment sample incubated under standard conditions would be mathematically related to the initial density of *Salmonella* cells. Advantage was taken of the moderate sensitivity (i.e., 10^2 cells per ml for a faint band on the electrophoresis gel in the BAX PCR system; results not shown but observed in the current study and consistent with those reported by Bailey *(1)*) but high specificity of PCR for *Salmonella (1),* the low density ($<10^2$ cells per ml) of *Salmonella* in preenrichment samples of chicken *(23, 26),* and the high repeatability of *Salmonella* growth under standard incubation conditions *(10, 17).*

Although repeatable under standard incubation conditions, the growth of *Salmonella* is affected by factors such as strain variation *(17, 19),* previous conditions that may result in sublethal injury *(6, 9, 14),* and the physicochemical and microbial composition of the food *(3, 22).* Any factor that affects the growth of *Salmonella* will also affect the PCR detection time score. In the current study, effects of serotype, type of chicken meat, and microbial competition on the mathematical relationship between PCR detection time score and the initial density of *Salmonella* cells inoculated into preenrichment samples of chicken were investigated.

When *Salmonella* Typhimurium was inoculated into sterile preenrichment samples composed of 25 g of cooked chicken breast or thigh meat and 225 ml of buffered peptone water, a linear relationship was observed between PCR detection time score and the initial density of *Salmonella* (Fig. 2). Comparison of the linear regression fits for breast and thigh meat (Table 5) with an F test using the Prism program indicated similar ($P > 0.05$) y-intercepts and slopes; thus, type of chicken meat did not affect the shape of the standard curve. Although cooked chicken thigh meat is higher in fat content *(2)* and has a higher pH (6.9 versus 6.1 *(21)*) than cooked chicken breast meat, 90% of the growth medium in the present study was buffered peptone water, whereas only 10% was chicken meat, which may explain the lack of effect of chicken meat type on the shape of the standard curve. However, Oscar *(20)* reported no difference in the growth of *Salmonella* Typhimurium on cooked chicken breast and thigh meat incubated at temperatures from 8 to 48°C.

In contrast to sterile preenrichment samples, when either *Salmonella* Typhimurium or *Salmonella* Worthington were inoculated into preenrichment samples composed of 25 g of naturally contaminated chicken breast skin and 225 ml of buffered peptone water, a nonlinear relationship was observed between PCR detection time score and the initial density of *Salmonella* (Fig. 3). In general, PCR detection time scores were similar at high initial density ($>10^2$ cells per 25 g) but were lower at low initial density ($<10^2$ cells per 25 g) of inoculated *Salmonella* than were the PCR detection time scores in sterile preenrichment samples (Fig. 2). In a recent study, Tamplin *(24)* reported that the growth of *Escherichia coli* O157:H7 in sterile ground beef was

TABLE 2. *Formulas used in the simulation model (Table 1) to predict the incidence and distribution of* Salmonella *contamination on chicken as a function of PCR detection time score and sample size*

Model cell address[a]	Formula	Description
A3:A22		PCR detection time scores
B3:B22		Frequency of the PCR detection time scores among the test samples
C4:C22	=RiskTriang (minimum, most likely, maximum) or =RiskPert (minimum, most likely, maximum)	Predicted log number of *Salmonella* cells per 25 g of chicken as a function of PCR detection time score from the final standard curve and its 95% prediction interval (Fig. 1). The input settings of the triangle and pert distributions for the individual PCR detection time scores are those shown in Table 4.
D4:D22	=Round(Power(10,#REF),0)	*Salmonella* cells were treated as discrete entities in the model and thus fractions of *Salmonella* cells were rounded to whole numbers; #REF refers to the adjacent cell in column C. The Power function takes the antilog of the adjacent number in column C.
E3:E22		A 500-g serving of chicken was divided into 20 25-g sectors and a level of *Salmonella* contamination was assigned to each sector by @Risk during simulation of the model. A discrete distribution was used to model the frequency of different levels of *Salmonella* contamination per 25-g sector.
F3:F22	=RiskDiscrete(D3:D22,B3:B22)	Discrete distribution for predicting the frequency of different levels of *Salmonella* contamination per 25-g sector
F23	=RiskOutput() + SUM(F3:F22)	Output cell for the total predicted number of *Salmonella* cells for the size of sample simulated from 25 to 500 g in 25-g increments. The total number of *Salmonella* cells per sample was obtained by summing the number in the relevant 25-g sectors, which were designated by defining the appropriate range in the SUM function, e.g., F3 to F6 for a 100-g sample or F3 to F22 for a 500-g sample.
G3:G22		The model was designed to simulate different sample sizes from 25 to 500 g in 25-g increments. Larger samples sizes can be simulated by adding additional discrete distributions in column F (i.e., additional sectors).

[a] Letters and numbers refer to columns and rows, respectively, in Table 1.

TABLE 3. *PCR detection time scores for naturally contaminated chicken samples that were not inoculated with* Salmonella

Sample no.	Type of chicken sample	PCR detection time score
1	Thigh skin	0
2	Thigh skin	0
3	Thigh skin	1
4	Breast skin	0
5	Breast skin	0
6	Breast skin	0
7	Breast meat	0
8	Breast meat	0
9	Breast meat	0
10	Breast meat	4
11	Breast meat	0
12	Breast meat	0

suppressed to a greater extent at high (100:1) than at low (1:1) initial ratios of spoilage bacteria to *E. coli* O157:H7. Thus, a likely explanation for the current results is that microbial competition suppressed the growth of *Salmonella* at low but not at high initial densities of inoculation.

Comparison of the quadratic polynomial fits with an F test in the Prism program indicated similar ($P > 0.05$) regression coefficients (Table 3) and thus similar standard curves for *Salmonella* Typhimurium and *Salmonella* Worthington in nonsterile preenrichment samples (Fig. 3). In previous studies, growth of *Salmonella* Typhimurium and *Salmonella* Worthington in BHI broth incubated at 40°C *(17)* and on sterile cooked chicken breast meat incubated at 25°C *(19)* were similar, consistent with the similar PCR detection time scores for these two serotypes in the current study. In general, variation in growth among serotypes or

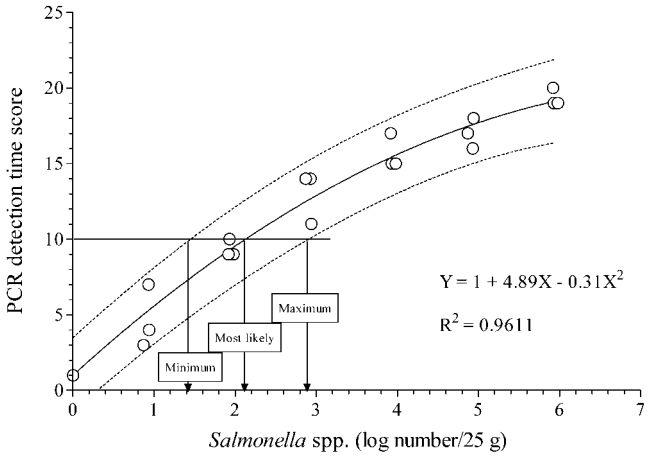

FIGURE 1. *Final standard curve and its 95% prediction interval for enumeration of* Salmonella *cells on chicken as a function of PCR detection time score. Minimum, most likely, and maximum values for log number of* Salmonella *cells per 25 g of chicken were interpolated from the standard curve and its 95% prediction interval as shown and were used to define triangle and pert distributions for the individual PCR detection time scores in the simulation model (Table 1).*

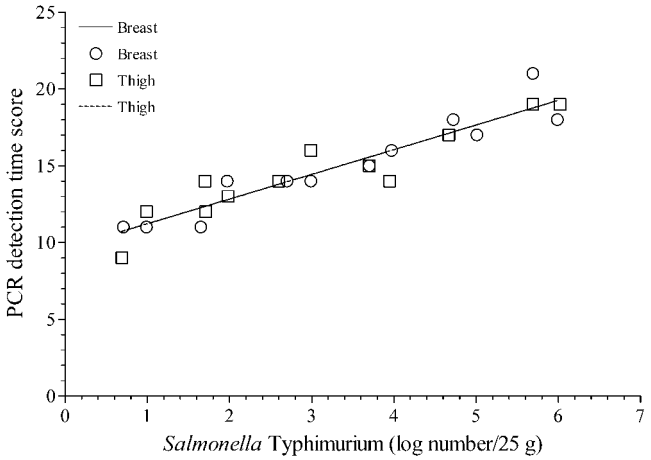

FIGURE 2. *Standard curve for PCR detection time score as a function of initial density of* Salmonella *Typhimurium inoculated into sterile preenrichment samples composed of 25 g of cooked chicken breast or thigh meat and 225 ml of buffered peptone water.*

strains of *Salmonella* in broth medium incubated at optimal growth temperatures is small. For example, Fehlhaber and Kruger *(10)* reported a coefficient of variation for generation time of 3.8% among 45 strains of *Salmonella* Enteritidis growing in broth medium incubated at 37°C. Likewise, Oscar *(17)* found little variation over three log cycles of growth at 40°C in BHI broth among 14 strains of *Salmonella* isolated from chicken operations.

The PCR detection time scores of 12 naturally contaminated chicken samples that were not inoculated with *Salmonella* were determined (Table 3). Ten of the samples were not contaminated with *Salmonella* and thus had PCR detection time scores of zero. For the two contaminated samples, PCR detection time scores of 1 and 4 were obtained. To determine the number of *Salmonella* cells for these samples, the data for *Salmonella* Typhimurium and *Salmonella* Worthington (Fig. 3) were combined to generate a single standard curve (Fig. 1). The *y*-intercept for the initial fit of this standard curve was 0.6. However, because a *Salmonella*-positive sample would have a minimum PCR detection time score of 1, equal to a single faint band at 24

TABLE 4. *Simulation model (Table 1) input settings for the distributions that defined the uncertainty of the final standard curve predictions of log number of* Salmonella *cells per 25 g of chicken as a function of PCR detection time score*

PCR detection time score	Minimum[a]	Most likely	Maximum	Type of distribution
1	0.00	0.00	0.55	triangle
2	0.00	0.20	0.73	pert
3	0.00	0.40	1.00	pert
4	0.12	0.65	1.22	pert
5	0.30	0.89	1.48	pert
6	0.53	1.12	1.75	pert
7	0.75	1.35	2.00	pert
8	1.00	1.60	2.30	pert
9	1.20	1.85	2.60	pert
10	1.45	2.12	2.90	pert
11	1.70	2.40	3.25	pert
12	1.97	2.72	3.61	pert
13	2.22	3.05	3.98	pert
14	2.50	3.39	4.38	pert
15	2.86	3.75	4.98	pert
16	3.20	4.20	5.60	pert
17	3.50	4.60	6.00	pert
18	3.92	5.20	6.00	pert
19	4.35	5.80	6.00	pert

[a] Most likely, minimum, and maximum values for the triangle and pert distributions were determined from the best-fit standard curve (Fig. 1) and its upper and lower 95% prediction interval curves, respectively.

TABLE 5. *Standard curves for enumeration of* Salmonella *(Y; log number of cells per 25 g) on chicken as a function of PCR detection time score (X)*

Experiment	Serotype	Type of chicken sample[a]	No. of samples	Standard curve	R^2
I	Typhimurium	sBM	12	$Y = 9.42 + 1.67X$	0.898
	Typhimurium	sTM	12	$Y = 9.79 + 1.54X$	0.861
II	Typhimurium	BS	12	$Y = 0.78 + 5.27X - 0.39X^2$	0.948
	Worthington	BS	6	$Y = -0.95 + 5.58X - 0.34X^2$	0.977

[a] sBM, sterile cooked chicken breast meat; sTM, sterile cooked chicken thigh meat; BS, naturally contaminated chicken breast skin.

h of incubation, the curve fit was repeated with the *y*-intercept constrained to a value of 1. The R^2 for the standard curve fit changed from 0.9616 to 0.9611 when the *y*-intercept was held constant at 1 and thus represented only a minor adjustment that resulted in a more logical prediction of the number of *Salmonella* cells as a function of PCR detection time score.

To incorporate the uncertainty of the standard curve predictions into the enumeration method, triangle and pert distributions derived from the best-fit line for the standard curve and its 95% prediction interval (Fig. 1) were generated for the PCR detection time scores in the simulation model (Table 1). A triangle distribution was used for a PCR detection time score of 1 because the minimum and most likely values were zero and this resulted in an error when the pert distribution was used. For the contaminated sample with a PCR detection time score of 1, the triangle distribution settings (minimum, most likely, maximum) were 0, 0, and 0.55 log per 25 g of chicken, respectively, whereas for the contaminated sample with a PCR detection time score of 4 the pert distribution settings were 0.12, 0.65, and 1.22 log per 25 g of chicken, respectively (Table 4). The two positive samples were not enough to accurately predict the distribution of *Salmonella* contamination on chicken; however, they were sufficient to demonstrate how the method can be used to enumerate *Salmonella* cells on chicken as a function of sample size.

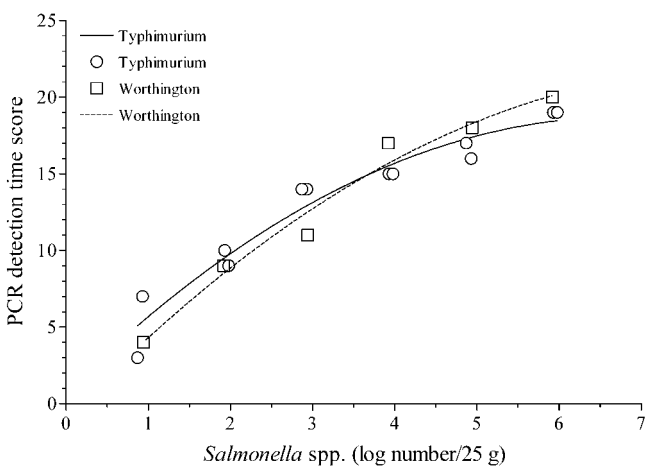

FIGURE 3. *Standard curve for PCR detection time score as a function of initial density of* Salmonella *Typhimurium and* Salmonella *Worthington inoculated into preenrichment samples composed of 25 g of naturally contaminated chicken breast skin and 225 ml of buffered peptone water.*

The size of the food sample is an important determinant of the incidence and distribution of pathogen contamination. To demonstrate this, the scenario shown in Table 1, which was based on the PCR detection time scores of 12 naturally contaminated samples of chicken, was simulated for samples sizes of 25, 50, 100, 200, 400, and 500 g. Results of the simulations (Table 6) indicated that the incidence and distribution of *Salmonella* contamination on chicken increased in a nonlinear manner as a function of size of sample and that when sample size was large enough the incidence of *Salmonella* contamination approached 100%, as would be expected. It is a common practice in risk assessments to express pathogen number on a per gram basis even though enumeration data are usually obtained with 25-g samples; this extrapolation is done by incorrectly assuming that pathogens are uniformly distributed in the food *(4, 5, 16, 27).* Once expressed on a per gram basis, risk assessors fit the enumeration data to probability distributions such as custom *(5, 16),* Poisson *(4),* or truncated exponential *(27).* Results of the current study indicate that such a linear extrapolation of enumeration data is not valid. Rather, pathogen number should be expressed as a nonlinear function of the size of the sample used to obtain the enumeration data on which the risk assessment is based.

Since the completion of this study, the format of the BAX system has changed from gel analysis of PCR products to real time PCR. It is now possible to quantify the PCR using the cycle threshold value rather than visual scoring. Thus, future PCR detection time scores could be based

TABLE 6. *Effect of size of sample on the predicted incidence and distribution of* Salmonella *contamination on naturally contaminated chicken samples*

Sample size (g)	Incidence (%)	Distribution of contamination (no. of *Salmonella* cells)		
		Minimum	Median	Maximum
25	16.7	1	2	16
50	30.8	1	3	22
100	51.5	1	3	33
200	76.7	1	5	42
400	94.1	1	8	70
500	97.4	1	9	81

on the sum of the cycle threshold values for the eight subsamples from the preenrichment sample. This approach should reduce the uncertainty of the standard curve predictions (i.e., reduce the width of the 95% prediction interval of the standard curve fit; see Fig. 1) because a PCR detection time score based on cycle threshold values should be more accurate that one based on visual scoring of the density of the PCR product bands in an electrophoresis gel.

The standard curve method for enumeration of *Salmonella* cells on chicken reduces the time and cost of enumeration by eliminating the selective enrichment, selective plating, and confirmation steps of the traditional MPN method for evaluation of *Salmonella* contamination. Use of a three-tube MPN method and PCR to enumerate *Salmonella* cells in preenrichment samples (i.e., nontraditional MPN method for *Salmonella*) would also not be as cost-effective as the current PCR detection time score and standard curve method because it would require from 9 PCR tests per sample for an enumeration range of <0.3 to $>1{,}100$ MPN per g to 18 PCR tests per sample for an enumeration range of <0.3 to $>10^6$ MPN per g. The latter range is comparable to the enumeration range of the method developed in the current study, which only required eight PCR tests per sample. Moreover, enumeration results for larger sample sizes can be obtained without further time or cost because the present method uses simulation modeling to extrapolate results obtained with one size of sample to samples of other sizes. The shape of the standard curve was not affected by the type of chicken meat or strain variation but was affected by microbial competition, which indicated that it was important to construct the standard curve in the presence of the natural microflora of chicken to use the new method to accurately enumerate *Salmonella*. Enumeration results obtained with the new method are useful for risk assessment because they consider the uncertainty of the standard curve predictions and because they provide predicted distributions of *Salmonella* contamination for different size samples of chicken that can be directly used in risk assessment.

ACKNOWLEDGMENTS

The author appreciates the outstanding technical assistance of Jacquelyn Ludwig and the excellent administrative assistance of Patricia Shannon of the U.S. Department of Agriculture Agricultural Research Service and the University of Maryland Eastern Shore for providing laboratory and office space and research support services as well as a professional environment in which to conduct the research.

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