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Research Paper

Risk of Salmonellosis from Chicken Parts Prepared from Whole Chickens Sold in Flow Pack Wrappers and Subjected to Temperature Abuse

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ABSTRACT

The flow pack wrapper is a popular packaging choice for retail sale of whole chickens. However, it may provide a favorable environment for growth and spread of *Salmonella* within the package, leading to an outbreak of salmonellosis. To investigate this possibility, a process risk model was developed that predicted the risk of salmonellosis from chicken parts prepared from whole chickens sold in flow pack wrappers and subjected to proper storage (6 h at 4°C) or improper storage (72 h at 15°C) before preparation. The model had four unit operations (pathogen events): (i) preparation (contamination), (ii) cooking (death), (iii) serving (cross-contamination), and (iv) consumption (dose-response). Data for prevalence, number, and serotype of *Salmonella* on chicken parts were obtained by whole sample enrichment, real-time PCR. Improper storage increased ($P < 0.05$) prevalence of *Salmonella* on raw chicken parts from 10.6% (17 of 160) to 41.2% (66 of 160) and incidence of cross-contamination of cooked chicken from 10% (4 of 40) to 52.2% (24 of 46). Improper storage also increased ($P < 0.05$) the number (mean ± standard deviation) of *Salmonella* from 0.017 ± 0.030 to 3.51 ± 1.34 log per raw chicken part and from 0.048 ± 0.089 to 3.08 ± 1.50 log per cooked chicken part. The predominant serotypes isolated ($n = 111$) were Typhimurium (34.2%), Typhimurium var 5- (20.7%), Kentucky (12.6%), Enteritidis (11.7%), and Heidelberg (8.1%). When chicken was properly stored before preparation, the model predicted that risk of salmonellosis was low and sporadic with only six cases per 100 simulations of 10^5 chicken parts. However, when 0.1 to 1% of chickens were improperly stored before preparation, the model predicted that salmonellosis would increase ($P < 0.05$) linearly from a median of 7 (range, 1 to 15) to a median of 72 (range, 52 to 93) cases per 10^5 chicken parts. These results indicated that the flow pack wrapper provided a favorable environment for growth and spread of *Salmonella* within the package and that even when only a small percentage of packages were subjected to improper storage before preparation, the risk and size of an outbreak of salmonellosis from chicken parts increased significantly.

Key words: Chicken parts; Flow pack wrapper; Process risk model; Risk assessment; *Salmonella*; Salmonellosis

Salmonella is a leading cause of foodborne illness in the United States and worldwide. Sporadic cases and outbreaks of salmonellosis occur and sometimes are attributed to chicken meat and meat products *(2, 6, 21)*. Salmonellosis is characterized as gastroenteritis with symptoms of fever, vomiting, headache, stomachache, diarrhea, dehydration, and in severe cases, bacteremia and death.

Prevalence of *Salmonella* on chicken is a common measure of food safety. Processing plants in the United States with a prevalence of *Salmonella* that exceeds the established standard (e.g., 7.5%) are classified as producing unsafe chicken and are subject to recalls and removal of federal inspection *(1)*. However, this metric does not consider number and serotype of *Salmonella* or other potential risk factors (e.g., consumer demographics, eating habits, and food handling practices) in its evaluation of food safety and therefore can be improved.

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The flow pack wrapper, which is hermetically sealed, is a popular method for packaging whole chickens for retail sale in the United States. Although chicken parts prepared from whole chickens sold in flow pack wrappers have low prevalence (<20%) and levels (<1 log) of *Salmonella (15–17)*, the flow pack wrapper may provide a favorable environment for growth and spread of *Salmonella* within the package during improper storage by consumers. Specifically, the flow pack wrapper causes meat juices to form a mobile layer around the whole chicken that may facilitate the growth and spread of *Salmonella* throughout the package. However, the effect of improper storage of whole chickens sold in flow pack wrappers on levels of *Salmonella* and risk of salmonellosis from commonly consumed chicken parts (i.e., wings, breasts, thighs, and drumsticks) have not been investigated.

In the present study, a process risk model that considers *Salmonella* prevalence, number, and serotype and other potential risk factors was developed and used to compare the risk of salmonellosis from chicken parts prepared from

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Node	Unit Operation	Pathogen Event	Consumer Event	Discrete	Pert	*Salmonella*				**Illness dose (log_{10}) sub-model**					
2	0	Refrigeration	Growth/Spread	**Proper Storage**	0						Discrete distribution (porportion, outcome value)					
3	1	Meal Preparation	Contamination	Proper storage test results	0	0.01	0	!				Proportion per risk category				
4				Improper storage test results	1	2.95	895			Factor	low-low	low	normal	high	high-high	
5							0			Meal	5.00	15.00	30.00	40.00	10.00	
6	2	Cooking	Death	**Proper Cooking**	0	-6.71	0			*Salmonella*	*0.00*	*22.50*	*0.00*	*8.10*	*69.40*	
7	3	Serving	Cross-contamination	Proper storage test results	0	0.03	0	!		Consumer	5.00	15.00	60.00	15.00	5.00	
8				Improper storage test results	1	2.24	175					Outcome value per risk category				Discrete
9				**Improper handling**	1		0			Factor	low-low	low	normal	high	high-high	output
10	4	Consumption	Salmonellosis	**Dose**			0			Meal	0.5	1	1.5	2	2.5	1.5
11				**Illness Dose**		3.07	1,167			*Salmonella*	1	2	3	4	5	4.0
12				Meal	normal					Consumer	1	2	3	4	5	4.0
13				*Salmonella*	high										lookup value →	9.5
14				Consumer	high						lookup value	Pert output	Pert distribution (log_{10})			
15				**No Illness**			**0**						minimum	median	maximum	
16											12.5	0.19	0.0	1.0	2.0	
17					Discrete		Pert (log_{10})				12.0	1.66	0.4	1.4	2.4	
18	Node	Unit Operation	Pathogen Event	Consumer Event	0 = no	1 = yes	minimum	median	maximum		11.5	1.85	0.8	1.8	2.8	
19	1	Meal Preparation	Contamination	Proper storage test results	89.37	*10.63*	*0.000*	*0.003*	*0.114*		11.0	2.51	1.2	2.2	3.2	
20				Improper storage test results	58.75	*41.25*	*0.000*	*3.466*	*6.778*		10.5	3.06	1.6	2.6	3.6	
21				**Improper storage**	99.80	0.20					10.0	2.64	2.0	3.0	4.0	
22	2	Cooking	Death	**Improper cooking**	90.00	10.00	-7	-6	0	!	9.5	3.07	2.4	3.4	4.4	
23	3	Serving	Cross-contamination	Proper storage test results	90.00	*10.00*	*0.000*	*0.006*	*0.181*		9.0	4.34	2.8	3.8	4.8	
24				Improper storage test results	47.83	*52.17*	*0.291*	*3.148*	*6.192*		8.5	4.08	3.2	4.2	5.2	
25				**Improper handling**	72.00	28.00					8.0	4.15	3.6	4.6	5.6	
26											7.5	5.80	4.0	5.0	6.0	
27											7.0	5.24	4.4	5.4	6.4	
28											6.5	5.96	4.8	5.8	6.8	
29											6.0	6.59	5.2	6.2	7.2	
30											5.5	6.80	5.6	6.6	7.6	
31											5.0	7.34	6.0	7.0	8.0	
32											4.5	7.01	6.4	7.4	8.4	
33											4.0	7.04	6.8	7.8	8.8	
34											3.5	8.84	7.2	8.2	9.2	
35											3.0	8.28	7.6	8.6	9.6	
36											2.5	8.52	8.0	9.0	10.0	

FIGURE 1. *Process risk model for predicting risk of salmonellosis from chicken parts prepared from whole broiler chickens sold in flow pack wrappers and subjected to proper storage or improper storage before preparation. See text for details.*

whole chickens sold in flow pack wrappers and subjected to proper storage or improper storage before preparation of commonly consumed chicken parts. Data for model development were obtained by whole sample enrichment, real-time PCR (WSE-qPCR), which has a lower limit of detection of one cell of *Salmonella* per chicken part *(16)*.

MATERIALS AND METHODS

Process risk model (general description). A process risk model (Fig. 1) that predicts the cases of salmonellosis from chicken parts prepared from whole broiler chickens sold in flow pack wrappers was developed in an Excel spreadsheet (Excel 2013, Microsoft Corporation, Redmond, WA) and was simulated with @Risk (version 6.3.1, Palisade Corporation, Ithaca, NY). The model consisted of four unit operations (pathogen events): (i) preparation (contamination), (ii) cooking (death), (iii) serving (cross-contamination), and (iv) consumption (dose-response). Specifically, the model simulated the initial contamination of raw chicken parts with *Salmonella* at preparation following proper (i.e., 6 h at 4°C) or improper (i.e., 72 h at 15°C) storage, death of *Salmonella* on chicken parts during cooking, cross-contamination of cooked chicken with *Salmonella* during serving following proper or improper storage before preparation, and dose-response of consumers following consumption of the cooked chicken parts. The model predicted prevalence and number of *Salmonella* on chicken parts after each unit operation (pathogen event) as well as the cases of salmonellosis from the batch of chicken parts simulated.

The full model contained 36 input distributions, formula, and six output distributions. The reduced model was the same except it only had one output distribution. Data for prevalence, number, and serotype of *Salmonella* on chicken parts were obtained by WSE-qPCR, as described below. The cells in the model where the data obtained by WSE-qPCR were entered had purple, italic, and underlined font to distinguish them from other cells in the model.

Data for consumer food handling behavior (i.e., incidence of improper storage, incidence of improper cooking, extent of improper cooking, and incidence of improper serving), consumer eating habits (i.e., incidence of low-low, low, normal, high, and high-high risk meals), and consumer demographics (i.e., incidence of low-low, low, normal, high, and high-high risk consumers) were not collected. Therefore, a broad range of these variables were simulated in the model using scenario analysis to evaluate their effects on the risk of salmonellosis. Values for consumer food handling behavior, eating habits, and demographics were entered in the model using red, normal font to distinguish them from data collected by WSE-qPCR.

Color coding was used for other types of information as well. A blue, normal font was used for cells that contained formula that performed calculations. Purple, bold font was used for output cells. An orange font was used for food handling outcomes, whereas exclamation marks were used to highlight the input value used for the current iteration when two input values were possible (e.g., level of contamination at preparation following proper storage or improper storage). The complete code used in the model was too complex to include in the text, tables, or figures. However, the model and its coding are available free of charge at www.ars.usda.gov/nea/errc/PoultryFARM.

WSE-qPCR. Data for prevalence, number, and serotype of *Salmonella* on chicken parts were obtained by WSE-qPCR, as described previously *(16)*. In brief, whole broiler chickens ($n = 51$) in flow pack wrappers were purchased from a local retail store

(Princess Anne, MD) at the rate of one per visit from 4 June 2013 to 18 May 2015. Chickens were stored for 6 h at 4°C (proper storage) or for 72 h at 15°C (improper storage) before preparation of chicken parts (wings, breasts, thighs, and drumsticks). Because of the time and cost of WSE-qPCR, only two storage scenarios were investigated and modeled. Based on predictions of a model for growth of *Salmonella* Typhimurium DT104 on chicken with native microflora *(11),* an improper storage scenario (i.e., 72 h at 15°C) was identified that was expected to result in substantial multiplication of the pathogen and thus provide a good test of the hypothesis that the flow pack wrapper would provide a good environment for the growth of spread of *Salmonella* in the package during temperature abuse.

To initiate WSE, 400 mL of prewarmed (40°C) buffered peptone water was added to a chicken part in a plastic stomacher bag. The WSE was performed for 8 h at 40°C and 80 rpm followed by 8 to 10 h at 6°C and 80 rpm. After WSE, 1-mL samples were collected for enumeration of *Salmonella* by qPCR and for isolation of *Salmonella* by a cultural method that involved further incubation (24 h at 40°C) in buffered peptone water followed by incubation (24 h at 42°C) in Rappaport-Vassiliadis R10 broth and then incubation (24 h at 40°C) on xylose lysine Tergitol 4 agar. Serotyping was performed by a *Salmonella* Reference Laboratory (U.S. Department of Agriculture, Animal and Plant Health Inspection Service, National Veterinary Services Laboratory, Ames, IA).

Because endogenous *Salmonella* lower the cycle threshold (C_T) values obtained by qPCR, two standard curves were developed: one curve for proper storage where low levels of endogenous *Salmonella* were present and one curve for improper storage where higher levels of endogenous *Salmonella* were present. Both standard curves were developed with raw and cooked chicken parts inoculated with different doses of *Salmonella* Typhimurium var 5-. This serotype was used for standard curve development because it was a predominant serotype isolated in the current study. For reasons discussed previously *(17),* maximum C_T values for each inoculated dose were used to develop the standard curves:

$$Y = Y_o - \left(\frac{X}{a}\right)^b$$

where Y was the predicted C_T value at inoculated dose X (log per chicken part), Y_o was the maximum C_T value at 0 log per chicken part (fixed parameter), a was a regression coefficient, and b was a shape parameter. Standard curves were developed with Prism (version 6.07, GraphPad Software, La Jolla, CA), and the interpolation function of Prism was used to convert the C_T values into log *Salmonella* per chicken part.

Modeling. The initial distribution of *Salmonella* among chicken parts at preparation and after proper or improper storage was simulated using a rare events modeling method, as described previously *(8, 10, 12).* In brief, a discrete distribution for prevalence of *Salmonella* was linked to a pert distribution, which was defined by minimum, median, and maximum values, for extent of *Salmonella* contamination (log per chicken part), to simulate both contaminated and noncontaminated chicken parts together. This was important because when only contaminated chicken parts are simulated and results are adjusted for prevalence after the fact, risk of salmonellosis from chicken parts is overestimated and the variability and uncertainty of the risk prediction is underestimated *(17).* An additional discrete distribution was included to simulate the percentage of chicken parts subjected to proper storage or improper storage before preparation.

Initial distribution of *Salmonella* on chicken parts at preparation was altered by two unit operations and pathogen events before consumption: (i) death during cooking and (ii) cross-contamination during serving. Cooking reduced prevalence and number of *Salmonella* on chicken parts, whereas cross-contamination, which followed cooking, increased prevalence and number of *Salmonella* on chicken parts.

A rare events modeling method was also used to simulate death of *Salmonella* on chicken parts during cooking. Here, the discrete distribution simulated the percentage of chicken parts that were properly cooked or improperly cooked. When a chicken part was properly cooked, it was assumed that all the *Salmonella* died and therefore the *Salmonella* load of the chicken part after cooking was zero. In contrast, when a chicken part was improperly cooked, there were three possible outcomes: (i) no death, (ii) partial death, or (iii) complete death. The outcome during undercooking depended on the initial number of *Salmonella* present before cooking and the log reduction of *Salmonella* during undercooking.

Data for the time and temperature of cooking of the chicken parts in this study were not available. Thus, to simulate death during cooking, the following assumptions were made. It was assumed that all *Salmonella* were located in the cold spot of the chicken part, that the size of the cold spot was 1 g, and that the cold spot of the chicken part was the 1 g that received the least amount of cooking. It was also assumed that undercooking, by definition, occurred when the time and temperature of cooking resulted in less than a 7-log reduction of *Salmonella* in the cold spot. In the baseline scenario (Fig. 1), it was assumed that undercooking occurred 10% of the time. In addition, it was assumed that 50% of the time when consumers undercooked their chicken that the cold spot received a time and temperature of cooking that resulted in a 6-log reduction of *Salmonella*. Finally, it was assumed that on rare occasion the cold spot of the chicken part was still raw after cooking or received a time and temperature of cooking that resulted in a 0-log reduction of *Salmonella*. Thus, in the baseline scenario (Fig. 1), a pert distribution with values of –7-log, –6-log, and 0-log reduction was used to simulate the extent of *Salmonella* death during undercooking, whereas a discrete distribution with values of 90% for proper cooking and 10% for improper cooking was used to simulate the incidence of undercooking. Therefore, if the log reduction during undercooking was greater than the log number of *Salmonella* on the chicken part before cooking, then all the *Salmonella,* if present, died; otherwise, some or none of them died depending on the number present and the log reduction during undercooking.

Unlike data for time and temperature of cooking, data for cross-contamination of cooked chicken parts with *Salmonella* from unwashed utensils used to prepare raw chicken parts for cooking were available and were obtained as described previously *(16).* In brief, the unwashed cutting board, knife, and gloves used to prepare raw chicken parts from whole chickens sold in flow pack wrappers were used to cut an autoclaved, cooked chicken breast into two parts, which were then used to swab the cutting board. Prevalence, number, and serotype of *Salmonella* on these cooked chicken parts were determined by WSE-qPCR, as described above.

Cross-contamination at serving was simulated as a rare event similar to contamination at preparation with two differences. First, incidence of cross-contamination was adjusted for how often consumers made this food handling mistake (i.e., used unwashed utensils to serve cooked chicken). Based on consumer surveys summarized in Oscar *(10),* it was assumed that incidence of this food handling mistake was 28% in the baseline scenario (Fig. 1).

TABLE 1. *Characteristics and associated risk category for determining the percentage of food, pathogen, and consumer events in the low-low, low, normal, high, and high-high risk categories of the dose-response model*[a]

Disease triangle factor	Risk factor	Risk category
Host	Very young	High
	Very old	High
	Diabetic	High
	Liver disease	High
	Immunocompromised (e.g., human immunodeficiency virus/AIDS)	High
	Pregnant	High
	Malnutrition	High
	Stress	High
	Healthy teen to middle-aged adult	Normal
	Vaccination (natural or synthetic)	Low
	Probiotic	Low
Pathogen	Top human clinical isolate	
	1–5	High-high
	6–10	High
	11–20	Normal
	>20	Low
	Animal host adapted (e.g., Pullorum, Gallinarum)	Low-low
Food	High fat	High
	Acidic beverage	Low
	Alcoholic beverage	Low
	Anti-acid pill	High

[a] The risk category for the disease triangle factor is equal to the sum of the risk factors. For example, two or more high risk factors in a disease triangle factor equals high-high risk, one high risk factor equals high risk, two high risk factors plus one low risk factor equals high risk, and vice versa for low risk factors.

Second, if the raw chicken part simulated was from a chicken that was properly stored before preparation, then cross-contamination was also from a chicken that was properly stored before preparation and vice versa.

In the current process risk model, the dose consumed with a chicken part was equal to the number of *Salmonella* that survived cooking plus the number of *Salmonella* that cross-contaminated the cooked chicken part during serving. If this number was less than the illness dose, then no illness occurred; otherwise, an illness occurred. Thus, dose-response was simulated as a discrete event (i.e., illness or no illness) rather than as a probabilistic event because when a person ingests a dose of *Salmonella* they do not have a probability of getting ill; they either become ill or they do not.

The illness dose was the result of the interaction between the meal (i.e., chicken part, beverage[s], and other foods), *Salmonella,* and consumer or the disease triangle factors. The source of the illness dose in the model was an array of 21 pert distributions (Fig. 1). During each iteration of the model, an illness dose was selected from this array by using a randomly determined lookup value. The lookup value was equal to the sum of the outputs from three randomly sampled discrete distributions: one distribution for the meal, one distribution for the *Salmonella,* and one distribution for the consumer. These discrete distributions simulated the percentage of meals, *Salmonella,* and consumers in five categories of risk: low-low, low, normal, high, and high-high. The output value from the discrete distribution for meals was weighted at half of the output values of the discrete distributions for *Salmonella* and consumers resulting in 21 possible lookup values from 2.5 to 12.5 in 0.5 increments. For example, if a chicken part was consumed with a normal risk meal (value = 1.5) and was contaminated with a normal risk serotype of *Salmonella* (value = 3) and was consumed by a consumer with a normal risk of acquiring salmonellosis (value = 3), then the lookup value was $7.5 = 1.5 + 3 + 3$, corresponding to a pert distribution for illness dose of 4.0 log (minimum), 5.0 log (median), and 6.0 log (maximum).

Serotype data obtained by WSE-qPCR *(16)* and criteria shown in Table 1 were used to help determine the percentage of meals, *Salmonella,* and consumers in each risk category. However, survey data for consumer food handling, eating habits, and demographics were not available for the chicken parts simulated in the model. Therefore, the following values were arbitrarily used in the baseline scenario: percentage of meals in the low-low, low, normal, high, and high-high categories of risk were 5, 15, 30, 40, and 10, respectively, and the percentage of consumers in the low-low, low, normal, high, and high-high categories of risk were 5, 15, 60, 15, and 5, respectively. It should be stated that dark meat (i.e., thigh or drumstick), which is higher in fat content and which composed 50% of the chicken parts simulated, was considered high risk, whereas white meat (i.e., wing or breast), which is lower in fat content and which composed 50% of the chicken parts simulated, was considered normal risk.

Model simulation. Effect of improper storage on risk of salmonellosis was evaluated by altering the discrete distribution for improper storage from 0 to 1% in 0.1% increments. These 11 scenarios were simulated using the reduced model (i.e., only one output distribution) with @Risk settings of 100,000 iterations or chicken parts, 100 simulations, Latin Hypercube sampling, Mersenne Twister, and a random selection of a different random number generator seed for each simulation. The output per simulation was cases of salmonellosis per 100,000 chicken parts. The 100,000 value was selected for two reasons: (i) to generate enough cases for statistical comparison because salmonellosis was a rare event and (ii) for comparison with public health data because the Centers for Disease Control and Prevention reports disease rates per 100,000 people. Additional "what-if" scenarios were run with the reduced model to evaluate effects of other potential risk factors (i.e., improper cooking, improper serving, consumer eating habits, and consumer demographics) on risk of salmonellosis.

Statistical analysis. A 2×2 contingency table and Fisher's Exact test were used to compare prevalence of *Salmonella* on chicken parts subjected to proper storage or improper storage, whereas a t test was used to compare the mean log number of *Salmonella* on chicken parts subjected to proper storage or improper storage. The effect of improper storage and other variables on the risk of salmonellosis was evaluated by linear regression. Statistical significance was at $P < 0.05$, and all statistical tests were performed in Prism (GraphPad Software, Inc.).

RESULTS AND DISCUSSION

After proper storage (Fig. 2), 7 (35%) of 20 chickens examined had one or more parts test positive for *Salmonella,* whereas after improper storage (Fig. 3), 12 (60%) of 20 chickens examined had one or more parts test positive for *Salmonella*. These prevalence values may have been higher if the shell had not been excluded from the analysis. Nonetheless, these prevalence values exceed the current standard (7.5%) used in the chicken industry to establish

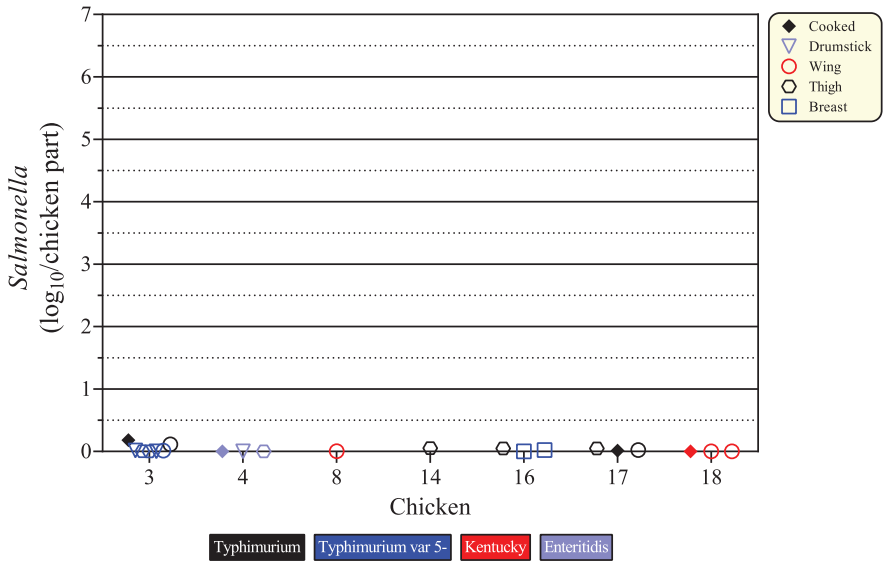

FIGURE 2. *Number and serotype of* Salmonella *on chicken parts prepared from whole broiler chickens sold in flow pack wrappers and subjected to proper storage before preparation. Symbol shape indicates type of chicken part, whereas symbol color indicates serotype.*

food safety, but they are consistent with those of other studies that used WSE *(15–18)*. Moreover, these results suggest that the whole carcass rinse method used by the chicken industry underestimates the prevalence of *Salmonella* on chickens in the United States as it can be reasonably assumed that the chickens examined in this study had a whole carcass rinse prevalence of <7.5% because they obviously passed inspection to reach the marketplace. In fact, several studies *(4, 19, 20)* have shown that the WSE method is more sensitive than the whole carcass rinse method for detection of *Salmonella* on whole chickens, especially when low levels of *Salmonella* are present. Thus, to better assess chicken safety, the chicken industry should consider switching from the whole carcass rinse method to the WSE method.

The standard curve for enumeration of *Salmonella* on chicken parts by WSE-qPCR after proper storage is shown in Figure 4A, and the standard curve for enumeration of *Salmonella* on chicken parts by WSE-qPCR after improper storage is shown in Figure 4B. In both cases, there was a mathematical relationship between the maximum C_T values and the doses of *Salmonella* inoculated onto chicken parts. However, it should be noted that the C_T values were higher for chicken parts subjected to improper storage, which indicated less growth of *Salmonella* during WSE. For example, at an inoculated dose of 3 log per chicken part, the standard curves predicted a C_T of 32.05 cycles for improper storage and 16.37 cycles for proper storage. A higher number of competing microorganisms in WSE of chicken parts subjected to improper storage may have reduced growth of *Salmonella* and resulted in the higher C_T values. Whether this is the correct or only explanation, the results indicated that it was important to develop two standard curves: one curve for proper storage and one curve for improper storage.

The standard curves shown in Figure 4 were used to obtain the enumeration results presented in Figures 2 and 3 and in Table 2. These data were used to model *Salmonella* contamination of raw chicken parts at preparation and *Salmonella* cross-contamination of cooked chicken parts at serving. For example, the discrete distribution for prevalence of *Salmonella* contamination of raw chicken parts at preparation after proper storage was ({0,1}, {143,17}), where 0 is the output for a noncontaminated raw chicken part, 1 is the output for a contaminated raw chicken part, 143 is the proportion of noncontaminated raw chicken parts, and 17 is the proportion of contaminated raw chicken parts. Likewise, the pert distribution for the log number of *Salmonella* per raw chicken part at preparation after proper storage was (0.000, 0.003, 0.114), where 0.000 is the minimum log number of *Salmonella* per raw chicken part, 0.003 is the median log number of *Salmonella* per raw chicken part, and 0.114 is the maximum log number of *Salmonella* per raw chicken part.

Prevalence of *Salmonella* on raw chicken parts at preparation was higher ($P < 0.05$) after improper storage (41.2%) than after proper storage (10.6%; Table 2). The log number (mean ± standard deviation) of *Salmonella* per raw chicken part at preparation was also higher ($P < 0.05$) after improper storage (3.51 ± 1.34) than after proper storage (0.017 ± 0.030). Thus, improper storage of whole chickens in flow pack wrappers resulted in growth and spread of *Salmonella* throughout the package and resulted in a higher prevalence and number of *Salmonella* on raw chicken parts (wings, breast, thighs, and drumsticks) before cooking.

Similar to raw chicken parts, prevalence of *Salmonella* on cooked chicken parts at serving was higher ($P < 0.05$) after improper storage (52.2%) than after proper storage (10%; Table 2). The log number (mean ± standard deviation) of *Salmonella* per cooked chicken part at serving was also higher ($P < 0.05$) after improper storage (3.08 ± 1.50) than after proper storage (0.048 ± 0.089). Thus, improper storage of whole chickens in flow pack wrappers before preparation of raw chicken parts resulted in growth and spread of *Salmonella* throughout the package and resulted in a higher incidence and extent of *Salmonella* cross-contamination of cooked chicken parts from unwashed utensils (i.e., cutting board, knife, and latex gloves) used to prepare raw chicken parts for cooking.

In addition to prevalence and number, the type of *Salmonella* present can affect the risk of salmonellosis because the ability to cause this disease in humans differs

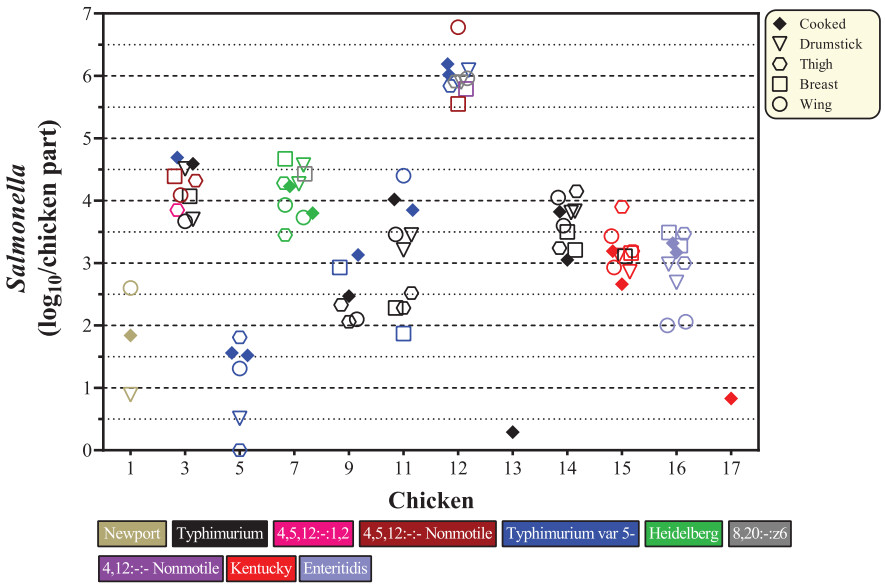

FIGURE 3. *Number and serotype of* Salmonella *on chicken parts prepared from whole broiler chickens sold in flow pack wrappers and subjected to improper storage before preparation. Symbol shape indicates type of chicken part, whereas symbol color indicates serotype.*

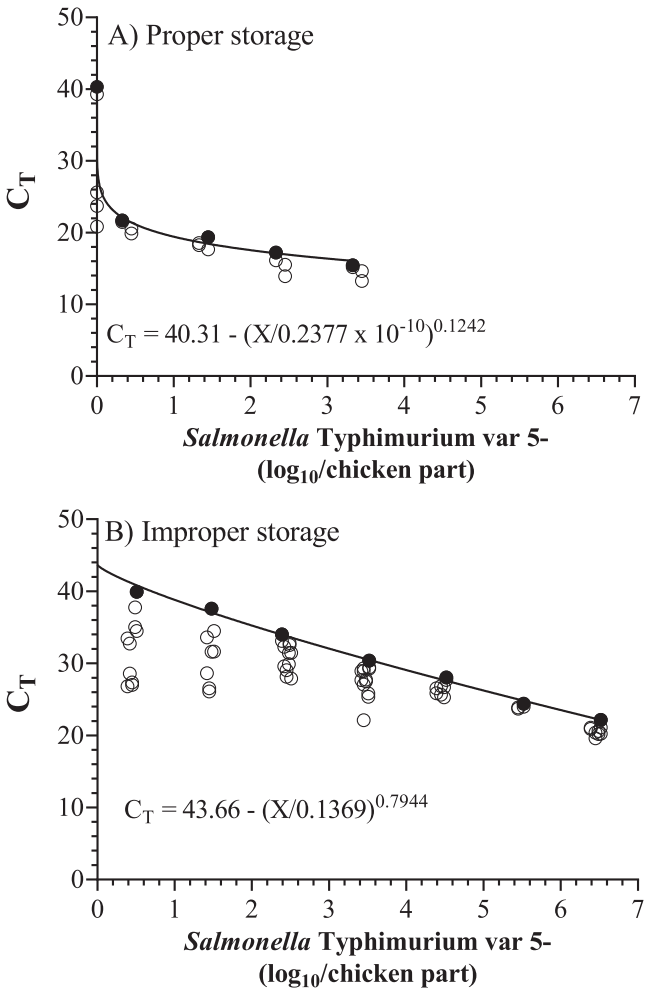

FIGURE 4. *Standard curves for enumeration of* Salmonella *by whole sample enrichment, real-time PCR on chicken parts prepared from whole broiler chickens sold in flow pack wrappers and subjected to (A) proper storage or (B) improper storage before preparation.*

among strains and serotypes of *Salmonella (9)*. In the present study, 10 different serotypes of *Salmonella* were isolated from raw and cooked chicken parts (Figs. 2 and 3, and Table 3). The predominant serotypes ($n = 111$) were Typhimurium (34.2%), Typhimurium var 5- (20.7%), Kentucky (12.6%), Enteritidis (11.7%), and Heidelberg (8.1%). Most serotypes (77.48%) were in the top 10 of human clinical isolates *(3)* and were classified as high risk (top 6 to 10) or high-high risk (top 5) for dose-response modeling purposes. The results presented in Table 3 were used in the model to define the discrete distribution for the percentage of *Salmonella* in each of the five risk categories used in the dose-response model.

Following proper storage, one or two cells of *Salmonella* were randomly distributed among multiple parts from the same chicken, and in one case more than one serotype was found to contaminate parts from the same chicken (Fig. 2). These results agree with previous studies using similar methods *(15–17)*. However, after improper storage (Fig. 3), high levels (maximum = 6.78 log per chicken part) of *Salmonella* were present on all or multiple parts from the same chicken. There were two occasions where no raw chicken parts were contaminated, but one cooked chicken part was cross-contaminated with a low number (i.e., <1 log) of *Salmonella*. In many cases, more than one serotype was found on parts from the same chicken, and both cooked chicken parts were cross-contaminated with *Salmonella* at levels similar to those found on raw chicken parts. These results indicated that the flow pack wrapper provided a favorable environment for growth and spread of *Salmonella* within the package and led to a high risk of cross-contamination of cooked chicken parts once the package was opened.

A previously published disease triangle modeling method *(12–14)* was modified and used to simulate dose-response in the current study. Two modifications made in the present study were that instead of two categories of risk per disease triangle factor and eight pert distributions for illness dose, five categories of risk per disease triangle factor, and 21 pert distributions for illness dose were used

TABLE 2. *Prevalence and number of* Salmonella *on raw and cooked chicken parts subjected to proper storage (6 h at 4°C) or improper storage (72 h at 15°C) before preparation*

		Prevalence			No. (log/chicken part)		
Storage	Part	Positive	Total	%	Minimum	Median	Maximum
Proper	Raw	17	160	10.63	0.000	0.003	0.114
	Cooked	4	40	10.00	0.000	0.006	0.181
Improper	Raw	66	160	41.25	0.000	3.466	6.778
	Cooked	24	46	52.17	0.291	3.148	6.192

(Fig. 1). Figure 5 shows that these 21 pert distributions did a good job of predicting published outbreak data *(22)* and human feeding trial data *(9)* for *Salmonella* that have been used to develop dose-response models for *Salmonella*.

It should be stated that the leftmost dose-response curve in Figure 5 represents consumption of a high-high risk serotype of *Salmonella* (e.g., Typhimurium) in a high-high risk meal (e.g., a chicken thigh plus an anti-acid pill) and by a high-high risk consumer (e.g., a 75-year-old man with diabetes). Conversely, the rightmost dose-response curve in Figure 5 represents consumption of a low-low risk serotype of *Salmonella* (e.g., Pullorum) in a low-low risk meal (e.g., chicken breast with an alcoholic beverage plus an acidic beverage) by a low-low risk consumer (e.g., a healthy individual with natural immunity to *Salmonella* and who routinely consumes a probiotic). These examples are provided to illustrate the thought process behind the modified disease triangle modeling method used in the current study. For a further description and discussion of this dose-response modeling method, please see previous studies *(12–14)*.

To begin the assessment of the risk of salmonellosis from chicken parts prepared from whole chickens sold in flow pack wrappers, the full model with six output distributions was used to simulate the baseline scenario described in the "Materials and Methods." The full model was simulated with @Risk settings of 100,000 iterations or chicken parts, Latin Hypercube sampling, Mersenne Twister, two simulations, and random number generator seeds of 1 and 23. In these two simulations, six cells (purple, normal font) were designated as output cells (Fig. 1). This was done to demonstrate the type of results that can be obtained from the model when all the output cells are used. In addition, it was done to demonstrate why it is important to run multiple simulations of the same scenario. The simulation results shown in Table 4, which were filtered to remove noncontaminated chicken parts for calculation of prevalence results, demonstrated that the model can predict both prevalence and number of *Salmonella* on chicken parts after each unit operation (pathogen event) in the risk pathway because both contaminated and noncontaminated chicken parts were simulated together.

The full model can also predict results for individual chicken parts as shown in Table 5. Here, the chicken parts from simulations of the baseline scenario with seeds of 1 and 23 that resulted in cases of salmonellosis were profiled. Examination of these results indicated that improper storage of whole chickens in flow pack wrappers before preparation of chicken parts followed by improper handling of cooked chicken (i.e., serving with unwashed utensils used to prepare the raw chicken parts) resulted in exposure of consumers to *Salmonella* and salmonellosis. In all of these cases, no *Salmonella* survived cooking. Thus, cross-contamination of cooked chicken with *Salmonella* from raw chicken during serving was the primary route of exposure and cause of salmonellosis. These results agree with those of a previous study *(17)* that used a similar process risk model for

TABLE 3. *Prevalence of* Salmonella *serotypes isolated from raw and cooked chicken parts and sorted by risk category for dose-response*

Serotype	Risk	No.	Percentage
Typhimurium	High-high	38	34.23
Typhimurium var 5-	High-high	23	20.72
Newport	High-high	3	2.70
Enteritidis	High-high	13	11.71
Heidelberg	High	9	8.11
4,5,12:−:1,2	Low	1	0.90
Kentucky	Low	14	12.61
4,5,12:Nonmotile	Low	5	4.50
4,12:Nonmotile	Low	1	0.90
8,20:−:z_6	Low	4	3.60
Total		111	100.00
	High-high	77	69.37
	High	9	8.11
	Normal	0	0.00
	Low	25	22.52
	Low-low	0	0.00

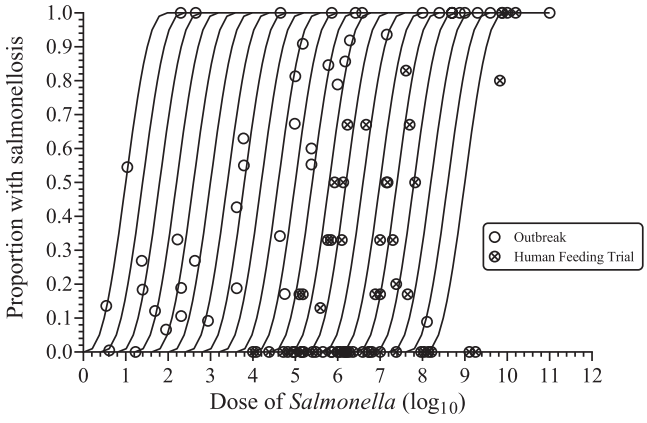

FIGURE 5. *Dose-response curves for predicting salmonellosis as a function of dose, serotype, consumer eating habits, and consumer demographics. See text for details.*

TABLE 4. *Statistical summary of results from two simulations of the baseline scenario for risk of salmonellosis from chicken parts prepared from chickens sold in flow pack wrappers and subjected to proper or improper storage before preparation*

Seed	Statistic	Initial contamination (no./chicken part)	Survived cooking (no./chicken part)	Cross-contamination (no./chicken part)	Dose consumed (no./chicken part)	Illness dose (no.)	Salmonellosis
1	Minimum	1	1	1	1	1	1
	Maximum	1,005,297	56	95,516	95,516	721,109,100	1
	Mean	228	29	94	94	861,873	1
	Median	1	1	1	1	4,292	1
	Filtered	89,346	99,998	97,205	97,205	0	99,995
	Prevalence	10.654	0.002	2.795	2.795	100.000	0.005
23	Minimum	1	0	1	1	2	1
	Maximum	2,064,809	0	194,407	194,407	601,740,400	1
	Mean	617.61	0	123.83	123.83	849,519	1
	Median	1	0	1	1	4,335	1
	Filtered	89,335	100,000	97,217	97,217	0	99,990
	Prevalence	10.665	0.000	2.783	2.783	100.000	0.010

Salmonella and chicken parts prepared from whole chickens sold in flow pack wrappers and subjected to proper storage before preparation of raw chicken parts. These simulations also showed that the cases of salmonellosis predicted by the model were variable and uncertain with 5 cases when a seed of 1 was used and 10 when a seed of 23 was used. This variability and uncertainty was due to the rare, random, variable, and uncertain nature of events in the risk pathway.

To better characterize the variability and uncertainty of the risk of salmonellosis, the baseline scenario was simulated using the reduced model where only one output cell (the one for dose-response in cell G15) was used. The same @Risk settings used to simulate the full model were used to simulate the reduced model except that 100 simulations were run and a different and randomly selected random number generator seed was used to initiate each simulation. The reduced model simplified data handling and reduced runtime so that it was possible to complete 100 simulations in a reasonable amount of time (i.e., ~20 min). The reduced model and its simulation approach were used for the baseline scenario as well as a number of what-if scenarios that are described below.

Simulation results for the baseline scenario and the reduced model indicated that the risk of salmonellosis was variable and uncertain and increased ($P < 0.05$) in a linear manner from a median of 7 (range, 1 to 15) to a median of 72 (range, 52 to 93) cases per 10^5 chicken parts as incidence of improper storage increased from 0.1 to 1% (Fig. 6A). However, when improper storage was 0%, risk of salmonellosis was low and sporadic with only 6 cases per 100 simulations of 10^5 chicken parts. These results indicated that chicken sold in flow pack wrappers had a low risk of salmonellosis when properly stored before preparation of chicken parts. However, if even a small percentage of chickens sold in flow pack wrappers were improperly stored before preparation of chicken parts, risk and size of an outbreak of salmonellosis increased significantly and proportionally.

The reduced model was next used to simulate a series of what-if scenarios for the purpose of evaluating effects of other potential risk factors on salmonellosis. Specifically, effects of incidence of improper cooking (0 to 100% in 10% increments), extent of improper cooking (median of –6.5-log to 0.5-log reduction in 0.5-log increments), incidence of

TABLE 5. *Results for individual iterations from two simulations of the baseline scenario that resulted in cases of salmonellosis*

Seed	Iteration (chicken part)	Initial contamination (no./chicken part)	Survived cooking (no./chicken part)	Cross-contamination (no./chicken part)	Dose consumed (no./chicken part)	Illness dose (no.)
1	2,771	0	0	23,413	23,413	1,612
	16,245	0	0	95,516	95,516	493
	39,081	23,182	0	2,281	2,281	299
	57,308	0	0	76,547	76,547	2,446
	71,917	0	0	33,552	33,552	508
23	5,529	510	0	54,188	54,188	125
	30,806	0	0	22,032	22,032	632
	32,334	4,222	0	1,670	1,670	385
	35,192	0	0	1,709	1,709	574
	45,668	201,731	0	12,547	12,547	2,599
	50,886	178,776	0	31,032	31,032	17,185
	55,127	0	0	18,756	18,756	1,302
	58,602	0	0	950	950	199
	77,563	0	0	194,407	194,407	118,290
	90,470	0	0	3,988	3,988	368

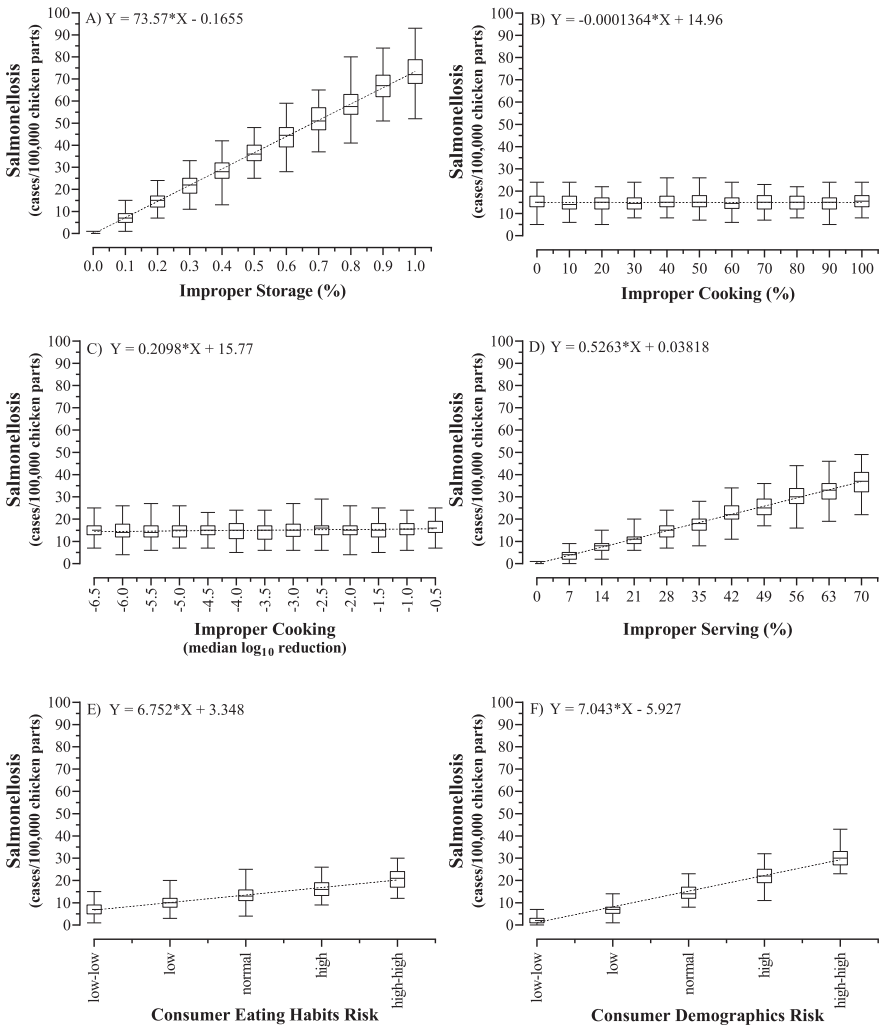

FIGURE 6. *Predicted risk of salmonellosis from chicken parts prepared from whole broiler chickens sold in flow pack wrappers as affected by (A) incidence of improper storage, (B) incidence of improper cooking, (C) extent of improper cooking, (D) incidence of improper serving, (E) consumer eating habits, and (F) consumer demographics. Whisker plots show 0, 25th, 50th, 75th, and 100th percentiles.*

improper handling (0 to 56% in 7% increments), consumer eating habits (from 100% low-low to 100% high-high risk), and consumer demographics (100% low-low to 100% high-high risk) were evaluated. Incidence of improper storage was fixed at 0.2% for all these simulations, whereas input values for the test variables were fixed at those in Figure 1 except when they were being evaluated.

Results of these simulations indicated that the risk of salmonellosis was not affected by incidence of improper cooking (Fig. 6B) or extent of improper cooking (Fig. 6C) as the slopes of the linear regression lines for these variables were not different ($P > 0.05$) from zero. In contrast, the risk of salmonellosis increased ($P < 0.05$) linearly in response to increases in improper handling or cross-contamination during serving (Fig. 6D). Likewise, positive and linear relationships were observed between risk of salmonellosis and consumer eating habits (Fig. 6E) and consumer demographics (Fig. 6F). Thus, in addition to improper storage, improper serving and consumer eating habits and demographics were important risk factors to consider when evaluating the microbiological safety of chicken sold in flow pack wrappers.

Chicken sold in flow pack wrappers and improperly stored had a strong odor of spoilage when opened. Although consumers would hopefully discard such a spoiled product and not consume it, they would not be aware of this situation until they opened the package. The mere act of opening the package would likely cross-contaminate the food preparation environment with *Salmonella* and potentially result in significant cross-contamination of ready-to-eat food and consumer exposure to *Salmonella* from the chicken. Thus, the risk of salmonellosis would not be eliminated by simply discarding the spoiled product after opening the package.

Consumer surveys indicate that on occasion temperatures in domestic refrigerators reach 15°C or above *(5, 7)*. Thus, it is possible that the studied scenario of improper storage (72 h at 15°C) could occur in the real world. Therefore, it may be important for the chicken industry to include some sort of time and temperature indicator in flow pack wrappers with instructions to discard the product before opening when the indicator signals expiration. This should be done to reduce the chance of cross-contamination of the food preparation environment and reduce the risk of consumer exposure to *Salmonella* and risk of an outbreak of salmonellosis from chicken sold in this packaging system.

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Mention of trade names or commercial products is solely for providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

REFERENCES

1. Anonymous. 2011. New performance standards for *Salmonella* and *Campylobacter* in young chicken and turkey slaughter establishments: response to comments and announcement of implementation schedule. *Fed. Regist*. 76:15282–15290.
2. Anonymous. 2013. Outbreak of *Salmonella* Heidelberg infections linked to a single poultry producer — 13 states, 2012–2013. *Morb. Mortal. Wkly. Rep*. 62:553–556.
3. Anonymous. 2014. Foodborne diseases active surveillance network (FoodNet): FoodNet surveillance report for 2012 (final report). U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta.
4. Berrang, M. E., N. A. Cox, D. E. Cosby, J. G. Frye, and C. R. Jackson. 2016. Detection of *Salmonella* serotypes by overnight incubation of entire broiler carcass. *J. Food Saf*. 37:e12298. doi:10.1111/jfs.12298.
5. Evans, E. W., and E. C. Redmond. 2016. Time-temperature profiling of United Kingdom consumers' domestic refrigerators. *J. Food Prot*. 79:2119–2127.
6. Hedican, E., B. Miller, B. Ziemer, P. LeMaster, S. Jawahir, F. Leano, and K. Smith. 2010. Salmonellosis outbreak due to chicken contact leading to a foodborne outbreak associated with infected delicatessen workers. *Foodborne Pathog. Dis*. 7:995–997.
7. James, S. J., J. Evans, and C. James. 2008. A review of the performance of domestic refrigerators. *J. Food Eng*. 87:2–10.
8. Oscar, T. P. 1998. The development of a risk assessment model for use in the poultry industry. *J. Food Saf*. 18:371–381.
9. Oscar, T. P. 2004. Dose-response model for 13 strains of *Salmonella*. *Risk Anal*. 24:41–49.
10. Oscar, T. P. 2004. A quantitative risk assessment model for *Salmonella* and whole chickens. *Int. J. Food Microbiol*. 93:231–247.
11. Oscar, T. P. 2006. Validation of a tertiary model for predicting variation of *Salmonella* Typhimurium DT104 (ATCC 700408) growth from a low initial density on ground chicken breast meat with a competitive microflora. *J. Food Prot*. 69:2048–2057.
12. Oscar, T. P. 2011. Plenary lecture: innovative modeling approaches applicable to risk assessments. *Food Microbiol*. 28:777–781.
13. Oscar, T. P. 2012. Food risk analysis, p. 175–187. *In* O. A. Oyarzabal and S. Backert (ed.), Microbial food safety. Springer, New York.
14. Oscar, T. P. 2012. Innovative modeling approaches for risk assessments in food safety, p. 389–422. *In* X. Yan, V. K. Juneja, P. M. Fratamico, and J. L. Smith (ed.), Omics, microbial modeling and technologies for foodborne pathogens. DEStech Publications, Inc., Lancaster, PA.
15. Oscar, T. P. 2013. Initial contamination of chicken parts with *Salmonella* at retail and cross-contamination of cooked chicken with *Salmonella* from raw chicken during meal preparation. *J. Food Prot*. 76:33–39.
16. Oscar, T. P. 2014. Use of enrichment real-time PCR to enumerate *Salmonella* on chicken parts. *J. Food Prot*. 77:1086–1092.
17. Oscar, T. P. 2016. Acquisition of data by whole sample enrichment, real-time polymerase chain reaction for development of a process risk model for *Salmonella* and chicken parts. *J. Nutr. Food Sci*. 6:538. doi:10.4172/2155-9600.1000538.
18. Oscar, T. P., G. K. Rutto, J. B. Ludwig, and S. Parveen. 2010. Qualitative map of *Salmonella* contamination on young chicken carcasses. *J. Food Prot*. 73:1596–1603.
19. Simmons, M., D. L. Fletcher, M. E. Berrang, and J. A. Cason. 2003. Comparison of sampling methods for the detection of *Salmonella* on whole broiler carcasses purchased from retail outlets. *J. Food Prot*. 66:1768–1770.
20. Simmons, M., D. L. Fletcher, J. A. Cason, and M. E. Berrang. 2003. Recovery of *Salmonella* from retail broilers by a whole-carcass enrichment procedure. *J. Food Prot*. 66:446–450.
21. Smith, K. E., C. Medus, S. D. Meyer, D. J. Boxrud, F. Leano, C. W. Hedberg, K. Elfering, C. Braymen, J. B. Bender, and R. N. Danila. 2008. Outbreaks of salmonellosis in Minnesota (1998 through 2006) associated with frozen, microwaveable, breaded, stuffed chicken products. *J. Food Prot*. 71:2153–2160.
22. Teunis, P. F., F. Kasuga, A. Fazil, I. D. Ogden, O. Rotariu, and N. J. Strachan. 2010. Dose-response modeling of *Salmonella* using outbreak data. *Int. J. Food Microbiol*. 144:243–249.