



Research Paper

Poultry Food Assess Risk Model for *Salmonella* and Chicken Gizzards: III. Dose Consumed Step

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ABSTRACT

The Dose Consumed step of the Poultry Food Assess Risk Model (PFARM) for *Salmonella* and chicken gizzards was presented and compared to the Exposure Assessment step of Quantitative Microbial Risk Assessment (QMRA). The specific objectives were 1) to demonstrate the dose consumed step of PFARM for *Salmonella* and chicken gizzards; 2) to compare *Salmonella* dose consumed from cooked chicken gizzards to that from cross-contaminated and temperature-abused lettuce; 3) to determine if *Salmonella* dose consumed changed over time in a production chain; and 4) to compare PFARM and QMRA predictions of *Salmonella* dose consumed. The PFARM and QMRA were developed in an Excel notebook and simulated with @Risk. *Salmonella* prevalence and number data ($P = 100$) for chicken gizzards (56 g) and scenario analysis were used to address objectives 1, 2, and 4, whereas running windows of 60 consecutive chicken gizzard samples and scenario analysis were used to address objective 3. A lot size of 1,000 kg of chicken gizzards was simulated. Mean portion size was 168 g resulting in the simulation of 5,952 meals per lot. Of these, $3.69 \pm 0.32\%$ and $0.49 \pm 0.07\%$ (mean \pm SD) resulted in *Salmonella* dose consumed of ≥ 1 per meal from cooked chicken gizzards and lettuce, respectively. However, the total *Salmonella* dose consumed per lot from cooked chicken gizzards (272 ± 27) was less ($P \leq 0.05$) than from lettuce ($6,050 \pm 4,929$) because of a few highly contaminated (> 310 *Salmonella*) lettuce portions at consumption. Over time in the production chain, *Salmonella* prevalence and total dose consumed per lot changed ($P \leq 0.05$) but the patterns differed. The QMRA predicted higher ($P \leq 0.05$) *Salmonella* dose consumed per meal than PFARM. In part, this was because QMRA only simulated contaminated grams, whereas PFARM simulated contaminated and non-contaminated meals. However, other factors, which are discussed, also contributed to the overestimation of *Salmonella* dose consumed by QMRA.

Quantitative Microbial Risk Assessment (QMRA) is a four-step process that has been used to assess the risk of salmonellosis from poultry food (FAO/WHO, 2002; Oh et al., 2023; Smadi & Sargeant, 2013; Zhu et al., 2017). The four steps are 1) hazard identification; 2) hazard characterization; 3) exposure assessment; and 4) risk characterization.

Like QMRA, the Poultry Food Assess Risk Model (PFARM) is a four-step process that has been used to assess the risk of salmonellosis from poultry food (Oscar, 1998, 2004a, 2016, 2017b, 2018b, 2019, 2020b). The four steps are 1) initial contamination; 2) illness dose; 3) dose consumed; and 4) consumer response. The susceptibility of consumers to *Salmonella* or consumer health and immunity in PFARM is simulated in the illness dose (Oscar, 2023c) and consumer response steps of PFARM.

In the present study, the dose consumed step of a PFARM for *Salmonella* and chicken gizzards was described, demonstrated, and compared to the exposure assessment step of QMRA. Previous studies in

this series demonstrate the initial contamination (Oscar, 2023b) and illness dose (Oscar, 2023c) steps of this PFARM. A future study in this series will demonstrate the consumer response step of this PFARM.

Two routes of consumer exposure to *Salmonella* from poultry food are recognized (Khalid et al., 2020; Luber, 2009). First, from under-cooked poultry food (Sampedro et al., 2018; Smadi & Sargeant, 2013). Second, from cross-contamination of ready-to-eat food during the preparation of raw poultry for cooking (Pouillot et al., 2012; Zhu et al., 2017). These two routes of consumer exposure to *Salmonella* were simulated in the current PFARM and QMRA with lettuce as the ready-to-eat food.

In the initial contamination step of this PFARM (Oscar, 2023b), the distribution of *Salmonella* among portions (56, 112, 168, 224, or 280 g) of chicken gizzards at the start of meal preparation was investigated and simulated and was found to change over time in the simulated production chain. In the dose consumed step of PFARM (this

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study), how the distribution of *Salmonella* among chicken gizzard and lettuce portions changed during meal preparation was simulated. Together, the initial contamination and dose consumed steps of PFARM are like the exposure assessment step of QMRA but also different. Here are thirteen differences.

First, in the exposure assessment step of QMRA, rinse, swab, and sponge methods are used to collect samples for determination of *Salmonella* prevalence and number (Bemrah et al., 2003; Pouillot et al., 2012). However, these methods do not recover all *Salmonella* from the poultry food (Lillard, 1988; Simmons et al., 2003). Therefore, in the initial contamination step of PFARM (Oscar, 2023b), whole sample enrichment was used to determine *Salmonella* prevalence and number because it can detect, enumerate, and isolate a single viable cell of *Salmonella* on or in the sample regardless of whether it is unattached, attached, or entrapped in the poultry food matrix (Oscar, 2014, 2017b).

Second, in the exposure assessment step of QMRA, a split sample method is used to obtain data for *Salmonella* prevalence and number (Bemrah et al., 2003; Jung et al., 2019). However, this method uses a different sample and a different size sample to determine *Salmonella* prevalence and number (Oscar, 2021). This results in confounded data for *Salmonella* prevalence and number that are not good for risk assessment. Therefore, in the initial contamination step of PFARM (Oscar, 2023b), a whole sample enrichment, quantitative polymerase chain reaction method is used to obtain non-confounded data for *Salmonella* prevalence and number by using the same sample and the same size of sample (Oscar, 2019, 2020b, 2023b).

Third, in the exposure assessment step of QMRA, *Salmonella* prevalence is not simulated throughout the production chain (Casulli et al., 2019; Jeong et al., 2018). However, in the initial contamination (Oscar, 2023b) and dose consumed (this study) steps of PFARM, *Salmonella* prevalence is simulated throughout the production chain using a rare event modeling method that was first published in 1998 (Oscar, 1998, 2004b). This method uses a DISCRETE distribution to simulate *Salmonella* serotype prevalence and a linked PERT distribution to simulate *Salmonella* number. Simulation of *Salmonella* prevalence throughout the production chain is important for model validation and accuracy.

Fourth, in the exposure assessment step of QMRA, *Salmonella* number is simulated per gram until consumption when it is multiplied by serving size (linear increase) and *Salmonella* prevalence to obtain *Salmonella* dose consumed (Casulli et al., 2019; Moller et al., 2015). This method is not used in PFARM because *Salmonella* prevalence and number increase in a nonlinear manner as a function of serving size (Oscar, 2019, 2020b, 2021). Instead, in the initial contamination and dose consumed steps of PFARM, *Salmonella* prevalence and number are expressed and simulated as a nonlinear function of the size of sample analyzed (Oscar, 2019, 2020b, 2021) to better estimate *Salmonella* dose consumed.

Fifth, in the exposure assessment step of QMRA, only grams contaminated with *Salmonella* are simulated (Pouillot et al., 2012; Stathas et al., 2024). Thus, the worst-case scenario like maximum temperature abuse or maximum undercooking always happens to a contaminated gram, which is not what occurs in nature, resulting in an overestimation of *Salmonella* dose consumed. Therefore, to better simulate what occurs in nature and to avoid overestimation of *Salmonella* dose consumed, both contaminated and noncontaminated servings are simulated together in the initial contamination and dose consumed steps of PFARM using the rare event modeling method (Oscar, 1998, 2004a).

Sixth, in the exposure assessment step of QMRA, only a fraction of the lot is simulated until consumption (Smadi & Sargeant, 2013; Stathas et al., 2024), which is not what occurs in nature. This prevents the prediction of *Salmonella* prevalence and number per serving at each step of the production chain, which is needed for model validation and accuracy. Therefore, to better simulate what occurs in nature

and to predict *Salmonella* prevalence and number per serving at each step in the production chain for model validation and accuracy, the same lot size is simulated throughout the production chain in the initial contamination and dose consumed steps of PFARM using a rare event modeling method (Oscar, 1998, 2004a).

Seventh, in the exposure assessment step of QMRA, *Salmonella* number is simulated as an integer and fraction (Smadi & Sargeant, 2013; Stathas et al., 2024), which is not what occurs in nature, and it overestimates *Salmonella* dose consumed because *Salmonella* number is always >0 per g. Thus, to better simulate what occurs in nature and to make better predictions of *Salmonella* dose consumed, servings, portions, and meals where the *Salmonella* number is zero are included and the *Salmonella* number is simulated as a whole number in the initial contamination and dose consumed steps of PFARM (Oscar, 2019, 2020b).

Eighth, predictive models for growth and inactivation of *Salmonella* in the exposure assessment step of QMRA are validated using conventional methods (Jia et al., 2020; Oh et al., 2023). However, there are 12 reasons why the PFARM, Acceptable Prediction Zones method should be used instead of conventional methods (bias factor, accuracy factor, root mean square error) when validating predictive models for growth and inactivation of *Salmonella* in poultry food (Oscar, 2020c). Thus, in the dose consumed step of PFARM, predictive models for growth (Oscar, 2020a) and thermal inactivation (Oscar, 2017a) of *Salmonella* were validated using the Acceptable Prediction Zones method.

Ninth, in the exposure assessment step of QMRA, thermal inactivation of *Salmonella* to elimination as a minority member of the native microflora of the poultry food during cooking and cooling is not simulated (Bemrah et al., 2003) resulting in all simulated grams having a *Salmonella* number >0 and an overestimation of *Salmonella* dose consumed. Thus, to better simulate what occurs in nature and to avoid an overestimation of *Salmonella* dose consumed, in the initial contamination and dose consumed steps of PFARM, the *Salmonella* number is simulated as a whole number and a line of death method is used to simulate thermal inactivation of *Salmonella* to elimination as a minority member of the native microflora of poultry food during cooking and cooling (Oscar, 2020b).

Tenth, in the exposure assessment step of QMRA, cross-contamination of ready-to-eat food with *Salmonella* as a minority member of the native microflora of poultry food is not simulated (Pouillot et al., 2012; Ravishankar et al., 2010) resulting in an overestimation of *Salmonella* dose consumed. Thus, to better simulate what occurs in nature and to avoid an overestimation of *Salmonella* dose consumed, a line of transfer method that simulates *Salmonella* as a minority member of the native microflora of poultry food during cross-contamination of ready-to-eat food during meal preparation is used in the dose consumed step of PFARM (this study).

Eleventh, in QMRA, a fixed number of iterations like 10,000 are simulated per replicate simulation of a scenario (Akil & Ahmad, 2019; Moller et al., 2015). However, when mean serving size differs among scenarios, results are confounded by differences in lot size. Therefore, in PFARM, the number of iterations simulated per replicate simulation of a scenario is based on lot size and mean serving size. Consider the following example. If the mean serving size is 100 g in Scenario A and 125 g in Scenario B, the lot size simulated in QMRA is 1,000 kg ($10,000 \times 0.1$ kg) for Scenario A and 1,250 kg ($10,000 \times 0.125$ kg) for Scenario B. In contrast, in PFARM, 10,000 iterations are conducted in Scenario A and 8,000 iterations are conducted in Scenario B so that lot size is 1,000 kg in both scenarios.

Twelfth, in QMRA, the probability of *Salmonella* in a one-gram sample is corrected for prevalence at consumption (Casulli et al., 2019; Smadi & Sargeant, 2013), whereas in PFARM, no such correction is needed because noncontaminated and contaminated servings are simulated together throughout the risk pathway. In QMRA, when a fixed number of iterations like 10,000 are simulated and a correction for prevalence or noncontaminated servings is made at consumption,

the comparison of results among scenarios is confounded by differences in lot size due to differences in mean serving size and prevalence. Continuing with the example, if the prevalence is 10% in Scenario A and 5% in Scenario B, the lot size simulated is 10,000 kg in Scenario A ($0.1 \text{ kg} * 10,000 * 10$) and 25,000 kg in Scenario B ($0.125 \text{ kg} * 10,000 * 20$).

Thirteenth, in QMRA, because only contaminated grams are simulated, the simulation will reach convergence after a fixed number of iterations like 10,000 and the coefficient of variation among replicate simulations will be small (Jeong et al., 2018; Stathas et al., 2024), whereas in PFARM (Oscar, 2019, 2020b), because both contaminated and noncontaminated servings are simulated and because a contaminated serving is a rare event, the simulation will not reach convergence after 10,000 iterations and the coefficient of variation among replicate simulations will be large. Thus, QMRA misses an important characteristic of the risk of foodborne illness: namely, that it is a rare and perfect storm event with high variability and uncertainty.

These differences between the initial contamination and dose consumed steps of PFARM and the exposure assessment step of QMRA are important because they result in different predictions of *Salmonella* dose consumed (this study) and risk and severity of salmonellosis (next study). Because the initial contamination and dose consumed steps of PFARM better simulate what occurs in nature and do not overestimate *Salmonella* dose consumed like QMRA, it is right to conclude that PFARM, although imperfect like all models, provides a better prediction of *Salmonella* dose consumed than QMRA. This conclusion will be supported by results of the current study, which had four objectives: 1) to demonstrate the dose consumed step of PFARM for *Salmonella* and chicken gizzards; 2) to compare *Salmonella* dose consumed from cooked chicken gizzards and cross-contaminated lettuce; 3) to determine if *Salmonella* dose consumed changed over time in a production chain; and 4) to compare PFARM and QMRA predictions of *Salmonella* dose consumed.

The reasons for using chicken gizzards in this study were discussed in a companion paper (Oscar, 2023b). In brief, chicken gizzards are an edible byproduct of chicken processing that are less often consumed but are often sold with whole chickens where they can be a source of cross-contamination during temperature abuse of whole chickens like those sold in flow-pack wrappers (Oscar, 2017b). Also, chicken gizzards, although sold with whole chickens, are not included in samples used to test whole chickens for *Salmonella* at the processing plant. Thus, they represent an unaccounted risk of salmonellosis for the consumer that needs to be assessed and managed.

Materials and methods

Poultry Food Assess Risk Model. The PFARM and QMRA for *Salmonella* and chicken gizzards were developed in an Excel notebook (Office 365, MicroSoft Corporation) and were simulated with @Risk (version 8.2, Decision Tools Suite, Palisade Corporation). The PFARM and QMRA had the following unit operations (pathogen events): 1) hatch-to-meal preparation (initial contamination); 2) preparation of chicken gizzards for cooking (cross-contamination of kitchen fomites); 3) preparation of ready-to-eat food (cross-contamination of lettuce); 4) holding of ready-to-eat food (growth on lettuce); 5) cooking and cooling of chicken gizzards (death and survival); 6) consumption of the meal (dose consumed from chicken gizzards and lettuce); and 7) post-consumption (consumer response).

In the present study, unit operations (pathogen events) 2–6, which comprised the dose consumed step, were simulated in spreadsheets (!) 2–6 of PFARM, which are described below. Figure 1 shows the dose consumed (DC) step of PFARM for *Salmonella* and chicken gizzards and how it flows from and to the other steps in the PFARM process.

Nomenclature. An exclamation mark (!) refers to a spreadsheet in the Excel notebook of PFARM. A subscript refers to an output from a

spreadsheet in PFARM. For example, N_2 would be the number of *Salmonella* that were transferred from chicken gizzards to kitchen fomites during the preparation of chicken gizzards for cooking, which was simulated in spreadsheet 2 (2!) of PFARM (see below). Also, dose consumed is synonymous with N_6 or the number of *Salmonella* at consumption, which was simulated in spreadsheet 6 (6!) of PFARM.

Native microflora. Data for native microflora (NM) of chicken gizzards in the simulated production chain were not collected or available. However, they were needed to simulate cross-contamination and thermal inactivation of *Salmonella* (see below). To fill this data gap, model inputs were based on published data for native microflora levels in chicken meat (Cox et al., 2010; Russell et al., 1997; Smith & Berrang, 2006).

The native microflora (log/g) at the start of meal preparation (NM_1) was simulated using a PERT distribution ($PERT_{NM}$) from @Risk (Palisade Corp.):

$$= (NM_{\min}, NM_{\text{mode}}, NM_{\max})$$

where NM_{\min} was the minimum level of native microflora, NM_{mode} was the most likely level of native microflora, and NM_{\max} was the maximum level of native microflora. Inputs for $PERT_{NM}$ were entered in the data input spreadsheet (D!) of PFARM (Fig. 2). They were 2.5, 4.5, and 7 log per g, where 7 log per g was assumed to be the minimum threshold for spoilage.

The $PERT_{NM}$ (one per potential serving in the portion) was randomly sampled by @Risk to determine NM_1 for each potential serving (56 g) in a portion (56, 112, 168, 224, or 280 g). However, only servings consumed were used to simulate the *Salmonella* dose consumed. For example, for a 168 g portion (3 servings) NM_1 on servings 4 and 5 were ignored in the calculations because they were not consumed.

Before simulation, the $PERT_{NM}$ was adjusted ($+1.75 = \log$ of 56) for the size of sample analyzed, which was 56 g. It was 4.25, 6.25, and 8.75 log per 56 g. In addition, the number of native microflorae on chicken gizzards before cooking ($NM_1 - NM_2$) was adjusted for the number of native microflorae transferred to kitchen fomites (NM_2).

Consumer survey. To simulate the dose consumed step of PFARM, data were needed for meal preparation practices like hygiene, meal preparation time, kitchen temperature, and cooked temperature. Consumer survey data were not collected. However, the survey tool was developed, linked to PFARM, and demonstrated using “What if” data (see below).

The survey tool was developed in spreadsheet S of this PFARM (Oscar, 2023c). Each query in the main survey body had five option buttons that corresponded to categories of risk from very low (1) to very high (5). DISCRETE distributions were used to simulate “What if” survey results:

$$= \text{RiskDiscrete}(\{1, 2, 3, 4, 5\}, \{5, 15, 60, 15, 5\})$$

where the first bracket was the risk categories, and the second bracket was their frequency of occurrence. For demonstration purposes, this DISCRETE distribution was used to simulate “What if” survey results for all meal preparation practices and time-periods. This was done to keep the demonstration simple and to obtain a non-confounded comparison of the relationship between initial *Salmonella* serotype prevalence, number, and zoonotic potential, and *Salmonella* dose consumed.

During the simulation of PFARM, the DISCRETE distributions for meal preparation practices were randomly sampled, and the selected risk category was used as the lookup value in the VLOOKUP function of Excel that returned a UNIFORM distribution from the array spreadsheet (A!) in PFARM (not shown). The UNIFORM distribution was randomly sampled by @Risk, and the output value was used as an input value in the appropriate predictive model in PFARM (see below). In this way, survey results were linked to and simulated in PFARM. The hybrid input distributions from this two-step process were NORMAL distributions because the distribution of “What if” survey results

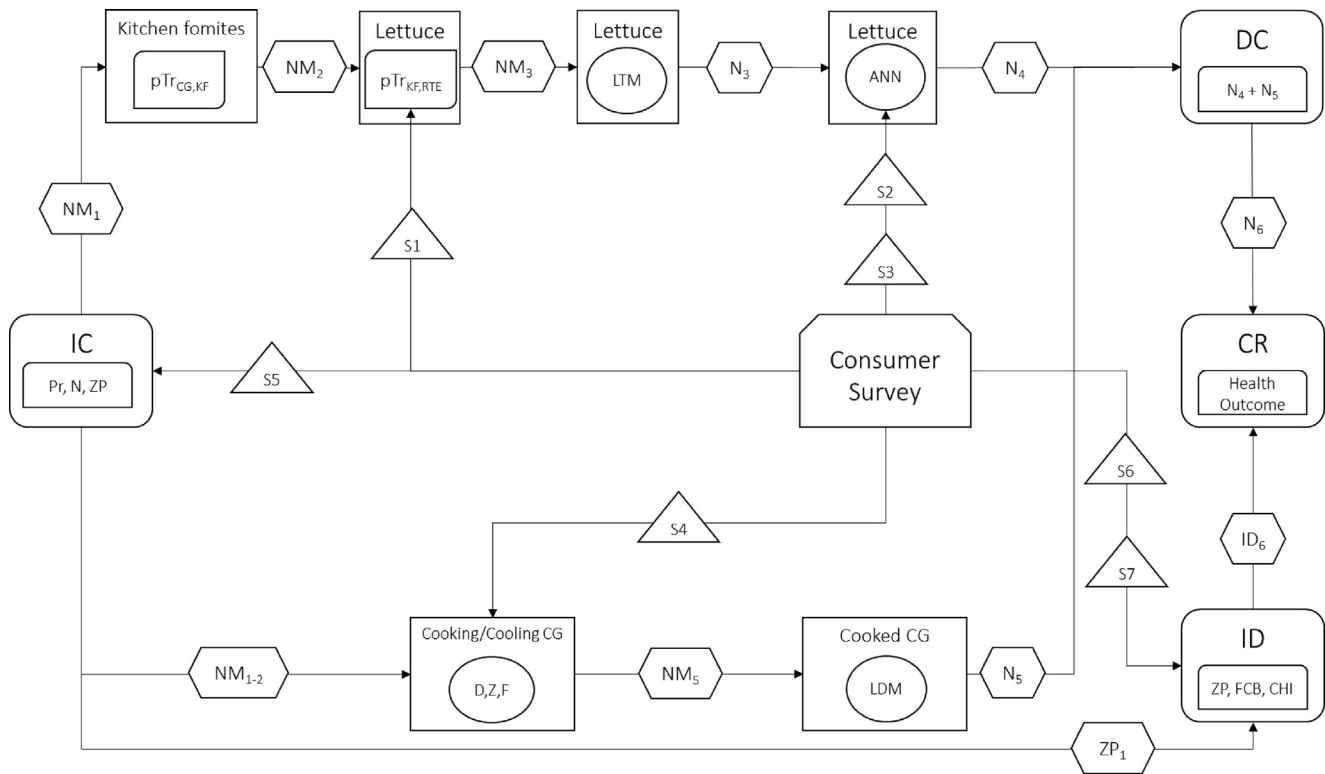


Figure 1. Diagram of the Dose Consumed (DC) step of the Poultry Food Assess Risk Model (PFARM) for Salmonella and chicken gizzards (CGs). Abbreviations: IC = initial contamination; Pr = prevalence; N = number; ZP = zoonotic potential; NM = native microflora; pTr = proportion of transfer; KF = kitchen fomite; RTE = ready-to-eat food (lettuce); LTM = line of transfer method; ANN = artificial neural network; S1 = consumer survey result for hygiene; S2 = consumer survey result for meal preparation time; S3 = consumer survey result for kitchen temperature; S4 = consumer survey result for final cooked temperature; S5 = consumer survey result for portion size; S6 = consumer survey result for food consumption behavior (FCB); S7 = consumer survey result for consumer health and immunity (CHI), D = D-value, Z = Z-value, F = F-value; LDM = line of death method; ID = illness dose; and CR = consumer response. See text for further details.

Data Input					Time (weeks)															
Sample size	56	g			Salmonella															
Mean Portion size	168	g			1 to 10	1 to 6	2 to 7	3 to 8	4 to 9	5 to 10										
Lot size	1,000	kg			Minimum	Minimum	Minimum	Minimum	Minimum	Minimum	Mode	Mode	Mode	Mode	Mode	Mode				
Portions	5,952	iterations			Maximum	Maximum	Maximum	Maximum	Maximum	Maximum	Maximum	Maximum	Maximum	Maximum	Maximum	Maximum				
Salmonella Testing	Minimum	Mode	Maximum	Unit																
Native Microflora	2.500	4.500	7.000	log/g																
Salmonella	0.000	0.417	2.788	log/sample																
Code	0.0	1.0	2.0	3	4	5														
Serotype	None	Kentucky	Infantis	Enteritidis	Typhimurium	Thompson														
Prevalence	65.0	16.0	9.0	6.0	3.0	1.0														
Zoonotic Potential	0.0	1.0	4.5	5.0	4.9	3.4														
Consumer Survey	61	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
Hygiene	3	1	4	3	3	3	3	4	3	4	3	3	3	3	4					
Meal preparation time	3	2	2	4	4	2	3	3	3	3	2	5	4	4	4					
Kitchen temperature	3	2	3	3	3	2	3	2	3	3	3	3	3	3	3					
Cooked Temperature	2	4	3	4	3	3	4	3	4	3	3	2	3	3	3					
Portion size	1	3	3	5	3	3	3	3	3	4	4	3	3	3	2					
Food Consumption Behavior	4	4	4	3	1	3	1	3	3	3	2	4	3	2	3					
Consumer Health & Immunity	3	3	3	2	4	2	5	3	2	2	4	3	3	2	2					

Figure 2. The data input spreadsheet (D!) in the Poultry Food Assess Risk Model (PFARM) for Salmonella and chicken gizzards. Data for native microflora (NM) and Salmonella prevalence (Pr), number (N), and serotype/zoonotic potential (ZP) and “What if” consumer survey data for hygiene, meal preparation time, kitchen temperature, cooked temperature, portion size, food consumption behavior, and consumer health and immunity were entered in D!. In addition, the size of sample analyzed (56 g) and lot size (1,000 kg), and overall (weeks 1 to 10) and time-period (running windows of 60 samples or six weeks) data for Salmonella serotype prevalence and number were entered in spreadsheet D!. See text for additional details.

among the five risk categories followed a NORMAL distribution, as shown above.

Cross-contamination of kitchen fomites. The chicken gizzards were sold in plastic-wrapped trays. At the start of meal preparation, the package was opened, and two chicken gizzards were combined to form a serving. This resulted in the transfer of native microflora from chicken gizzards to hands, the cutting board, and other surfaces or kitchen fomites (KFs).

This step was simulated in 2! of PFARM (Fig. 3). Here, the proportion of native microflora transferred from chicken gizzards (CGs) to

kitchen fomites ($pTr_{CG,KF}$) was simulated with a UNIFORM distribution from @Risk:

$$= RiskUniform(min, max)$$

where min was the minimum transfer rate ($pTr_{CG,KF}$), which was 0.005, and max was the maximum transfer rate ($pTr_{CG,KF}$), which was 0.02 (Pouillot et al., 2012; Ravishankar et al., 2010). This UNIFORM distribution was randomly sampled by @Risk to determine the transfer rate ($pTr_{CG,KF}$) for the simulated serving of chicken gizzards. Thus, native


Contamination from Chicken Gizzards to Kitchen Fomites			
NM	Unit		
4.58	log number		
Serving 1			
Native Microflora (NM)		<i>Salmonella</i>	
NM ₁	NM ₂	N ₁	N ₂
2,145,416	26,818	5	1
Transfer rate →	0.013		
	<i>Salmonella</i>	Transferred (0 = no; 1 = yes)	Rand
	1	0	409,521
	2	1	24,581
	3	0	755,171
	4	0	1,380,348
	5	0	801,615
	6		501,891

Figure 3. Spreadsheet 2 (2!) in the Poultry Food Assess Risk Model (PFARM) for *Salmonella* and chicken gizzards simulated cross-contamination of kitchen fomites (hands, cutting board, and other) with native microflora (NM) and *Salmonella* (N) from chicken gizzards. Results for a single serving and iteration are shown. See text for additional information.

microflora transferred from chicken gizzards to kitchen fomites (NM₂) were:

$$= NM_1 * pTr_{CG,KF}$$

No denominator was defined because no assumptions were made as to the distribution of native microflora on kitchen fomites. Rather, in the next step, the native microflora transferred to lettuce resulted from contact between kitchen fomites and lettuce without assumptions about the size of the area of contact or time of contact as multiple combinations of these factors could result in the same amount of bacterial transfer.

Salmonella was a minority member of the native microflora of chicken gizzards at the start of meal preparation. Thus, transfer to kitchen fomites only occurred if by random chance *Salmonella* were located on a contact surface between chicken gizzards and kitchen fomites. To simulate this scenario, the position of *Salmonella* in the native microflora during the preparation of chicken gizzards for cooking or in the line of transfer was randomly assigned using the RAND function of Excel and then, if by random chance *Salmonella* was in the population of native microflora transferred (i.e., its' position in the line of transfer was ≤ the position of the last native microorganism transferred) it was transferred to kitchen fomites (N₂); otherwise, it was not (Fig. 3).

Cross-contamination of lettuce. Transfer of native microflora and *Salmonella* from kitchen fomites to lettuce was assumed to depend on consumer hygiene during meal preparation as assessed by the consumer survey (S1 in Fig. 1). Determination of hygiene involved three steps (Oscar, 2023c). First, two questions with pull-down menus were used to query about consumer hygiene practices for the cutting board and hands. Second, five true or false questions with check boxes were used to query about hygiene practices including one for other surfaces. Third, a hygiene score was calculated:

$$= ROUND(AVERAGE(P3, P4, SUM(P6 : P10)), 1)$$

where P3 was the risk category (1, 2, 3, 4, or 5) for the cutting board question, P4 was the risk category for the hands' question, and P6 to P10 were answers to the true or false questions for consumer hygiene practices where 0 = true and 1 = false. The ROUND function of Excel was used to round the result to one decimal place. Capital letters fol-

lowed by numbers in the formula were cell addresses in the survey spreadsheet (S!) of PFARM (Oscar, 2023c).

A DISCRETE distribution from @Risk (see above) was used to simulate a "What if" scenario for hygiene (DISCRETE_{S1}) in PFARM. During simulation of PFARM, DISCRETE_{S1} was randomly sampled by @Risk and the risk category for hygiene was used as the lookup value in the VLOOKUP function of Excel to identify the appropriate UNIFORM distribution in spreadsheet A! of PFARM for the proportion of native microflora transferred from kitchen fomites to ready-to-eat (RTE) food (lettuce; pTr_{KF,RTE}) where category 1 = 0.00 to 0.00; category 2 = 0.00 to 0.005; category 3 = 0.005 to 0.02; category 4 = 0.02 to 0.2; and category 5 = 0.2 to 0.4 (Pouillot et al., 2012; Ravishankar et al., 2010). Cross-contamination of ready-to-eat food (lettuce) with native microflora from kitchen fomites during meal preparation (NM₃) was:

$= NM_2 * pTr_{KF,RTE}$ where NM₂ was native microflora of chicken gizzards that cross-contaminated kitchen fomites (cutting board, hands, other surfaces) during meal preparation and pTr_{KF,RTE} was the proportion of native microflora transferred from kitchen fomites to ready-to-eat food (lettuce). Transfer of *Salmonella* from kitchen fomites to ready-to-eat food (lettuce; N₃) was simulated using the line of transfer method as described above for transfer of *Salmonella* from chicken gizzards to kitchen fomites. Cross-contamination from kitchen fomites to ready-to-eat food (lettuce) was simulated in spreadsheet 3! of PFARM (Fig. 4).

Growth on lettuce. Growth of *Salmonella* on ready-to-eat food (lettuce) after cross-contamination was simulated in spreadsheet 4! of PFARM (Fig. 5) and depended on meal preparation time (S2) and kitchen temperature (S3) from the consumer survey (Oscar, 2023c). DISCRETE distributions (see above) of "What if" scenarios for meal preparation time (DISCRETE_{S2}) and kitchen temperature (DISCRETE_{S3}) simulated the frequency of occurrence of risk categories (1–5) for these variables.

During the simulation of PFARM, DISCRETE_{S2} for meal preparation time and DISCRETE_{S3} for kitchen temperature were randomly sampled, and the selected risk categories were used in the VLOOKUP function of Excel to return the appropriate UNIFORM distribution from spreadsheet A! in PFARM. For meal preparation time, they were: 1 = 0 to 1 h; 2 = 1 to 2 h; 3 = 2 to 4 h; 4 = 4 to 6 h; and 5 = 6


Cross-contamination from Kitchen Fomites to Lettuce			
3	0.005	0.020	proportion
Serving 1			
Native Microflora		Salmonella	
NM ₂	NM ₃	N ₂	N ₃
26,818	335	1	0
Transfer rate →	0.013		
	<i>Salm</i>	Transferred (0 = no; 1 = yes)	Rand
 PFARM 3!	1	0	16,508
	2		1,031
	3		20,875
	4		10,354
	5		381
	6		20,243

Figure 4. Spreadsheet 3 (3!) in the Poultry Food Assess Risk Model (PFARM) for Salmonella and chicken gizzards simulated cross-contamination of ready-to-eat food (lettuce) with native microflora (NM) including Salmonella (N) from kitchen fomites (hands, cutting board, and other). Results for a single serving and iteration are shown. See text for additional information.


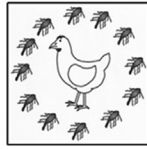
Growth on Lettuce after Cross Contamination				 PFARM 4!	Temperature Abuse	
Serving 1					Before	After
Input	Unit	Range	Value	Serving	N ₃	N ₄
Temperature	°C	16 to 40	20.7	1	1	3
Time	h	0 to 8		2	0	0
0.00	h	1.02		3	0	0
7.75	h	1.58		4		
	log Δ →	0.56	 PFARM 4!	5		
	antilog Δ →	3.63		Portion	1	3
Text String						
20.70						
20.77.75						
Meal preparation time	Risk category	5				
	Minimum	6.00	3			
	Maximum	8.00	h			
Kitchen temperature	Risk category	2				
	Minimum	20				
	Maximum	25	C			

Figure 5. Spreadsheet 4 (4!) in the Poultry Food Assess Risk Model (PFARM) for Salmonella and chicken gizzards simulated the growth of Salmonella on lettuce after cross-contamination from kitchen fomites (hands, cutting board, and other). Results are for a single serving and iteration. See text for additional information.

to 8 h, whereas for kitchen temperature, they were: 1 = 16 to 20°C; 2 = 20 to 25°C; 3 = 25 to 30°C; 4 = 30 to 34°C; and 5 = 34 to 40°C.

An artificial neural network model for *Salmonella* Newport and Romaine lettuce was used to predict growth as a function of meal preparation time (0–8 h) and kitchen temperature (16–40°C) (Oscar, 2020a). The most probable number of data used to develop the artificial neural network were collected under dynamic conditions of time and temperature that simulated the warming of the lettuce from refrigerated to kitchen temperatures. The lower limit of enumeration of the automated, miniature, most probable number assay used to collect the growth kinetic data for development and validation of the artificial neural network was 0 log per size of sample (0.2 g) used in the storage trials (Oscar, 2018a, 2020a). The MPN data, which were free of values below or above the detection and enumeration range of the auto-

mated, miniature, most probable number assay, were used to train and test the artificial neural network using Excel and NeuralTools (version 8.2, Palisade Corp.), which is an Excel add-in program. The artificial neural network was validated for goodness-of-fit and interpolation using the test data, model performance, and model validation criteria of the Acceptable Prediction Zones method (Oscar, 2005a, 2005b, 2020c, 2023a).

The number of *Salmonella* on a lettuce serving at consumption (N₄) was:

$$= N_3 * 10^{\Delta}$$

where N₃ was the number of *Salmonella* transferred to a lettuce serving from kitchen fomites and 10^Δ was the antilog of the predicted log increase of *Salmonella* number per serving during growth on lettuce between cross-contamination and consumption, which was the meal

preparation time. It was assumed that the serving of lettuce was associated with the serving of chicken gizzards that was the source of the *Salmonella*.

The CONCATENATE function of Excel and the randomly selected values for meal preparation time and kitchen temperature were used to create text strings (Fig. 5) that were used as lookup values in the VLOOKUP function of Excel to return values for predicted log number of *Salmonella* from an array in the spreadsheet A! of PFARM for zero time and the randomly selected meal preparation time, and kitchen temperature. The array (7,956 rows × four columns; not shown) was based on predictions of the artificial neural network for growth of *Salmonella* Newport on Romaine lettuce (Oscar, 2020a). The inclusion of the artificial neural network, which was developed with compatible software (i.e., NeuralTools, Palisade Corp.) in the PFARM would have slowed the simulation speed of @Risk because it would have limited CPU simulations in @Risk to 1 instead of 10. Thus, an array of predictions from the artificial neural network was used instead.

Cooking and cooling. Thermal inactivation of *Salmonella* during cooking and cooling of poultry food is a well-studied process (Jarvis et al., 2016). The conventional method used is to inoculate a poultry food with a high initial number of *Salmonella* and then heat the poultry food at constant temperatures to obtain D-values and then a Z-value (Juneja et al., 2012; Murphy et al., 2002). These values are used to calculate the cumulative process lethality value (F) during cooking and cooling, which is then used to calculate the cumulative log reduction (F/D) for the process. This approach was used in the present PFARM with one addition. Namely, the inclusion of a thermal line of death method (Oscar, 2020b) to simulate the random death and survival of *Salmonella* as a minority member of the native microflora of chicken gizzards.

The thermal inactivation of native microflora including *Salmonella* on and in chicken gizzards was simulated in spreadsheet 5! of PFARM (Fig. 6). Here, the chicken gizzards were cooked and cooled under a single scenario of cooking and cooling where cooking occurred at 350°F (177°C) in a conventional domestic oven and cooling occurred at room temperature 68°F (20°C). When the interior cold spot of the chicken gizzards reached a final cooked temperature of 170°F (77°

C), they were removed from the oven and cooled to <120°F (49°F) before serving and consumption. Collecting temperature profile data from the cold spot during cooking and cooling and using it to simulate thermal inactivation likely underestimates the log reduction of native microflora including *Salmonella* because most native microflora including *Salmonella* are likely on the surface of the chicken gizzards (McMeekin & Thomas, 1979; Thomas & McMeekin, 1981).

The temperature profile for this scenario is shown in Figure 7A. The temperature profile was based on eight replications of a cooking and cooling experiment. The chicken gizzard servings used in these experiments weighed 62 ± 4 g (mean ± standard deviation; SD) and had an initial temperature of 46 ± 2°F (mean ± SD; 7.8 ± 0.3°C). The kitchen temperature during cooling was 68°F (20°C) for all replicate experiments.

The temperature profile was used to calculate the cumulative process lethality (F, min) during cooking and cooling of the chicken gizzards for recorded cooking and cooling temperatures from 109 to 174°F (43–79°C):

$$F = \int_0^t 10^{(T(t)-(T_{ref})/z)} dt$$

where T(t) was temperature (°F) at time t, T_{ref} was 145°F (62.8°C), and Z was °F needed to change D (time for a 1-log reduction) by a factor of 10. A Z-value of 13.55°F (7.6°C) and a D-value of 2.94 min for thermal inactivation of *Salmonella* on chicken patties were used to calculate F (Murphy et al., 2002) because Z- and D-values for native microflora of chicken gizzards were not available.

The log change of native microflora per g (Δ) of chicken gizzards during cooking and cooling was calculated as F/D. The Δ were log-transformed and graphed as a function of temperature (Fig. 7B) and then fitted to a linear model (version 9.3, Prism, GraphPad Software, Inc.).

The linear model and interpolation function of Prism were used to establish the five UNIFORM distributions for Δ (see below) that were used to simulate the thermal inactivation of native microflora as a function of the five risk categories for cooked temperature from the consumer survey (S4) (Oscar, 2023c). These data-driven approach for simulating thermal inactivation provided an objective but imper-

Cooking (Death, Survival, NM, <i>Salmonella</i>)				
Category	min	max	Log reduction	
3	2.06	3.58	-2.82	
Serving 1				
Native Microflora			<i>Salmonella</i>	
NM ₁ - NM ₂	NM ₅	Last Microbe to Die	N ₁ - N ₂	N ₅
2,118,598	3,195	2,115,403	5	0
	<i>Salmonella</i>	Survivor (0 = no; 1 = yes)	Rand	
	1	0	365,781	
	2	0	1,753,078	
	3	0	1,515,288	
	4	0	155,405	
	5	0	1,672,950	
	6		1,920,823	
	7		1,366,116	



PFARM 5!

Figure 6. Spreadsheet 5 (5!) in the Poultry Food Assess Risk Model (PFARM) for *Salmonella* and chicken gizzards simulated death and survival of native microflora (NM) including *Salmonella* (N) on chicken gizzards during cooking and cooling. Results are for a single serving and iteration. See text for additional information.

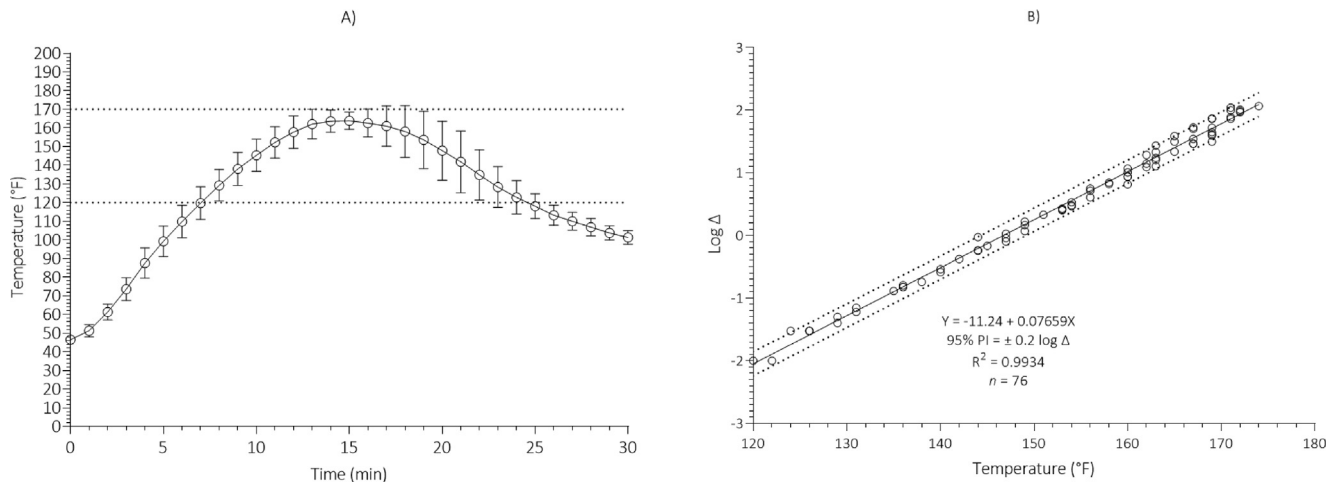


Figure 7. A) Temperature profile of chicken gizzards during cooking and cooling in a conventional oven set at 350°F. Symbols and error bars are means and standard deviations, respectively, of eight replicate experiments. Horizontal dashed lines indicate the target final cooked temperature (170°F) and the target final cooling temperature (120°F). B) Linear regression model for predicting the log of the log reduction (Δ) of native microflora on chicken gizzards during cooking and cooling in a conventional oven set at 350°F. Temperature (X-axis) is the final cooked temperature. Abbreviations: PI = prediction interval; and R^2 = coefficient of determination.

fect assessment of thermal inactivation because it did not consider other cooking methods and cooking and cooling scenarios that may have different thermal inactivation properties. Nonetheless, the relative comparisons among scenarios in the present study were credible.

During simulation of the current PFARM, DISCRETE_{S4} for cooked temperature was randomly sampled by @Risk and the selected risk category (1, 2, 3, 4, or 5) was used as the lookup value in the VLOOKUP function of Excel that returned a UNIFORM distribution for Δ (log/g) from spreadsheet A! in PFARM where 1 = 6.5 to 96; 2 = 1.1 to 16.4; 3 = 0.2 to 2.8; 4 = 0.03 to 0.48; and 5 = 0.01 to 0.08.

Variability and uncertainty of the cooking temperature profile were simulated in the UNIFORM distributions for Δ using the 95% prediction interval of $\pm 0.2 \log \Delta$ from the linear model (Fig. 7B). For example, for risk category 1 with a minimum cooked temperature of $>160^\circ\text{F}$, the most likely $\log \Delta$ was $-11.24 + (0.07659 \times 160)$, which was 1.01. Next, the minimum and maximum $\log \Delta$ at 160°F were $1.01 - 0.2$ or 0.81 and $1.01 + 0.2$ or 1.21, respectively. The antilogs of these values were taken to obtain Δ values of 6.52, 10.34, and 16.38 log reductions per gram, which were used to define a PERT distribution for the minimum value in the UNIFORM distribution for risk category 1. These calculations were repeated with a cooked temperature of 170°F to obtain a PERT distribution (38.05, 60.30, 95.57) for the maximum value of the UNIFORM distribution of Δ for risk category 1. Thus, the UNIFORM distribution for risk category 1 was:

$$\text{Risk category 1 (160, } 170^\circ\text{F)} = \text{UNIFORM}(\min_{\text{PERT}(6.52, 10.34, 16.38)}, \max_{\text{PERT}(38.05, 60.30, 95.57)})$$

with a range from 6.5 to 96 as reported above. These calculations were repeated for the other four risk categories to obtain the following UNIFORM distributions of Δ for simulating thermal inactivation:

$$\begin{aligned} \text{risk category 2 (150, } 160^\circ\text{F)} &= \text{UNIFORM}(\min_{\text{PERT}(1.12, 1.77, 2.81)}, \max_{\text{PERT}(6.52, 10.34, 16.38)}) \\ \text{risk category 3 (140, } 150^\circ\text{F)} &= \text{UNIFORM}(\min_{\text{PERT}(0.19, 0.3, 0.48)}, \max_{\text{PERT}(1.12, 1.77, 2.81)}) \\ \text{risk category 4 (130, } 140^\circ\text{F)} &= \text{UNIFORM}(\min_{\text{PERT}(0.03, 0.05, 0.08)}, \max_{\text{PERT}(0.19, 0.3, 0.48)}) \\ \text{risk category 5 (120, } 130^\circ\text{F)} &= \text{UNIFORM}(\min_{0.01}, \max_{\text{PERT}(0.03, 0.05, 0.08)}) \end{aligned}$$

Before @Risk randomly sampled the selected UNIFORM distribution for Δ , it was adjusted ($+1.75 = \log$ of 56) for the size of sample

analyzed, which was 56 g. The randomly selected $\Delta_{56\text{g}}$ from this UNIFORM distribution was then used to determine the number of native microflora that survived the cooking and cooling (NM_5) of the serving:

$$= \text{NM}_3 * 10_{56\text{g}}^{-\Delta}$$

where NM_3 ($\text{NM}_1 - \text{NM}_2$) was the number of native microflora on the chicken gizzards serving before cooking (after adjustment for cross-contamination of kitchen fomites), and $10_{56\text{g}}^{-\Delta}$ was the antilog of the negative log change per 56 g during cooking and cooling.

The ROUNDOWN function of Excel was used to convert integers to whole numbers because it was not possible to have a fraction of a *Salmonella*. Thus, when the number of native microflora on a serving of chicken gizzards after cooking (NM_5) was >0 but <1 , the result was 0 and the serving had no surviving native microflora or *Salmonella*. In other words, it was properly cooked. On the other hand, a serving or portion (1–5 servings) was undercooked when the number of native microflora was ≥ 1 after cooking and cooling.

Salmonella was simulated as a minority member of native microflora during cooking and cooling using the thermal line of death method of Oscar (2020b). To do this, the RAND function of Excel was used to randomly assign *Salmonella* to positions in the thermal line of death (Fig. 6) and if the position was greater than the last native microorganism killed, *Salmonella* survived (N_5); otherwise, it died. No assumptions were made about where *Salmonella* was in the chicken gizzard matrix. However, it is logical to assume that *Salmonella* near the front of the thermal line of death were on the surface of the chicken gizzard near the heat source, whereas those located near the end of the thermal line of death were close to or in the cold spot and away from the heat source.

Dose consumed. Changes in native microflora and *Salmonella* number from the start of meal preparation to consumption were tracked in spreadsheet 6! of PFARM (Fig. 8). This figure shows a dimension of PFARM not previously demonstrated. Namely, PFARM simulates changes in native microflora and *Salmonella* number of five potential servings that could comprise a portion or meal. For clarity of presentation, results for only one serving in a portion were shown in previous Figures. Note that PFARM simulates values of 0 and makes no attempt to log transform them, which would result in errors by @Risk.

During the simulation of PFARM, DISCRETE_{S5} for portion size was randomly sampled by @Risk and the selected portion size was used to

PFARM 6!	Consumption (Exposure)						
		Salmonella	None	None	Kentucky		
		Portion	Serving 1	Serving 2	Serving 3	Serving 4	Serving 5
Raw Gizzard before Preparation	N ₁	19	0	0	19		
Raw Gizzard after Preparation	N ₂	19	0	0	19		
Kitchen Fomites after Preparation	N ₂	0	0	0	0		
Lettuce after Preparation	N ₃	0	0	0	0		
Lettuce at Consumption	N ₄	0	0	0	0		
Cooked Gizzard at Consumption	N ₅	1	0	0	1		
		NM					
Exposure		Portion	Serving 1	Serving 2	Serving 3	Serving 4	Serving 5
Raw Gizzard before Preparation	NM ₁	15,192,072	3,013,976	2,405,312	9,772,784		
Raw Gizzard after Preparation	NM ₂	15,007,650	2,995,388	2,387,169	9,625,093		
Kitchen Fomites after Preparation	NM ₂	181,794	18,401	17,954	145,439		
Lettuce after Preparation	NM ₃	2,628	187	189	2,252		
Cooked Gizzard at Consumption	NM ₅	185,303	24,121	11,352	149,830		

Figure 8. Spreadsheet 6 (6!) in the Poultry Food Assess Risk Model (PFARM) for Salmonella and chicken gizzards tracked changes in native microflora (NM) including Salmonella (N) on the servings in a simulated portion of chicken gizzards and lettuce during meal preparation. Results are for a single portion and iteration. See text for additional information.

determine which servings were used to calculate the change in native microflora and *Salmonella* number from the start of meal preparation to consumption for the simulated meal or consumer.

The *Salmonella* dose consumed or N₆ per meal or consumer was:

= N₄ + N₅ where N₄ was the sum of *Salmonella* number among servings of lettuce at consumption, and N₅ was the sum of *Salmonella* number among servings of cooked chicken gizzards at consumption.

Quantitative Microbial Risk Assessment. Because PFARM and QMRA are similar, it was easy to create a QMRA for *Salmonella* and chicken gizzards in PFARM that simulated the same risk pathway and data (Fig. 9). This was done to compare the rare event modeling method of PFARM to the probabilistic modeling method of QMRA for *Salmonella* dose consumed.

The QMRA simulates dose consumed by expressing *Salmonella* number per g until consumption when it is multiplied by serving size (56, 112, 168, 224, or 280 g), and then *Salmonella* prevalence per 56 g to determine *Salmonella* dose consumed (N₆ per meal or consumer). Unlike PFARM, QMRA does not simulate *Salmonella* serotype prevalence and zoonotic potential.

Scenarios 01 to 04. Data (n = 100 for weeks 1–10 in Fig. 2) for *Salmonella* number, and serotype prevalence and zoonotic potential, of chicken gizzards (Oscar, 2023b) were used to address objectives 1, 2, and 4. They were entered in spreadsheet D (Fig. 2) of PFARM and simulated with PFARM and QMRA using @Risk settings of Latin Hypercube sampling, Mersenne Twister generator, 5,952 iterations,

and initial seeds of 1, 2, 3, or 4. The lot size was 1,000 kg of chicken gizzards. Mean portion size was 168 g. Thus, 5,952 meals or consumers were simulated.

Scenarios 1 to 5. This scenario analysis addressed objective 3. Here, data (n = 100) for *Salmonella* serotype prevalence and zoonotic potential, and number (Oscar, 2023b) were simulated using a moving window of 60 consecutive samples of chicken gizzards (Fig. 2). This resulted in five time-period scenarios: 1) weeks 1–6; 2) weeks 2–7; 3) weeks 3–8; 4) weeks 4–9; and 5) weeks 5–10. They were simulated in PFARM with the same @Risk settings as those used for scenarios 01–04 except that 16 additional and randomly selected initial seeds were used to initiate each replication simulation for a total of 20 replicate simulations per time-period scenario. The number of replicate simulations was a function of simulation time (20 min) and convergence of the statistical analysis.

Statistical analysis. Simulation results for scenarios 1–5 (objective 3) were analyzed by one-way, analysis of variance to determine if *Salmonella* prevalence per 5,952 meals and total *Salmonella* dose consumed per lot (1,000 kg of chicken gizzards) changed over time in the production chain. When one-way, analysis of variance was significant (P ≤ 0.05), the means of 20 replicate simulations were compared using Tukey’s multiple comparison test at P ≤ 0.05. On the other hand, simulation results for scenarios 01–04 (objective 4) were compared within a replicate simulation using a nonparametric test (Mann-Whitney) to determine if the mean rank of *Salmonella* dose consumed

QMRA Model for <i>Salmonella</i> and Chicken Gizzards					
Unit Operation	Pathogen Event	Node	Result	Unit	FORMULA
Hatch to Meal Preparation	Initial Contamination of Chicken Gizzards	N1	2.62E-01	P _{N1} /g	=POWER(10,'1'!C4)/56
Opening Package and Partitioning	Cross-contamination of Kitchen Fomites	N2	3.23E-03	P _{N2} /g	=D3*2!B8
Preparation of Salad	Cross-contamination of Lettuce	N3	2.33E-05	P _{N3} /g	=D4*3!B7
Holding	Growth on Lettuce	N4	2.32E-05	P _{N4} /g	=D5*POWER(10,'4'!C88)
After Partitioning	Contamination of Chicken Gizzards before Cooking	N1 - N2	2.59E-01	P _{N1 - N2} /g	=(POWER(10,'1'!C4)/56)-D4
Cooking and Cooling	Death and Survival on Chicken Gizzards	N5	1.85E-05	P _{N5} /g	=D7*POWER(10,'5'!E3)
Consumption	Dose Consumed	N6	2.18E-03	P _{N6} /serving	=(D6+D8)*('1'!A10*56)*((100-D!B11)/100)
After Consumption	Consumer Response	CR	5.60E-06	P _{CR} /serving	=1-(1+(D9/51.45)^-0.1324)

Figure 9. Spreadsheet QMRA (QMRA!) in the Poultry Food Assess Risk Model (PFARM) for Salmonella and chicken gizzards contained a Quantitative Microbial Risk Assessment (QMRA) model that simulated the probability of Salmonella in one gram of chicken gizzards and lettuce until consumption when it simulated probability of Salmonella in a meal of chicken gizzards and lettuce. Results are for a single iteration of a gram and then a portion of chicken gizzards and lettuce. See text for additional information.

per meal differed ($P \leq 0.05$) between PFARM and QMRA. All statistical analyses were performed in Prism (version 9.5.1, GraphPad Software, Inc.).

Results

Objective 1. This objective was to demonstrate the dose consumed step of the PFARM for *Salmonella* and chicken gizzards. This was done by providing examples of individual iterations for the events simulated in spreadsheets 2! to 6! of PFARM.

Spreadsheet 2! simulated transfer of native microflora including *Salmonella* from chicken gizzards to kitchen fomites (Fig. 3). In this example, there were 2,145,416 cells of native microflora on the simulated chicken gizzard serving. Of these, 5 were *Salmonella*. The bacterial transfer rate from chicken gizzards to kitchen fomites was 0.013. Thus, 26,818 cells of native microflora were transferred from chicken gizzards to kitchen fomites. However, by random chance only 1 of the 5 *Salmonella* cells was transferred from chicken gizzards to kitchen fomites because its location in the line of transfer (i.e., 24,581) was $< 26,818$, which was the last microbe transferred.

Spreadsheet 3! simulated transfer of native microflora including *Salmonella* from kitchen fomites to lettuce (Fig. 4). In this example, the hygiene category from the consumer survey for the simulated chicken gizzard serving was 3, which corresponded to a transfer rate from 0.005 to 0.02. The randomly selected transfer rate was 0.013. Thus, of the 26,818 cells of native microflora on kitchen fomites, 335 were transferred to lettuce. By random chance, the *Salmonella* cell on the kitchen fomite was not transferred to lettuce because its location in the line of transfer (i.e., 16,508) was > 335 , which was the last microbe transferred.

Spreadsheet 4! simulated growth of *Salmonella* on lettuce after cross-contamination from kitchen fomites (Fig. 5). In this example, the portion size from the consumer survey was three servings or 168 g. However, only serving #1 was contaminated with *Salmonella* and at a level of just 1 cell. The category for meal preparation time from the consumer survey for this simulated meal or consumer was 5, which corresponded to a meal preparation time of 6–8 h. The category for kitchen temperature from the consumer survey for this simulated meal or consumer was 2, which corresponded to a kitchen temperature of 20–25°C. The randomly selected value from the identified UNIFORM distribution for meal preparation time was 7.75 h, and the randomly selected value from the identified UNIFORM distribution for kitchen temperature was 20.7°C. The artificial neural network model for the growth of *Salmonella* on lettuce in PFARM predicted 0.56 logs of growth for the simulated time (7.75 h) and temperature (20.7°C) scenario, which increased the *Salmonella* number on serving #1 from 1 to 3 cells. Because no *Salmonella* were on lettuce servings #2 and #3 in the meal, no growth occurred on them and thus, the *Salmonella* dose consumed (N_4) from the simulated lettuce portion was three cells.

Spreadsheet 5! simulated the death and survival of native microflora including *Salmonella* during the cooking and cooling of chicken gizzards (Fig. 6). In this example, the category for cooked temperature from the consumer survey for the simulated meal or consumer was 3, which corresponded to a cooked temperature from 140 to $< 150^\circ\text{C}$ and a log per g reduction of 0.31–1.83, and a log per 56 g reduction of 2.06–3.58. The randomly selected log per 56 g reduction from the identified UNIFORM distribution was 2.82. Thus, of the 2,118,598 cells of native microflora on and in the serving of chicken gizzards, 3,195 survived the cooking and cooling process. By random chance, none of the five *Salmonella* on and in the serving of chicken gizzards survived cooking and cooling because by random chance their location in the line of death was before that of the last microbe to die (i.e., 2,115,403). Thus, the *Salmonella* dose consumed from this undercooked serving of chicken gizzards (N_5) was 0.

Spreadsheet 6! simulated *Salmonella* dose consumed (N_6) from undercooked chicken gizzards (N_5) and lettuce (N_4) (Fig. 8). In this example, the portion size from the consumer survey was three servings or 168 g. Serving #3 was contaminated with 19 cells of *Salmonella* Kentucky at the start of meal preparation, whereas servings #1 and #2 were not contaminated with *Salmonella*. The lettuce portion was contaminated with 2,629 cells of native microflora from chicken gizzards but none of them by random chance were *Salmonella*. All three servings in the portion were undercooked (i.e., native microflora > 0) resulting in the consumption of 185,303 cells of native microflora of which one was a *Salmonella* Kentucky.

Objective 2. This objective was to compare *Salmonella* dose consumed from lettuce (N_4) to *Salmonella* dose consumed from cooked chicken gizzards (N_5). Four replicate simulations of a single scenario were conducted (Table 1). The initial *Salmonella* prevalence among chicken gizzard portions (mean = 168 g) was $70.8 \pm 0.7\%$ (mean \pm SD) per 5,952 meals, whereas the total initial *Salmonella* number (N_1) was $66,837 \pm 1,792$ per 1,000 kg of chicken gizzards.

After preparation of the chicken gizzards for cooking, the *Salmonella* prevalence of cross-contaminated lettuce portions (N_3) was $0.49 \pm 0.07\%$ per 5,952 meals and the total *Salmonella* number on lettuce portions (N_3) was 31 ± 4 per 1,000 kg of chicken gizzards. Holding of the cross-contaminated lettuce during cooking and cooling of the chicken gizzards resulted in the growth of *Salmonella* so that at consumption, the total number of *Salmonella* consumed from lettuce (N_4) was $6,050 \pm 4,929$ per 1,000 kg of chicken gizzards.

On the other hand, after cooking and cooling the chicken gizzards, the prevalence of *Salmonella* among cooked chicken gizzard portions was $3.69 \pm 0.32\%$ per 5,952 meals and the total *Salmonella* number on cooked chicken gizzards at consumption (N_5) was 272 ± 27 per 1,000 kg. Thus, most of the *Salmonella* exposures (89.1%) were from undercooked chicken gizzards, whereas most of the *Salmonella* consumed (95.7%) were from cross-contaminated and temperature-abused lettuce.

Results in Figure 10 provide further insight. Here, the *Salmonella* transferred from chicken gizzards to lettuce portions (N_3) was low, from 1 to 3 cells. Likewise, *Salmonella* that survived cooking and cooling of chicken gizzard portions (N_5) was low, from 1 to 6 cells. Thus, most of the *Salmonella* dose consumed (N_6) was from a few meals in which *Salmonella* grew to high levels on lettuce portions ($N_4 > 310$) because of poor hygiene practices during meal preparation followed by long meal preparation (holding) times and high kitchen temperatures. This worst-case scenario was a rare event that occurred from 0 to 3 times per 5,952 meals or per lot (1,000 kg) of chicken gizzards.

Another interesting finding is shown in Figure 11. Here, the worst-case scenario (N_6 per meal > 310) was most often associated with a chicken gizzard portion with a lower (N_1 per portion < 100) rather than a higher (N_1 per portion > 100) initial dose. This occurred because chicken gizzard portions with low initial doses occurred more often than chicken gizzard portions with high initial doses. Thus, it was more likely for the worst-case scenario or simultaneous occurrence of multiple risk factors (poor hygiene, long meal preparation time, and high kitchen temperature) to occur with an initial dose near the mode than the maximum.

Objective 3. This objective was to determine if *Salmonella* dose consumed changed over time in the simulated production chain. Results in Figure 12A indicated that *Salmonella* prevalence per 5,952 meals at consumption changed ($P \leq 0.05$) over time in the production chain and from highest to lowest was period 1 $> 2 > 5 > 3 > 4$. Likewise, the results in Figure 12B indicated that total *Salmonella* dose consumed (N_6) per 1,000 kg of chicken gizzards changed ($P \leq 0.05$) over time in the production chain and from highest to lowest was period 1 = 2 = 5 \geq 3 = 4. Thus, the pattern of change was different between *Salmonella* prevalence and total dose consumed because among replicate simulations ($n = 20$) of the scenarios, total *Salmonella* dose consumed per lot was more variable and uncertain than *Salmonella*

Table 1
Comparison of *Salmonella* prevalence (Pr) and total number (N) from lettuce and undercooked chicken gizzards^a

Scenario	Metric	Node					Unit
		N ₁	N ₃	N ₄	N ₅	N ₆	
01	Pr	70.61%	0.39%	0.39%	3.88%	4.25%	5,952 meals
02	Pr	70.97%	0.55%	0.55%	3.65%	4.13%	5,952 meals
03	Pr	69.98%	0.52%	0.52%	3.26%	3.75%	5,952 meals
04	Pr	71.71%	0.49%	0.49%	3.98%	4.44%	5,952 meals
	Mean	70.82%	0.49%	0.49%	3.69%	4.14%	5,952 meals
	SD	0.72%	0.07%	0.07%	0.32%	0.29%	5,952 meals
	CV	1.02%	14.90%	14.90%	8.69%	7.03%	
Scenario	Metric	N ₁	N ₃	N ₄	N ₅	N ₆	Unit
01	N	66,369	26	74	290	364	1,000 kg
02	N	67,383	35	11,427	269	11,696	1,000 kg
03	N	64,664	32	4,339	234	4,573	1,000 kg
04	N	68,933	31	8,358	293	8,651	1,000 kg
	Mean	66,837	31	6,050	272	6,321	1,000 kg
	SD	1,792	4	4,929	27	4,928	1,000 kg
	CV	2.68%	12.07%	81.47%	10.01%	77.97%	

^a N₁ = start of meal preparation; N₃ = lettuce after cross-contamination; N₄ = lettuce after growth or at consumption; N₅ = undercooked chicken gizzards after cooking and cooling or at consumption; N₆ = lettuce + undercooked chicken gizzards at consumption; SD = standard deviation; and CV = coefficient of variation.

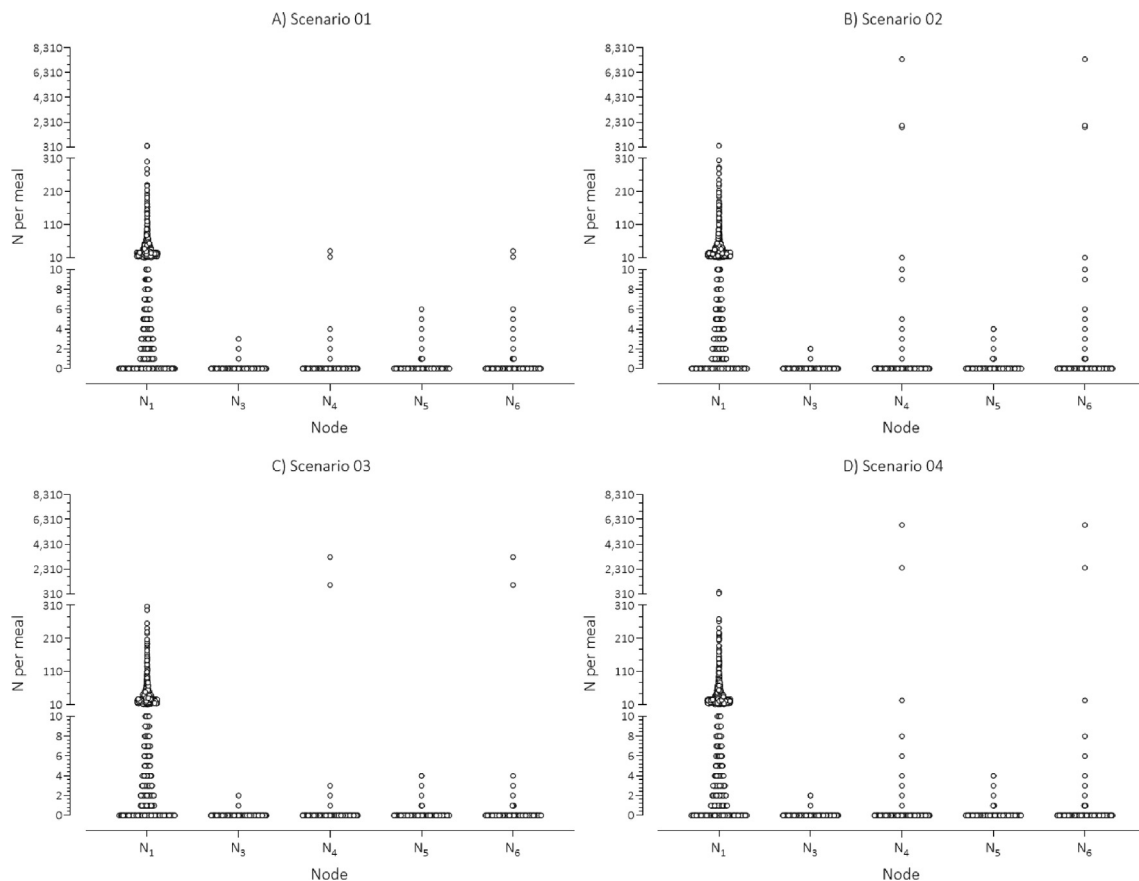


Figure 10. Simulation results for the distribution of *Salmonella* number (N) among meals for four replicate simulations of a single scenario in the Poultry Food Assess Risk Model (PFARM) for *Salmonella* and chicken gizzards. Symbols represent *Salmonella* number per meal ($n = 5,952$). The simulated nodes were 1) chicken gizzard portions at the start of meal preparation (N₁); 2) lettuce portions after cross-contamination (N₃); 3) lettuce portions after growth of transferred *Salmonella* (N₄); 4) chicken gizzard portions after cooking and cooling (N₅); and 5) dose consumed per meal (N₆ = N₄ + N₅). A lot size of 1,000 kg of chicken gizzards was simulated. Mean portion size was 168 g and thus, 5,952 meals were simulated. The *Salmonella* serotype prevalence and number data simulated in this scenario were from weeks 1 to 10 of this study as presented in Figure 2.

prevalence per 5,952 meals. This occurred because total *Salmonella* dose consumed was influenced by a worst-case (“perfect storm”) scenario that required simultaneous occurrence of multiple risk factors (initial contamination, cross-contamination, poor hygiene, long meal preparation time, and high kitchen temperature).

Objective 4. This objective was to compare predictions of PFARM and QMRA for *Salmonella* dose consumed. This was done by simulating scenarios 01 to 04 and comparing the mean rank of *Salmonella* dose consumed among meals at consumption within replicate simulations using a nonparametric test (Mann-Whitney). Results (Fig. 13) indi-

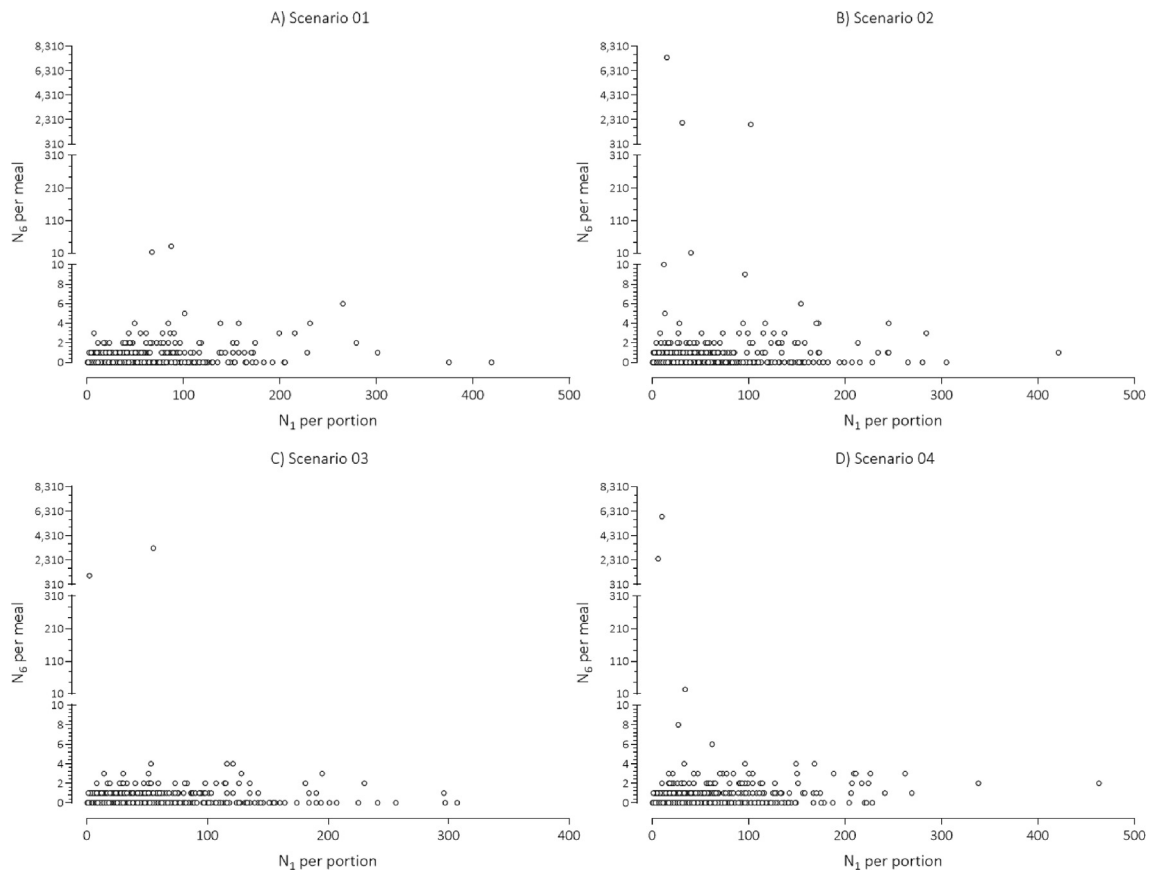


Figure 11. Dose consumed (N_6) per meal versus initial dose (N_1) per chicken gizzard portion at the start of meal preparation for the four replicate simulations of the scenario simulated in Figure 10 using the Poultry Food Assess Risk Model (PFARM) for *Salmonella* and chicken gizzards.

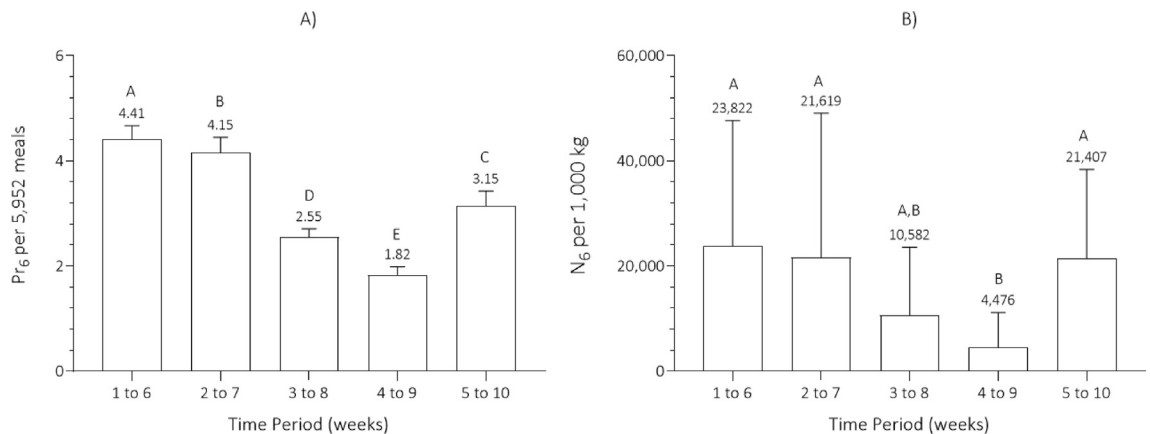


Figure 12. Simulation results for A) *Salmonella* prevalence at consumption (Pr_6) per 5,952 meals; and B) total *Salmonella* dose consumed (N_6) per 1,000 kg of chicken gizzards over time in the production chain per the Poultry Food Assess Risk Model (PFARM) for *Salmonella* and chicken gizzards. The effect of time on *Salmonella* prevalence and total dose consumed was simulated using running windows of 60 samples of chicken gizzards as shown in Figure 2. Bars are means \pm standard deviations for 20 replicate simulations of the five time-period scenarios. Bars within a graph panel with different letters differ at $P \leq 0.05$ per one-way, analysis of variance followed by Tukey’s multiple comparison test at $P \leq 0.05$.

cated that the mean rank of *Salmonella* dose consumed per meal was higher ($P \leq 0.05$) for QMRA than PFARM. The reasons for this difference in results are discussed above (see “Introduction”) and below.

Discussion

The first objective of this study was to demonstrate the dose consumed step of a PFARM for *Salmonella* and chicken gizzards.

This involved a perspective review of relevant PFARM studies, a detailed description of the PFARM modeling methods used, and a provision of simple examples from individual iterations of the PFARM to show how the PFARM works. The intention of the perspective review and detailed description and demonstration of the modeling methods was so that this study can serve as a reference for future studies that use the PFARM approach. This will allow the publication of one instead of four papers per PFARM. To better understand the methods, results, and conclusions of this study, it is important to

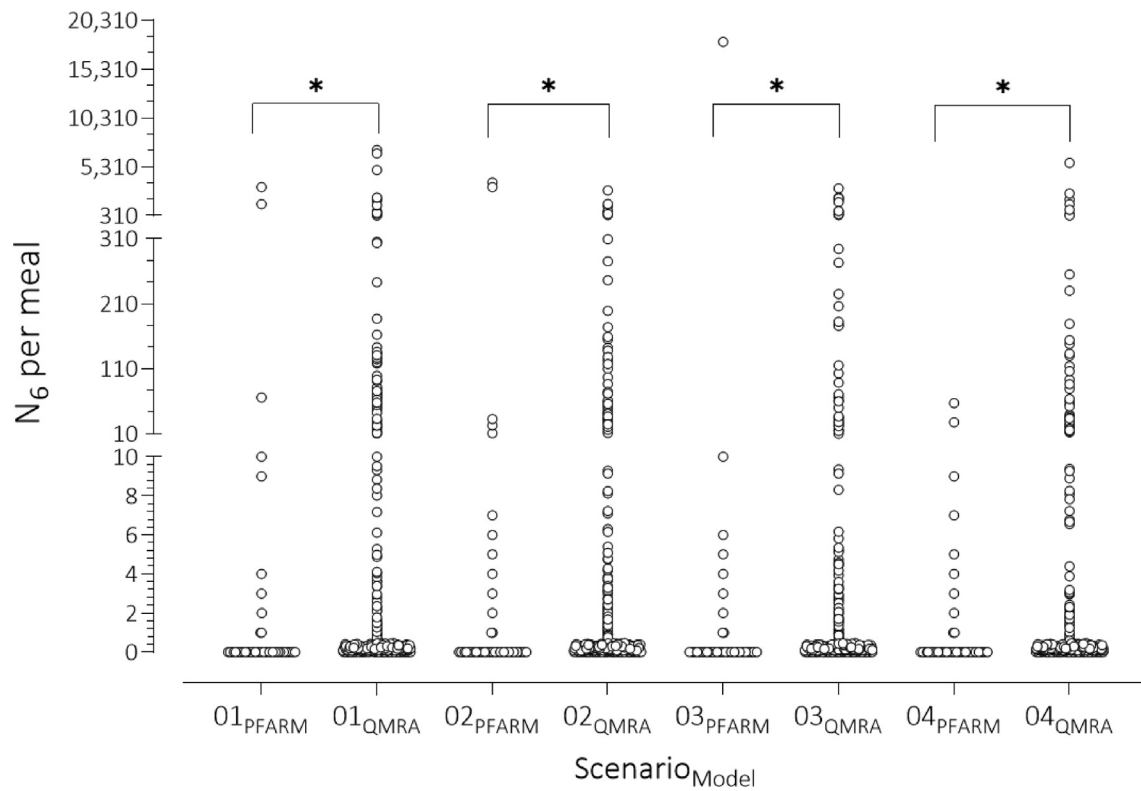


Figure 13. Comparison of Quantitative Microbial Risk Assessment (QMRA) and Poultry Food Assess Risk Model (PFARM) for prediction of *Salmonella* dose consumed (N_6) per meal. Results are for four replicate simulations of the scenario described in Fig. 10. Distributions within a replicated simulation with * differ at $P \leq 0.05$ per the Mann-Whitney test of mean ranks.

read the companion papers (Oscar, 2023b, 2023c) and to view the recorded presentation at: <https://ars-usda.app.box.com/s/ezg9pe0klo3u3xmxwbwjl0m94lstos3ub>.

The coding and debugging of the current PFARM for *Salmonella* and chicken gizzards were performed by the author and were too complex to provide in a traditional table format for this type of modeling study. Therefore, after publication of the fourth and final paper in this series, the PFARM will be open access on the PFARM project website: <https://www.ars.usda.gov/nea/errc/PoultryFARM>. This should make it possible for others to understand and repeat this work.

The second objective of this study was to compare two routes of consumer exposure to *Salmonella* from chicken gizzards. Here, it was found that although *Salmonella* exposure occurred more often from undercooked chicken gizzards, cross-contaminated lettuce was the main *Salmonella* dose-consumed pathway. This occurred because of a few lettuce portions per lot of chicken gizzards that had high levels (> 310) of *Salmonella* at consumption because multiple risk factors (i.e., initial contamination, poor hygiene, long meal preparation time, and high kitchen temperature) occurred at the same time. Thus, *Salmonella* dose consumed per lot of chicken gizzards was highly variable and uncertain because it was influenced by a few rare and random worst-case scenario and perfect storm events. This has important implications for the risk and severity of salmonellosis as will be shown in the next study in this series that addresses the consumer response step of this PFARM.

Although cooked chicken gizzards were not the main *Salmonella* dose consumed pathway in the present study, it does not mean that they cannot be it just may take a higher initial number of *Salmonella* in the raw poultry food at the start of meal preparation. For example, in a 2017 PFARM study (Oscar, 2017b), it was found that improper storage of whole chickens sold in flow pack wrappers at 15°C for 72 h resulted in *Salmonella* levels at the start of meal preparation of

up to 7 log per chicken part. Such a high number of *Salmonella* in raw poultry food could result in much greater exposure to *Salmonella* from undercooked poultry food.

In the present study, there was evidence of temperature abuse before meal preparation in some packages of chicken gizzards, but the highest initial number of *Salmonella* observed was just 2.8 logs per 56 g of chicken gizzards (Oscar, 2023b). This was not enough for a high number to survive the cooking and cooling process simulated in the current PFARM. Nonetheless, it is important to consider both routes of consumer exposure to *Salmonella* when assessing the risk and severity of salmonellosis from poultry food (Luber, 2009).

A third objective of the current study was to determine if *Salmonella* dose consumed changed over time in the simulated production chain. It was found that both *Salmonella* prevalence per 5,952 meals and *Salmonella* dose consumed per 1,000 kg of chicken gizzards changed over time in the production chain but that the pattern of change differed because of differences in the variability and uncertainty of *Salmonella* prevalence and dose consumed. More specifically, the *Salmonella* dose consumed per lot was more variable and uncertain because it was influenced by a few lettuce portions per lot that had high *Salmonella* number at consumption. These high *Salmonella* dose-consumed meals were rare and random events because they required multiple risk factors (initial contamination, poor hygiene, long meal preparation time, and high kitchen temperature) to occur at the same time.

Other important findings in the current study were that initial *Salmonella* dose and *Salmonella* dose consumed per meal were not correlated and that initial *Salmonella* doses near the mode rather than the maximum were more often associated with the highest *Salmonella* doses consumed per meal. This occurred because it was by random chance which chicken gizzards were served with lettuce that had been cross-contaminated and then held under the right conditions of time

and temperature for the growth of *Salmonella* to high levels just before consumption. In other words, the simultaneous occurrence of multiple risk factors was more likely at initial doses of *Salmonella* that occur most often (mode) than those that occur less often (maximum).

Random location of *Salmonella* on and in poultry food occurs when it is present at low levels. In the present study, most servings of chicken gizzards (56 g) were contaminated with no or low levels (1–10 cells) of *Salmonella* at the start of meal preparation (Oscar, 2023b). Being a minority member of the native microflora has important implications for the simulation of cross-contamination and thermal inactivation events. In the present study, natural ecology of *Salmonella* in the chicken gizzard matrix was simulated using a line of transfer method for cross-contamination and a line of death method for thermal inactivation (Oscar, 2020b).

In the line of transfer method for cross-contamination, *Salmonella* on or in the chicken gizzard serving were randomly assigned a position in the line of transfer. No specific mechanism was attached to this position. Rather, it was assumed that multiple mechanisms could account for a particular position. For example, a position near the front of the line of transfer could be due to the *Salmonella* cell being unattached to the chicken gizzard or kitchen fomite matrix and within the contact surface zone between the chicken gizzard and kitchen fomite or between the kitchen fomite and lettuce. On the other hand, a position beyond the end of the line of transfer could be due to the *Salmonella* cell being firmly attached to the chicken gizzard or kitchen fomite matrix and/or outside the contact surface zone between the chicken gizzard and kitchen fomite or between the kitchen fomite and lettuce.

To better simulate cross-contamination in the future, data are needed for transfer rates of native microflora on and in poultry food to kitchen fomites and then to ready-to-eat food (e.g., lettuce). Current transfer rates are based on inoculation studies with high levels of freshly inoculated *Salmonella* (Gorman et al., 2002; Ravishankar et al., 2010), which simulates an artificial ecology.

Likewise, in the line of death method for thermal inactivation, position of a *Salmonella* cell in the line of death can be due to multiple possible mechanisms. For example, the location near the front of the line could be from low thermal resistance and/or from being close to the heat source. On the other hand, the location of a *Salmonella* cell beyond the line of death could be from high thermal resistance and/or from being located far from the heat source like in the cold spot.

To better simulate thermal inactivation during cooking and cooling in the future, data are needed for D-values and Z-values for native microflora in and on poultry food. Current D- and Z-values are based on inoculation studies with high levels of freshly inoculated *Salmonella* (Juneja et al., 2013; Murphy et al., 2004), which simulates an artificial ecology.

Information about meal preparation practices (hygiene, meal preparation time, kitchen temperature, cooked temperature, portion size) is needed in the dose-consumed step of PFARM. In a previous study (Oscar, 2023c), a consumer survey tool to obtain this information was developed. In addition, a method was devised to link and simulate the meal preparation practices data in PFARM. This involved two steps. First, DISCRETE distributions were used to simulate the distribution of consumer survey responses among five categories of risk from very low (1) to very high (5). Second, UNIFORM distributions were used to simulate variability and uncertainty of the risk factor within a consumer survey category except for portion size, which had discrete values of 56, 112, 168, 224, and 280 g.

What may not be obvious is that this simulation method results in a hybrid input distribution for the risk factor that can vary in shape. It can be a uniform, normal, or a right- or left-skewed lognormal distribution depending on the distribution of survey responses among the five categories of risk. In the present study, it was a normal distribution because all factors for meal preparation practices were simulated using DISCRETE distributions with a normal distribution of consumer survey responses among categories of risk.

The PFARM for *Salmonella* is a work in progress. There were some data gaps in the current PFARM that might appear disqualifying but were not. First, “What if” survey data were used to demonstrate the PFARM. Second, DISCRETE distributions for meal preparation practices risk factors were the same. Third, surrogate data for native microflora were used. Fourth, distributions for meal preparation practices and native microflora were the same across time periods. Fifth, only one cooking and cooling method was simulated.

These data gaps can be addressed by collecting data for meal preparation practices in the production chain of interest using the PFARM consumer survey and data for native microflora using conventional microbiological methods like total plate counts. Despite these limitations, the current PFARM was successfully described and demonstrated and the finding that *Salmonella* dose consumed changed over time in the production chain would likely be the same regardless of whether “What if” or real data for meal preparation practices and surrogate or real data for native microflora were simulated because it was the result of *Salmonella* prevalence, number, and serotype/zoonotic potential data collected with chicken gizzards in the initial contamination step of this PFARM (Oscar, 2023b). What might change is the pattern of change of *Salmonella* dose consumed among periods if, as expected, meal preparation practices and native microflora change over time in the production chain due to circumstances like seasonal changes in temperature or cooking and cooling methods.

One reason for holding meal preparation practices and native microflora constant as a function of time in the production chain was to evaluate the hypothesis that the initial dose of *Salmonella* was a good indicator of *Salmonella* dose consumed. This hypothesis was rejected because it was shown that the highest initial doses of *Salmonella* were not associated with the highest *Salmonella* doses consumed. Rather, the most frequently occurring initial *Salmonella* doses, which were at lower initial doses, were associated with the highest *Salmonella* doses consumed. Thus, it was concluded that a performance standard based on initial number of *Salmonella* alone (prevalence + number) would not be a good indicator of *Salmonella* dose consumed or poultry food safety. Rather, identification of higher-risk production chains may require a performance standard for *Salmonella* that is based on multiple risk factors like *Salmonella* serotype prevalence, number, and zoonotic potential, and meal preparation practices, food consumption behavior, and consumer health and immunity. These results support a 2020 PFARM study (Oscar, 2020b) indicating that *Salmonella* prevalence alone (single risk factor) was not a good indicator of poultry food safety.

The next step in PFARM for *Salmonella* and chicken gizzards is consumer response. In the consumer response step, the initial contamination (Oscar, 2023b), illness dose (Oscar, 2023c), and dose consumed (this study) steps will be combined to predict risk and severity (no exposure, no response, infection, illness, hospitalization, and death) of salmonellosis. Multiple potential performance standards for salmonellosis that are based on multiple risk factors (*Salmonella* prevalence, number, and zoonotic potential, and meal preparation practices, food consumption behavior, and consumer health and immunity) will be evaluated with the goal of identifying one that will be a best fit for use in the poultry industry to better identify higher risk production chains and lots of poultry food, improve poultry food safety, and possibly reduce annual cases of salmonellosis from poultry food.

Comparison of PFARM and QMRA. The fourth objective of this study was to compare PFARM and QMRA for predicting *Salmonella* dose consumed. This was done using “What if” data for meal preparation practices, and food consumption behavior, and scenario analysis for *Salmonella* prevalence, number, and zoonotic potential (serotype) data for chicken gizzards. These comparisons showed that QMRA predicted a higher *Salmonella* dose consumed per meal than PFARM. This occurred because QMRA only simulated contaminated grams, whereas PFARM simulated contaminated and noncontaminated servings. Thus, the worst-case scenario (i.e., cross-contamination and substantial

growth of *Salmonella* on lettuce) that resulted in a high *Salmonella* dose consumed always occurred to a contaminated gram in QMRA but most often occurred to a noncontaminated serving in PFARM. Other differences between QMRA and PFARM contributed to this difference in results as discussed above (see “Introduction”) and below.

The rest of this discussion provides a comparison of three recent QMRA studies and the current PFARM. The intention is twofold. First, to point out similarities between the QMRA studies and the current PFARM to build confidence in the results of the current study. Second, to point out differences between the QMRA studies and the current PFARM study to better explain and justify the novel methods used and results obtained in this study.

In the QMRA of Straver et al. (2007), chicken fillets were obtained from supermarkets and butcher shops in the Netherlands. The chicken fillets varied in size from 129 to 151 g with a mean of 182 g. In comparison, in the current PFARM, the chicken gizzards were obtained from a local supermarket in the United States (Oscar, 2023b). The sample size analyzed in the present study was two chicken gizzards, which together ranged in size from 42 to 66 g with a mean of 53 g. The amount of chicken gizzards simulated in the current PFARM was 56, 112, 168, 224, or 280 g with a mean portion size of 168 g. Thus, the amount of chicken gizzards per meal simulated in the present study was like the size of chicken fillets simulated in the QMRA of Straver et al. (2007).

In the QMRA of Straver et al. (2007), the chicken fillets were rinsed in 280 mL of buffered peptone water for 10 s. To determine *Salmonella* prevalence and number, the chicken fillet rinse was divided into two samples (split sample method). One sample (200 mL) was used to determine *Salmonella* prevalence by pooling (five samples or 1,000 mL per pool) and cultivation, whereas a portion (33.3 mL) of the other sample (80 mL) was used to determine *Salmonella* number by a most probable number method. Thus, in the QMRA of Straver et al. (2007), a sampling method (rinsing) was used that does not recover all the *Salmonella* from the chicken fillet and a different sample and a different size of sample (split sample method) were used to determine *Salmonella* prevalence and number.

As discussed in a previous PFARM study (Oscar, 2021), the rinse sampling method and split sample methods do not provide accurate *Salmonella* prevalence and number data for risk assessment because of completeness and computational errors. On the other hand, the methods (whole sample enrichment-quantitative polymerase chain reaction-Monte Carlo simulation) used in PFARM to determine *Salmonella* prevalence and number do provide accurate data for risk assessment without completeness and computational errors (Oscar, 2019, 2020b).

Like the current PFARM, the QMRA of Straver et al. (2007) simulated cross-contamination of a kitchen fomite (cutting board) and then lettuce. However, the QMRA of Straver et al. (2007) did not simulate the growth of *Salmonella* on lettuce after cross-contamination, which was the main *Salmonella* dose consumed pathway in the current study. In addition, the QMRA of Straver et al. (2007) did not simulate cooking and cooling of the chicken fillets. Rather, it was assumed that cooking resulted in the elimination of *Salmonella*, which was not the case in the current study. Thus, the only *Salmonella* exposure route in the QMRA of Straver et al. (2007) was from cross-contaminated lettuce without temperature abuse.

In the QMRA of Straver et al. (2007), the growth of *Salmonella* on chicken fillets during home storage was simulated. In the current PFARM, the growth of *Salmonella* during home storage was not simulated because it was shown in a previous PFARM study (Oscar, 2017b) that the growth of *Salmonella* during home storage results in unpredictable changes in *Salmonella* prevalence, number, and serotypes among servings of chicken in the package. Therefore, to provide a better simulation of *Salmonella* dose consumed in the current PFARM, data for initial *Salmonella* prevalence, number, and serotype were collected after the package was opened and partitioned into chicken gizzards

portions for cooking at the start of meal preparation (Oscar, 2023b). This was done to better capture and simulate the effects of transit and home storage on *Salmonella* growth on chicken gizzards.

In the QMRA of Straver et al. (2007), the model used to predict *Salmonella* growth on chicken fillets during home storage did not consider lag time and was validated using conventional methods. In contrast, in the current PFARM, the model (Oscar, 2020a) used to predict *Salmonella* growth on lettuce after cross-contamination considered lag time and was validated using the Acceptable Prediction Zones method, which provides a more complete evaluation of model performance than conventional methods (Oscar, 2005b, 2020c).

In the QMRA of Straver et al. (2007), the transfer rates for *Salmonella* from chicken fillets to kitchen fomites (cutting board) were simulated using a LOGNORMAL distribution with a mean of -0.89 and a standard deviation of 0.34, which was truncated at 0. Thus, the range (± 2 standard deviations) of transfer rates was from 0.027 to 1.00. In comparison, in the current PFARM, the transfer rates of native microflora from chicken gizzards to kitchen fomites (hands, cutting board, and other) were simulated with a UNIFORM distribution that ranged from 0.005 to 0.02 and a line of transfer method that simulated *Salmonella* as a minority member of the native microflora of chicken gizzards.

In the QMRA of Straver et al. (2007), the transfer rates for *Salmonella* from kitchen fomites to ready-to-eat food (lettuce) were simulated using a LOGNORMAL distribution with a mean of -1.21 and a standard deviation of 0.6, which was truncated at 0. Thus, the range (± 2 standard deviations) of transfer rates was 0.0039–1.00. In comparison, in the current PFARM, the transfer rates of native microflora from kitchen fomites (hands, cutting board, and other) to ready-to-eat food (lettuce) were simulated with a combination of DISCRETE and UNIFORM distributions that resulted in a range of transfer rates from 0.0 to 0.4 with most (60%) being from 0.005 to 0.02. Also, the line of transfer method that simulated *Salmonella* as a minority member of the native microflora was used in this PFARM to simulate the natural ecology of *Salmonella* transfer from kitchen fomites to lettuce.

In the QMRA of Straver et al. (2007), only chicken fillets and lettuce that were contaminated with *Salmonella* were simulated and prevalence of cross-contamination was not simulated. In contrast, in the present PFARM, noncontaminated and contaminated servings of chicken gizzards and lettuce were simulated, and the prevalence of cross-contamination events was simulated. These differences between the QMRA of Straver et al. (2007), and the present PFARM can help explain the difference in results and conclusions obtained. Notably, in the QMRA of Straver et al. (2007), it was found that the fillets with the highest initial levels of *Salmonella* accounted for most of the *Salmonella* exposures, whereas in the current PFARM, the most abundant initial levels of *Salmonella* (those near the mode and not those near the maximum) accounted for most of the *Salmonella* dose consumed or exposures.

In the QMRA of Pouillot et al. (2012), data for *Salmonella* prevalence and number of chicken neck skin ($n = 149$) were collected at markets in Dakar, Senegal in 2005 and 2006. In comparison, in the present PFARM, data for *Salmonella* prevalence, number, and serotype of chicken gizzards ($n = 100$) were collected from a supermarket in Salisbury, Maryland, USA in 2018 (Oscar, 2023b).

In the QMRA of Pouillot et al. (2012), data ($n = 72$) for food handling practices were collected using a consumer survey, observational studies, and time and temperature loggers in 2008. Thus, the consumer survey data in the QMRA of Pouillot et al. (2012) were collected two or more years after the data for *Salmonella* prevalence and number of the chicken neck skin. In contrast, in the present PFARM, it was deemed important to collect the data for *Salmonella* prevalence and the number of chicken gizzards at the same time as the data for meal preparation practices. However, this was not possible because the consumer survey for meal preparation practices was developed after the data for *Salmonella* prevalence and number of chicken gizzards were

collected. Consequently, the current PFARM was limited to describing and demonstrating the data collection and modeling methods using “What if” data and scenarios for meal preparation practices.

In the QMRA of Pouillot et al. (2012), most consumers did not have access to cold storage. Thus, the median precooking temperature was 29.7°C (85°F), whereas the median postcooking temperature was 30°C (86°F). In comparison, in the present PFARM, the temperature of chicken gizzards before meal preparation ranged from 4 to 6°C (39 to 43°F), whereas the simulated kitchen temperature ranged from 16 to 40°C (61 to 104°F) but was 20°C (68°F) during cooling of cooked chicken gizzards.

In the QMRA of Pouillot et al. (2012), the storage conditions resulted in growth of *Salmonella* on chicken before and after cooking, whereas in the present study, the growth of *Salmonella* on chicken gizzards before cooking was captured in the *Salmonella* prevalence and number data and simulated in the PFARM (Oscar, 2023b), as explained above, whereas the postcooking growth of *Salmonella* on cooked chicken gizzards was not simulated for the reasons provided below.

In the QMRA of Pouillot et al. (2012), cross-contamination of ready-to-eat food with *Salmonella* from the chicken was simulated using 29 input distributions based on results of the consumer surveys and observational studies. In comparison, in the present PFARM, cross-contamination of ready-to-eat food (lettuce) with *Salmonella* from chicken gizzards was simulated in two steps using six input distributions. In step one, a DISCRETE distribution was used to simulate the frequency of occurrence of five categories of risk that were based on “What if” responses to consumer survey queries for hygiene practices during meal preparation. In step two, variability, and uncertainty of cross-contamination within each risk category were simulated using UNIFORM distributions ($n = 5$) for transfer rates of native microflora. In this way, the current PFARM, like the QMRA of Pouillot et al. (2012), simulated a wide range of consumer behaviors and transfer rates that could lead to cross-contamination of ready-to-eat food during the preparation of raw poultry for cooking.

In the QMRA of Pouillot et al. (2012), chicken neck skin samples (10 g) were stomached in buffered peptone water (90 mL) and subsamples of the buffered peptone water were used to determine *Salmonella* prevalence (0.1 mL) and number (6 mL). *Salmonella* prevalence was determined by a standard culture method (ISO 6579), whereas *Salmonella* number was determined by a mini-most probable number method (Pavic et al., 2010). The lower limits of detection and enumeration of these methods were not reported but were > 0 log per size of sample analyzed.

In contrast, in the present PFARM, the same sample and same size of sample (56 g) were used to determine *Salmonella* prevalence, number, and serotype (Oscar, 2023b) and the lower limit of detection and enumeration was 0 log per size of sample analyzed (Oscar, 2016, 2017b). In addition, *Salmonella* prevalence and number were simulated as a function of the size of sample analyzed to obtain data for larger sample sizes (Oscar, 2023b) using a validated Monte Carlo simulation method (Oscar, 2004b, 2021).

In the QMRA of Pouillot et al. (2012), *Salmonella* prevalence and number results for chicken neck skin, a normally unconsumed part of the carcass, were extrapolated to the whole carcass by making assumptions (i.e., Poisson distribution) and using statistical methods. However, in a 2010 PFARM study (Oscar et al., 2010) that mapped the distribution of *Salmonella* among parts of the chicken carcass, it was shown that the pattern of *Salmonella* contamination was diverse, random, and did not follow a Poisson distribution. Thus, rather than use assumptions, statistical methods, and unproven extrapolation methods to fill data gaps in risk assessments, the approach used in PFARM is to go to the laboratory and develop the methods needed to fill the data gaps in the split sample method (Oscar, 2023b).

In the QMRA of Pouillot et al. (2012), cross-contamination of cooked chicken with *Salmonella* followed by growth was simulated, whereas cross-contamination of other ready-to-eat food with *Sal-*

monella followed by growth was not simulated. In contrast, in the present PFARM, cross-contamination of cooked chicken with *Salmonella* followed by growth was not simulated, whereas cross-contamination of other ready-to-eat food (lettuce) with *Salmonella* followed by growth was simulated.

In the present PFARM, cross-contamination of cooked chicken gizzards with *Salmonella* followed by growth was not simulated for several reasons. First, because cross-contamination likely would have occurred while the cooked chicken gizzards were still hot enough to kill or injure the transferred *Salmonella*. Second, the simulated time between cross-contamination and consumption of cooked chicken gizzards was too short (10–20 min) for *Salmonella* growth to occur, as lag time of *Salmonella* on cooked chicken ranges from 1.5 h at 40°C to 9 h at 16°C (Oscar, 1999a, 1999b, 2000, 2002), which was the range of simulated kitchen temperatures. Third, *Salmonella* that survived cooking and cooling would likely be injured and thus, would have extended lag times (Mackey & Derrick, 1982).

In the QMRA of Pouillot et al. (2012), the number of consumers who shared a meal was simulated and ranged from 1 to 14. Also, it was assumed that the *Salmonella* on the ready-to-eat food and cooked chicken in the meal were shared equally among the consumers. In contrast, in the present PFARM, one consumer ate each meal. Thus, if consumers shared a meal together, the *Salmonella* dose consumed would differ among the consumers, which is what occurs in nature.

In the QMRA of Bemrah et al. (2003) for turkey cordon bleu, *Salmonella* prevalence and number were determined on samples ($n = 325$) from 21 caterers, eight retailers, and 65 batches in a region of France in 1998 and 1999. In contrast, in the current PFARM, for chicken gizzards, *Salmonella* prevalence and number were determined on samples ($n = 100$) from one brand, 20 packages, and one retailer in Salisbury, Maryland, USA in 2018 (Oscar, 2023b).

In the QMRA of Bemrah et al. (2003), a turkey cordon bleu was divided into two parts and one part was refrozen and the other was thawed at room temperature. Next, 25 g of the thawed turkey cordon bleu was homogenized in buffered peptone water and was used to determine *Salmonella* prevalence per a standard culture method (French Standard NF V08-052). If the sample tested positive for *Salmonella* in the prevalence assay, the other sample from the original turkey cordon bleu was thawed and a 25 g sample of it was homogenized in 100 mL of buffered peptone water. Next, 16 mL of the homogenate or 3.2 g (25 g * (16 mL/125 mL)) was used to determine the *Salmonella* number using a miniature-most probable number method. Thus, a different sample and a different size sample of turkey cordon bleu was used to determine *Salmonella* prevalence and number (split sample method).

In the QMRA of Bemrah et al. (2003), the sample size used to determine *Salmonella* prevalence was 25 g, whereas the sample size used to determine *Salmonella* number was 3.2 g. When a different sample and a different sample size are used to determine *Salmonella* prevalence and number, the results are confounded and not accurate for risk assessment because of completeness and computational errors (Oscar, 2021). This occurs because *Salmonella* in poultry food is a minority member of the native microflora, are not uniformly distributed, and *Salmonella* prevalence and number increase in a nonlinear manner as a function of sample size (Oscar, 2019, 2020b). Thus, for accurate exposure and risk assessment, it is important to express and simulate *Salmonella* prevalence and number as a function of the size of sample analyzed and not per gram and to obtain them using the same sample and same size of the sample (Oscar, 2021).

The latter conclusions are supported by application of the one pathogen cell test (Oscar, 2021) to turkey cordon bleu in the QMRA of Bemrah et al. (2003), as now explained. If the turkey cordon blue weighs 100 g, is contaminated with one cell of *Salmonella*, is divided in two, and analyzed for *Salmonella* prevalence and number by the methods used in the QMRA of Bemrah et al. (2003), there is only a 25% chance of detecting the *Salmonella* cell in the prevalence test

and a 0% chance of detecting the *Salmonella* cell in the miniature-most probable number assay because only samples testing positive for *Salmonella* in the prevalence test are analyzed for number in the miniature-most probable number assay. Therefore, if a sample tests positive for *Salmonella* in the prevalence test it will assess negative for *Salmonella* in the miniature-most probable number assay because the *Salmonella* cell cannot be in both samples at the same time, which although from the same original sample are now different samples.

Moreover, in the QMRA of Bemrah et al. (2003), even if all the set-aside samples were used in the miniature-most probable number assay, there would only be a 3.2% chance that a single cell of *Salmonella* would be enumerated because the miniature-most probable number assay used only 3.2 g of the original 100 g sample of turkey cordon bleu. In contrast, in the present PFARM, *Salmonella* prevalence and number of chicken gizzards were determined using the same sample and the same sample size (56 g) and the methods (whole sample enrichment, quantitative polymerase chain reaction, and Monte Carlo simulation) used (Oscar, 2023b) passed the one pathogen cell test (Oscar, 2021).

In the QMRA of Bemrah et al. (2003), 19 of 65 batches of turkey cordon bleu tested positive for *Salmonella*. Among the 95 turkey cordon bleu from the 19 batches that tested positive for *Salmonella*, 36 tested positive for *Salmonella* in the prevalence assay. However, only two of the 36 set-aside samples tested positive for *Salmonella* in the miniature-most probable number assay. This occurred because a different sample and a much smaller sample were used in the miniature-most probable number assay than in the prevalence assay, as described above.

In the QMRA of Bemrah et al. (2003), D-values and Z-values for simulating thermal inactivation of *Salmonella* during cooking and post-cooking storage were obtained experimentally using turkey cordon bleu and *Salmonella* serotypes Hadar and Typhimurium, which were isolated from turkey cordon bleu. The D-values ranged from 18 min at 55°C to 0.56 min at 62°C for serotype Hadar and from 21 min at 55°C to 0.64 min at 62°C for serotype Typhimurium. The Z-values were 4.66°C for serotype Hadar and 4.8°C for serotype Typhimurium. In comparison, in the present PFARM, the D-values and Z-value for simulating thermal inactivation of native microflora during cooking and cooling were obtained from a published study using chicken patties and *Salmonella* (Murphy et al., 2002). The D-values ranged from 27 min at 55°C to 0.32 min at 70°C and the Z-value was 7.6°C.

In the QMRA of Bemrah et al. (2003), two cooking (oven and frying) methods were simulated for turkey cordon bleu. The oven temperatures used to cook turkey cordon bleu in catering establishments ranged from 133 to >200°C (271 to >392°F). The cooked temperature of turkey cordon bleu in the QMRA of Bemrah et al. (2003) ranged from 74 to 97°C (165 to 207°F) with a mean of 83°C (181°F). In comparison, in the present PFARM, the oven temperature simulated was 350°F (177°C) and the simulated cooked temperature of chicken gizzards ranged from 109 to 170°F (43 to 77°C).

In the QMRA of Bemrah et al. (2003), the cooked turkey cordon bleu was stored in an oven at 54–81°C (129–178°F) with a mean of 69°C (156°F) before consumption. In comparison, in the present PFARM, cooked chicken gizzards were cooled at a kitchen temperature of 68°F (20°C) to <120°F (49°C) for 10–20 min before consumption.

In the QMRA of Bemrah et al. (2003), the cooking time ranged from 13 to 31 minutes with a mean of 23 min, whereas the postcooking storage time ranged from 17 to 90 min with a mean of 60 min. In comparison, in the present PFARM, the simulated cooking time ranged from 6 to 20 min and the cooling time ranged from 11 to 17 min.

In the QMRA of Bemrah et al. (2003), the decimal reduction of *Salmonella* during oven cooking and postcooking storage of turkey cordon bleu ranged from 6.4×10^2 to 2.2×10^{12} with a median of 4×10^6 . In comparison, in the present PFARM, the log reduction (log/g) of native microflora including *Salmonella* during oven cooking and room

temperature cooling of chicken gizzards ranged from 0 to 96 with a most likely value (60%) from 0.2 to 2.8.

In the QMRA of Bemrah et al. (2003), visits to catering establishments were used to collect data on meal preparation practices including time and temperature profiles of the cooking and cooling process. In comparison, in the present PFARM, “What if” data based on a consumer survey tool were used to simulate meal preparation practices except for the time and temperature profile for cooking and cooling, which was obtained experimentally.

In the QMRA of Bemrah et al. (2003), cross-contamination and growth of *Salmonella* on ready-to-eat food including cooked turkey cordon bleu was not simulated. In contrast, in the present PFARM, cross-contamination and growth of *Salmonella* on ready-to-eat food (lettuce) was simulated and was found to be the main *Salmonella* dose consumed pathway from chicken gizzards.

In the QMRA of Bemrah et al. (2003), the *Salmonella* number was simulated using Poisson distributions with λ of 0.5, 2, and 10. This approach was used because data for *Salmonella* number were only obtained for two samples. Thus, there were not enough data to define a probability distribution for simulating *Salmonella* number from turkey cordon bleu.

In contrast, in the present PFARM, data for *Salmonella* number were obtained for 35 of 100 samples analyzed and it was possible to define PERT distributions for *Salmonella* number over time in the production chain and to simulate how the distribution of *Salmonella* number among portions of chicken gizzards changed from the start of meal preparation to consumption.

In addition, it has been found in previous PFARM studies and the current one that *Salmonella* number among servings and portions of ground chicken (Oscar, 2019), ground turkey (Oscar, 2020b), chicken liver (Oscar, 2021), chicken gizzards (Oscar, 2023b), and chicken parts (Oscar, 2013, 2016, 2017b) is random and does not follow a Poisson distribution.

In conclusion, the results of these comparisons between QMRA and PFARM for *Salmonella* exposure or dose consumed from poultry food show that the two approaches to exposure assessment are similar in some ways and different in others. Nonetheless, a goal of these comparisons was to increase confidence in the methods, assumptions, and results of the current PFARM. Hopefully, that was achieved.

In addition, it was shown that the rinse, split sample, and probabilistic methods of QMRA have completeness and computational errors, whereas the whole sample enrichment-quantitative polymerase chain reaction-Monte Carlo simulation and rare event modeling methods of PFARM do not (Oscar, 2021). Thus, it is right to have confidence that PFARM provides a better prediction of *Salmonella* exposure or dose consumed than QMRA.

Finally, the importance of these differences between QMRA and PFARM will not be fully realized until the fourth and final paper in this series is published. In the consumer response step of PFARM for *Salmonella* and chicken gizzards, the initial contamination (Oscar, 2023b), illness dose (Oscar, 2023c), and dose consumed (this study) steps will be combined to predict the risk and severity (no exposure, no response, infection, illness, hospital, or death) of salmonellosis over time in the production chain.

Moreover, results of the consumer response step in this PFARM for *Salmonella* and chicken gizzards will be compared with those from QMRA over time in the simulated production chain to show why the differences in the two approaches are so important. Namely, it will be shown that the diagnostic tests and diagnosis methods used in QMRA result in an inaccurate assessment and management of the risk and severity of salmonellosis from poultry food because they do not adequately simulate what occurs in nature, whereas PFARM, although not perfect, uses more accurate and complete diagnostic tests and diagnosis methods and does a better job of simulating what occurs in nature.

CRedit authorship contribution statement

Thomas P. Oscar: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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