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Research Paper

Poultry Food Assess Risk Model for *Salmonella* and Chicken Gizzards: I. Initial Contamination

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ABSTRACT

The Poultry Food Assess Risk Model (PFARM) project was initiated in 1995 to develop data collection and modeling methods for simulating the risk of salmonellosis from poultry food produced by individual production chains. In the present study, the Initial Contamination (IC) step of PFARM for *Salmonella* and chicken gizzards (CG) was conducted as a case study. *Salmonella* prevalence (Pr), number (N), and serotype/zoonotic potential (ZP) data (n = 100) for one sample size (56 g) of CG were collected at meal preparation (MP), and then Monte Carlo simulation (MCS) was used to obtain data for other sample sizes (112, 168, 224, 280 g). The PFARM was developed in Excel and was simulated with @Risk. Data were simulated using a moving window of 60 samples to determine how *Salmonella* Pr, N, and ZP changed over time in the production chain. The ability of *Salmonella* to survive, grow, and spread in the production chain and food, and then cause disease in humans was ZP, which was based on U. S. Centers for Disease Control and Prevention data for salmonellosis. Of 100 CG samples tested, 35 were contaminated with *Salmonella* with N from 0 to 0.809 (median) to 2.788 log per 56 g. *Salmonella* serotype Pr per 56 g was 16% for Kentucky (ZP_{mode} = 1.1), 9% for Infantis (ZP_{mode} = 4.4), 6% for Enteritidis (ZP_{mode} = 5.0), 3% for Typhimurium (ZP_{mode} = 4.9), and 1% for Thompson (ZP_{mode} = 3.7). Results from MCS indicated that *Salmonella* Pr, N, and ZP among portions of CG at MP changed ($P \leq 0.05$) over time in the production chain. Notably, the main serotype changed from Kentucky (low ZP) to Infantis (high ZP). However, the pattern of change for *Salmonella* Pr, N, and ZP differed over time in the production chain and by the statistic used to characterize it. Thus, a performance standard (PS) based on *Salmonella* Pr, N, or ZP at testing or MP will likely not be a good indicator of poultry food safety or risk of salmonellosis.

A Pathogen Reduction (PR) and Hazard Analysis and Critical Control Point (HACCP) program, and a performance standard (PS) based on *Salmonella* prevalence (Pr) are used in the United States to improve poultry food safety by identifying poultry processing plants that pose a higher risk of salmonellosis (Ebel & Williams, 2015, 2020; Williams et al., 2022). The PR/HACCP/PS approach has reduced *Salmonella* Pr of poultry food (Williams et al., 2014, 2020). However, annual cases of salmonellosis from poultry food have stayed the same (Williams et al., 2022). There are several reasons why this may be happening.

First, *Salmonella* Pr is one of several factors that determine the risk of salmonellosis from poultry food (Akil & Ahmad, 2019; Bemrah et al., 2003; Jeong et al., 2019; Oscar, 2019, 2020; Smadi & Sargeant, 2013; Zhu et al., 2017). Other important risk factors are *Salmonella* number (N), *Salmonella* serotype/zoonotic potential (ZP), meal preparation practices (MPPs), food consumption behavior (FCB), and consumer health and immunity (CHI). Thus, to identify poultry production chains with a higher risk of salmonellosis, a *Salmonella* PS based on multiple risk factors (Pr, N, ZP, MPP, FCB, CHI) may be needed.

Second, when a nationwide Quantitative Microbial Risk Assessment (QMRA) is performed and the poultry industry is treated as a single production chain, a reduction in *Salmonella* Pr will result in a reduction of salmonellosis because all other risk factors are held constant (Ebel & Williams, 2015; Oscar, 2018b; Williams & Ebel, 2012). Thus, results of a nationwide QMRA do not indicate that a PS based on *Salmonella* Pr will be an accurate predictor of poultry production chains that pose a higher risk of salmonellosis (Oscar, 2020). Rather, they indicate that *Salmonella* Pr is an important risk factor for salmonellosis. Thus, the use of a nationwide QMRA in which all other risk factors were held constant as the scientific basis for use of *Salmonella* Pr as the PS in PR/HACCP programs may have led to the false expectation that a reduction in *Salmonella* Pr would lead to a reduction in salmonellosis from poultry food. This may help explain why the current PR/HACCP/PS approach has reduced *Salmonella* Pr but not

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salmonellosis cases from poultry food. The remedy is a PS for *Salmonella* in PR/HACCP that is based on multiple risk factors (Pr, N, ZP, MPP, FCB, CHI) in the local poultry production chains.

Third, less exposure of consumers to *Salmonella* in poultry food from PR/HACCP/PS would be expected to reduce consumer resistance to salmonellosis over time (Havelaar & Swart, 2014; McCullough & Eisele, 1951b; Swart et al., 2012). Thus, in the longer-term, PR/HACCP/PS may increase rather than decrease cases of salmonellosis from poultry food as shown in a recent study where this scenario was simulated (Oscar, 2018b). When combined with active surveillance and product recalls (Batz et al., 2012; Flockhart et al., 2017; Tack et al., 2020) that blunt outbreaks and reduce cases of salmonellosis, the net result of PR/HACCP/PS in the longer-term may be the observed stasis of salmonellosis cases from poultry food. Thus, it may be important to implement PR/HACCP/PS along with measures (e.g., vaccination) that maintain consumer resistance to salmonellosis in the face of reduced consumer exposure to *Salmonella* from poultry food for there to be an actual reduction in cases of salmonellosis from poultry food in the longer-term (Oscar, 2018b). Monitoring of blood antibodies to *Salmonella* may be one way of monitoring consumer exposure and resistance to salmonellosis in local poultry production chains (McCullough & Eisele, 1951a, 1951b, 1951c, 1951d).

Fourth, the carcass rinse aliquot (CRA) method used in PR/HACCP/PS purports to provide accurate information about *Salmonella* Pr of whole carcasses. However, carcass rinsing followed by culture isolation from only 30 of the 400 mL of carcass rinse underestimates *Salmonella* Pr as demonstrated when the CRA method is compared to the whole sample enrichment (WSE) method (Simmons, Fletcher, Berrang, & Cason, 2003a; Simmons, Fletcher, Cason, & Berrang, 2003b). In fact, when WSE was used to test chickens that passed PR/HACCP/PS with a *Salmonella* Pr of ≤7.5% per the CRA method, the observed *Salmonella* Pr by WSE was 65% to 90% (Oscar et al., 2010; Parveen et al., 2007). Thus, underestimation of *Salmonella* Pr by the CRA method could result in an inaccurate identification of poultry processing plants that pose a higher risk of salmonellosis and help explain the observed stasis in salmonellosis cases from poultry food.

Fifth, the main critical control point (CCP) in the poultry production chain for *Salmonella* may be in the consumer's home rather than on the farm or at the processing plant. Thus, even if the poultry industry were to deliver products to consumers that were contaminated with a single cell of *Salmonella*, that cell could reach high and dangerous levels if the products were packaged in a way that allowed the growth and spread of *Salmonella* throughout the package when improperly stored and handled by the consumer. In fact, when whole chickens sold in flow pack wrappers, a widely used packaging platform in the poultry industry, were improperly stored (72 h at 15°C), *Salmonella* Pr increased from 10.6% to 41.6% per chicken part, mean *Salmonella* N increased from 0.0 to 3.5 log per chicken part, maximum *Salmonella* N increased from 0.2 to 6.8 log per chicken part, and rates of salmonellosis increased from <1 to 70 cases per 100,000 chickens parts when the incidence of improper storage increased from 0% to just 1% (Oscar, 2017c). Thus, the flow pack wrapper used to deliver the chicken to the consumer provided an environment that allowed for the growth and spread of *Salmonella* throughout the package during temperature abuse.

Another important consideration is that salmonellosis from poultry food is a rare, random, variable, and uncertain event that occurs when by random chance multiple risk factors occur at the same time. Thus, to predict this perfect storm, a rare event modeling method is needed to properly simulate and predict consumer exposure and response to *Salmonella* contamination in the poultry production chain (Oscar, 1998a, 2004a, 2017c, 2019, 2020). A rare event modeling method simulates both contaminated and noncontaminated portions of poultry food by integrating data for *Salmonella* Pr, N, and ZP and MPP, FCB, and CHI into a prediction of risk of salmonellosis using Monte Carlo simulation (MCS) methods.

Active surveillance leading to detection, attribution, and blunting of outbreaks by recall and destruction of unsafe products is one approach that reduces cases of salmonellosis from poultry food (Batz et al., 2012; Flockhart et al., 2017; Tack et al., 2020). Even better would be an active surveillance system that detects unsafe poultry food before it is consumed and thus reduces sporadic cases of salmonellosis as well as cases of salmonellosis from outbreaks. This could be accomplished using a PS for *Salmonella* that is based on multiple risk factors (Pr, N, ZP, MPP, FCB, CHI) instead of just one (Pr) (Oscar, 2020). Moreover, it would be important to use the best available methods for collecting and simulating the data on which the PS is based.

The Poultry Food Assess Risk Model (PFARM) project was initiated in 1995 to develop data collection and modeling methods for assessing the risk of salmonellosis from poultry food produced by local production chains using a PS based on multiple risk factors (Pr, N, ZP, MPP, FCB, CHI) instead of one (Pr). The PFARM process (Fig. 1) has four steps as follows: 1) initial contamination (IC); 2) illness dose (ID); 3) dose consumed (DC); and 4) consumer response (CR). The four steps are like the four steps of QMRA as follows: 1) hazard identification; 2) hazard characterization; 3) exposure assessment; and 4) risk characterization. However, different terminology is used for these steps in PFARM to differentiate it from QMRA.

In the present study, the IC step of PFARM for *Salmonella* and chicken gizzards (CGs) was conducted using locally acquired data for *Salmonella* Pr, N, and ZP. The CGs were selected as the case study because they are an edible byproduct of chicken processing that harbor *Salmonella* (Duc et al., 2018; Raji et al., 2021) but have not been widely studied. They can also serve as a source of cross-contamination of other chicken parts with *Salmonella* when they are sold in packages of whole chickens (Oscar et al., 2010).

Data for *Salmonella* Pr, N, and ZP of CG were collected at meal preparation (MP) at a rate of 10 samples per week for 10 straight weeks. A moving window of 60 samples, like the one used in PR/HACCP/PS, was then used to simulate changes in *Salmonella* Pr, N, and ZP of CG at MP over time in a local production chain using the rare event modeling method of PFARM (Oscar, 1998a, 2004a). The objectives were to conduct the IC step of PFARM for *Salmonella* and CG as a case study and to provide a perspective review of the data collection and modeling methods used in the IC step of PFARM in the hopes of facilitating understanding and adoption of this approach to poultry food safety.

Materials and methods

Poultry food assess risk model

Poultry FARM for *Salmonella* and CG was created in an Excel notebook (Office 365, MicroSoft Corp., Redmond, WA) and was simulated with @Risk (version 8.2, Palisade Corp., Ithaca, NY). The Excel notebook had ten spreadsheets (!) and simulated the risk pathway shown in Figure 2. Once published, it will be open access on the PFARM website: www.ars.usda.gov/nea/errc/PoultryFARM.

Chicken gizzards

A single brand of CG from a single processing plant was obtained from a local retail store (Salisbury, MD) between 15 October 2018 and 18 March 2019. They were sold in plastic-wrapped trays, sometimes with hearts. Two packages were obtained per week because there were not enough CGs in one package to obtain ten samples. They were transported to the laboratory (20–40 min) at ambient temperature (20–24°C) where they were stored for 4–5 h at 4–6°C before analysis. This was a typical consumer transport scenario for fall and winter seasons in the simulated production chain.

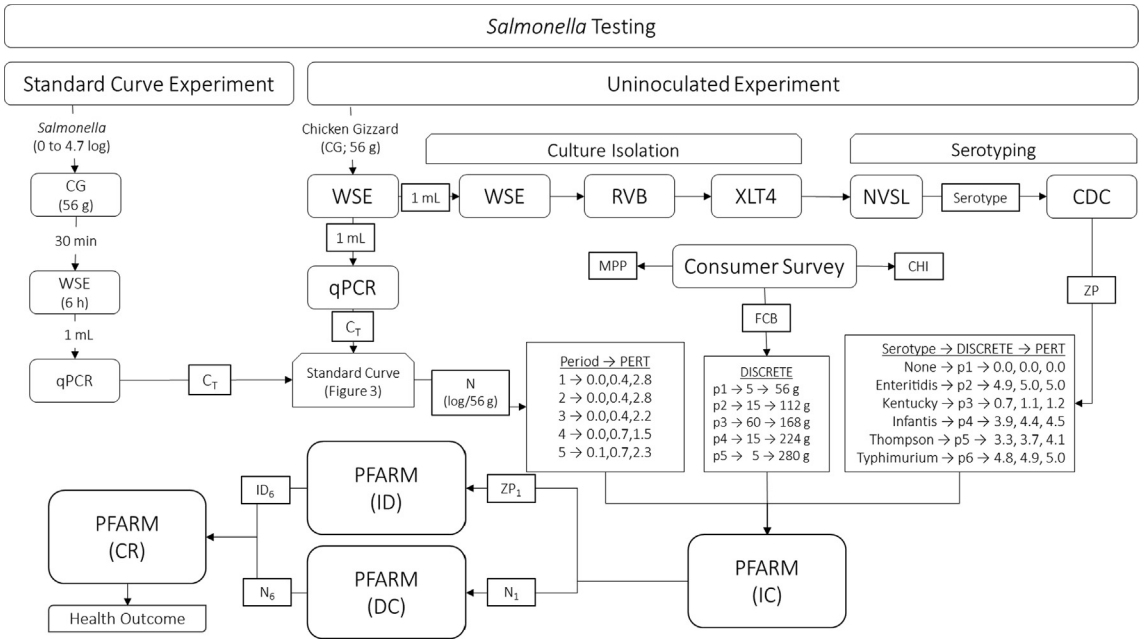


Figure 1. Schematic diagram of the Initial Contamination (IC) step of the Poultry Food Assess Risk Model for *Salmonella* and chicken gizzards (CG) and its relationship to the Illness Dose (ID), Dose Consumed (DC), and Consumer Response (CR) steps of PFARM. Other abbreviations: WSE = whole sample enrichment; qPCR = real-time polymerase chain reaction; CT = cycle threshold; N = number; RVB = Rappaport Vassiliadis Broth; XLT4 = xylose lysine tergitol 4 agar; NVSL = National Veterinary Services Laboratory; CDC = Centers for Disease Control and Prevention; p = probability of occurrence; ZP = zoonotic potential; MPPs = meal preparation practices; CHI = consumer health and immunity; and FCB = food consumption behavior. See texts for further details.

	A	B	C	D	E	F
1	Spreadsheet (!)	Description		Hatch-to-Meal Preparation		Risk Pathway
2	C	Table of Contents		(Contamination)		
3	S	Consumer Survey		↓		
4	D	Data Input		Kitchen Fomites	→	Lettuce
5	1	Contamination at Meal Preparation		(Contamination)		(Contamination)
6	2	Cross-contamination of Kitchen Fomites		↓		↓
7	3	Cross-contamination of Lettuce		Cooking		Lettuce
8	4	Growth on Lettuce		(Survival)		(Growth)
9	5	Death during Cooking		↓		↓
10	6	Dose-Response		Consumption		
11	A	Arrays		(Dose-Response)		
12				↓		↓
13				No Exposure		Exposure
14				PFARM C!		↓
15						No Response
16						↓
17						Infection
18						↓
19				Poultry Food Assess Risk Model (PFARM) for Salmonella and Chicken Gizzards		Illness
20						↓
21						Hospital
22						↓
23						Death

Figure 2. Spreadsheet C (C!) in the Excel notebook for the Poultry Food Assess Risk Model (PFARM) for *Salmonella* and chicken gizzards (CGs) showing the Table of Contents and risk pathway.

To determine IC with *Salmonella* at MP, two CGs were placed in a plastic weigh boat to form a sample that was simulated in PFARM as a serving. Mean weight ± standard deviation (SD) of CG samples was 53.3 ± 3.8 g (range: 44.9–66.0 g; n = 100).

Samples and weigh boats were placed in stomacher bags (177 by 304 mm, Seward, London, UK) for WSE. For clarity of presentation, a sample size of 56 g was assumed from this point forward.

Ten samples (56 g) of CG were tested per week. Samples A1 to A5 were from package A, and samples B1 to B5 were from package B (Table 1).

Whole sample enrichment

Four-hundred mL of prewarmed (40°C) buffered peptone water (BPW, Microbiology International, Frederick, MD) was added to sam-

Table 1
Salmonella contamination of chicken gizzards at meal preparation in a single production chain over time

Week	*Salmonella*	Sample									
		A1	A2	A3	A4	A5	B1	B2	B3	B4	B5
1	Log number			1.286				1.012	1.566		0.333
	Serotype[a]			E				Th	E		E
2	Log number	0.912								2.778	
	Serotype	I								K	
3	Log number	1.046	0.958	0.612	1.251	0.562	1.446	2.163	0.994	1.661	1.832
	Serotype	K	K	K	K	K	T	K	K	K	K
4	Log number			0.243	0.000			0.606	0.417	0.417	
	Serotype			K	T			K	K	T	
5	Log number		0.100								
	Serotype		K								
6	Log number										
	Serotype										
7	Log number	0.661	0.431		0.433						
	Serotype	I	K		K						
8	Log number				0.706						
	Serotype				E						
9	Log number			0.976			0.248		0.544	1.475	
	Serotype			E			I		I	E	
10	Log number						2.345	0.544	0.347	1.843	1.563
	Serotype						I	I	I	I	I

[a] E = Enteritidis; K = Kentucky; I = Infantis; Th = Thompson; and T = Typhimurium.

ples of CG followed by placement of samples in a prewarmed (40°C), programmable, refrigerated, and orbital shaking incubator (Innova 42, New Brunswick Scientific, Edison, NJ). Separate incubators were used for sample sets A and B.

Incubators were programmed to incubate samples for 6 h at 40°C and 80 revolutions per minute (rpm) and then to cool and hold them at 6°C and 80 rpm until sampling ended. Under these holding conditions (6°C, 16–24 h), *Salmonella* N stays the same (Oscar, 2014a, 2015). There is nothing special about 6°C other than it is one of several temperatures (4–8°C) that the samples could have been held at to preserve *Salmonella* N.

Real-time polymerase chain reaction

After 6 h of incubation (Fig. 1), a 1-mL sample of WSE from each sample of CG (10 per run) was collected into a 1.5-mL microcentrifuge tube for real-time polymerase chain reaction (qPCR; iQ-Check for *Salmonella* II, Bio-Rad, Hercules, CA), as described in a previous study (Oscar, 2014b). When a sample was positive for *Salmonella*, a cycle threshold value (C_T) was obtained. It was the qPCR cycle where *Salmonella* was first detected. The lower limit of detection of the qPCR assay was 2 log per mL (Lauer, 2015; Lauer et al., 2009).

Standard curve experiment for enumeration

Samples of CG were inoculated with different levels (0–4.7 log per 56 g) of *Salmonella* in a series of six weekly runs from 4 February 2019 to 18 March 2019 (Fig 1). Each run had ten samples of two CGs: five from package A (A1 to A5) and five from package B (B1 to B5).

Frozen (−80°C) stock cultures of *Salmonella* were thawed at room temperature. After gentle shaking, 5 μL was transferred to 1 mL of BPW in a 1.5-mL microcentrifuge tube. Cultures were incubated for 72 h at 22°C to obtain stationary phase cells for inoculation of CG (Fig. 1).

Salmonella serotypes Kentucky, Enteritidis, and Typhimurium, which were isolated from CG in the current study, were used for standard curve development. Levels of *Salmonella* serotypes in undiluted inoculation cultures were determined using an automated, miniature, most probable number (MPN) method (Oscar, 2017a). Concentration (log/mL) of *Salmonella* serotypes in inoculation cultures before dilution was (mean ± SD; n = 2) 7.60 ± 0.03 for Kentucky, 8.83 ± 0.20 for Enteritidis, and 8.71 ± 0.24 for Typhimurium.

Just before inoculation, cultures were serially diluted (1:10) in BPW. Samples of CG were then spot inoculated on their surface with 5 μL of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, or 10^{-6} dilutions. Dilutions were applied once within a run to samples from package A and once within a run to samples from package B. Inoculated samples were held for 30 min at room temperature in a biohood before being placed in stomacher bags and incubated as described above.

Standard curve for enumeration

After qPCR, C_T (Y) were graphed as a function of dose (log/56 g) of *Salmonella* inoculated. The resulting curve (Fig. 3) was fitted (version 9.3, Prism, GraphPad Software, Inc., San Diego, CA) to the Weibull model:

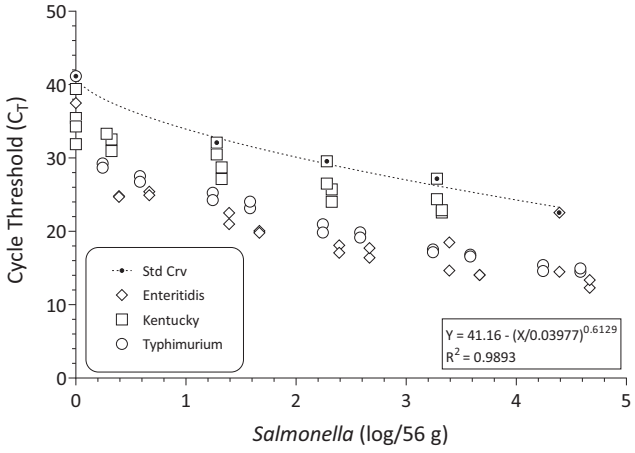


Figure 3. Standard curve for enumeration of *Salmonella* on chicken gizzards (CGs) by whole sample enrichment (WSE), real-time polymerase chain reaction (qPCR). Open symbols are observed cycle threshold values (C_T) for *Salmonella* serotypes Kentucky, Enteritidis, and Typhimurium per the legend. The dashed line is the standard curve, and open symbols with a black dot in the center are observed values used to develop the standard curve. The standard curve equation and its coefficient of determination (R^2) are shown. The standard curve predicts C_T as a function of the log dose of *Salmonella* inoculated onto CG.

$$Y = Y_0 - \left(\frac{X}{a}\right)^b$$

where Y_0 was C_T at 0 log N per sample (56 g), X was log N per sample (56 g), *a* was a regression coefficient, and *b* was a shape parameter.

The Y_0 was fixed to the maximum observed C_T, which was 41.16. This ensured that *Salmonella* N was ≥0 log per sample (56 g). Also, because samples may contain native *Salmonella* that lower C_T, especially at low inoculated doses, maximum C_T at each inoculated dose was used in curve-fitting to produce a fail-safe standard curve, as described in previous studies (Oscar, 2017c, 2020).

Uninoculated experiment for enumeration

The C_T for uninoculated samples (56 g) of CG with native *Salmonella* were obtained with the same WSE-qPCR method (Fig. 1). Weekly runs of ten samples (56 g) of naturally contaminated CG occurred from 15 October 2018 to 17 December 2018. The C_T for these samples were converted to log N ±95% confidence interval (CI) per 56 g using the standard curve interpolation function of Prism. Median 95% CI was ±0.45 log per 56 g.

Because of time and cost, a single standard curve was used to enumerate native *Salmonella* on and in CG. Because *Salmonella* N is not affected by serotype when this approach is used (Oscar, 2021), enumeration data for all serotypes were combined and simulated together in PFARM, as described below.

Culture isolation

For samples (56 g) of CG that tested positive for *Salmonella* by WSE-qPCR, a second sample (1 mL) of WSE (6°C) was collected and used for culture isolation of *Salmonella* (Fig. 1), as described in a previous study (Oscar, 2014b). In brief, samples were incubated in BPW for a further 16 h at 40°C followed by selective enrichment for 24 h at 42°C in Rappaport Vassiliadis (RV) broth (Becton Dickinson, Sparks, MD) and then selective plating and growth for 24 h at 40°C on xylose lysine tergitol 4 (XLT4) agar. One presumptive colony was picked per XLT4 agar plate and was stored at −80°C in tryptic soy broth with 15% glycerol.

Serotyping

Isolates were shipped to a *Salmonella* Reference Center (U. S. Department of Agriculture, National Veterinary Services Laboratory, Ames, IA) for serotyping (Fig. 1). Only samples testing positive by WSE-qPCR, culture isolation, and serotyping were considered positive for *Salmonella*.

Monte Carlo simulation

Collection of data for *Salmonella* Pr, N, and ZP of CG was time-consuming and expensive. Thus, to save time and money, Monte Carlo simulation of data for one sample size (56 g) was used to obtain data for other sample sizes (112, 168, 224, 280 g) as described in previous studies (Oscar, 1998a, 2004a). This was accomplished in spreadsheet 1 (1!) of PFARM (Fig. 4), which simulated *Salmonella* Pr, N, and ZP at MP as a function of portion size from one to five servings or from 56 to 280 g in increments of 56 g. Data for simulation of *Salmonella* Pr, N, and ZP and portion size were entered in spreadsheet D (D!) of PFARM (Fig. 5).

Simulation of portion size

Portion size was simulated in PFARM 1! (Fig. 4) using a DISCRETE distribution from @Risk (Fig. 1):

$$= \text{RiskDiscrete}(\{X_1, X_2, X_3, X_4, X_5\}, \{p_1, p_2, p_3, p_4, p_5\})$$

where X was portion size, *p* was probability of occurrence, and 1 was 56 g; 2 was 112 g; 3 was 168 g; 4 was 224 g; and 5 was 280 g. During simulation of PFARM, this distribution was randomly sampled to determine portion size of CG. For example, in Figure 4, the portion size of CG was three servings or 168 g.

Simulation of Salmonella prevalence

Five serotypes of *Salmonella* were isolated from CG in the present study (Table 1). *Salmonella* serotype Pr was simulated in PFARM (Fig. 4) using a DISCRETE distribution from @Risk (Fig. 1):

$$= \text{RiskDiscrete}(\{X_0, X_1, X_2, X_3, X_4, X_5\}, \{p_0, p_1, p_2, p_3, p_4, p_5\})$$

where X was a code (Fig. 5), p was probability of occurrence, and 0 was None, 1 was Kentucky, 2 was Infantis, 3 was Enteritidis, 4 was Typhimurium, and 5 was Thompson.

During simulation of PFARM, this distribution was randomly sampled for each serving (56 g) of CG in the simulated portion. For example, in Figure 4, which had a portion size of three servings, serving 1 had a code of 0 for None, serving 2 had a code of 1 for Kentucky,

	A	B	C	D	E	F	G	H
1	Hatch-to-Meal Preparation (Contamination)							
2	Portion Size		*Salmonella* Number (N)		Zoonotic Potential (ZP)			
3	Frequency	Serving	Log N	N	Code	Name	Composite	ZP
4	1	1	0.70	0	0	None	0.0	0.0
5	1	2	0.64	4	1	Kentucky	0.4	1.0
6	1	3	0.73	5	5	Thompson	2.0	3.6
7		4						
8		5						
9	Portion Size		N_1		 PFARM 1!		ZP_1	
10	3		9				2.4	
11								

Figure 4. Spreadsheet 1 (1!) in the Excel notebook for the Poultry Food Assess Risk Model (PFARM) for *Salmonella* and chicken gizzards (CGs), which simulates the distribution of *Salmonella* contamination (prevalence, Pr; number, N; and serotype/zoonotic potential, ZP) among portions of CG at meal preparation (MP). Results are for a single portion (3 servings or 168 g) of CG. The subscript 1 indicates a model output from 1! in PFARM. See text for further details.

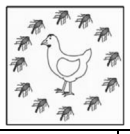
Data Input						
Sample size	56	g		PFARM D!		
Mean Portion size	168	g				
Lot size	1,000	kg				
Portions	5,952	iterations				
Salmonella Testing	Minimum	Mode	Maximum	Unit		
Native Microflora	2.500	4.500	7.000	log/g		
Salmonella	0.000	0.417	2.788	log/sample		
Code	0	1	2	3	4	5
Serotype	None	Kentucky	Infantis	Enteritidis	Typhimurium	Thompson
Prevalence	63.3	23.3	1.7	5.0	5.0	1.7
Zoonotic Potential	0.0	1.1	4.3	5.0	4.9	3.7
Consumer Survey	20	1	2	3	4	5
Hygiene	2	1	4	3	3	3
Meal preparation time	2	2	2	4	4	2
Kitchen temperature	3	2	3	3	3	2
Cooked Temperature	3	4	3	4	3	3
Portion size	4	3	3	5	3	3
Food Consumption Behaivor	3	4	4	3	1	3
Consumer Health & Immunity	3	3	3	2	4	2

Figure 5. Spreadsheet D (D!) in the Excel notebook for the Poultry Food Assess Risk Model (PFARM) for *Salmonella* and chicken gizzards (CGs). Data for *Salmonella* prevalence (Pr), number (N), and serotype obtained from *Salmonella* testing for Initial Contamination (IC) are entered in this spreadsheet. See text for further details.

and serving 3 had a code of 5 for Thompson. Thus, the portion (168 g) was contaminated with two serotypes of *Salmonella* at MP.

Simulation of Salmonella serotype

The next step (Fig. 1) was to convert serotype to ZP, which had a value from 0.1 to 5.0 in increments of 0.1 and was based on epidemiological data for cases of human salmonellosis (Table 2) from the U. S. Centers for Disease Control and Prevention (CDC) (Anonymous, 2018):

$$= 5.1 - 0.1r \text{ IF } r \leq 20$$

$$= 3.1 * (c/c_{20}) \text{ IF } r > 20$$

where r was the epidemiological rank of the serotype from 1 to 20, c was cases of salmonellosis attributed to the serotype, and c_{20} was cases of salmonellosis attributed to the 20th ranked serotype (Table 2) *[57, 58]*.

The first equation calculated ZP for serotypes ranked in the top 20 of human clinical isolates, whereas the second equation calculated ZP for serotypes ranked outside the top 20 of human clinical isolates. In the current study, *Salmonella* serotype ZP ranged from 0.7 to 1.1 (mode) to 1.2 for Kentucky, 3.9 to 4.4 (mode) to 4.5 for Infantis, 4.9 to 5.0 (mode) to 5.0 for Enteritidis, 4.8 to 4.9 (mode) to 5.0 for Typhimurium, and 3.3 to 3.7 (mode) to 4.1 for Thompson (Table 2).

To simulate variation of ZP within a serotype of *Salmonella*, a PERT distribution from @Risk was used (Fig. 1) as follows:

$$\text{PERT}_{ZP} = \text{RiskPert}(X_{min}, X_{mode}, X_{max})$$

where X_{min} was minimum ZP, X_{mode} was most likely ZP, and X_{max} was maximum ZP based on CDC data from 2007 to 2016 (Table 2). During simulation of PFARM, PERT_{ZP} for the *Salmonella* serotype was randomly sampled to determine ZP for the simulated serving in the portion. For example, in Figure 4, the randomly selected ZP were 1.0 for serotype Kentucky and 3.6 for serotype Thompson.

Simulation of Salmonella number

This was a multiple-step process. The first step was to simulate log N (N_{log}) of *Salmonella* on CG at MP. This was done using a PERT distribution for each potential serving ($n = 5$) in a portion:

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

where min was minimum N_{log}, mode was most likely N_{log}, and max was maximum N_{log}.

This distribution is used in PFARM because it is easy to define, it can be used with small sets of data, and it can vary in shape from a normal distribution to a log-normal distribution that is skewed to the right or left. Thus, it is simple, robust, and flexible.

Next, the IF, POWER (antilog), and ROUNDDOWN (to whole numbers) functions of Excel were used to convert N_{log} to N by linking results for Pr, ZP, and N:

$$N = 0 \text{ IF Pr or ZP} = 0$$

$$N \geq 1 \text{ IF Pr or ZP} > 0$$

where N was 0 when the serving was not contaminated with *Salmonella*, and N was ≥ 1 when the serving was contaminated with *Salmonella*.

Salmonella N per portion at MP (N_1) was then calculated as the sum of N for all servings in the portion. For example, in Figure 4, in the simulated portion, N was 0, 4, and 5 for servings 1, 2, and 3, respectively, for an N_1 of 9 per 168 g. Subscript 1 refers to the spreadsheet in PFARM (Fig. 4) from which the output (Pr_1, ZP_1, or N_1) originated.

When the portion was contaminated with multiple serotypes of *Salmonella*, a composite ZP_1 was calculated. For example, in Figure 4, the simulated portion was contaminated with serotype Kentucky (N = 4; ZP = 1.0) and serotype Thompson (N = 5; ZP = 3.6). Thus, the composite ZP_1 was 2.4 = {(4/9) * 1.0} + {(5/9) * 3.6}.

Scenario analysis

A scenario is a unique set of inputs in PFARM or similar models. Altering just one input creates a new scenario. In the present study,

Table 2
Epidemiological data for human salmonellosis from the U. S. Centers for Disease Control and Prevention that were used to simulate zoonotic potential of *Salmonella* serotypes[a]

	Kentucky			Infantis			Enteritidis		
Year	*C*	*r*	ZP	*c*	*r*	ZP	*c*	*r*	ZP
2007	95	> 20	1.0	517	12	3.9	6056	2	4.9
2008	93	> 20	0.8	633	12	3.9	7197	1	5.0
2009	73	> 20	0.9	626	12	3.9	7122	1	5.0
2010	94	> 20	1.1	807	10	4.1	8896	1	5.0
2011	101	> 20	1.1	901	9	4.2	7546	1	5.0
2012	113	> 20	1.2	1106	7	4.4	7095	1	5.0
2013	92	> 20	1.1	1310	7	4.4	6815	1	5.0
2014	93	> 20	0.9	1357	7	4.4	8895	1	5.0
2015	87	> 20	0.7	1057	8	4.3	9150	1	5.0
2016	63	> 20	0.8	1281	6	4.5	7830	1	5.0
	Typhimurium			Thompson			20th Ranked Serotype		
Year	*C*	*r*	ZP	*c*	*r*	ZP	*c*	Name	
2007	6152	1	5.0	406	17	3.4	285	Hadar	
2008	6485	2	4.9	411	18	3.3	351	Schwarzengrund	
2009	6087	2	4.9	473	13	3.8	266	Hadar	
2010	6104	1	5.0	480	14	3.7	271	Poona	
2011	6120	2	4.9	534	14	3.7	282	Anatum	
2012	5702	2	4.9	818	12	3.9	301	Berta	
2013	5745	2	4.9	627	13	3.8	259	Berta	
2014	5041	2	4.9	626	12	3.9	307	Agona	
2015	4943	2	4.9	723	14	3.7	394	Paratyphi B var. L (+) tartrate+	
2016	4581	3	4.8	792	10	4.1	257	Anatum	

[a] Abbreviations: c = cases of salmonellosis; r = epidemiological rank of the serotype; and ZP = zoonotic potential.

scenario analysis was used to evaluate the effect of time on the distribution of *Salmonella* contamination (Pr_1, N_1, and ZP_1) among portions of CG at the start of MP in a local production chain.

A consumer survey was developed to obtain data for amount of CG consumed. However, it was not administered to consumers in the production chain. Therefore, in the absence of data for amount of CG consumed and to demonstrate the IC step of PFARM for *Salmonella* and CG, a single distribution of portion sizes that was based on expert opinion (the author's) was simulated using a DISCRETE distribution with probability of occurrences of 5, 15, 60, 15, and 5% for portion sizes of 56, 112, 168, 224, and 280 g, respectively (Fig. 1). Although the consumption pattern of CG could change over time in the production chain, one consumption pattern was simulated to control this variable and make it easier to interpret simulation results for *Salmonella* Pr_1, N_1, and ZP_1 over time in the production chain. Other distributions of portion size were simulated (results not shown) and yielded similar results and the same conclusions but are not presented to maintain clarity of presentation.

In PFARM, a fixed amount of poultry food is simulated, and the amount simulated is determined by the end-user. Here, for demonstration purposes, a lot size of 1000 kg of CG was simulated. Mean portion size for the simulated consumption pattern was 168 g (=(0.05 * 56) + (0.15 * 112) + (0.6 * 168) + (0.15 * 224) + (0.05 * 280)). Thus, the number of portions simulated was 5952 (1000 kg/0.168 kg) per lot.

A moving window of 60 samples was used to assess the effect of time on *Salmonella* contamination of CG at MP in the production chain. This resulted in five scenarios or time periods (Table 3): A) 1–6 weeks; B) 2–7 weeks; C) 3–8 weeks; D) 4–9 weeks; and E) 5–10 weeks. For clarity of presentation, from this point forward, these scenarios will be referred to as periods 1, 2, 3, 4, and 5, respectively.

Scenarios were simulated with @Risk settings of Latin Hypercube sampling, Mersenne Twister generator, initial seeds of 1, 2, 3, and 4, and 5952 iterations. Different initial seeds were used to replicate the simulations and to provide values of Pr_1, ZP_1, and N_1 for statistical analysis.

Data analysis

The mean rank from a nonparametric test (Kruskal-Wallis or K-W) was used to evaluate the distribution of *Salmonella* ZP_1 and N_1 among portions of CG at MP over time in the production chain (Steel and

Table 3
Data used to simulate the effect of time on *Salmonella* contamination of chicken gizzards at meal preparation

		Time (weeks)					
Salmonella		1 to10	1 to 6	2 to 7	3 to 8	4 to 9	5 to 10
Log number	Minimum	0.000	0.000	0.000	0.000	0.000	0.100
	Mode	0.417	0.417	0.417	0.417	0.706	0.706
	Maximum	2.788	2.788	2.788	2.163	1.475	2.345
Serotype	None	65.0	63.3	65.0	66.7	76.7	76.7
	Kentucky	16.0	23.3	26.7	25.0	10.0	5.0
	Infantis	9.0	1.7	3.3	1.7	5.0	13.3
	Enteritidis	6.0	5.0	0.0	1.7	5.0	5.0
	Typhimurium	3.0	5.0	5.0	5.0	3.3	0.0
	Thompson	1.0	1.7	0.0	0.0	0.0	0.0

Torrie, 1980). The K-W mean rank was determined as follows. Values of *Salmonella* ZP_1 or N_1 from a replicate simulation (initial seed of 1, 2, 3, or 4) of the five scenarios were combined, sorted from smallest to largest, and then ranked from first to last. The K-W mean rank for each scenario (time period) was then calculated as the sum of ranks divided by 5952. In addition to K-W mean ranks, total ZP_1 and total N_1 per replicate simulation of a scenario (time period) were calculated by summing all 5952 values.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the effect of time on *Salmonella* Pr_1, K-W mean ranks for ZP_1 and N_1, and total ZP_1 and N_1. When ANOVA was significant ($P \leq 0.05$), means were compared using Tukey's multiple comparison test at $P \leq 0.05$. All statistical analyses were conducted in Prism (GraphPad).

Results

Salmonella prevalence

A sample was positive for *Salmonella* when it tested positive by WSE-qPCR, culture isolation, and serotyping. Of 100 CG samples (56 g) examined, 35 tested positive for *Salmonella* (Table 1) for a Pr of 35% per 56 g (weeks 1–10 in Table 3). Over time (moving window of 60 samples), *Salmonella* Pr decreased from 36.7% to 35.0% to 33.3% to 23.3% to 23.3% per 56 g in periods 1, 2, 3, 4, and 5, respectively (sum of Pr for serotypes in Table 3).

Salmonella serotype prevalence

Five serotypes of *Salmonella* were isolated from CG in the present study (Table 1). They were Kentucky (Pr = 16% per 56 g), Infantis (Pr = 9% per 56 g), Enteritidis (Pr = 6% per 56 g), Typhimurium (Pr = 3% per 56 g), and Thompson (Pr = 1% per 56 g). Over time, the main serotype changed from Kentucky in period 1 to Infantis in period 5 (Table 3).

Zoonotic potential of Salmonella serotypes

Epidemiological data from CDC for human cases of salmonellosis (Table 2) were used to convert serotype to ZP. Most likely ZP were 1.1 for Kentucky, 4.4 for Infantis, 5.0 for Enteritidis, 4.9 for Typhimurium, and 3.7 for Thompson (Fig. 1).

Standard curve for Salmonella enumeration

The standard curve used to enumerate *Salmonella* on CG as a function of cycle threshold (C_T) values from WSE-qPCR (Y-axis) and inoculated dose of *Salmonella* (X-axis) is shown in Figure 3. It had an enumeration range from 0 to 4.7 log per 56 g of CG.

The C_T from the standard curve experiments varied among serotypes of *Salmonella* (Fig. 3). In general, they were higher for Kentucky than for Enteritidis and Typhimurium, which had similar C_T.

The standard curve was based on the highest C_T within an inoculated dose of *Salmonella*. This resulted in a fail-safe standard curve. The C_T used to develop the standard curve were from serotypes Typhimurium ($n = 1$), Kentucky ($n = 3$), and Enteritidis ($n = 1$) and are shown in Figure 3 as symbols with black dots in their centers.

Salmonella number

Among the 35 samples of CG that were contaminated with *Salmonella*, N_{log} ranged from 0 to 2.778 log with a median of 0.809 log per 56 g (Table 1). Minimum, mode, and maximum N_{log} changed over time in the production chain as shown in Table 3.

Potential temperature abuse

In week 3 (Table 1), all samples from packages A and B tested positive for *Salmonella* with N_{log} above the median (0.809 log/56 g) in 8 of 10 samples. Also, in week 10, all samples from package B tested positive for *Salmonella* with N_{log} above the median in 3 of 5 samples. Thus, 3 of 20 packages showed signs of potential temperature abuse before MP. This was captured in the data and simulated in PFARM.

Simulation results for Salmonella prevalence

Simulation results indicated that Pr_1 changed ($P \leq 0.05$) over time in the production chain (Fig. 6A) and from high to low by period was $1 > 2 > 3 > 4 = 5$. When simulation results for *Salmonella* Pr_1 (Fig. 6A) were compared to test results for *Salmonella* Pr (Table 3), the pattern of change over time was the same but Pr_1 was higher than Pr in all time periods. This occurred because mean portion size was 168 g for Pr_1 and 56 g for Pr. However, Pr_1 was not three times higher than Pr because *Salmonella* Pr increases in a nonlinear manner as a function of portion size (Oscar, 2019, 2020, 2021). Thus, it is important to express Pr as a function of the size of sample analyzed or simulated.

Simulation results for zoonotic potential of Salmonella

The K-W mean rank of ZP_1 (Fig. 6B) was affected ($P \leq 0.05$) by time and from high to low by period was $1 > 5 > 2 = 3 = 4$. Likewise, total ZP_1 (Fig. 6C) was affected ($P \leq 0.05$) by time and from high to low by period was $5 > 1 > 4 > 2 = 3$. These patterns over time in the production chain differed from each other and from those for Pr (Table 3) and Pr_1 (Fig. 6A). Thus, how *Salmonella* contamination of CG changed over time depended on the type of contamination and statistic used to evaluate it.

The ZP can be used to identify the serotype on a portion, and it can be used to identify portions with multiple serotypes. For example, in Figure 7, at a ZP of 0.7, the serotype was Kentucky, whereas at a ZP of 2.0, the portion was contaminated with serotype Kentucky and one or more of the other serotypes.

Figure 7 also shows that the main serotype shifted from Kentucky (low ZP) in period 1 to Infantis (high ZP) in period 5. The ability to identify the serotype on a simulated poultry food portion is a novel feature of PFARM that is possible because of the simulation method for ZP.

Simulation results for Salmonella number

Figure 8 shows the distribution of N_1 among portions of CG at MP over time in the production chain. Simulation results indicated that the K-W mean ranks for N_1 were affected ($P \leq 0.05$) by time and from high to low by period were $1 > 2 > 3 > 5 > 4$ (Fig. 6D). Likewise, total N_1 per lot of CG (Fig. 6E) was affected ($P \leq 0.05$) by time and from high to low was $1 > 2 > 5 > 3 > 4$. These patterns differed from each other and from those for Pr (Table 3), Pr_1 (Fig. 6A), and ZP_1 (Fig. 6B, C). Thus, how *Salmonella* contamination of portion of CG at MP changed over time in the production chain depended on the type of contamination and the statistic used to evaluate it.

Simulation results summary

Together, *Salmonella* test results for Pr and PFARM simulation results for Pr_1, ZP_1, and N_1 indicated that IC of portions of CG with *Sal-*

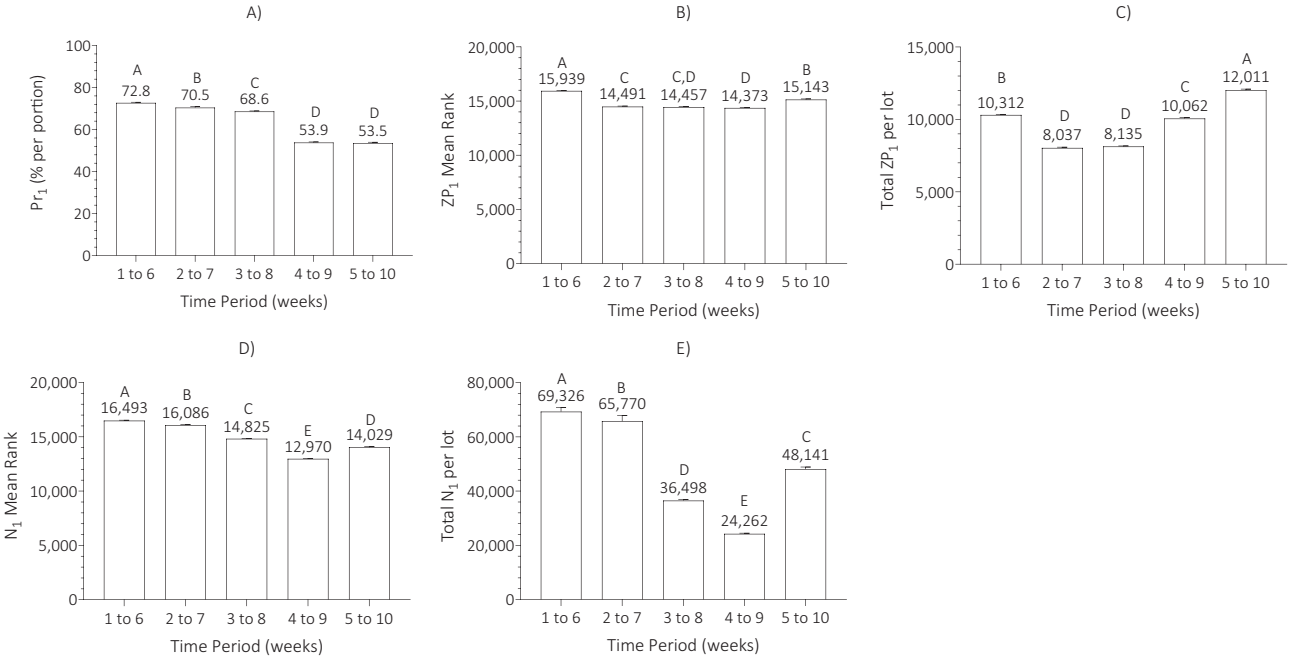


Figure 6. Simulation results of the Poultry Food Assess Risk Model (PFARM) for *Salmonella* and chicken gizzards (CGs) showing the effect of time on: A) *Salmonella* prevalence (Pr_1); B) Kruskal-Wallis (K-W) mean rank of zoonotic potential (ZP_1) of *Salmonella*; C) total ZP_1 of *Salmonella*; D) K-W mean rank of *Salmonella* number (N_1); and E) total *Salmonella* N_1 of CG at the start of meal preparation (MP). Bars are means ± standard deviations of four replicate simulations of PFARM for *Salmonella* and CG. Bars with different capital letters within a panel differ at $P \leq 0.05$ per one-way, analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

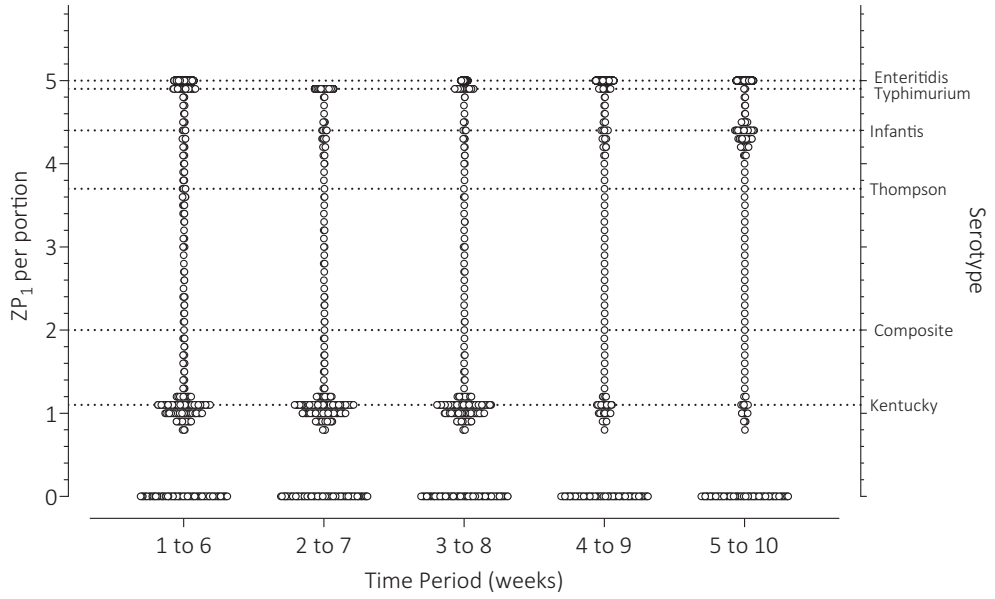


Figure 7. Simulation results of the Poultry Food Assess Risk Model (PFARM) for *Salmonella* and chicken gizzards (CGs) showing the effect of time on zoonotic potential (ZP_1) of portions of CG contaminated with *Salmonella* serotypes Enteritidis, Typhimurium, Infantis, Thompson, and Kentucky at the start of meal preparation (MP) in a single production chain. Results are for a single replicate simulation (initial seed = 1) of PFARM for *Salmonella* and CG. Horizontal lines are the most likely ZP for the indicated serotype of *Salmonella* and portions contaminated with two or more serotypes of *Salmonella* (i.e., Composite).

monella at the start of MP in the simulated production chain changed over time but the pattern of change depended on the type of *Salmonella* contamination examined and the statistic used to evaluate it. Thus, a PS based on one of these measures of *Salmonella* contamination will likely not be a good indicator of poultry food safety or risk of salmonellosis.

Discussion

A next step for PR/HACCP/PS in the poultry industry could be to add a PS for *Salmonella* N or serotype. However, like PS for *Salmonella* Pr, they may not reduce rates of salmonellosis because they do not consider other risk factors like MPP, FCB, and CHI. In addition, as shown

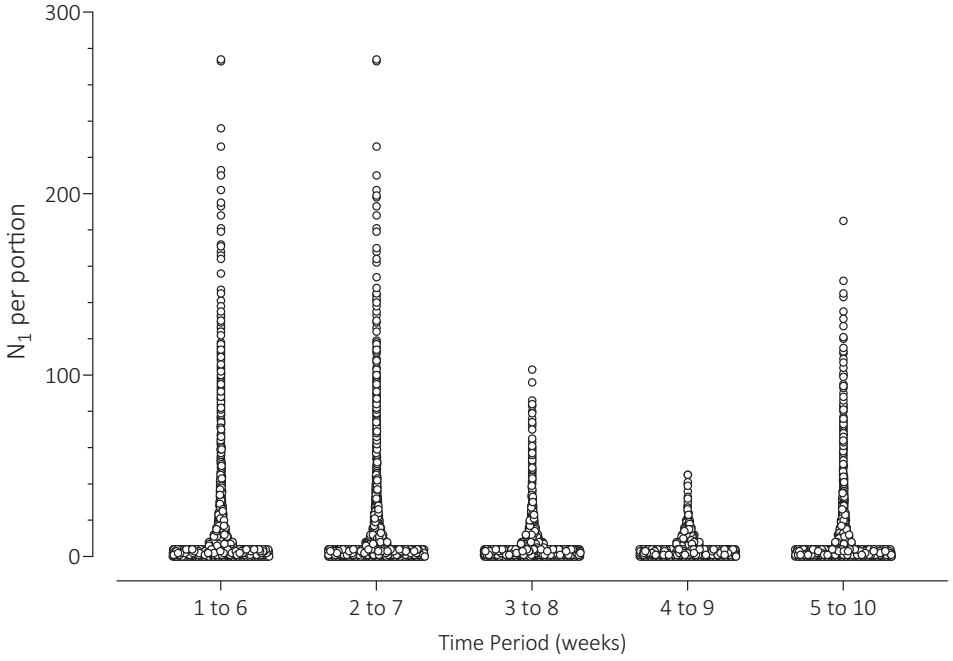


Figure 8. Simulation results of the Poultry Food Assess Risk Model (PFARM) for *Salmonella* and chicken gizzards (CGs) showing the effect of time on number (N_1) of *Salmonella* on portions of CG at the start of meal preparation (MP) in a single production chain. Results are for a single replicate simulation (initial seed = 1).

in the present study, Pr, N, and ZP (serotype) of *Salmonella* on CG changed in different ways over time in the production chain and their pattern of change depended on the statistic used to evaluate them. Therefore, a method like PFARM that integrates *Salmonella* Pr, N, and ZP, and MPP, FCB, and CHI into a single PS for risk of salmonellosis may be needed to properly identify higher risk production chains as part of the PR/HACCP/PS approach to poultry food safety.

Because PFARM predictions are only as good as the data and simulation methods used to make them, it is important to use data collection and modeling methods that provide the best possible data and simulation of consumer exposure and response to *Salmonella* contamination in the poultry food production chain. This has required, within the umbrella of the PFARM project circa 1995, modification of existing methods and the development of new methods for *Salmonella* testing and Monte Carlo simulation (MCS). Here, the data collection and MCS methods used in the IC step PFARM for *Salmonella* will be reviewed. This is a perspective review with considerable self-citation because the PFARM methods, although vetted through the peer-review process, have not been understood and adopted by others.

The best place to start is at the beginning. The first PFARM was a concept paper and conference proceeding published in 1997 (Oscar, 1997). It was a hatch-to-table PFARM for *Salmonella* and chicken that had 28 steps in the risk pathway. However, it was realized that this PFARM was not researchable because of the time, cost, and technical difficulties of deploying *Salmonella* testing of chicken at multiple points in the hatch-to-table pathway.

Therefore, in the next PFARM, which was for *Salmonella* and whole chickens (Oscar, 1998a), the IC step was conducted at one point in the risk pathway, which was at the processing plant exit. Here, it was envisioned that predictive models for *Salmonella* growth, survival, death, and cross-contamination could be used to determine how *Salmonella* Pr, N, and serotypes among whole chickens would change from the processing plant exit to the table. This PFARM was still a hatch-to-table model because the *Salmonella* Pr, N, and serotype data collected at the processing plant exit captured all previous steps in the hatch-to-processing plant exit portion of the risk pathway.

Subsequent PFARM for *Salmonella* and chicken or turkey (Oscar, 2004a, 2011, 2012a, 2012b, 2016, 2017c, 2018b, 2019, 2020) also performed the IC step at the processing plant exit or at retail. However, this changed when it was found (Oscar, 2017b) that when whole chickens sold in flow pack wrappers were temperature abused during cold storage, *Salmonella* grew and spread throughout the package resulting in unpredictable increases in Pr, N, and serotypes. This finding was contrary to the previous PFARM assumption that temperature abuse of packaged poultry food would only increase *Salmonella* N and not Pr or serotypes. Thus, in the present study, the IC step of PFARM was moved to the start of MP to capture and simulate the effect of previous temperature abuse on the distribution of *Salmonella* Pr, N, and ZP (serotype) among portions of CG.

Early in the PFARM project, it was realized that existing data collection methods were not designed to obtain the data needed to properly simulate consumer exposure and response to *Salmonella* contamination in the poultry food production chain. Notably, existing methods could not detect, enumerate, and isolate a single cell of *Salmonella* from the same sample or from any size of sample that had or did not have bones. Therefore, research was initiated to address these and other limitations of existing methods.

In 1995, when the PFARM project was initiated, the most widely used sampling method for *Salmonella* detection was the carcass rinse method (Bokanyi et al., 1990; Cox & Blankenship, 1975; D'Aoust et al., 1982; Jetton et al., 1992). This involved placing a chicken carcass in a plastic bag with 400 mL of BPW followed by shaking for one minute to recover *Salmonella* into BPW and then using a 30 mL aliquot of carcass rinse for culture isolation of *Salmonella* and determination of Pr.

Early studies with the carcass rinse method showed that a small percentage of bacteria are recovered using this method (Lillard, 1988) and that *Salmonella* Pr depended on the volume of carcass rinse used for culture isolation (Surkiewicz et al., 1969). In addition, *Salmonella* Pr was lower than that observed when the whole carcass sample was used for culture isolation (Simmons et al., 2003b). Thus, a modified version of the carcass rinse method was developed for PFARM.

In PFARM, after addition of 400 mL of BPW, the whole sample is incubated with shaking (continuous rinsing) for an extended time (6 h for enumeration and 22 h for culture isolation) instead of incubat-

ing an aliquot (30 mL) of sample rinse (1 min) for an extended time (24 h). This WSE method is used in PFARM because it can detect a single viable cell of *Salmonella* on (unattached in the surface water layer) or in (attached or entrapped) the sample (Oscar, 2004b, 2013a, 2014b). In addition, it can be linked to other methods like qPCR, culture isolation, and serotyping that provide information about *Salmonella* N and serotype on or in the boneless or bone-in sample.

The idea to use WSE-qPCR in PFARM to enumerate *Salmonella* on and in poultry food was from studies (Blackburn, 1991; Firstenberg-Eden, 1983; Gibson, 1988; Russell et al., 1995) that used impedance technology to enumerate bacteria in foods. Like WSE-qPCR, a standard curve is used in the impedance method to enumerate the target organism. However, instead of C_T, standard curves use detection time by impedance to enumerate the target organism in naturally contaminated samples. Impedance is not used in PFARM because it is not as specific for *Salmonella* as qPCR and cannot be used with bone-in samples like chicken legs.

Like other enumeration methods for *Salmonella*, WSE-qPCR is expensive. One way to reduce its' cost is to only use it at one point in the hatch-to-table chain. In the present study, enumeration data for *Salmonella* contamination of CG were only collected at the start of MP.

A second way to reduce the cost of WSE-qPCR is to collect data with one sample size and then use MCS to obtain data for other sample sizes (Oscar, 2004b, 2019, 2020, 2021). In the present study, data for *Salmonella* Pr, N, and ZP of CG were collected with one sample size (56 g) and then, MCS was used to obtain these data for other sample sizes (112, 168, 224, 280 g). In these ways, the IC step of PFARM was made more affordable.

Although the IC step for *Salmonella* and CG was done at the start of MP, the current PFARM still simulated the whole production chain because the data for *Salmonella* Pr, N, and ZP at MP captured all previous steps in the hatch-to-MP pathway. Thus, PFARM for *Salmonella* and CG is a hatch-to-table model that can be used to evaluate the effects of preharvest and postharvest interventions on poultry food safety. It just requires collection of *Salmonella* Pr, N, and ZP data for control and test scenarios at MP.

Implementation of PFARM at the start of MP presents the challenge of gaining access to samples in the consumer's kitchen. However, because poultry food can be stored at −20°C (temperature of a typical domestic freezer compartment) for up to 8 days without a change in *Salmonella* N or a change in subsequent growth kinetics (Oscar, 2013b, 2014a), a short delay between MP and sample collection could be accommodated and help with the logistics of implementing PFARM in the poultry food production chain. In addition, collection of samples for the IC step of PFARM for *Salmonella* could be coordinated with collection of consumer survey data for MPP, FCB, and CHI that are needed to simulate these risk factors in PFARM. Thus, logistics of implementing PFARM at MP should not only be possible but desirable as well.

Implementing PFARM at MP has advantages over implementing PFARM at the processing plant exit or at retail. Because the IC step of PFARM is closer to consumption, fewer steps need to be simulated and unpredictable changes in *Salmonella* Pr, N, and ZP due to temperature abuse in the cold chain can be captured in the data and simulated in PFARM. In addition, implementation at MP reduces the cost of data collection and simplifies the PFARM making it easier to understand and use. Furthermore, because the IC step for *Salmonella* is after the final partitioning event, the poultry food is in the form consumed making the simulation of consumer exposure and response more straightforward with fewer assumptions, which reduces the uncertainty of PFARM predictions for risk of salmonellosis.

The size of sample used in the IC step for *Salmonella* in PFARM is an important consideration. First, the sample needs to be large enough for *Salmonella* to be found at a reasonable rate so that *Salmonella* testing does not become too costly in terms of time, labor, and materials invested in processing negative samples. Second, the size of sample analyzed cannot be too large that it prevents a reasonable simulation of the amount poultry food consumed. For example, a 325 g sample is larger than a typical serving size and thus would increase rates of *Salmonella* detection but would not result in as realistic of a simulation of FCB and consumer exposure and response.

Other important considerations are how the *Salmonella* contamination data are expressed and simulated. In PFARM, *Salmonella* Pr and N data are expressed and simulated together as a function of the size of sample analyzed because they increase in a nonlinear manner as a function of sample size analyzed and serving size simulated (Oscar, 2019, 2020, 2021). In contrast, in the per-gram method, *Salmonella* Pr is expressed without a denominator and not simulated, and *Salmonella* N is expressed and simulated on a per-gram basis. Why is this important? To answer this question, consider the following example. Four samples (56 g) of CG were tested and found to harbor 0, 0, 0, and 56 *Salmonella* per 56 g or 0, 0, 0, and 1 *Salmonella* per g. If the portion size was 224 g, *Salmonella* N would be 56 per portion by the PFARM method and 224 per portion by the per-gram method. Thus, the PFARM method provides an accurate prediction of *Salmonella* N whereas the per-gram method does not because it falsely assumes a linear increase of *Salmonella* N as a function of portion size, and it does not simulate *Salmonella* Pr. In fact, the per-gram method is only accurate when the portion size is equal to the size of sample analyzed. A more detailed proof of these results using the one pathogen cell test can be found in a previous study (Oscar, 2021).

In the present study, the size of sample analyzed and simulated in PFARM was two chicken gizzards (56 g). This was a successful choice because *Salmonella* was found in 35 of 100 samples examined, which was enough positive results to define distributions for *Salmonella* N and serotype Pr over time in the production chain. Thus, the IC step for *Salmonella* and CG was not too costly in terms of time, labor, and materials used to process negative samples. Second, the sample size was small enough (56 g) that it allowed a realistic simulation of *Salmonella* contamination as a function of portion size from 56 to 280 g in increments of 56 g. A typical portion size for chicken is 100 g.

When using qPCR for enumeration, it is important to consider dead cells, which under the right conditions could inflate C_T values and enumeration results. In the present study, WSE-qPCR in 400 mL of BPW was used to enumerate *Salmonella* on and in CG. For *Salmonella* on and in CG to be detected and enumerated by this method, they would have to be released from CG into BPW followed by growth to the limit of detection (LOD) of the qPCR method, which is 2 log per mL (Lauer, 2015; Lauer et al., 2009). Because dead cells do not grow, it would require a high initial number (>4.6 log per 56 g) on and in CG for them to be detected and quantified by qPCR. The highest number of native *Salmonella* observed in this study was 2.8 log per 56 g of CG, making the quantification of dead cells an unlikely scenario. Thus, because WSE-qPCR is a growth-based assay and because dead cells do not grow, it would require extraordinary circumstances for dead cells of *Salmonella* to be enumerated by this method (Kramer et al., 2011).

Salmonella growth during WSE occurs in three phases as follows: 1) lag; 2) exponential; and 3) stationary (Rhodes et al., 1985). During exponential phase, there is a mathematical relationship between the inoculated dose of *Salmonella* and C_T from qPCR, whereas during lag and stationary phases, there is not (Kramer et al., 2011). For example, if poultry food samples were inoculated with 0, 1, 2, 3, 4, and 5 logs of *Salmonella* and then incubated for 6 h at 40°C in 400 mL of BPW and lag phase of *Salmonella* was 1 h and the growth rate of *Salmonella* was 1 log/h (Oscar, 1998b, 1999c, 2018a), after 6 h of WSE, the concentration of *Salmonella* in BPW would be 2.4, 3.4, 4.4, 5.4, 6.4, and 7.4 log/mL, respectively. Thus, all samples would reach the LOD of qPCR and generate a C_T value and there would be a mathematical relationship between dose of *Salmonella* inoculated and C_T from qPCR.

In contrast, if WSE was 1 h, the concentration of *Salmonella* in BPW would be <0, <0, <0, 0.4, 1.4, and 2.4 log per mL. Thus, only the

highest inoculated dose of *Salmonella* would reach the LOD of qPCR and generate a C_T value resulting in insufficient data for a standard curve.

On the other hand, if WSE was 24 h, the concentration of *Salmonella* would be the same for all inoculated doses and equal to the maximum population density. Thus, all samples would reach the LOD of qPCR and generate a similar C_T value and thus, there would not be a mathematical relationship between C_T and the inoculated dose of *Salmonella*. Therefore, when using WSE-qPCR to enumerate *Salmonella*, it is important to collect WSE samples for qPCR during the exponential phase of growth.

In the present study and based on previous studies (Oscar, 2016, 2017c, 2019, 2020, 2021), samples for qPCR were collected at 6 h of WSE, and a nonlinear mathematical relationship was observed between C_T from qPCR and dose of *Salmonella* inoculated. This relationship was used to develop a standard curve for the enumeration of native *Salmonella* on and in CG as a function of C_T from qPCR. The standard curve had a range of enumeration from 0 to 4.7 log per 56 g that was broad enough to enumerate levels of native *Salmonella* found on all CGs examined, which had *Salmonella* N that ranged from 0 to 0.809 (median) to 2.788 log per 56 g.

An important feature of the IC step of PFARM for *Salmonella* is that data collected with one sample size can be used to obtain data for other sample sizes (Oscar, 2004b, 2019, 2020, 2021). This is accomplished using Monte Carlo simulation (MCS) and a rare event modeling method. In this way, the lower and upper limits of enumeration are extended to larger sample sizes. Also, although it was not done in this study, MCS results can be used to develop nonlinear regression models that predict *Salmonella* N at continuous sample sizes (Oscar, 2021). In other words, at increments <56 g. Thus, WSE-qPCR-MCS method is a robust and low-cost method for predicting *Salmonella* Pr, N, and ZP as a function of portion size or amount of poultry food consumed.

The IC step of PFARM for *Salmonella* (Fig. 1) is versatile. It has been used for samples that range in size from 25 g (ground chicken) to 375 g (chicken breast) (Oscar, 2016, 2017c). In addition, it has been applied to both boneless (Oscar, 2019, 2020, 2021) and bone-in chicken parts (Oscar, 2013a, 2014b, 2016, 2017c). Yet, it is not a perfect method. Here, a brief review is provided of what has been done in the PFARM project to develop and improve this method and what can be done in the future to make it an even better method.

Effects of size (25–375 g) and type (boneless, bone-in, ground, byproduct) of poultry food (chicken parts, ground chicken and turkey, chicken liver, CG), serotype, and native microflora (NM) on the growth of *Salmonella* during WSE and resulting standard curves for enumeration by qPCR or other methods (viable counts, PCR) have been investigated in the PFARM project (Oscar, 2004b, 2008, 2013a, 2014b, 2016, 2017c, 2019, 2020, 2021). Thus far, the size and type of poultry food have not affected standard curves for enumeration (Oscar, 2004b, 2014b). If this holds, it may be possible to use one standard curve for multiple sizes and types of poultry food, which would save considerable time and money.

In contrast, differences in standard curves among serotypes of *Salmonella* occur (Oscar, 2013a, 2016). In the present study, C_T for serotype Kentucky were, in general, higher than C_T for serotypes Typhimurium and Enteritidis, which were similar, indicating less growth of serotype Kentucky during WSE. This agrees with other studies in the PFARM project (Oscar, 1998b, 2000, 2003, 2013a) in which the growth of *Salmonella* on poultry food segregates into two general groups: slower-growing serotypes (Kentucky, Enteritidis, 8, 20:-:z_6) and all others.

Producing standard curves for all serotypes isolated from the poultry food of interest could be time-consuming and cost-prohibitive and it may not result in better results because other factors (native microflora, previous history) also may affect the standard curve. One approach that might work and be time- and cost-effective would be to use a slow-growing reference strain, like the isolate of serotype Kentucky in the present study, to produce a fail-safe standard curve for the enumeration of *Salmonella*.

Salmonella enumeration by WSE-qPCR is affected by native microflora (NM), which change standard curves from linear to nonlinear (Oscar, 2004b). Also, high NM increase C_T (less growth of *Salmonella* during WSE) for chicken parts (Oscar, 2017c), which agrees with studies (Beckers et al., 1987; Jameson, 1962; Rhodes et al., 1985) showing NM reduce the growth of *Salmonella* during WSE. Thus, the inclusion of NM as an independent variable in future standard curves may improve the method by reducing uncertainty in standard curve predictions.

Native *Salmonella* affect C_T and standard curves for enumeration by WSE-qPCR. When levels of native *Salmonella* are high (Oscar, 2017c), larger variations of C_T occur at low (<2 log/chicken part) than at higher inoculated doses (>2 log/sample) of *Salmonella* indicating that native *Salmonella* can significantly lower C_T and create a fail-dangerous standard curve. This can be understood by considering that 10 cells of native *Salmonella* would represent 90.9% of *Salmonella* on samples inoculated with 0 log of *Salmonella* but only 0.99% cells on samples inoculated with 3 logs of *Salmonella*. Thus, the 10 cells of native *Salmonella* would change C_T more at the low than at the high dose of inoculated *Salmonella*.

Previous history affects the physiological state of *Salmonella* and growth during WSE (Oscar, 1999a, 1999b, 1999c, 2013b, 2018a). Thus, it likely affects C_T, and standard curves for the enumeration of *Salmonella* by WSE-qPCR (Kramer et al., 2011). For example, injury of *Salmonella* extends lag phase and likely increases C_T from WSE-qPCR (Mackey & Derrick, 1982). Effect of previous history on enumeration of *Salmonella* by WSE-qPCR needs to be investigated further and perhaps included in future protocols by including an adaptation step prior to WSE-qPCR to standardize physiological states of native and inoculated *Salmonella*.

On the other hand, the physiological state of native *Salmonella* may affect ZP. Thus, it may be better not to standardize the physiological state in the suggested manner. For instance, if *Salmonella* cells in a sample were injured (lower ZP), their lag phase would be extended, their C_T would be higher, their number would be underestimated, and this would indirectly capture the lower ZP. Thus, by using a growth-based assay (WSE-qPCR) in PFARM, effects of the poultry production chain on *Salmonella* fitness (physiological state/ZP) are captured and simulated in the enumeration data, which is a desirable feature for risk assessment.

In summary, previous studies (Oscar, 2004b, 2008, 2013a, 2014b, 2016, 2017c, 2019, 2020, 2021) indicate that when all other variables are constant, highest C_T within an inoculated dose of *Salmonella* during standard curve development for enumeration by WSE-qPCR is associated with the slowest growing serotype, highest level of NM, lowest level of native *Salmonella*, and longest lag time. Thus, use of the highest C_T within inoculated doses is the current approach used in PFARM and results in a fail-safe standard curve for enumeration of *Salmonella* by WSE-qPCR. Although results are biased in the fail-safe direction, it is better to err in this direction when using data and models (standard curves) to predict poultry food safety (Ross, 1996). However, if this method is used across poultry production chains, the relative comparisons would not be biased.

The serotype of *Salmonella* that contaminates a poultry food is important information for the simulation of consumer response to *Salmonella* exposure. Here, the culture isolation and serotyping methods used in PFARM are briefly reviewed and reasons for using them instead of traditional methods are explained along with ideas for improving them.

Culture isolation of *Salmonella* from poultry food is time-consuming, labor-intensive, and expensive. Some ways to make it more affordable are to use fewer media, lower volumes of media, and process fewer presumptive colonies per sample. Thus, instead of two selective broths and two selective agars, one selective broth

(RV) and one selective agar (XLT4) are used in the PFARM method and instead of 10 mL of selective broth, 1 mL of selective broth is used in the PFARM method. Also, instead of five presumptive colonies per plate, one presumptive colony per plate is tested in the PFARM method and instead of two confirmation media, one rapid test is used in the PFARM method (Oscar, 2014b).

Savings of time, labor, and materials from this reductive approach to culture isolation of *Salmonella* in the IC step of PFARM increases the number of samples analyzed, reduces experimental error by using a less complex method, and thus, increases rather than decreases the amount and quality of data obtained, and increases rather that decreases probability of detecting more rather than less serotypes in the poultry food of interest.

On rare occasion in the present study, two morphologies of presumptive colonies of *Salmonella* were observed on an XLT4 plate. Frequency of picking of the different morphologies depended on their relative abundance among samples in the set. This method can be improved in the future by picking all colony morphologies on a plate and recording and then simulating their relative abundance. This will result in a small but important improvement in the simulation of *Salmonella* serotype Pr in PFARM.

Enrichment bias occurs when the enrichment media provides conditions that favor the growth of one *Salmonella* serotype over another. However, for it to occur, the sample must be contaminated with two or more serotypes of *Salmonella*. In previous studies (Oscar et al., 2010; Parveen et al., 2007), when samples were obtained from local poultry production chains, only one or two serotypes of *Salmonella* were isolated. In the present study, when CGs were obtained from the same local production chain over time, most packages of CG were contaminated with only one or two serotypes of *Salmonella*. Thus, contamination of CG with two or more serotypes of *Salmonella* was a rare event at best.

Enrichment bias also requires that the serotypes present in the same sample have different growth kinetics in the enrichment media. However, most serotypes of *Salmonella* have similar growth kinetics in different matrices (Oscar, 1998b, 2000, 2015). In the present study, the slower-growing *Salmonella* serotype Kentucky was the predominant serotype isolated from CG, which would not be expected if enrichment bias was a major event.

Enrichment bias at whatever level it occurred was not viewed as a problem in the present study. Rather, it was viewed as acceptable because it would have resulted in a fail-safe prediction of poultry food safety because the fastest growing and most fit serotype in the sample would have been isolated and simulated in PFARM.

Knowing the serotypes of *Salmonella* present in a poultry food is important in the ID step of PFARM. However, it took about 30 days to obtain these data from an outside laboratory. In the future, it may be possible to obtain serotyping data in a timelier manner using an in-house method like automated ribotyping (Oscar, 1998c), light scattering sensor (Abdelhaseib et al., 2019), or CRISPR-SeroSeq (Rasamsetti et al., 2022).

An important feature of the PFARM method demonstrated in the present study and previous studies (Oscar, 2019, 2020, 2021) was the simulation of portions of CG that were contaminated with multiple serotypes of *Salmonella*. This was accomplished using a rare event modeling method and calculation of a composite value for ZP of *Salmonella* that was based on the number of each serotype present in the portion and their ZP. This is an important and novel feature of PFARM.

The PFARM for *Salmonella* is a work in progress. Improvements reported for the first time in the current study were as follows: 1) moving the IC step of PFARM for *Salmonella* to the start of MP; 2) use of the K-W mean rank statistic to evaluate the distribution of *Salmonella* N and ZP among portions of CG at MP; 3) acquisition of new data for *Salmonella* Pr, N, and ZP (serotype) of CG; and 4) simulation of *Salmonella* Pr, N, and ZP of CG over time in a production chain.

Important findings in this study were that *Salmonella* Pr, N, and ZP (serotype) of CG changed over time in the simulated production chain but how they changed depended on the type of contamination (Pr, N, ZP) and the statistic (K-W mean rank, percentage, total sum) used to evaluate it. Thus, results indicated that a PS based on *Salmonella* Pr, N, or ZP (serotype) alone will not likely be a good indicator of poultry food safety. Rather, a method like PFARM may be needed to predict a single PS (risk of salmonellosis) that is based on multiple risk factors (Pr, N, ZP, MPP, FCB, and CHI).

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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