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Use of Enrichment Real-Time PCR To Enumerate *Salmonella* on Chicken Parts†

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ABSTRACT

Salmonella bacteria that survive cooking or that cross-contaminate other food during meal preparation and serving represent primary routes of consumer exposure to this pathogen from chicken. In the present study, enrichment real-time PCR (qPCR) was used to enumerate *Salmonella* bacteria that contaminate raw chicken parts at retail or that cross-contaminate cooked chicken during simulated meal preparation and serving. Whole raw chickens obtained at retail were partitioned into wings, breasts, thighs, and drumsticks using a sterilized knife and cutting board, which were then used to partition a cooked chicken breast to assess cross-contamination. After enrichment in buffered peptone water (400 ml, 8 h, 40°C, 80 rpm), subsamples were used for qPCR and cultural isolation of *Salmonella*. In some experiments, chicken parts were spiked with 0 to 3.6 log of *Salmonella* Typhimurium var. 5− to generate a standard curve for enumeration by qPCR. Of 10 raw chickens examined, 7 (70%) had one or more parts contaminated with *Salmonella*. Of 80 raw parts examined, 15 (19%) were contaminated with *Salmonella*. Of 20 cooked chicken parts examined, 2 (10%) were cross-contaminated with *Salmonella*. Predominant serotypes identified were Typhimurium (71%) and its variants (var. 5−, monophasic, and nonmotile) and Kentucky (18%). The number of *Salmonella* bacteria on contaminated parts ranged from one to two per part. Results of this study indicated that retail chicken parts examined were contaminated with low levels of *Salmonella,* which resulted in low levels of cross-contamination during simulated meal preparation and serving. Thus, if consumers properly handle and prepare the chicken, it should pose no or very low risk of consumer exposure to *Salmonella*.

Growth-based systems (e.g., impedance) can be used to detect and enumerate total bacteria and specific bacteria (e.g., *Escherichia coli*) on chicken *(5)*. These systems operate on the principle that bacteria, as they grow in liquid media, produce chemical changes that can be detected as an optical or electrical signal. Before a signal can be detected, bacteria must grow to a level of 6 to 7 log cells per ml *(7)*.

In growth-based assays for enumeration, detection time (DT) is inversely related to the initial number of bacteria in the sample *(6)*. Standard curves that relate bacterial plate counts to DT under standard conditions in a growth-based system can be used to convert DT of unknown samples into bacterial counts *(2)*. Bacterial counts obtained in this manner are relative to DT, which, in turn, is a function of lag time and generation time of reference bacteria used to develop the standard curve *(7)*.

The current approach used in the chicken industry to test for *Salmonella* is to rinse the whole carcass for 1 min with 400 ml of buffered peptone water (BPW) and then analyze an aliquot (30 ml) of the rinse sample for the pathogen. Because the rinse aliquot (RA) method does not recover all of the *Salmonella* bacteria on the carcass *(12),* use of this method in growth-based assays would result in an underestimation of prevalence and number of *Salmonella* bacteria.

A more complete recovery of *Salmonella* from chicken can be realized by whole carcass enrichment (WCE) in BPW *(26)*. Although WCE is not compatible with current growth-based enumeration systems on the market, it can be used to detect and enumerate *Salmonella* by developing a standard curve that relates concentration of *Salmonella* in BPW at an early time of enrichment (6 h) to initial number of *Salmonella* spiked onto the sample *(19)*.

Malorny et al. *(15)* demonstrated that there is a linear relationship between concentration of *Salmonella* in BPW and cycle threshold (C_T) value for detection of *Salmonella* by real-time PCR (qPCR). Thus, it should be possible to use enrichment qPCR rather than plate counts to develop a standard curve for enumerating *Salmonella* on chicken parts. In fact, an enrichment qPCR approach has been used to enumerate *Campylobacter* in chicken rinse samples *(8)*.

Salmonella bacteria that survive cooking or that cross-contaminate other food during meal preparation and serving represent primary routes of consumer exposure to

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this pathogen from chicken. Consequently, the present study was undertaken to use enrichment qPCR to detect and enumerate *Salmonella* that contaminate raw chicken parts at retail or that cross-contaminate cooked chicken during simulated meal preparation and serving.

MATERIALS AND METHODS

Stock culture. The standard curve for enumeration was developed with *Salmonella enterica* serotype Typhimurium var. 5−, which was the most common serotype isolated in the current study. Stock cultures of this organism were maintained at −80°C in brain heart infusion broth (BD, Sparks, MD) that contained 15% (vol/vol) glycerol (Sigma, St. Louis, MO).

Chicken parts. Whole broiler chickens (1,958 ± 132 g; $n = 12$) from a single processing plant were obtained at retail from 8 February to 9 May 2012. They were partitioned (one per week), using a sterilized knife and cutting board, into wings (without tips), boneless breasts (with skin), thighs, and drumsticks. The unwashed cutting board, knife, and latex gloves were immediately used to partition an autoclaved (121°C for 15 min; BioClave, Biomega Research Products, Edison, NJ) chicken breast into two parts. This was done to study cross-contamination of cooked chicken with *Salmonella* from raw chicken during simulated meal preparation and serving.

Chicken part inoculation. To obtain cells of *Salmonella* Typhimurium var. 5− for inoculation of chicken parts, 5 μl of stock culture was added to 9 ml of BPW (BD) in a dilution tube with cap. After incubation (22°C, 72 h, 0 rpm), serial dilutions (1:10) of the culture were prepared in BPW. Raw chicken parts were then surface inoculated with 5 μl of 10^{-3}, 10^{-4}, 10^{-5}, or 10^{-6} dilutions of the culture.

Each run of the experiment consisted of 10 samples (eight raw chicken parts and two cooked chicken breast parts); over the course of two runs, each type of raw chicken part was inoculated once with each serial dilution of the culture. In addition, the cooked chicken breast parts were inoculated with 5 μl of the 10^{-7} dilution of the culture in each run of the experiment. Before transfer to plastic bags (177 by 304 mm; Seward, London, UK), inoculated chicken parts were held at room temperature for 30 min to allow attachment of *Salmonella*.

Concentration of *Salmonella* in the undiluted culture was determined by spiral plating (Whitley automatic spiral plater, Microbiology International, Frederick, MD) 50 μl of the 10^{-5} and 10^{-6} dilutions onto xylose lysine Tergitol 4 (XLT4) agar (BD). Spiral plates were incubated for 24 h at 40°C before automated counting (ProtoCol, Microbiology International) of colonies.

Number of *Salmonella* bacteria inoculated onto cooked chicken breast parts from the 10^{-7} dilution was determined by drop plating 5-μl samples ($n = 13$ per run) onto XLT4. Drop plates were incubated for 24 h at 40°C before manual counting of colonies.

Whole part enrichment. Four hundred milliliters of 40°C BPW was added to chicken parts in plastic bags, and then samples were incubated (Innova 4230, New Brunswick Scientific, Edison, NJ) for 8 h at 40°C and 80 rpm. At the end of enrichment, a 1-ml sample was collected into a 1.5-ml centrifuge tube (sample A) and a 1-ml sample was collected into a dilution tube with 9 ml of BPW (sample B). Sample A was used for qPCR, and sample B was used for *Salmonella* isolation.

Real-time qPCR. An AOAC International–approved qPCR kit (iQ-Check *Salmonella* II, Bio-Rad, Hercules, CA) was used, per manufacturer's instructions. In brief, ''A'' samples were centrifuged for 5 min at 11,000 rpm (Mini Spin Plus, Eppendorf, Hamburg, Germany), supernatant was removed, and then 200 μl of lysis reagent with glass beads was added to cell pellets. Next, samples were vortexed for 3 min at 3,000 rpm (Disrupter Genie, Scientific Industries, Bohemia, NY) and heated for 10 min at 95°C (ThermoStat Plus, Eppendorf). After centrifugation (5 min, 11,000 rpm), 5 μl of supernatant per sample was added to 45 μl of PCR mix. The qPCR reaction, which included internal standards and positive and negative controls, was performed in a thermal cycler (Mini Opticon, Bio-Rad) per manufacturer's protocol. When a sample was positive for *Salmonella,* a C_T value was obtained.

***Salmonella* isolation.** After ''B'' samples were incubated for 24 h at 40°C, 100 μl was transferred to 10 ml of Rappaport Vassiliadis broth (BD), which was then incubated (42°C, 23 h, 0 rpm). After incubation, 200 μl of Rappaport Vassiliadis broth culture was tested for *Salmonella* using an AOAC International–approved lateral flow assay (Reveal 2.0, Neogen, Lansing, MI). Samples that tested positive for *Salmonella* were serially diluted (1:10) in BPW, and then 50-μl aliquots of the 10^{-4} and 10^{-5} dilutions were spiral plated onto XLT4 agar. After incubation (40°C, 24 h), a suspect colony (black) of *Salmonella* was picked, regrown, and sent to a reference laboratory (U.S. Department of Agriculture, Animal and Plant Health Inspection Service, National Veterinary Services Laboratory, Ames, IA) for identification by serotyping.

Standard curve for enumeration. The C_T obtained by qPCR (Y) was graphed as a function of the number of *Salmonella* bacteria spiked onto chicken parts (X; log CFU per part). The resulting curve was fitted (Prism 6 for Windows, GraphPad, San Diego, CA) to the following equation:

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

where Y_0 is the predicted C_T at 0 log CFU per part, a is a regression coefficient, and b is a shape parameter. The interpolation function of Prism was used to convert C_T for unknown samples into log CFU of *Salmonella* per chicken part; each value was then converted to its antilog and rounded to the nearest whole number.

RESULTS

Experiment 1. To establish the relationship between C_T obtained by qPCR and concentration of *Salmonella* in BPW (log CFU per milliliter), an experiment consisting of two runs was conducted. In this experiment, cultures of *Salmonella* Typhimurium var. 5− (8.82 ± 0.06 log CFU/ml [mean ± SD]; $n = 2$) were serially diluted (1:10) in BPW to 10^{-6} (run 1) or 10^{-9} (run 2). One-milliliter samples (A and B) of each dilution were collected and subjected to qPCR and lateral flow assays as described under ''Materials and Methods.'' The sample from the 10^{-9} dilution was negative for *Salmonella* by qPCR and lateral flow assays, whereas all other samples (10^0 to 10^{-8} in 10^{-1} increments) were positive for *Salmonella* by both assays.

A graph (Fig. 1) of C_T versus concentration (log CFU per milliliter) of *Salmonella* Typhimurium var. 5− in BPW resulted in a slightly concave line, with Y_0 (mean ± SE) = 40.97 ± 0.31, $a = 0.611 \pm 0.047$, shape parameter (b) = 1.23 ± 0.032, and coefficient of determination (R^2) = 0.999.

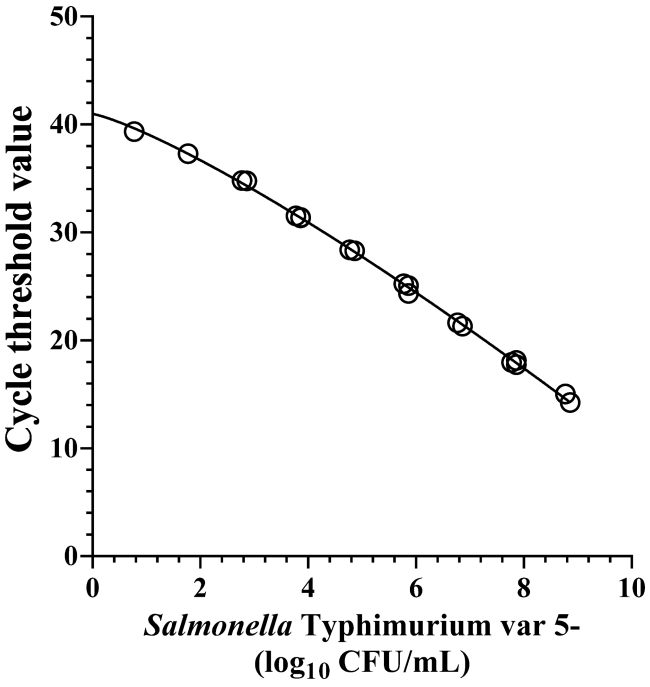

FIGURE 1. *Standard curve relationship for cycle threshold value as a function of the concentration of* Salmonella *Typhimurium var. 5− in buffered peptone water.*

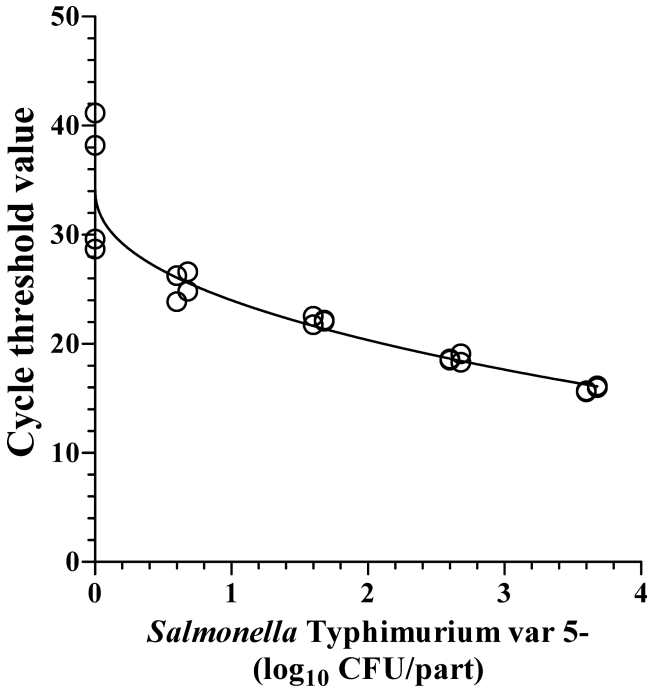

FIGURE 2. *Standard curve relationship for cycle threshold value as a function of the dose of* Salmonella *Typhimurium var. 5− per chicken part.*

There was close agreement between replicate runs of the experiment. Coupled with subsequent results (see below) in which C_Ts up to 43.39 were observed for culture-confirmed *Salmonella*-positive samples, it was concluded that the qPCR assay was capable of detecting a single CFU of *Salmonella* Typhimurium var. 5− in a 1-ml sample of BPW.

Experiment 2. The next experiment was designed to develop a standard curve for enumeration of *Salmonella* on chicken parts. In this experiment, whole chickens were obtained at retail and were partitioned into eight raw parts, as described under ''Materials and Methods.'' Next, raw parts were inoculated with 5 μl of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions of the culture (8.96 ± 0.06 log CFU/ml [mean ± SD]; $n = 2$) of *Salmonella* Typhimurium var. 5−. Over the course of two runs, each type of raw chicken part (wings, breasts, thighs, drumsticks) was inoculated once with the 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions. The mean calculated number of *Salmonella* Typhimurium var. 5− inoculated onto raw chicken parts was 3.64, 2.64, 1.64, and 0.64 log CFU for 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions, respectively.

After enrichment (40°C, 8 h, 80 rpm) in 400 ml of BPW, 1-ml samples (A and B) were collected and subjected to qPCR (sample A) and lateral flow (sample B) assays, as described under ''Materials and Methods.'' The C_Ts (mean ± SD; $n = 4$) for the 0.64, 1.64, 2.64, and 3.64 log CFU per part inoculation levels were 25.4 ± 1.3, 22.1 ± 0.3, 18.6 ± 0.3, and 15.8 ± 0.3, respectively (Fig. 2). These C_Ts corresponded to final concentrations of *Salmonella* Typhimurium var. 5− of 5.71, 6.67, 7.66, and 8.44 log CFU/ml, as interpolated from the standard curve in Figure 1. All ($n = 16$) ''B'' samples in this experiment tested positive for *Salmonella* in the lateral flow assay.

The C_Ts for each inoculation level ($n = 4$) were obtained with four different types of raw chicken parts (wing, breast, thigh, and drumstick). Although raw chicken parts differed in weight (Table 1) and were incubated in the same volume (400 ml) of BPW, there was little variation in C_T within an inoculation level (Fig. 2). In fact, coefficients of variation (CVs; mean/SD) for the 0.64, 1.64, 2.64, and 3.64 log CFU doses were 5.0, 1.5, 1.8, and 1.6%, respectively. These results indicated that type of chicken part had little effect on qPCR results. This finding also suggested that the type of chicken part does not contribute significantly to the total volume of the enrichment. If it did, then one would expect C_T to be lower for larger chicken parts because of a dilution effect. These results agree with those of previous studies *(18, 19),* in which type of chicken part did not affect standard curves in growth-based assays involving chicken sample enrichment.

As another component of this experiment, four cooked chicken breast portions ($n = 2$ per run) were inoculated with 5 μl of the 10^{-7} dilution (1.96 log CFU/ml or 91 CFU/ml) of the culture of *Salmonella* Typhimurium var. 5−. The calculated CFU of *Salmonella* Typhimurium var. 5− in the inoculation volume was 0.455 CFU/5 μl. Thus, not all of these inoculations were expected to result in contamination of cooked chicken parts with the pathogen.

Results of the drop plate assay described under ''Materials and Methods,'' which simulated the spiking protocol, indicated that 17 (65%) of 26 of these simulated inoculations would result in no *Salmonella* Typhimurium var. 5− being inoculated, whereas 9 (35%) of 26 of these simulated inoculations would result in 1 CFU of *Salmonella* Typhimurium var. 5− being inoculated. Thus, it was expected that one or two of the four parts of cooked chicken

TABLE 1. *Prevalence, number, and serotypes of* Salmonella *on chicken parts obtained at retail*[a]

Part	Wt (g)		Prevalence, % (no./total no.)			No.		Serotypes
	Mean	SD	qPCR	LF	XLT4	C_T	CFU/part	
Wing	85	8	20 (4/20)	20 (4/20)	15 (3/20)	36, 35, 30, 39	1, 1, 1, 1	Typhimurium (2); 4,5,12:nonmotile (1); not identified (1)
Breast	260	27	25 (5/20)	25 (5/20)	20 (4/20)	32, 28, 31, 42, 33	1, 2, 1, 1, 1	Typhimurium (1); 4,5,12:nonmotile (1); Typhimurium var. 5− (1); 4,12:i:− (1); not identified (1)
Thigh	138	15	15 (3/20)	15 (3/20)	15 (3/20)	40, 31, 31	1, 1, 1	Typhimurium var. 5− (1); Kentucky (1); 4,12:nonmotile (1)
Drumstick	107	12	15 (3/20)	15 (3/20)	15 (3/20)	35, 32, 30	1, 1, 1	Typhimurium var. 5− (2); Kentucky (1)
Cooked	86	16	10 (2/20)	10 (2/20)	10 (2/20)	30, 34	1, 1	Kentucky (1); 4,12:i:− (1)

[a] SD, standard deviation; qPCR, real-time PCR; LF, lateral flow; C_T, cycle threshold.

breast in this experiment would be positive for *Salmonella*. However, the cooked chicken breasts used in this experiment were partitioned into two parts using the knife, cutting board, and latex gloves that had been used to partition the raw chicken. Thus, it was possible that they could have been cross-contaminated with *Salmonella* from raw chicken.

After enrichment (40°C, 8 h, 80 rpm) of cooked chicken breast parts in 400 ml of BPW, 1-ml samples (A and B) were collected and subjected to the qPCR (sample A) and lateral flow (sample B) assays as described under ''Materials and Methods.'' All four samples tested positive for *Salmonella* by qPCR, with C_Ts of 41.15, 29.59, 28.67, and 38.18. These C_Ts corresponded to final concentrations of *Salmonella* Typhimurium var. 5− in the samples of 0.00, 4.42, 4.71, and 1.41 log CFU/ml, as interpolated from the standard curve in Figure 1. However, only two of four ''B'' samples tested positive for *Salmonella* in the lateral flow assay. The two samples with the highest C_Ts (41.15 and 38.18) were negative for *Salmonella* in the lateral flow assay.

To construct the standard curve for enumeration of *Salmonella* on chicken parts, it was assumed that type of chicken part did not affect C_T, as discussed above, and based on results of the drop plate assay, it was assumed that the dose of *Salmonella* Typhimurium var. 5− inoculated onto cooked chicken breast portions that tested positive for *Salmonella* (in this case all four) was 0 log CFU. The resulting standard curve (Y_0 [mean ± SE] = 34.34 ± 1.355, a = 0.004677 ± 0.007518, and b = 0.4357 ± 0.1046) had high goodness of fit, except for the region corresponding to the lowest dose (0 log CFU) of inoculation, where variation of C_T among samples was high (CV = 18%) resulting in an R^2 of 0.8675.

A tailing of standard curves at high DT or, in this case, high C_T is a common observation in growth-based assays *(4)*. A possible explanation is that, at low inoculation sizes, variation of lag time among individual cells is high and results in high variation of DT *(2)* or, in this case, C_T. In fact, Koutsoumanis and Lianou *(10)* reported that variation of lag times among individual cells of *Salmonella* increases as the initial population size decreases from 100 cells to 1 cell.

Experiment 3. In the final experiment, the standard curve (Fig. 2) was used to enumerate *Salmonella* bacteria on chicken parts. In this experiment, 10 whole chickens obtained at retail (one per week) were partitioned into eight raw parts, as described under ''Materials and Methods.'' In addition, two cooked chicken breast parts were included in each run (n = 10) of the experiment to characterize cross-contamination of cooked chicken with *Salmonella* from raw chicken during simulated meal preparation and serving. Again, two 1-ml samples (A and B) were collected after whole part enrichment (40°C, 8 h, 80 rpm) in 400 ml of BPW. ''A'' samples were used for qPCR, and ''B'' samples were used for the lateral flow assay as well as for *Salmonella* isolation and identification by serotyping.

Seven (70%, whole chicken prevalence) of 10 chickens examined had one or more raw or cooked parts that were contaminated with *Salmonella*. There were six different patterns of contamination. Four chickens were contaminated on one part: left wing (n = 1), right drumstick (n = 1), or right thigh (n = 2). Two chickens were contaminated on three parts (Figs. 3 and 4), and one chicken was contaminated on seven parts (Fig. 5).

Prevalence, number, and serotype of *Salmonella* bacteria on chicken parts are shown in Table 1. Prevalence by qPCR was 15 (19%) of 80 for raw chicken parts and 2 (10%) of 20 for cooked chicken breast parts. The serotypes identified were Typhimurium (4,[5],12:i:1,2) and its variants (i.e., var. 5−, monophasic, and nonmotile) and Kentucky.

Two samples positive by qPCR and lateral flow resulted in no isolate on XLT4. These isolates might not have produced hydrogen sulfide and, thus, may have been missed because only black, hydrogen sulfide–producing colonies were picked for serotyping in this experiment.

In a subsequent study *(20)*, three samples were encountered that were positive by qPCR and lateral flow but that did not produce typical black colonies on XLT4. However, suspect colonies (white) were picked and subsequently identified as *Salmonella* Enteritidis.

Results of qPCR for the 17 *Salmonella*-positive samples indicated a mean C_T of 33.54 (range, 28.16 to 42.31). These C_Ts were all above 25.41, which was the mean C_T for raw chicken parts inoculated with 0.64 log CFU (4 CFU) of *Salmonella* Typhimurium var. 5−. However, these C_Ts were similar to those in qPCR results for cooked chicken parts inoculated with 0 log CFU (1 CFU), which had a mean C_T of 34.4 (range, 28.67 to 41.15).

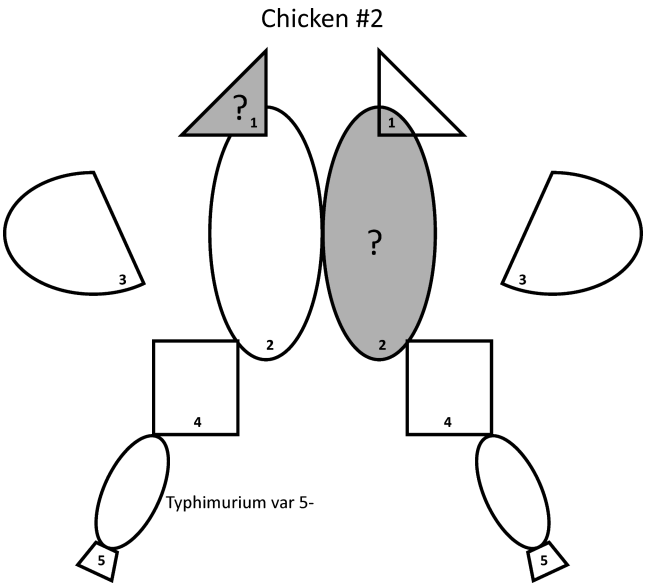

FIGURE 3. *Distribution of* Salmonella *contamination on 1, wings; 2, breast; 3, cooked; 4, thigh; and 5, drumstick of chicken no. 2. Two isolates (?) were not identified, as explained in the text.*

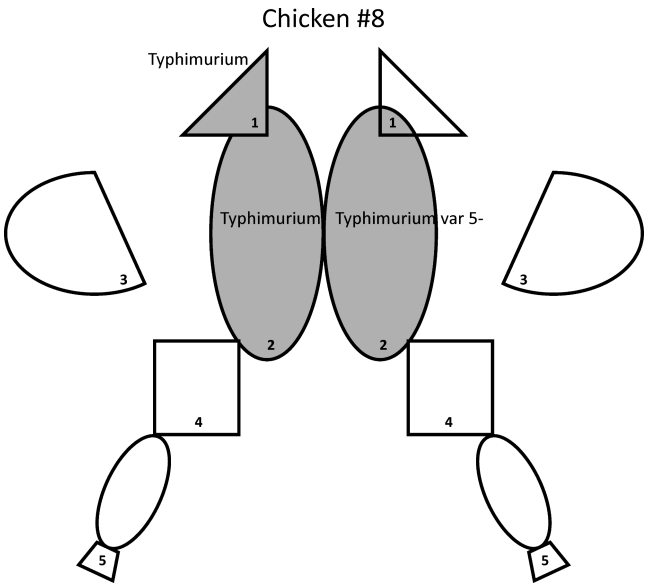

FIGURE 5. *Distribution of* Salmonella *contamination on 1, wings; 2, breast; 3, cooked; 4, thigh; and 5, drumstick of chicken no. 8.*

When the standard curve (Fig. 2) was used to enumerate the 17 *Salmonella*-positive samples from noninoculated chicken parts obtained at retail, 16 were found to have 1 CFU and 1 was found to have 2 CFU (Table 1). Thus, the distribution of *Salmonella* contamination among chicken parts in the current study could be described by a simple discrete distribution (Fig. 6). In a previous study *(19)* that used a standard curve based on plate counts at an early time of whole part enrichment (i.e., 6 h), four positive chicken parts obtained at retail were reported to be contaminated with 1 ($n = 3$) or 4 ($n = 1$) CFU of *Salmonella*.

DISCUSSION

Salmonella that survive cooking or that cross-contaminate other food during meal preparation and serving represent the primary routes of consumer exposure to this pathogen from chicken. Consequently, a protocol was used in the present study to provide new information on prevalence, number, and serotype of *Salmonella* on raw chicken parts and on cooked chicken exposed to utensils used to process raw chicken during simulated meal preparation and serving.

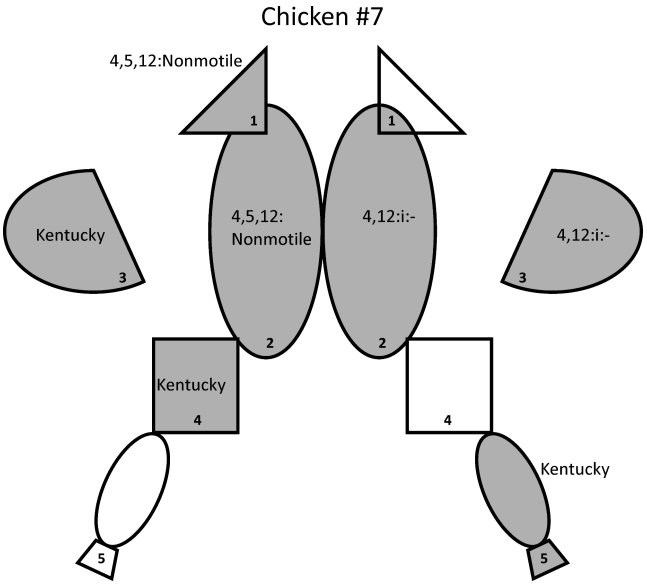

FIGURE 4. *Distribution of* Salmonella *contamination on 1, wings; 2, breast; 3, cooked; 4, thigh; and 5, drumstick of chicken no. 7.*

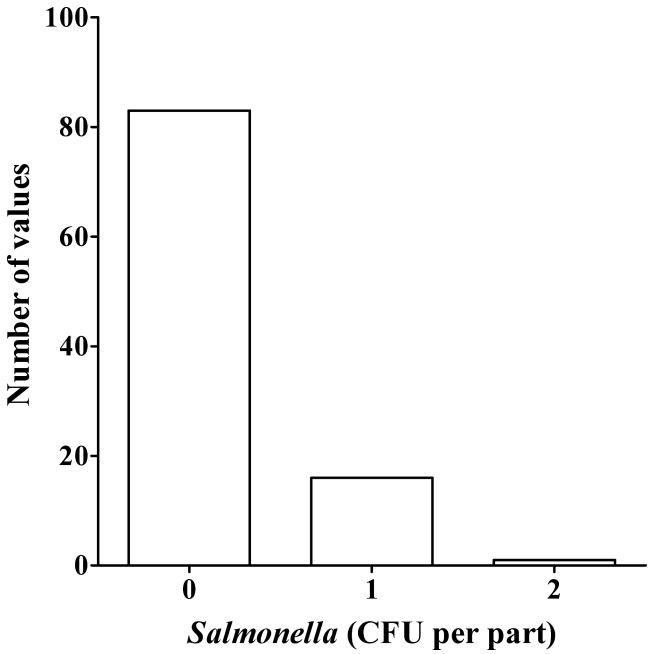

FIGURE 6. *Discrete distribution for* Salmonella *contamination on chicken parts at retail.*

The protocol involved a growth-based assay that used whole part enrichment (40°C, 8 h, 80 rpm) in BPW and qPCR to detect and enumerate *Salmonella* on raw chicken parts and cooked chicken breast parts and that used selective enrichment in Rappaport Vassiliadis broth and selective plating on XLT4 agar to detect and isolate *Salmonella* for subsequent identification by serotyping.

The protocol was applied to whole chickens ($n = 10$) from a single processing plant and obtained at retail. Results of the survey indicated a prevalence of *Salmonella* contamination for raw chicken parts ($n = 80$) of 19% and a prevalence of *Salmonella* cross-contamination for cooked chicken ($n = 20$) of 10%. The number of *Salmonella* per chicken part was one or two cells per part as determined by enrichment qPCR. The serotypes found were Kentucky and Typhimurium (4,5,12:i:1,2) and its variants: var. 5− (4,12:i:1,2), monophasic (4,12:i:−), 4,5,12:nonmotile (4,5,12:−:−), and 4,12:nonmotile (4,12:−:−).

Although most of the *Salmonella* serotypes found were top human clinical isolates (Typhimurium and its variants), only one or two cells were present per chicken part. Risk of getting salmonellosis from exposure to a single cell of *Salmonella* is about 2.3×10^{-3} *(23),* whereas risk of severe illness (3.1×10^{-4}) or death (7.5×10^{-6}) is even lower *(*24*)*. Nonetheless, results of risk assessments for *Salmonella* and chicken show that lightly contaminated chicken at the end of processing *(16)* or at retail *(17)* can pose a higher risk of salmonellosis than highly contaminated chicken at the end of processing or at retail, if by random chance they are temperature abused during distribution, undercooked by consumers, and consumed by someone from the high-risk population (e.g., an immunocompromised person).

Seven (70%) of 10 whole broiler chickens examined in the present study had one or more raw or cooked parts that were positive for *Salmonella* by enrichment qPCR. The raw parts examined accounted for only 60% of the weight of the whole chickens. Had all parts of the chickens been examined, prevalence would likely have been higher than 70%. This is important because all chickens tested in the present study came from the same processing plant, which, to the best of the author's knowledge, was considered to be in compliance with the current *Salmonella* performance standard (5 positives in a 51-sample set). Thus, the current results suggest that the RA method used to test for *Salmonella* in the chicken industry may be underreporting the number of chickens that are actually contaminated with *Salmonella,* resulting in a false sense of security.

A similar conclusion was reached by Cox and Blankenship *(3)* nearly 40 years ago. They examined chickens from four commercial plants, using the WCE and RA methods. They reported a *Salmonella* prevalence of 46% for the WCE method and a *Salmonella* prevalence of 4% for the RA method. In addition, the WCE method identified 10 serotypes of *Salmonella,* whereas the RA method identified only two serotypes of *Salmonella* in the sample set. Moreover, an experiment with spiked samples demonstrated that the WCE method was more sensitive than the RA method for detecting *Salmonella* on chicken. To achieve a detection rate of 100%, the WCE method required inoculation of 8 cells of *Salmonella,* whereas the RA method required inoculation of 52 cells of *Salmonella*. In the current study, the enrichment qPCR method required only a single cell of *Salmonella* for a detection rate of 100%.

Simmons et al. *(25)* also compared WCE and RA methods for detection of *Salmonella* on chicken. They purchased 100 chickens at retail and observed a *Salmonella* prevalence of 38% for the WCE method and 13% for the RA method. In a subsequent study, they *(26)* used the WCE method to examine 251 chickens at retail from 14 processing plants and found a *Salmonella* prevalence of 34%. They concluded that the WCE method was better than the RA method at detecting *Salmonella* on chicken when low numbers were present. In the current study and in a previous study *(19),* chicken parts obtained at retail that tested positive for *Salmonella* by enrichment qPCR or by enrichment plate counts had low numbers of *Salmonella* (i.e., 1 to 4 CFU per part).

Other studies that used the WCE method reported high prevalences of *Salmonella* on chicken. Parveen et al. *(22)* collected chickens ($n = 480$) before and after chilling in a commercial processing plant. They observed a *Salmonella* prevalence of 88% for prechill carcasses and 84% for postchill carcasses. Oscar et al. *(21)* obtained young chickens ($n = 70$) in the Cornish game hen class at retail from a single processing plant. They reported a *Salmonella* prevalence of 22% for parts and 57% for whole chickens.

In addition to the present study, there is only one other report *(21),* to the author's knowledge, of the distribution of *Salmonella* on the chicken carcass. In the current study, there were six different patterns of *Salmonella* contamination among seven contaminated broiler chickens, for a differential pattern rate of 86%. This result is similar to that of Oscar et al. *(21),* who observed 37 different patterns of *Salmonella* contamination among 40 contaminated chickens in the Cornish game hen class, for a differential pattern rate of 92%.

The numbers of contaminated parts per anatomical location on the carcass were not sufficient in the current study to draw conclusions about the specific distribution of *Salmonella* on the carcass. However, Oscar et al. *(21)* were able to identify ''hot spots'' of contamination in their study with young chickens. They observed that the sacral and rib backs were the most highly contaminated parts. In addition, *Salmonella* contamination was higher on the front of the carcass (wings, breast, and rib back) than on the back of the carcass (thigh, drumsticks, and sacral back), presumably due to the carcass being hung by the legs and washed extensively from back to front during commercial processing. Also, the right drumstick was more often contaminated with *Salmonella* than the left drumstick, presumably because the viscera were hung over the right drumstick for subsequent examination by the inspector.

Enrichment qPCR has great potential for determining the distribution (i.e., carcass mapping), number, and types of *Salmonella* on the chicken carcass. Such information is valuable for inspection, hazard analysis and critical control point, risk assessment, and application of interventions designed to reduce or eliminate this pathogen during processing. Use of qPCR to enumerate *Salmonella* in food

requires that a standard curve be developed. Growth phase of cells affects C_T obtained *(9)*. C_T values are three to four cycles less for exponential than stationary-phase cells *(15)*. In the present study, qPCR samples for standard curve development and for enumeration were collected at a standard time (i.e., 8 h) of whole chicken part enrichment at 40°C. Thus, cells of *Salmonella* used in qPCR for standard curve development and unknown samples should have been in the same phase of growth (i.e., exponential).

The C_T obtained in enrichment qPCR is likely affected by several factors: previous history, microbial competition, serotype, initial number, and attachment to food matrix *(11)*. Stressors such as heat, cold, drying, gamma radiation, and low pH can injure *Salmonella* and extend lag phase *(13, 14)*, resulting in a higher C_T. Microbial competition (number and types) can suppress growth of *Salmonella* in BPW *(1)*, resulting in a higher C_T. Differences in growth among serotypes of *Salmonella* during whole part enrichments can result in significant differences in standard curves based on DT by plate counts *(19)* and, by inference, C_T. Individual cell lag times exhibit more variation as initial number decreases *(10)*, resulting in greater variation of C_T at low initial levels of *Salmonella*. Finally, if *Salmonella* cells grow attached to chicken parts, this could result in no C_T or a lower C_T than expected. In the present study, chicken parts were constantly agitated (80 rpm) during enrichment in hopes of rapidly releasing the first daughter cells from attached or entrapped parent cells for an accurate and consistent determination of C_T and number of *Salmonella*.

High C_T and low number of *Salmonella* observed in the current study for ''naturally contaminated'' samples could be explained by none, one, or more of the aforementioned factors. Further research is needed to determine the effects of previous history, microbial competition, serotype, initial number, and attachment or entrapment on the results of enrichment qPCR. It may turn out that none of these factors have a large influence on C_T and that enumeration results obtained here are accurate and valid; only time will tell.

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