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 (*Ct*) 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 10⁻⁶, 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 Cᵥ 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 10⁻⁷ 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 *Cᵥ* 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 *Y₀* is the predicted *Cᵥ* 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 *Cᵥ* 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 *Cᵥ* 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 (10⁻⁶ to 10⁻⁸ in 10⁻⁵ increments) were positive for *Salmonella* by both assays.

A graph (Fig. 1) of *Cᵥ* versus concentration (log CFU per milliliter) of *Salmonella* Typhimurium var. 5– in BPW resulted in a slightly concave line, with *Y₀* (mean ± SE) = 40.97 ± 0.31, *a* = 0.611 ± 0.047, shape parameter (*b*) = 1.23 ± 0.032, and coefficient of determination (*R²*) = 0.999,
There was close agreement between replicate runs of the experiment. Coupled with subsequent results (see below) in which C;S 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;S (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;S 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;S 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; 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; 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 ml. 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 retaila

<table>
<thead>
<tr>
<th>Part</th>
<th>Wt (g)</th>
<th>Prevalence, % (no./total no.)</th>
<th>No.</th>
<th>C, CFU/part</th>
<th>Serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>qPCR</td>
<td>LF</td>
<td>XLT4</td>
</tr>
<tr>
<td>Wing</td>
<td>85</td>
<td>8</td>
<td>20 (4/20)</td>
<td>20 (4/20)</td>
<td>15 (3/20)</td>
</tr>
<tr>
<td>Breast</td>
<td>260</td>
<td>27</td>
<td>25 (5/20)</td>
<td>25 (5/20)</td>
<td>20 (4/20)</td>
</tr>
<tr>
<td>Thigh</td>
<td>138</td>
<td>15</td>
<td>15 (3/20)</td>
<td>15 (3/20)</td>
<td>15 (3/20)</td>
</tr>
<tr>
<td>Drumstick</td>
<td>107</td>
<td>12</td>
<td>15 (3/20)</td>
<td>15 (3/20)</td>
<td>15 (3/20)</td>
</tr>
<tr>
<td>Cooked</td>
<td>86</td>
<td>16</td>
<td>10 (2/20)</td>
<td>10 (2/20)</td>
<td>10 (2/20)</td>
</tr>
</tbody>
</table>

a SD, standard deviation; qPCR, real-time PCR; LF, lateral flow; C, 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, s of 41.15, 29.59, 28.67, and 38.18. These C, s 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, s (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, 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 (Y₀ [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, among samples was high (CV = 18%) resulting in an R² of 0.8675.

A tailing of standard curves at high DT or, in this case, high C, 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,. 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, of 33.54 (range, 28.16 to 42.31). These C, were all above 25.41, which was the mean C, for raw chicken parts inoculated with 0.64 log CFU (4 CFU) of Salmonella Typhimurium var. 5—. However, these C, were similar to those in qPCR results for cooked chicken parts inoculated with 0 log CFU (1 CFU), which had a mean C, of 34.4 (range, 28.67 to 41.15).
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.
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 \( 2.3 \times 10^{-3} \) (23), whereas risk of severe illness \( (3.1 \times 10^{-4}) \) or death \( (7.5 \times 10^{-6}) \) 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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