Initial Contamination of Chicken Parts with *Salmonella* at Retail and Cross-Contamination of Cooked Chicken with *Salmonella* from Raw Chicken during Meal Preparation†

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ABSTRACT

The current study was undertaken to acquire data on contamination of chicken parts with *Salmonella* at retail and to acquire data on cross-contamination of cooked chicken with *Salmonella* from raw chicken during meal preparation. Whole raw chickens (*n* = 31) were obtained from local retail stores and cut into two wings, two breasts without skin or bones, two thighs, and two drumsticks. Data for cross-contamination were obtained by cutting up a sterile, cooked chicken breast with the same board and knife used to cut up the raw chicken. The board, knife, and latex gloves used by the food handler were not rinsed or washed before cutting up the sterile, cooked chicken breast, thus providing a worst-case scenario for cross-contamination. Standard curves for the concentration of *Salmonella* bacteria in 400 ml of buffered peptone water after 6 h of incubation of chicken parts as a function of the initial log number of *Salmonella* bacteria inoculated onto chicken parts were developed and used to enumerate *Salmonella* bacteria. Standard curves were not affected by the type of chicken part but did differ (*P* < 0.05) among the five isolates of *Salmonella* examined. Consequently, *Salmonella* bacteria were enumerated on naturally contaminated chicken parts using a standard curve developed with the serotype of *Salmonella* that was isolated from the original sample. The prevalence of contamination was 3% (4 of 132), whereas the incidence of cross-contamination was 1.8% (1 of 57). The positive chicken parts were a thigh from chicken 4, which contained 3 CFU of *Salmonella enterica* serotype Kentucky, and both wings, one thigh, and one cooked breast portion from chicken 15, which all contained 1 CFU of serotype 8,20::−;8,20::−;8,20::−. These results indicated that the poultry industry is providing consumers in the studied area with chicken that has a low prevalence and low number of *Salmonella* bacteria at retail and that has a low incidence and low level of cross-contamination of cooked chicken with *Salmonella* from raw chicken during meal preparation under a worst-case scenario.

Although the poultry industry and regulatory agencies are doing their best to reduce or eliminate *Salmonella* from poultry farms and processing plants, ready-to-cook chickens sold at retail continue to be contaminated with *Salmonella* (5, 14, 17, 20). In addition, despite efforts to educate consumers, mistakes are still being made during meal preparation that can result in the cross-contamination of other foods with *Salmonella* from raw chicken (1). Thus, even though consumers may eliminate *Salmonella* on ready-to-cook chicken by proper cooking, they could still be exposed to and acquire a *Salmonella* infection from cross-contamination of other foods with *Salmonella* from raw chicken during meal preparation.

Even though there are recent published data for the prevalence of *Salmonella* contamination on chickens obtained at retail (5, 14, 17, 20), there is much less information about the number of *Salmonella* that are present (9). In addition, this information is from a time period before current production and processing practices were implemented by the poultry industry that have significantly reduced the prevalence and, probably, the number of *Salmonella* bacteria on chickens exiting the processing plant and purchased by consumers at retail stores. Thus, there is a need to determine the number of *Salmonella* bacteria on the edible parts of the chicken carcass at retail since the implementation of programs and procedures designed to reduce or eliminate this pathogen from poultry.

There are a few studies (2, 4, 8) that have examined the cross-contamination of other foods with *Salmonella* from naturally contaminated raw chickens during meal preparation. However, these studies are limited to incidence data and were not conducted in recent years. Thus, there is a need to determine the incidence and levels of *Salmonella* bacteria that are transferred from naturally contaminated chickens to other foods during meal preparation since the implementation of modern-day poultry production and processing procedures.

Recovery of *Salmonella* from chicken samples is an important consideration for accurate determination of the...
prevalence and level of Salmonella contamination. The current approach used by the poultry industry in the United States is to recover Salmonella from chicken samples by rinsing the whole chicken carcass for 1 min in 400 ml of buffered peptone water (BPW), followed by incubation of a 30-ml aliquot for 24 h at 37°C. However, rinsing for 1 min does not recover all of the Salmonella from chicken samples (10) and, thus, does not provide a complete assessment of the prevalence and levels of Salmonella contamination. In fact, Simmons et al. (18) compared the aliquot method with the method of incubating the whole chicken carcass in 500 ml of BPW for 24 h at 37°C. They found that the prevalence of Salmonella contamination for the same 100 raw chickens obtained at retail was 13% for the aliquot method and 38% for the whole-chicken incubation method. Thus, for higher and more complete recovery of Salmonella from chicken samples and more accurate determination of the prevalence and level of contamination, it is important to incubate the whole-chicken sample and not just an aliquot of a rinse sample from the whole-chicken sample.

In the present study, the concentration of Salmonella bacteria in BPW at an early time of incubation (6 h) of whole chicken parts was determined by spiral plating on xylose lysine Tergitol 4 (XLT4) agar and found to be proportional to the log number of Salmonella bacteria inoculated onto the chicken parts. The resulting standard curves were used to enumerate Salmonella bacteria on boneless and bone-in parts of whole broiler chickens (~42 days of age) obtained at retail. The effects of the chicken-part type and isolate of Salmonella on the standard curves and the ability of Salmonella to cross-contaminate cooked chicken during meal preparation were also investigated. The most-probable-number method, which requires a homogenous sample to enumerate bacteria in food samples, was not used to enumerate Salmonella in the present study because it was not possible to homogenize the chicken parts that contained bones.

MATERIALS AND METHODS

Salmonella. The isolates of Salmonella used in the current study were Salmonella enterica serotype Typhimurium DT104 ATCC 700408 (sT700408), Salmonella Typhimurium ATCC 14028 (sT14028), Salmonella enterica serotype Kentucky s167 (sk167), Salmonella Kentucky s361 (sk361), and Salmonella 8.20:–:z80, s362 (sz362). Isolates sT700408 and sT14028 were from a commercial source (American Type Culture Collection, Manassas, VA), isolate sk167 was obtained from a local poultry company, sk361 was isolated from a chicken thigh in the current study, and sz362 was isolated from a chicken wing in the present study. Stock cultures of these isolates were maintained at −70°C in brain heart infusion broth (BD, Sparks, MD) that contained 15% (vol/vol) glycerol (Sigma-Aldrich, St. Louis, MO).

Preparation of chicken parts. Whole raw chickens (n = 31) of three different brands were purchased fresh at local retail stores in the city of Salisbury, MD, and the town of Princess Anne, MD, between 16 November 2010 and 18 October 2011. The whole chickens were cut into two wings, two breasts without skin or bones, two thighs, and two drumsticks using a sterilized board made of plastic and a sterilized knife with a stainless steel blade.

One breast was cubed into two equal-sized portions, while the other breast was cooked (121°C for 15 min) to sterility in a tabletop autoclave (BioClave, Biomega Research Products, Inc., Edison, NJ). A sterilized, cooked breast from a previous chicken was cubed into two equal-sized portions with the board and knife used to cut up the raw chicken. The board, knife, and latex gloves used by the food handler were not rinsed or washed before they were used to cut up the sterilized cooked chicken breast. This was done to study cross-contamination of Salmonella from the raw chicken to cooked chicken during meal preparation under a worst-case scenario. The cross-contamination experiment was repeated with all 31 chickens using either one or two of the sterilized, cooked chicken breast portions for a total of 57 possible cross-contamination events.

Inoculation and incubation of chicken parts. Cultures of Salmonella were initiated by adding 5 μl of the appropriate stock culture to 9 ml of BPW (BD) in a glass dilution tube (16 by 125 mm) with a plastic cap. Cultures of Salmonella were incubated without shaking at 22°C for 72 h to obtain stationary-phase cells for inoculation of chicken parts. After incubation for 72 h, cultures of Salmonella were serially diluted (1:10) in BPW, and then chicken parts were spot inoculated on their surface with 2 μl (on one sample only) or 5 μl of the 10−2, 10−3, 10−4, 10−5, or 10−6 dilution of the Salmonella culture. This resulted in initial numbers of Salmonella bacteria that ranged from 0.36 to 4.86 log CFU per chicken part.

Chicken parts were placed individually into stomacher bags (177 by 304 mm; Seward, London, UK) and stored overnight at 7°C. This was done to allow the Salmonella on artificially contaminated chicken parts to become more closely associated with the chicken part, like the Salmonella on the naturally contaminated chicken parts. In addition, this was done to standardize the previous history of the Salmonella on the artificially contaminated chicken parts with the previous history of the Salmonella on the naturally contaminated chicken parts, which were treated in the same manner.

To initiate incubation of chicken parts, 400 ml of prewarmed BPW (42°C) was added to each bag, and then the chicken parts, whether they were artificially contaminated, naturally contaminated, or not contaminated with Salmonella, were immediately incubated at 42°C for 6 h with shaking at 80 rpm (Innova 4230 orbital shaking refrigerated incubator, New Brunswick Scientific, Edison, NJ).

Sampling and spiral plating. At 6 h of incubation, a sample (4 ml) of BPW was collected, and 50 μl was immediately spiral plated (Whitley Automatic Spiral Plater, Microbiology International, Frederick, MD) onto XLT4 agar (BD). A second sample (1 ml) was collected and serially diluted (1:10) in BPW to 10−1, 10−2, 10−3, and 10−4, and 50 μl of each serial dilution was spiral plated onto XLT4 agar. Spiral plates were incubated for 24 h at 42°C, and then black colonies that formed were counted using an automated colony counter (Protocol Automated Colony Counter, Microbiology International).

Isolation and identification of Salmonella. At 6 h of incubation, a third sample (0.1 ml) of BPW from the chicken part incubation was collected and used to inoculate 10 ml of Rappaport Vassiliadis broth (BD), which was then incubated for 23 h at 42°C without shaking. Next, a 0.2-ml sample of the Rappaport Vassiliadis broth incubate was used in a commercial test kit (Reveal 2.0, Neogen, Lansing, MI) to determine whether Salmonella bacteria were present in the sample. The test kit uses an AOAC International approved method involving a test strip that contains antibodies that specifically detect the presence of Salmonella antigens in the sample as it wicks through the test strip.
Samples testing positive for *Salmonella* were serially diluted (1:10) in BPW to $10^{-2}$, followed by spiral plating of 50 μl of the $10^{-2}$ and $10^{-3}$ dilutions onto XLT4 agar. After 24 h of incubation at 42 °C, an isolated black colony was selected, picked, and transferred to 9 ml of BPW in a dilution tube with cap and then incubated at 22 °C for 72 h. Aliquots (0.1 ml) of the 72-h culture were then transferred to storage vials that contained 0.9 ml of brain heart infusion broth with 15% (vol/vol) glycerol. These new stock cultures were stored at −70°C. Isolates were sent to the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, National Veterinary Services Laboratories (Ames, IA) for *Salmonella* serotyping.

**Standard curves.** The concentration of *Salmonella* bacteria in BPW at 6 h of incubation of chicken parts ($\gamma$; log CFU per milliliter) was graphed as a function of the log number of *Salmonella* bacteria inoculated ($\alpha$; log CFU per part) on chicken parts. These data were then fitted, using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, CA), to a linear regression model, $y = a + bx$, where $a$ was the $y$-intercept and $b$ was the slope. Pairwise comparisons of the standard curves were made in GraphPad Prism version 5.02 for Windows using an *F* test that determined whether $a$ and $b$ differed ($P < 0.05$) among the two standard curves being compared. These comparisons were made to determine whether the standard curves were providing similar predictions for unknown samples.

**RESULTS**

Of the 310 chicken parts included in this study, 121 were artificially contaminated with one of the five isolates of *Salmonella* examined, whereas 189 were not artificially contaminated with *Salmonella*. The prevalence of *Salmonella* contamination of parts obtained from chickens at retail that were not artificially contaminated with *Salmonella* was 3% (4 of 132), whereas the incidence of cross-contamination of cooked breast meat with *Salmonella* from raw chicken during simulated meal preparation under a worst-case scenario of food handling was 1.8% (1 of 57). The naturally contaminated chicken parts were a thigh from chicken 4 and both wings, one thigh, and one cooked breast portion from chicken 15 (Table 1). The isolate of *Salmonella* from chicken 4 was identified as serotype Kentucky, whereas all four isolates of *Salmonella* from chicken 15 were identified as serotype 8,20:−:z6, 8,20:−:z6, 8,20:−:z6, and 8,20:−:z6.

Artificially contaminated chicken parts were used to develop standard curves for enumerating *Salmonella* bacteria on naturally contaminated chicken parts obtained at retail or cross-contaminated with *Salmonella* from raw chicken during simulated meal preparation. Standard curves were developed for five isolates of *Salmonella* (sT700408, sT14028, sK167, sK361, and sz362) and four different types of chicken parts (wings, breasts without skin or bones, thighs, and drumsticks) for a total of 20 standard curves.

Within the data for each isolate of *Salmonella*, an *F* test was used to make a total of six pairwise comparisons (Fig. 1). No differences ($P > 0.05$) in y-intercepts ($a$) or slopes ($b$) of the standard curves were observed among the four types of chicken parts when they were compared in this way (results not shown). The six panels in Figure 1 show summary graphs of the pairwise comparisons made among the four different kinds of chicken parts, where the data in the graphs represent the combined data for all five isolates of *Salmonella*. The graphs in this figure illustrate that the concentrations of *Salmonella* bacteria in BPW at 6 h of incubation of chicken parts increased linearly as a function of the log number of *Salmonella* bacteria inoculated, that the standard curves were similar among all types of chicken parts, and that the standard curves had an enumeration range from $0 \rightarrow 5$ log CFU of *Salmonella* bacteria per chicken part.

Because the type of chicken part did not affect ($P > 0.05$) the parameters of the standard curves, data for the four types of chicken parts were combined, and standard curves were developed for each of the five isolates of *Salmonella* (Table 2). Next, the *F* test was used to make all possible pairwise comparisons of standard curves for the five isolates of *Salmonella* (Fig. 2). In contrast to the results for the four different types of chicken parts, there were significant differences observed in the y-intercepts ($a$) but not the slopes ($b$) of the standard curves for the five isolates of *Salmonella* (Table 3). These results indicated that some isolates of *Salmonella* grew differently in the whole-part incubations. Specifically, sT700408 and sT14028, the ATCC strains, grew faster (i.e., higher y-intercepts) than the chicken isolates sK167, sK361, and sK362, which grew in a similar manner. Thus, it was decided that it would be better to use individual standard curves rather than a combined standard curve for enumeration of *Salmonella* bacteria. The standard curve for sK361 was used to determine the level of initial contamination of serotype 8,20:−:z6 on both wings and thigh from retail chicken 15 and the level of cross-contamination of this serotype on the cooked breast portion, which were all 1 CFU per part (Table 1).

**TABLE 1. Serotypes and numbers of Salmonella bacteria on naturally contaminated chicken parts**

<table>
<thead>
<tr>
<th>Chicken</th>
<th>Date (mo/day/yr)</th>
<th>Brand</th>
<th>Part</th>
<th>Wt (g)</th>
<th>Isolate</th>
<th>Salmonella serotype</th>
<th>log CFU/ml</th>
<th>log CFU/part</th>
<th>No. of CFU/part</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>12/14/2010</td>
<td>C</td>
<td>Thigh</td>
<td>173</td>
<td>s361</td>
<td>Kentucky</td>
<td>2.34</td>
<td>0.41</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>4/12/2011</td>
<td>A</td>
<td>Wing</td>
<td>74</td>
<td>s362</td>
<td>8,20:−:z6</td>
<td>1.30</td>
<td>0.00</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>4/12/2011</td>
<td>A</td>
<td>Thigh</td>
<td>129</td>
<td>s363</td>
<td>8,20:−:z6</td>
<td>1.78</td>
<td>0.00</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>4/12/2011</td>
<td>A</td>
<td>Cooked breast</td>
<td>102</td>
<td>s364</td>
<td>8,20:−:z6</td>
<td>1.30</td>
<td>0.00</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>4/12/2011</td>
<td>A</td>
<td>Wing</td>
<td>78</td>
<td>s365</td>
<td>8,20:−:z6</td>
<td>1.60</td>
<td>0.00</td>
<td>1</td>
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DISCUSSION

When attempting to detect and enumerate Salmonella bacteria on parts of the chicken carcass, it is important to consider how the Salmonella bacteria are associated with the carcass tissues. Electron and confocal microscopy studies indicate that bacteria, including Salmonella, which contaminate the carcass of poultry are associated with the carcass in three main ways: (i) unattached in the water layer on the surface of carcass tissues—these cells are readily recovered by rinsing; (ii) attached to collagen fibers of the skin and muscle tissue—these cells are generally not recovered by rinsing; and (iii) entrapped in skin crevices, between muscle fibers, and in feather follicles—these cells may be difficult to recover by rinsing (7, 12, 19).

Bacterial cells that are attached to surfaces, whether meat surfaces or equipment surfaces, and are growing as biofilms release daughter cells into the surrounding water phase (3). Thus, it was hoped that by incubating chicken parts in BPW with agitation, it would be possible to detect all of the Salmonella bacteria associated with the chicken part whether they were unattached, attached, or entrapped. More specifically, it was hoped that all the cells of Salmonella associated

<table>
<thead>
<tr>
<th>TABLE 2. Standard curve parameters for different isolates of Salmonella inoculated onto chicken parts</th>
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<tr>
<td>Isolate</td>
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<tr>
<td>Salmonella Typhimurium DT104 ATCC 700408</td>
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<td></td>
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<tr>
<td>Salmonella Typhimurium ATCC 14028</td>
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<tr>
<td></td>
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<tr>
<td>Salmonella Kentucky s167</td>
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<td></td>
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<tr>
<td>Salmonella Kentucky s361</td>
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<tr>
<td></td>
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<tr>
<td>Salmonella 8,20:–:z6 s362</td>
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<td></td>
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</tbody>
</table>

$^a$ BFV, best-fit value; CI, 95% confidence interval; $R^2$, coefficient of determination; $a$ = y-intercept; $b$ = slope.
with the chicken part would be quickly released or quickly produce and release daughter cells that would be rapidly dispersed into the surrounding BPW, where they would find favorable conditions and rapidly grow to a concentration (1.3 log CFU/ml) that could be detected by spiral plating.

Another important consideration for detecting and enumerating the *Salmonella* bacteria associated with the chicken parts was to try to simulate the natural association (unattached, attached, and entrapped) of *Salmonella* bacteria on chicken parts in the standard curve experiments where chicken parts were artificially contaminated with *Salmonella*. The approach taken to accomplish this in the current study was to store the artificially contaminated parts overnight at 7°C to try to give them a chance to become more closely associated with the chicken part before incubation, detection, and enumeration in BPW.

Another reason for storing the artificially contaminated chicken parts overnight at 7°C was to try to standardize the

![Figure 2](http://meridian.allenpress.com/jfp/article-pdf/76/1/33/1685470/0362-028x_jfp-12-224.pdf)
immediately previous temperature history of the Salmonella on artificially contaminated chicken parts with the immediately previous temperature history of naturally contaminated chicken parts. The immediately previous temperature history has been shown to influence the subsequent growth kinetics of Salmonella bacteria (11, 13). Thus, not accounting for a similar effect in the current study could have resulted in an inaccurate estimation of the prevalence and level of Salmonella contamination, since the enumeration method was based on the growth kinetics of Salmonella bacteria in BPW during incubation of whole chicken parts.

Another important consideration in the present study was how long the chicken parts needed to be incubated in BPW for the proper detection and enumeration of Salmonella bacteria. Based on the results of a previous study (16), an incubation time of 6 h was selected. To validate the time of incubation, the protocol was first tried with isolate sT700408, and the resulting standard curve was found to have a y-intercept at x equals 0 log CFU per part of 2.7 log CFU/ml (95% confidence interval, 2.47 to 3.03 log CFU/ml), which was above the lower limit of detection of 1.3 log CFU/ml by spiral plating, as performed in this study. Thus, at 6 h of incubation, the protocol appeared more than adequate for detecting 1 CFU of Salmonella on a naturally contaminated chicken part even if the Salmonella bacteria were slower growing than expected, due to factors such as strain variation, microbial competition, and previous history.

Further confirmation of the 6-h incubation period was obtained in subsequent experiments with other isolates of Salmonella (sT14028, sK167, sK361, and sz362). Here, as with sT700408, all y-intercepts had best-fit values and 95% confidence intervals that were above the lower limit of detection of Salmonella of 1.3 log CFU/ml by spiral plating. Thus, it was concluded that 6 h of incubation in BPW was sufficient time to detect 1 viable CFU of Salmonella on a naturally contaminated chicken part.

The reason for acquiring data for contamination of chicken parts with Salmonella and cross-contamination of other foods with Salmonella from raw chicken during meal preparation was to fill important data gaps in risk assessments for Salmonella and chicken that are being performed by regulatory agencies in the United States, Europe, and throughout the world. These risk assessments are being used as the scientific basis to inform policy decisions aimed at protecting public health from this important human foodborne pathogen. For risk assessment purposes, it is not only useful to know the number of pathogens on and in a food sample but also their ability to grow and cause infection. Thus, an enumeration method such as the one used in the current study that is based on the growth kinetics of Salmonella bacteria during incubation of the food sample under favorable conditions for growth provides the type of quantitative data that is highly relevant for risk assessments.

Interestingly, the type of chicken part did not affect the parameters of the standard curve even though there were differences in size and shape and chemical and microbial composition of the chicken parts. This finding agrees with results from a previous study (16) conducted with whole, bone-in parts from young chickens in the Cornish game hen class. Thus, differences in the size and shape and differences in the chemical and microbial composition of chicken parts do not appear to have a significant effect on the growth kinetics of Salmonella bacteria in BPW during incubation of whole parts obtained from chickens at retail.

In contrast to the results for the different types of chicken parts, differences in the standard curves among the five isolates of Salmonella examined were observed in the current study, indicating that strain variation affects the growth kinetics of Salmonella bacteria in BPW during incubation of chicken parts. These results agree with previous studies (6, 15) reporting variation of growth kinetics among strains and serotypes of Salmonella growing in laboratory media. Thus, for the most accurate enumeration of Salmonella bacteria on chicken parts by the current method, it is best to use a standard curve developed with the serotype of Salmonella that was isolated from the naturally contaminated chicken part on which enumeration is being performed.

Although this is not the first study to use naturally contaminated chickens to study the incidence of cross-contamination of other foods with Salmonella from raw chicken during meal preparation (2, 4, 8), it is the first study to use naturally contaminated chickens to quantify the transfer of Salmonella from raw chicken to another food. By
using naturally contaminated chicken that typically contains only a few cells of *Salmonella* and by using an enumeration method that is capable of detecting a single CFU of viable *Salmonella* on or in the food sample, it was possible to determine not only the number of *Salmonella* bacteria transferred but also the incidence of this event for a particular food handling scenario. In the present study, a worst-case scenario was simulated in which the food handler used the same knife, cutting board, and latex gloves for preparing the raw chicken for cooking and for cutting up a cooked chicken breast without first rinsing or washing the cutting board, knife, or hands. However, even under this worst-case scenario, there was only a single cross-contamination event involving the transfer of a single CFU of *Salmonella* from the raw chicken to the cooked chicken out of 57 meal preparation events, for an incidence of cross-contamination of 1.8%.

The data acquired in the present study were from a limited number of brands marketed in a small geographical area near the author’s laboratory. Thus, they may not reflect the situation in other regions of the United States or in other nations of the world. Nonetheless, the results of this study indicate that in the studied area, the poultry industry is supplying consumers with chickens that are contaminated with very low levels of *Salmonella* bacteria, and thus, the risk of acquiring a *Salmonella* infection directly from chicken or indirectly from cross-contamination of other foods with *Salmonella* from raw chicken during meal preparation appears to be low, especially when proper food handling and cooking methods are followed.

**REFERENCES**


