INTRODUCTION

The microbiological profile of processed poultry has been a long-time concern for both spoilage and food safety reasons, as represented by an early publication reporting the effect of cold storage on bacteria associated with poultry carcasses and eggs (Wiley et al., 1908). Later research from commercial turkey processing plants found that surface bacteria on carcasses increased 10-fold during processing (Walker and Ayres, 1959). More recent research has also shown that *Salmonella* prevalence also tends to increase with early processing steps in commercial turkey plants through picking, although decreases are observed after washing and chilling (Nde et al., 2006). Test methods appropriate to determine levels of bacteria, especially pathogens, have evolved over time and are necessary to monitor product contamination.

Researchers have employed a variety of methods to study bacteria on poultry carcasses. Five different sample methods have been reported to produce different results, sponge sampling and whole carcass rinsing (WCR) were performed on turkey hen carcasses inoculated with a known amount of nalidixic acid-resistant *Salmonella enterica* serovar Enteritidis. Five turkey hen carcasses were collected from the shackle line in a commercial processing plant in each of 4 replicate trials. Carcasses were placed in a cooler with a small amount of ice and transported to the laboratory for approximately 1.5 h. *Salmonella* inoculum was applied by spreading 0.5 mL on the back and 0.5 mL on the thigh. After 10 min, the carcasses were sampled via a premoistened 4 × 8-cm sponge, swiping 10 times vertically and 10 horizontally on the back, and then repeating the same sequence on the thigh using a 10 × 5-cm template. After sponge sampling carcasses were placed in a clean plastic bag, 200 mL of buffered peptone was added, and bags were manually shaken for 60 s for a low volume whole carcass rinse (WCR). Liquid from stomached sponges and from WCR rinseate was serially diluted in 0.85% saline and plated onto Brilliant Green agar with sulfapyridine containing 200 ppm of nalidixic acid. Plates were incubated at 37°C for 24 h and colonies indicative of *Salmonella* were counted and transformed from cfu/mL to log cfu/cm². The low volume WCR recovered significantly more *Salmonella* than sponge sampling in trial 3 (log 3.1 vs. 2.3, respectively) and trial 4 (log 3.1 vs. 2.2, respectively). No differences were observed in trials 1 and 2 due to sample method. Low volume WCR is equal to or more effective than sponge sampling for recovering inoculated *Salmonella* from turkey carcasses.

Key words: turkey carcass, *Salmonella*, sponge sampling, whole carcass rinse

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1 Corresponding author: doug.smith@ncsu.edu
and swabbing, have been mandated for poultry carcass sampling in the United States by the USDA Food Safety Inspection Service (FSIS). Previously, FSIS had instituted a turkey carcass sampling program that specified a 600-mL WCR method (USDA FSIS, 2011b). Current programs for testing Salmonella prevalence in turkey plants use a 2-site carcass swabbing method, whereas broiler processing plants use a 400-mL WCR to sample carcasses (USDA FSIS, 2011a; 1998b). Supporting the 2-site swab method is a report noted that higher numbers of Escherichia coli were recovered from the back and thighs of postchill turkeys as compared with the breast when carcasses were sponge sampled (Bodnaruk et al., 1998). Also, Kotula (1966) found that broiler carcass swab counts were higher from thighs, which were higher than leg swabs, which were higher than breast. However, a report where skin samples were removed from various sites on broiler carcasses did not find a difference in numbers of bacteria due to sample location (Klinger et al., 1981). Additional research has shown that a 2-site swab method similar to the FSIS method produced a lower Salmonella prevalence than did a modified WCR method on turkey carcasses (McEvoy et al., 2005).

Although FSIS mandates a 400-mL rinse for chickens, and previously a 600-mL rinse for turkeys, lower volume rinses have been reported to provide equivalent results to higher volume rinses. A low volume WCR of 100 mL was as effective as a 300-mL volume for determining Salmonella prevalence on broiler chicken carcasses (Cox et al., 1981). A low volume WCR of 100 mL was conducted on turkey carcasses inoculated with 30 cells of a marker strain of Salmonella; all carcasses were positive, providing evidence that low volume WCR is appropriate for detecting even small numbers of bacterial contaminants (Dickens et al., 1986).

The USDA FSIS has mandated pathogen testing of raw poultry products in an effort to protect human health, with a swabbing method specified for monitoring Salmonella on carcasses at turkey processing plants. The objective of this study was to evaluate a method very similar to the FSIS-approved sponge sampling method and a low volume whole carcass rinse for enumerating Salmonella inoculated onto turkey hen carcasses.

MATERIALS AND METHODS

In each of 4 replicate trials conducted on different days, 5 eviscerated turkey hen carcasses were removed at random from the processing line before final carcass washer in the evisceration room at a commercial processing plant (n = 20). Carcasses were placed in a clean cooler with a small amount of ice and transported approximately 1.5 h to the laboratory. Average carcass weight was 6.0 kg. All 5 carcasses were inoculated with 1 mL of a Salmonella Enteritidis inoculum. A sterile L-shaped cell spreader (Fisher Scientific, Pittsburg, PA) was used to thoroughly spread approximately 0.5 mL onto the back and another 0.5 mL onto the right thigh. Carcasses were held uncovered for 10 min to allow bacterial attachment to the skin surface. The inoculum was a nalidixic acid-resistant strain of Salmonella Enteritidis, originally isolated from a poultry flock and maintained on nutrient agar slants (Remel, Lenexa, KS). The turkey carcass inoculum was prepared by cultivating bacteria from the slant in Brain Heart Infusion broth (Oxoid Ltd., Basingstoke, Hampshire, England) at 37°C for 6 h (to approximately $10^8$ log cfu/mL). Serial dilutions were conducted using 0.85% sterile saline to obtain a final estimated concentration of log 6.0 log cfu/mL in the inoculum. The inoculum from each trial was serially diluted, plated, and counted as described below to determine actual counts.

Sponge sampling of each of the 5 turkey carcasses was conducted per USDA Food Safety Inspection Service procedures (USDA FSIS, 1998b). Briefly, sterile sponges (approximately 4 × 8 cm) in a sterile bag (Biotrace International, Muncie, IN) were hydrated with 10 mL of 1.0% buffered peptone (Oxoid Ltd.). For each sample, using a sterile gloved hand, the sponge was used to swab a 5 × 10-cm area on the back of the carcass (area delimited with a sterile template), 10 times horizontally and 10 times vertically. The same sponge and template was then used to sample the right thigh of the same carcass. Sampling was conducted on the same back and thigh area where the inoculum was previously spread. The sponge was returned to the bag, another 10 mL of 1.0% peptone was added, and samples were stomached for 30 s. The low volume WCR was conducted on each of the same 5 carcasses immediately after the swab sample was taken. Each carcass was placed in a clean plastic bag and 200 mL of 1.0% buffered peptone was added and manually shaken for 1 min. One milliliter from each sample bag was serially diluted in 0.85% sterile saline, and 0.1 mL was plated onto duplicate plates of Brilliant Green agar (Neogen Corp., Lansing, MI) with 200 ppm of nalidixic acid (Fisher Scientific, Fair Lawn, NJ). Plates were incubated at 37°C for 24 h and colonies indicative of Salmonella were counted.

Counts were transformed to log$_{10}$ cfu/cm$^2$. Sponge sample numbers were converted to cm$^2$ by dividing the 20-mL sponge diluent by the 100-cm$^2$ sponge area; data were then transformed to log$_{10}$ cfu/cm$^2$. Data were an-
analyzed by SAS ANOVA (SAS Institute, 2004) by main effects of trial and method and tested for significance ($P < 0.05$) by residual error.

**RESULTS AND DISCUSSION**

The levels of *Salmonella* inoculum applied in each trial were as follows: trial 1, log 6.0; trial 2, log 6.1; and, trials 3 and 4 were log 5.8. The target inoculation level was 6.0, so there was variation between trials for numbers of applied bacteria.

Mean (±SEM) numbers of *Salmonella* recovered by WCR (3.1 ± 0.01 log cfu/cm²) was higher ($P = 0.002$) than numbers recovered by sponge sampling (2.7 ± 0.12 log cfu/cm²) conducted on 20 inoculated turkey hen carcasses. Numbers of bacteria recovered varied by trial; because different trials were conducted on different days (as would occur for *Salmonella* testing in the processing plant), data are also reported by trial in Table 1. The WCR recovered more *Salmonella* than sponge sampling in trial 3 (log 3.1 vs. 2.3, respectively), with similar results observed for trial 4 (log 3.1 vs. 2.2, respectively). There was no difference in bacteria recovered due to method in trials 1 and 2. A previous report showed that the WCR was more effective for detecting *Salmonella* prevalence on turkey carcasses than a 2-site swab (McEvoy et al., 2005). Alternatively, Fromm (1959) found alginate swabbing was more effective than WCR for recovering bacteria from chicken carcasses. The difference in recovery of *Salmonella* by WCR versus sponge sampling was less than one log in 2 of the trials and not significant in the other 2 trials. However, further examination of the raw data without mL to cm² or log-transformation is another potential measure of method efficacy.

The actual numbers derived from the log counts for each trial, which are also presented as percentage recovery of the original inoculation, are shown in Table 2. Across trials, WCR recovered from 10 to 16 times more bacteria (6.3 to 50.2%) from carcasses than did the sponge sampling (0.5 to 3.2%). Neither method was particularly effective at recovering numbers of bacteria; obvious variation was also observed among trials in percent recovery for both methods. A prior research report comparing several sample methods found that cotton swabs retained bacteria after carcass sampling, whereas the WCR method left bacteria on the carcass after rinsing; both circumstances likely caused under-estimation of bacterial numbers on chicken carcasses (Fromm, 1959). The FSIS has acknowledged that carcass swabbing may be under-representing *Salmonella* prevalence and levels on turkey carcasses (USDA FSIS, 2011b).

Differences between trials are also apparent; in trial 1, more than 50% of the original inoculum load was recovered, but the amount recovered decreased in each subsequent trial until only 6.8% of the original inoculum was recovered in trial 4. There was no obvious reason for this decline, as inoculation and recovery methods were not changed between trials. Potentially, the plant could have changed processing procedures, such as increasing chlorine in the evisceration equipment rinses, the residual of which may have affected recovery of applied *Salmonella*. However, this scenario is unlikely due to the short effective life of chlorine on organic tissue.

Although the WCR method recovered significantly more bacteria than did sponge sampling, as reported in Table 1, neither method efficiently recovered bacteria applied in the inoculum as shown in Table 2. A direct comparison of methods is problematic as one method samples a set area (100 cm²) while the other samples the entire carcass with a known volume of liquid (200 mL).

| **Table 1.** Mean numbers (log₁₀) of *Salmonella* recovered by either sponge sampling or low volume whole carcass rinse (WCR) from turkey hen carcasses inoculated with a nalidixic acid-resistant strain of *Salmonella* Enteritidis in 4 replicate trials |
|---|---|---|---|---|
| Item | Trial (log cfu/cm²) | 1 | 2 | 3 |
| Sponge¹ | 3.2 | 3.3 | 2.3 | 2.2 |
| WCR² | 3.2 | 3.2 | 3.1 | 3.1 |
| Pooled SEM | 0.03 | 0.04 | 0.14 | 0.16 |
| $P$-value | 0.812 | 0.090 | <0.001 | <0.001 |

¹$n = 5$.

| **Table 2.** Mean total cfu of *Salmonella* inoculated and recovered from turkey carcasses by sponge sampling or low volume whole carcass rinse (WCR) in each of 4 trials (recovered log counts multiplied by original sample dilution factor, that is, 20 mL for sponge and 200 mL for WCR samples) |
|---|---|---|---|---|
| Item | Trial | 1 | 2 | 3 |
| Inoculated | 1,000,000 | 100.0 | 1,258,925 | 100.0 | 630,957 | 100.0 |
| Recovered, sponge² | 31,700 | 3.2 | 39,900 | 3.2 | 4,000 | 0.6 |
| Recovered, WCR³ | 502,400 | 50.2 | 399,000 | 31.7 | 63,200 | 10.0 |
| Not recovered | 465,900 | 46.6 | 820,025 | 65.1 | 563,757 | 89.4 |
| Total recovered | 53.4 | 34.9 | 10.6 | |
| ²$n = 5$.

1Percent recovered by method = (cfu recovered/cfu inoculated) × 100.
prevalence data, but the WCR would provide more accurate enumeration data than sponge sampling for *Salmonella* given that WCR recovered more bacteria. In contrast, McEvoy et al. (2005) reported no difference between the 2-site swab and WCR for numbers of *E. coli* recovered from turkey carcasses between the 2 methods. Perhaps resident *E. coli* and inoculated *Salmonella* cells adhere to the carcass differently, resulting in a different recovery pattern.

Results from this study showed that a low volume WCR was generally more effective than a 2-site swab method similar to the FSIS method for recovering inoculated *Salmonella* from turkey carcasses. However, the variability and poor performance of results from this study, and reports from previous studies, shows that more research is required to determine the efficacy of turkey carcass sampling methods for *Salmonella*.

**REFERENCES**


