Recovery of Salmonellae from Chilled Broiler Carcasses as Affected by Rinse Media and Enumeration Method

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ABSTRACT

Distilled water (DW) and 0.85% NaCl (PS) were evaluated as carcass rinse media for recovery of total aerobic bacteria (APC), total coliforms (TC), *Escherichia coli*, and salmonellae from broiler carcasses. Salmonellae were enumerated by two methods, most-probable-number (MPN) and centrifugation-plating onto dulcitol novobiocin agar (DBN). Commercially processed chilled broiler carcasses (10/trial, 3 trials) were aseptically cut in half, and each half was rinsed (1 min) with either 250 ml DW or PS. Carcass rinses were recovered and analyzed for populations of APC, TC, *E. coli*, and salmonellae.

Recovery of APC, TC, and *E. coli* were not affected (P>.05) by rinse media; however, significant trial effects were present. Recovery of salmonellae was influenced by rinse media as well as by enumeration method. Using the MPN procedure, salmonellae were detected on 20 and 27% of carcass halves using PS and DW, respectively, whereas with DBN, salmonellae were recovered from 33% of PS-rinsed carcass halves and none of those rinsed with DW. Incidence of salmonellae on individual carcass halves did not correlate between either the two enumeration methods or rinse media. With both enumeration methods, the extent of salmonellae contamination was <1 CFU/ml of rinse media. Rinsing carcasses with PS offered no advantages for recovery of APC, TC, and *E. coli*; however, salmonellae recovery on DBN was enhanced by PS as compared to DW rinse.

*Salmonella* continues to be of major concern to the broiler industry, not only because of its public health significance, but also due to its ability to proliferate in the gastrointestinal tract of the chicken and subsequently survive on commercially processed broiler carcasses.

A number of control points for *Salmonella* contamination are available to the broiler industry during live production, including use of *Salmonella*-free feed, biosecurity, and dust and vector control (16). However, the ultimate control point is the processing phase, which has been the primary target in implementing Hazard Analysis Critical Control Point (HACCP) programs (28). In designing and implementing HACCP programs, reliable microbiological monitoring techniques are needed. The technique of choice must be rapid, reliable, objective, and quantitative.

Salmonella enumeration techniques are needed to identify contamination sources for the determination of critical control points (CCPs) (28). These techniques are also needed to account for significant reductions in *Salmonella* numbers when evaluating CCPs. Several sampling methods are in use today including the 25-g meat sample (25), skin maceration (6,23,24), carcass swabs (2,13,22), minced meat (11,17) and whole bird rinse (9,10). In the United States, the whole bird rinse sampling method has gained wide acceptance for sampling broiler carcasses. It is a noninvasive and rapid method, which leaves a completely usable carcass after sampling. However, it has been shown that this method does not remove all salmonellae from the surface of broiler carcasses (15,19).

Different rinse media [e.g., buffered solutions, microbiological media such as lactose broth (Blankenship and Cox, 4), and distilled water (7)] have been incorporated into the whole bird rinse technique to enhance microbial recovery. Physiological saline was used in several studies examining the attachment and detachment of bacterial cells to poultry meat surfaces. However, the efficacy of physiological saline in removing attached bacteria remains questionable, since both positive (3,27) and negative (18) results have been reported. The objectives of this study were to re-evaluate the influence of rinse media (physiological saline vs. distilled water) on recovery of salmonellae and other bacterial populations from broiler carcasses and to evaluate the influence of enumeration method on recovery of salmonellae.

MATERIALS AND METHODS

Carcasses

In each of three trials, 10 commercially chilled broiler carcasses were used. Carcasses were aseptically separated into halves. One-half of each carcass was rinsed in physiological saline (PS, 0.85% NaCl), and the other half was rinsed in distilled water (DW). Carcass halves were rinsed in sterile 42 x 63-cm bags according to USDA protocol (8). Rinse volume was increased to 250 ml in this study to ensure sufficient rinse action, although as low as 100 ml of rinse fluid has been shown to be effective in recovery of bacterial populations (7,9,10). Rinse suspensions were subjected to microbiological analyses (total aerobic plate, total coliform, *Escherichia coli*, and salmonellae counts).
Microbiological analyses

To obtain total aerobic plate (APC), total coliform, and *E. coli* counts, carcass rinses were serially diluted with sterile buffered peptone water (Difco Laboratories, Detroit, MI) and inoculated (1.0 ml) onto appropriate media utilizing duplicate plates for each dilution. Petrifilm™ plates were used to determine total aerobic plate counts, coliform, and *E. coli* counts, respectively. Inoculation procedures, incubation times and temperatures, and interpretation of results were conducted according to Petrifilm™ instructions.

**Salmonella enumeration**

Salmonellae were enumerated by two methods: a three-tube, most probable number method (MPN) (8) and the rinse-centrifugation plating onto dulcitol novobiocin agar method (DBN) (12). A volume of 40 ml of each rinse sample was used for each method.

The MPN method consisted of five steps: i) preenrichment in buffered peptone water (Difco) for 24 h at 37°C; ii) centrifugation (10,000 *g* for 15 min) of a subsample (40 ml) of the original rinse sample; iii) collection and resuspension of the pellet with 20 ml of 0.1 M potassium phosphate buffer (pH 7.0); iv) a second centrifugation (10,000 *g* for 15 min.), and v) final resuspension of the pellet in 1.0 ml of 0.1 M potassium phosphate buffer (pH 7.0). A sample (0.1 ml) of this suspension was spread-plate inoculated onto DBN agar in 15-cm petri dishes using a sterile bent glass rod. DBN agar (5.0 g dulcitol, 8.5 g bile salts No. 3, 0.00033 g brilliant green, 1.5 g ammonium ferric citrate, 8.5 g sodium thiosulfate, 0.025 g neutral red, 0.05 g yeast, 15 g agar, and 0.015 g sodium novobiocin, in 1.0 L distilled water) was prepared by dissolving all ingredients, except sodium novobiocin, by boiling, cooling to 50°C, adjusting the pH to 7.0 with NaOH, reheating to boil, cooling to 50°C and adding sodium novobiocin as a filter sterilized solution, and pouring into 15-cm petri dishes (12).

All enumeration data, with the exception of *Salmonella* data, were transformed to log_{10} CFU/ml and subjected to analysis of variance, using the General Linear Model procedure of Statistical Analysis Systems (26). The statistical model consisted of rinse media and trial (both considered as fixed effects) and their interaction. When significant differences occurred (*P*<0.05), means were separated by Tukey’s Studentized range test (5). *Salmonella* data were presented as incidence rates due to extremely low populations.

**RESULTS AND DISCUSSION**

Rinse media did not affect recovery of APC, coliform bacteria, or *E. coli*. No statistical differences (*P*>0.05) in populations of these bacteria, recovered from carcass halves rinsed with either PS or DW, were observed (Table 1). In this study, carcass halves were used to compare the rinse treatments. It has been reported that the variation in bacterial numbers between carcass halves was less than the variation between whole carcasses which have been treated similarly (14). A significant trial effect was observed for APC (*P*<0.001), coliform bacteria and *E. coli* (*P*<0.05). These differences were attributed to variation associated with flocks and preslaughter handling of birds processed (20). There were no significant (*P*>0.05) interactions between rinse media and trials.

**TABLE 1. Bacterial populations (log_{10} CFU/ml) recovered from chilled broiler carcass halves.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Aerobic plate counts</th>
<th>Total coliforms</th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinse media</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Saline</td>
<td>3.48</td>
<td>2.26</td>
<td>1.53</td>
</tr>
<tr>
<td>Distilled water</td>
<td>3.52</td>
<td>2.11</td>
<td>1.63</td>
</tr>
<tr>
<td>SEM (df = 30)</td>
<td>0.064</td>
<td>0.077</td>
<td>0.092</td>
</tr>
<tr>
<td>Trial</td>
<td><strong>3.82b</strong></td>
<td><strong>2.33a</strong></td>
<td><strong>1.81a</strong></td>
</tr>
<tr>
<td>1</td>
<td>3.40b</td>
<td>2.25b</td>
<td>1.53b</td>
</tr>
<tr>
<td>2</td>
<td>3.82b</td>
<td>2.33a</td>
<td>1.81a</td>
</tr>
<tr>
<td>3</td>
<td>3.28b</td>
<td>1.98b</td>
<td>1.42</td>
</tr>
<tr>
<td>SEM (df = 20)</td>
<td>0.078</td>
<td>0.097</td>
<td>0.11</td>
</tr>
<tr>
<td>Interaction</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1. Pooled standard error of mean (degrees of freedom).
2. NS = nonsignificant (*P*>0.05).

Enumeration methods (MPN and DBN) were compared and evaluated for *Salmonella* quantification. As compared to MPN, the DBN method is more rapid and less laborious. The DBN method consists of a highly selective plating medium for *Salmonella* with dulcitol, bile salts, novobiocin, and brilliant green contributing to its selectivity (12,21). For both MPN and DBN procedures, *Salmonella* counts were <1 CFU/ml and consequently statistical analysis was not possible. Therefore, incidence rates of salmonellae on carcass halves were used as criteria for determining the effect of rinse media (PS and DW) and detection method (MPN and DBN) on recovery. The number of *Salmonella*-positive carcass halves was similar between the two rinse media when the MPN method was used (Table 2). Salmonellae were detected on 6 carcass halves rinsed with PS and on 8 rinsed with DW. However, when DBN was used, salmonellae were found on 10 carcass halves rinsed with PS and none rinsed with DW (Table 2). It appears that the DW rinse may have adversely affected any *Salmonella* cells present and prevented them from growing in the presence of the selective agents found in DBN, but not in MPN media. This possibility is indirectly supported by the similarities observed between rinse media (PS and DW) for the MPN procedure (Table 2).

Recovery rates of salmonellae from carcass halves obtained with the two detection methods (MPN and DBN), independent of rinse media used, were compared for each trial (Table 3). In trial 1, salmonellae were detected on 9 of 10 carcass halves with the MPN method and on 3 of 10 using the DBN method. The three samples (1, 4, and 10) which were positive by the DBN method were also positive.

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TABLE 2. Effects of rinse media on recovery of salmonellae from broiler carcass halves using most-probable-number (MPN) and rinse-centrifugation plating onto dabcilitol novobiocin agar (DBN) methods.

<table>
<thead>
<tr>
<th>Samples</th>
<th>MPN</th>
<th>DBN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PS</td>
<td>DW</td>
</tr>
<tr>
<td>Number salmonellae positive samples</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Total number of samples</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Percent positive samples</td>
<td>20</td>
<td>27</td>
</tr>
</tbody>
</table>

1 Data from three trials combined.
2 0.85% NaCl rinse.
3 Distilled water rinse.

TABLE 3. Recovery of salmonellae from individual carcass half samples using MPN and rinse-centrifugation plating onto DBN methods.

<table>
<thead>
<tr>
<th>TRIAL</th>
<th>Sample</th>
<th>No.</th>
<th>MPN</th>
<th>DBN</th>
<th>MPN</th>
<th>DBN</th>
<th>MPN</th>
<th>DBN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>1+</td>
<td>+2</td>
<td>(.027)</td>
<td>2+</td>
<td>(.05)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>(.025)</td>
<td>(.025)</td>
<td>(.025)</td>
<td>(.04)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>(.025)</td>
<td>(.025)</td>
<td>(.025)</td>
<td>(.05)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>(.3)</td>
<td>(.3)</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>(.3)</td>
<td>(.3)</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>(.3)</td>
<td>(.3)</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>(.4)</td>
<td>(.4)</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>(.4)</td>
<td>(.4)</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>(.4)</td>
<td>(.4)</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

1 Carcass halves.
2 Salmonella detected.
3 Value in parentheses indicates MPN index.
4 Value in parentheses indicates recovered population (CFU/ml).
5 Salmonella not detected.

by the MPN method. In trial 2, salmonellae were detected on 3 of 10 with MPN and on 4 of 10 using the DBN method. Two samples were Salmonella positive by both methods. In trial 3, salmonellae were detected on 2 of 10 carcass halves using MPN and on 3 of 10 using DBN. In this trial none of the carcass halves were Salmonella positive by both methods. For the three trials, 45% of the carcass halves were Salmonella positive by both methods.

In this study, physiological saline offered no advantage for the recovery of aerobic bacteria, coliform bacteria, or E. coli from chilled broiler carcasses. However, Salmonella recovery on DBN was enhanced by the PS rinse as compared to the DW rinse. Although DBN has been shown to provide fully confirmed Salmonella counts within 48 h (12), this experiment does not provide evidence that these confirmed Salmonella counts would be equivalent to the conventional MPN procedure when examining commercially processed broiler carcasses. The incidence of Salmonella positive carcasses did not correlate between the two enumeration methods (MPN and DBN) or rinse media. It was also found that the extent of Salmonella contamination was <1 CFU/ml of carcass rinse, with both enumeration methods. Additional studies using the DBN procedure or modifications to it are needed before it will be suitable as a replacement for the more laborious MPN technique commonly used for enumerating Salmonella on broiler carcasses.

REFERENCES


