Using Indicator Bacteria and Salmonella Test Results from Three Large-Scale Beef Abattoirs over an 18-Month Period To Evaluate Intervention System Efficacy and Plan Carcass Testing for Salmonella

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ABSTRACT

To develop a process for predicting the likelihood of Salmonella contamination on beef carcasses, we evaluated the influence of several possible causative factors (i.e., year, abattoir, day of week, month, and intervention system components) on the risk of Salmonella and indicator organism contamination. Hide and carcass sponge samples were collected in 2005 to 2006 in six steps at three abattoirs in the East (A), Midwest (B), and Southwest (C) United States. Each abattoir used the same intervention system. Samples were analyzed for aerobic plate counts (APCs; \( n = 18,990 \)) and Enterobacteriaceae counts (EBCs; \( n = 18,989 \)) and the presence or absence of Salmonella (\( n = 5,355 \)). Our results demonstrated that many factors play a significant role in the level of microbial contamination of beef carcasses. Overall, Salmonella prevalence and EBC levels were significantly higher in 2006 than in 2005. APCs and EBCs were highest in abattoirs A (3.57 log CFU/100 cm\(^2\)) and B (1.31 log CFU/100 cm\(^2\)). The odds of detecting a positive Salmonella isolate were greatest in abattoir C and lowest in abattoir A. Across the three abattoirs, the overall intervention process effectively reduced microbiological contamination. Salmonella prevalence fell from 45% (preevisceration) to 0.47% (postchilled–lactic acid), and there were APC and EBC reductions of 5.43 and 5.28 log CFU/100 cm\(^2\), respectively, from hide-on to postchilled–lactic acid samples. At each abattoir, composites of three individual EBC-negative carcass samples yielded Salmonella-negative results 97 to 99% of the time. These results suggest the possibility of using indicator test results to accurately predict the absence of Salmonella in a beef carcass sample.

Each year in the United States, there are an estimated 1.4 million salmonellosis cases and 400 deaths caused by Salmonella infections (28). Ground beef has been implicated as the vehicle for transmission of Salmonella in multiple foodborne outbreaks (9–11, 13). The U.S. Food Safety and Inspection Service has documented a decrease in the prevalence of Salmonella in ground beef, from 7.5% of samples in 1996 to 1.6% in 2004 (24, 25); however, outbreaks of human Salmonella infections associated with ground beef continue to occur. Even though beef processing plant practices adhere to current U.S. Food Safety and Inspection Service production guidelines, with the history of multiple ground beef–associated outbreaks (9–11), and the recent first multistate outbreak of multidrug-resistant Salmonella Typhimurium phage type DT104 associated with consumption of store-bought ground beef (13), there are increased governmental concerns regarding whether current critical control points (i.e., preventive measures to control food safety hazards) and pathogen reduction strategies are adequate for Salmonella control (12).

Because Salmonella colonizes the gastrointestinal tracts of cattle, it is believed that carcasses can become contaminated with Salmonella during slaughter operations. To decrease carcass contamination, antimicrobial interventions have been put into place at commercial beef processing facilities. Many beef processors now apply multiple intervention technologies, i.e., water washes, organic acid washes, carcass trimming, and steam vacuuming (4), because the literature has demonstrated that the application of multiple interventions has greater potential to reduce microorganisms than interventions applied individually (7, 8, 14, 17, 19).

Even though pathogen contamination is typically the greatest food safety concern associated with beef slaughter, fabrication, and production of raw products, there are drawbacks to the use of pathogen testing for evaluating process control. These drawbacks include the low prevalence of pathogenic bacteria cells found on beef carcasses and raw products (6) and the costs associated with pathogen testing. Researchers have studied the reduction of indicator organisms, e.g., aerobic plate counts (APCs), generic E. coli, total coliforms, and Enterobacteriaceae counts (EBCs), as a measure of intervention system efficacy (7, 20). Others have reported that limited sampling data preclude the establishment of a direct relationship between indicator organisms and pathogens (3). Indicator bacteria analyses cost less than pathogen testing and may alert processors to situations in which fecal contamination is occurring, regardless of whether pathogens are involved.

In this study, on-line carcass sponge samples were collected from three commercial beef abattoirs located in geo-
Graphically different regions of the United States. Samples were collected at various locations throughout the beef production process over a 18-month period. Sample analyses included enumeration of indicator organisms (APCs and EBCs) and qualitative analysis for Salmonella. The overall objective of this project was to develop a predictive tool for plant-specific or perhaps widespread use that would allow operators of large-scale abattoirs to evaluate specific indicator test results and operational parameters in terms of the likelihood of Salmonella contamination. The specific objectives of the study were to (i) determine the relationships existing between the presence of Salmonella, the level of APCs, and the presence and levels of EBCs on beef carcasses, and (ii) determine the influence of several possible causative factors (i.e., geographic region, day of week, month, and intervention treatments) on the risk of Salmonella and indicator contamination.

MATERIALS AND METHODS

Sample collection steps and microbial interventions. Carcass sponge samples were collected from three commercial beef abattoirs located in graphically different regions of the United States: East (A), Midwest (B), and Southwest (C). As shown in Figure 1, samples were collected at six steps throughout the process: (1) after the hide was split at the midline but before complete hide removal = hide on, (2) after complete hide removal but prior to any antimicrobial intervention = preintervention, (3) after evisceration, on-line carcass trimming, and final inspection but before carcass washes = prepasteurization–lactic acid spray, (4) after the pasteurization and lactic acid sprays but before carcass chilling = postpasteurization–lactic acid, (5) after chilling the carcass for 36 to 48 h postmortem = postchill, and (6) after spraying the chilled carcasses with lactic acid before going into fabrication = postchilled–lactic acid.

At each of the three abattoirs, the microbial intervention systems were the same. Before hide-on sampling (step 1), the hide was opened on the rear shanks and midline, but no intervention was applied directly to the hide prior to sampling. Once the hide was completely removed, prior to sampling at step 2, carcass trimming occurred, and the hide removal cut pattern lines on the carcass were treated with steam vacuuming. Between sampling steps 2 and 3, the preeviscerated carcasses were sprayed with 4.0 to 5.0% lactic acid, eviscerated, and subjected to further steam vacuuming and trimming. Between sampling steps 3 and 4, the carcasses were treated with an ambient-temperature water wash and then a pasteurization water wash (target water temperature, >82.2°C) and a 4.0 to 5.0% lactic acid spray. Between sampling steps 4 and 5, the carcasses were in a cooler and subjected to intermittent spray chilling for 36 to 48 h. Between sampling steps 5 and 6, 4.0 to 5.0% lactic acid was again sprayed on the carcass immediately before it was moved to the fabrication room.

Sample collection procedure. Samples were collected daily over an 18-month period beginning the first week of March 2005 and ending mid-September 2006. On each sampling day, at each sample step, three carcasses were randomly selected and sampled. Each hide was sampled with one Speci-Sponge (Nasco, Fort Atkinson, Wis.) moistened with 25 ml of buffered peptone diluent (Difco, Becton Dickinson, Sparks, Md.), and each carcass was sampled with two Speci-Sponges, each moistened with 25 ml of buffered peptone diluent. Excess diluent was removed from each sponge inside the Speci-Sponge bag before sampling. Sampling was performed as described by Arthur et al. (3) and consisted of sampling an area approximately 8,000 or 100 cm² on the carcass or hide, respectively. To sample such a large area on the carcass, two sponges were used: sponge 1 sampled the inside and outside round (≈4,000 cm²), and sponge 2 sampled the navel-plate-brisket-shank area (≈4,000 cm²). Sample sponges were stored in insulated coolers containing ice packs and were transported to the laboratory. Microbiological analysis was done within 24 h of the samples being obtained at the plant.

Microbiological analysis. Samples from all steps (1 through 6) were analyzed for APCs and EBCs, but only those samples collected from the carcass (steps 2 through 6) were analyzed for Salmonella.

Sponges were pummeled by stomaching at normal speed for 1 min with a Stomacher 400 (Seward, Fisher Scientific, Itasca, Ill.). For carcass samples, the two sponges were placed in a single sample bag before stomaching. After stomaching, a 2-ml aliquot was removed, with 1 ml for APC and 1 ml for EBC analysis. Then, six sponges (two sponges per carcass × three carcasses per step) were placed in a single sample bag and enriched for Salmonella detection.

APCs were determined by the Spiral Plate Method as described in the U.S. Food and Drug Administration Bacteriological Analytical Manual (26). Spiral plating was performed by plating 100 µl of appropriately diluted samples on plate count agar (Difco)
co, Becton Dickinson) with an Autoplate 4000 (Spiral Biotech, Norwood, Mass.). The agar plates were then incubated at 35 ± 1°C for 48 ± 3 h. After incubation, enumeration was performed as instructed by the manufacturer. Total EBCs were determined by plating appropriate dilutions onto 3M Petrifilm Enterobacteriaceae Count Plates (3M Microbiology, St. Paul, Minn.) and incubating at 37 ± 1°C for 24 ± 2 h. After the Petrifilm was incubated, enumeration was performed by selecting plates with isolated colonies ranging in numbers from 10 to 150 and counting all red colonies that produced gas, acid (yellow zone surrounding colony), or both.

Salmonella detection was performed by first screening the samples (each of which consisted of liquid expressed from six sponges) with the automated VIDAS Salmonella enzyme-linked fluorescent assay (bioMérieux-USA, Durham, N.C.) (2). Each sample was enriched with 225 ml of buffered peptone diluent and incubated at 35 ± 1°C for 18 to 24 h. After initial enrichment, 0.1 and 1.0 ml were transferred in parallel to 10 ml of Rappaport-Vassiliadis broth (Difco, Becton Dickinson) and 10 ml of tetra-thionate broth supplemented with iodine (Difco, Becton Dickinson), respectively. Inoculated Rappaport-Vassiliadis broth and tetra-thionate broth supplemented with iodine were incubated at 41 to 42°C for 18 to 24 h. After incubation, 1.0 ml of Rappaport-Vassiliadis broth and 1.0 ml of tetra-thionate broth supplemented with iodine were each transferred into 9 ml of M-broth (Difco, Becton Dickinson) and incubated at 41 to 42°C for 6 to 8 h. After incubation, 1.0 ml from each M-broth sample was transferred into one test tube, heated for 15 ± 1 min in a water bath at 95 to 100°C, and allowed to cool; then, the automated VIDAS Salmonella assay was performed. Samples positive on the enzyme-linked fluorescent assay screen were confirmed as Salmonella (27) by first streaking onto brilliant green sulfa agar supplemented with 0.1% sodium sulfapyridine (Difco, Becton Dickinson) and xylose lysine Tergitol 4 (XLT4) agar (Difco, Becton Dickinson) from the Rappaport-Vassiliadis and tetra-thionate broth supplemented with iodine enrichments and incubating for 35 ± 2°C for 18 to 24 h. Three typical colonies from each of the incubated brilliant green sulfa agar supplemented with 0.1% sodium sulfapyridine and XLT4 agar plates were selected for biochemical analysis. Typical colonies on the brilliant green sulfa agar supplemented with 0.1% sodium sulfapyridine agar were pink and opaque with a smooth appearance and the entire edge surrounded by a red color in the medium. Crowded brilliant green sulfa agar supplemented with 0.1% sodium sulfapyridine plates will produce typical colonies that appear tan against the green background. Typical colonies selected from the XLT4 agar plates appeared black or red with or without black centers with a red rim around the colony. Biochemical analysis was performed with triple sugar iron (Difco, Becton Dickinson) and lysine iron agar (Difco, Becton Dickinson) slants. Inoculation of the triple sugar iron and lysine iron agar slants was performed in parallel with a single colony by stabbing the butt and streaking the top of the slant in one operation. Inoculated slants were incubated at 35 ± 2°C for 24 ± 2 h. Isolates that produced typical biochemical reactions on the triple sugar iron and lysine iron agar slants were subjected to serological analysis with polyvalent (O) antiserum (serogroups A through I; Difco, Becton Dickinson). Isolates were considered confirmed with positive agglutination from the polyvalent (O) antiserum.

Statistical analysis. Statistical analysis was performed on combined data collected at steps 1 through 6 from abattoirs A, B, and C. Main effects included the following: abattoir = P, year = Y, month = M, day of week = DW, and sample step = SS. The APC and EBC data were converted to log CFU/100 cm² prior to statistical analysis. For both APCs and EBCs, minimum detectable limits for carcass samples and hide samples were 0.625 and 25 CFU/100 cm², respectively. When carcass samples (steps 2 through 6) or hide samples (step 1) did not produce any colonies, values of −0.51 log/100 cm² (0.3125 CFU/100 cm² less than the detection limit) or 1.09 log/100 cm² (12.5 CFU/100 cm² less than the detectable limit) were used, respectively, for statistical analysis. For evaluating the influence of the factors tested and their interactions on the amount of microbial contamination, an analysis of variance was done (SAS PROC GLM, SAS Institute, Cary, N.C. (22)). For each factor, except for interactions involving both month and day of week, all pairwise comparisons were conducted by the least significant difference procedure. Odds ratios for Salmonella were determined by SAS PROC LOGISTIC (SAS Institute (22)) on the factors tested. For evaluating the relationship between the presence of Salmonella and the levels of APCs and EBCs, sample averages for APCs and EBCs were calculated for each sampling day at each step. To evaluate the relationship between the presence of Salmonella and the presence of Enterobacteriaceae, the averaged EBC values were converted to binary EBC data (positive = detected CFU; negative = no CFU). Sample steps in which Salmonella results did not correspond with an APC and EBC result were excluded from the analysis (n = 127 of 5,355 Salmonella data points were excluded). The relationships existing between the presence of Salmonella, the level of APC, and the presence and levels of EBC were evaluated and analyzed by SAS PROC LOGISTIC (SAS Institute (22)).

RESULTS AND DISCUSSION

APC and EBC enumeration. Most factors (abattoir = P, year = Y, month = M, day of week = DW, and sample step = SS) that were evaluated, and their interactions, had a significant effect on APC and EBC. When combined data (all three abattoirs) were analyzed, all main effects were significant (P < 0.05) for both APC and EBC, except the Y for APC (P = 0.18) and the DW for EBC (P = 0.73). All evaluated pairwise interactions for APC were significant (P < 0.05), except for Y × DW. Interactions that did not significantly affect EBC were P × DW, Y × DW, P × Y × DW, DW × SS, P × DW × SS, Y × DW × SS, and P × Y × DW × SS. In general, the main effects and their interactions had a significant effect on the amount of APC and EBC contamination of beef carcasses.

When data from all six sampling steps were pooled, significant differences (P < 0.05) in APCs and EBCs were found between all three abattoirs. The APC was highest (3.57 log CFU/100 cm²) in abattoir A and second highest (3.12 log CFU/100 cm²) in abattoir B (Table 1). In contrast to APC values, the EBC values were highest in abattoir B and second highest in abattoir A. For both APC and EBC, abattoir C had the lowest levels of APCs and EBCs (Table 1).

When data from all three abattoirs were pooled, no statistically significant difference in APC was seen between 2005 and 2006 (Table 2). The mean value of EBCs in 2005 was slightly, but significantly, less than the mean value from 2006 (Table 2). The relatively narrow APC and EBC 95% confidence intervals at the level of abattoir (Table 1), year, and month for all three abattoirs and sampling steps combined (Table 2) and the combination of sampling step and abattoir (Table 3) suggest that operational practices and
TABLE 1. Aerobic plate counts (APCs) and Enterobacteriaceae counts (EBCs) on beef carcasses across all years, months, and sampling steps: (1) hide on, (2) preintervention, (3) prepasteurization–lactic acid spray, (4) postpasteurization–lactic acid, (5) postchill, and (6) postchilled–lactic acid.

<table>
<thead>
<tr>
<th>Region</th>
<th>APCs</th>
<th>EBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>East</td>
<td>Mean 95% CI</td>
<td>Mean 95% CI</td>
</tr>
<tr>
<td>5,001</td>
<td>3.57 A</td>
<td>3.51–3.64</td>
</tr>
<tr>
<td>Midwest</td>
<td>6,340</td>
<td>3.12 b</td>
</tr>
<tr>
<td>Southwest</td>
<td>7,649</td>
<td>2.16 c</td>
</tr>
</tbody>
</table>

**Comparisons were made between abattoirs. Values for mean and 95% CI (confidence interval) are log CFU/100 cm².**

**Values in the same column with the same letter are not significantly different (P ≥ 0.05).**

When data were organized by sampling step and abattoir, several trends were apparent (Table 3). For sampling steps 2 through 6, the highest mean APC occurred at abattoir A. Abattoir C had the lowest mean APC for sampling steps 1 through 3, 5, and 6. Abattoir B had an intermediate mean APC for sampling steps 2, 3, 5, and 6. With the exception of abattoirs A and B at sampling step 1, all differences in mean APC between abattoirs were statistically significant (P < 0.05). In terms of mean EBC, abattoir C had the lowest value at sampling steps 2, 3, 5, and 6. Abattoirs A and B had the highest value for sampling steps 2, 4, and 6 and sampling steps 1, 3, and 5, respectively. Although the same intervention system was used at all three abattoirs, there were clearly important differences between abattoirs. These differences are likely not attributable to regional differences in animal suppliers, because there was considerable sourcing overlap between the three abattoirs. The largest differences between abattoirs in mean APC and mean EBC were at sampling steps 2 and 3, perhaps reflecting differences in hide removal, trimming, or steam vacuuming.

Figure 2 illustrates the hide and carcass microbial profiles (data combined for all three abattoirs) for APCs and EBCs at each of the six steps sampled in the process. For both APCs and EBCs, values were significantly different between all six processing steps, with the exception of similar EBC values at postpasteurization–lactic acid and postchilled–lactic acid (sampling steps 4 and 6; Fig. 2). These results show that the overall intervention process, from hide-on (sampling step 1) to postchilled–lactic acid samples (sampling step 6), effectively reduced microbiological contamination, as evidenced by average APC and EBC decreases of 5.43 and 5.28 log CFU/100 cm², respectively. Contamination of the sterile carcass surface during the hide removal process resulted in preintervention (sampling step 2) APC and EBC values of 3.10 and 1.04 log

**TABLE 2. Aerobic plate counts (APCs) and Enterobacteriaceae counts (EBCs) on beef carcasses across all abattoirs and sampling steps: (1) hide on, (2) preintervention, (3) prepasteurization–lactic acid spray, (4) postpasteurization–lactic acid, (5) postchill, and (6) postchilled–lactic acid.**

<table>
<thead>
<tr>
<th>Year</th>
<th>APCs</th>
<th>EBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>10,041</td>
<td>10,040</td>
</tr>
<tr>
<td>2006</td>
<td>8,949</td>
<td>8,949</td>
</tr>
</tbody>
</table>

**Comparisons were made between years and months. Values for mean and 95% CI (confidence interval) are log CFU/100 cm².**

**Values within a category in the same column with the same letter are not significantly different (P ≥ 0.05).**
TABLE 3. Aerobic plate counts (APCs) and Enterobacteriaceae counts (EBCs) on beef carcasses

<table>
<thead>
<tr>
<th>Sampling step</th>
<th>Abattoir</th>
<th>U.S. region</th>
<th>APCs</th>
<th>EBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95% CI&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>n</td>
</tr>
<tr>
<td>1</td>
<td>A East</td>
<td>840</td>
<td>7.34 A&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.29–7.39</td>
</tr>
<tr>
<td></td>
<td>B Midwest</td>
<td>1,028</td>
<td>7.40 A</td>
<td>7.35–7.45</td>
</tr>
<tr>
<td></td>
<td>C Southwest</td>
<td>1,271</td>
<td>6.18 B</td>
<td>6.14–6.22</td>
</tr>
<tr>
<td>2</td>
<td>A East</td>
<td>841</td>
<td>6.41 C</td>
<td>4.56–4.67</td>
</tr>
<tr>
<td></td>
<td>B Midwest</td>
<td>1,071</td>
<td>3.27 E</td>
<td>3.21–3.33</td>
</tr>
<tr>
<td></td>
<td>C Southwest</td>
<td>1,272</td>
<td>1.95 I</td>
<td>1.90–1.99</td>
</tr>
<tr>
<td>3</td>
<td>A East</td>
<td>841</td>
<td>3.49 D</td>
<td>3.43–3.55</td>
</tr>
<tr>
<td></td>
<td>B Midwest</td>
<td>1,081</td>
<td>3.25 E</td>
<td>3.19–3.31</td>
</tr>
<tr>
<td></td>
<td>C Southwest</td>
<td>1,272</td>
<td>1.95 I</td>
<td>1.90–1.99</td>
</tr>
<tr>
<td>4</td>
<td>A East</td>
<td>840</td>
<td>1.26 K</td>
<td>1.21–1.32</td>
</tr>
<tr>
<td></td>
<td>B Midwest</td>
<td>1,082</td>
<td>0.92 M</td>
<td>0.89–0.94</td>
</tr>
<tr>
<td></td>
<td>C Southwest</td>
<td>1,272</td>
<td>1.09 L</td>
<td>1.06–1.12</td>
</tr>
<tr>
<td>5</td>
<td>A East</td>
<td>820</td>
<td>2.65 F</td>
<td>2.56–2.74</td>
</tr>
<tr>
<td></td>
<td>B Midwest</td>
<td>1,038</td>
<td>2.48 G</td>
<td>2.42–2.54</td>
</tr>
<tr>
<td></td>
<td>C Southwest</td>
<td>1,281</td>
<td>1.26 K</td>
<td>1.22–1.30</td>
</tr>
<tr>
<td>6</td>
<td>A East</td>
<td>819</td>
<td>2.03 H</td>
<td>1.94–2.11</td>
</tr>
<tr>
<td></td>
<td>B Midwest</td>
<td>1,040</td>
<td>1.54 J</td>
<td>1.49–1.60</td>
</tr>
<tr>
<td></td>
<td>C Southwest</td>
<td>1,281</td>
<td>1.03 L</td>
<td>1.00–1.06</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data were combined across years and months, with comparisons made between abattoirs and sampling steps: (1) hide on, (2) preintervention, (3) prepasteurization–lactic acid spray, (4) postpasteurization–lactic acid, (5) postchill, and (6) postchilled–lactic acid.

<sup>b</sup>Log CFU/100 cm<sup>2</sup>.

<sup>c</sup>CI, confidence interval.

<sup>d</sup>Values in the same column and the same sampling step with the same letter are not significantly different (P ≥ 0.05).

<sup>e</sup>Parentheses denote negative numbers.

CFU/100 cm<sup>2</sup>, respectively. The subsequent interventions applied to the carcass after the hide was removed (i.e., preevisceration wash, pasteurization wash, and lactic acid wash) further reduced the APCs and EBCs on the carcass immediately prior to the fabrication room (sampling step 6) to 1.46 and −0.38 log CFU/100 cm<sup>2</sup>, respectively. Samples collected at pasteurization–lactic acid (sampling step 4) produced the lowest APC mean values; however, significantly larger APC values were found after chilling (sampling step 6), which suggested regrowth, additional contamination during the chilling process, or both. The 5.0% lactic acid treatment on the chilled carcass reduced the APC value to 1.46 log CFU/100 cm<sup>2</sup>, but the final APC level for the chilled carcasses was significantly higher than the APC level on the carcasses immediately prior to chilling (sampling step 4). A similar microbial pattern was seen with EBC counts, but the 5.0% lactic acid treatment on the chilled carcass (sampling step 6) reduced the mean EBC value to the same level seen immediately before chilling (sampling step 4). The same trends were also seen within each individual abattoir (Table 3).

Consistent with others’ research (16), both APC and EBC counts increased during chilling. The increase in mean APC value was greater (0.95 log CFU/100 cm<sup>2</sup>) than for
the mean EBC value (0.58 log CFU/100 cm²). The difference seen in increases in levels of EBC and APC is most likely attributed to either non-EBC bacteria recovering from any injury or growing at a faster rate than the EBC bacteria or the carcasses being contaminated with greater levels of non-EBC bacteria during chilling. Additional studies would have to be performed to effectively evaluate whether re-growth, contamination, or combinations of both caused the increased bacterial levels.

**Salmonella prevalence.** All main effects had a statistically significant ($P < 0.05$) influence on the prevalence of *Salmonella*, except for the day of the week ($P = 0.079$). *Salmonella* prevalence for abattoirs A, B, and C was 7.89, 17.08, and 19.61%, respectively (Table 4). Odds ratio analysis showed that a sample from abattoir C was 3.88 times and a sample from abattoir B was 2.88 times more likely to be *Salmonella* positive than a sample from abattoir A (Table 4). Others have reported a higher incidence of *Salmonella* in fecal samples on the pen floor from feedlots located in the Southwest than from feedlots located in the Midwest (15), suggesting an important correlation between feedlot environment and postharvest carcass contamination with *Salmonella*.

Individual abattoir data suggested that abattoir C experienced higher *Salmonella* incidence in the spring and fall (Fig. 3). Abattoirs A and B had prevalence primarily in the summer; however, samples collected in November and January produced unexpectedly high prevalence (Fig. 3), indicating both summer and winter seasons can be a problem. The causes of seasonal variation in the incidence of foodborne pathogens are poorly understood, although other researchers (5, 21, 23) have reported seasonal *Salmonella* prevalence patterns. Further investigations into climate variation and beef production operation practices (i.e., farm and feedlot hygienic practices and transportation conditions and distances) would be required to better understand the reasons for the occurrence of higher than expected incidences of *Salmonella* in the winter months at abattoirs A and B.

Figure 4 shows the *Salmonella* incidence rates for beef carcasses from combined data (all three abattoirs) at each of the sampled process steps. The intervention system, which included a preevisceration wash, a pasteurization wash, and lactic acid washes, proved very effective at reducing the prevalence of *Salmonella*. Carcasses sampled before the intervention system had a *Salmonella* incidence rate of 45.18%, whereas only 0.47% of the carcasses sampled after the intervention system were *Salmonella* positive (Fig. 4). As previously described, carcasses sampled at postchilled–lactic acid (sampling step 6) had the lowest

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**TABLE 4. Prevalence of *Salmonella* and Enterobacteriaceae on beef carcasses and the utility of Enterobacteriaceae-negative results as a negative screen for *Salmonella***

<table>
<thead>
<tr>
<th>Abattoir</th>
<th>Region</th>
<th>n</th>
<th>% positive</th>
<th>Odds ratio</th>
<th>n&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% positive</th>
<th>Utility as a negative screen for <em>Salmonella</em>&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>East</td>
<td>1,432</td>
<td>7.89</td>
<td>1.00 A&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1,412</td>
<td>66.6</td>
<td>99.2</td>
</tr>
<tr>
<td>B</td>
<td>Midwest</td>
<td>1,797</td>
<td>17.08</td>
<td>2.88 B</td>
<td>1,690</td>
<td>57.5</td>
<td>97.9</td>
</tr>
<tr>
<td>C</td>
<td>Southwest</td>
<td>2,126</td>
<td>19.61</td>
<td>3.88 C</td>
<td>2,126</td>
<td>52.1</td>
<td>97.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are combined across years, months, and sampling steps: (2) preintervention, (3) pasteurization–lactic acid spray, (4) postpasteurization–lactic acid, (5) postchill, and (6) postchilled–lactic acid. Comparisons were made between abattoirs.

<sup>b</sup> Number of EBC results with a corresponding *Salmonella* result.

<sup>c</sup> Percentage of Enterobacteriaceae-negative samples that were *Salmonella* negative.

<sup>d</sup> Values in the same column with same letter are not significantly different ($P \geq 0.05$).

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**FIGURE 3. Monthly *Salmonella* incidence rates for combined beef carcass samples collected at (2) preintervention, (3) pasteurization–lactic acid spray, (4) postpasteurization–lactic acid, (5) postchill, and (6) postchilled–lactic acid sampling steps from March 2005 to September 2006 in abattoirs A (East, left column), B (Midwest, center column), and C (Southwest, right column). There were no *Salmonella*-positive samples in February at abattoir A.**
FIGURE 4. Incidence of Salmonella on beef carcasses. Data are combined across abattoirs A (East), B (Midwest), and C (Southwest) between March 2005 and September 2006 at sampling steps (2) preintervention, (3) prepasteurization–lactic acid spray, (4) postpasteurization–lactic acid, (5) postchill, and (6) postchilled–lactic acid. Columns having the same letter are not significantly different (P ⩾ 0.05).

Probability of testing positive for Salmonella. The odds ratios of Salmonella detection increased in reverse order with the applied microbial interventions: postchill (sampling step 5), postpasteurization–lactic acid (sampling step 4), prepasteurization–lactic acid (sampling step 3), and preintervention (sampling step 2). Sampling at these steps was 2.22, 5.13, 96.06, and 226.30 times more likely to result in a Salmonella-positive carcass than sampling at postchilled–lactic acid (sampling step 6).

Unlike the APC and EBC carcass microbial profiles, there was no indication of regrowth or additional contamination during the chilling process for Salmonella. This suggests that although general microbial regrowth or additional contamination of the carcass may occur during chilling, Salmonella cells are not likely to repair or multiply during a normal 48-h chill period.

Relationship between Salmonella, APC, and EBC mean values. In an effort to develop tools for choosing pathogen testing frequency, the data were evaluated to determine the relationship between levels of indicator organisms (APCs and EBCs) and the presence or absence of Salmonella. It has been suggested that organism levels, such as APCs and EBCs, do not correlate with pathogen prevalence and therefore cannot be used to gauge the presence or absence of specific pathogens (18). Other researchers have taken caution when interpreting the relationship between indicator organisms and pathogen presence or absence because of limited numbers of samples (3). It has been suggested that levels of indicator organisms may be used successfully if sufficient data exist to establish a relationship between the presence and absence of a pathogen and the indicator organisms (1). The data set collected and reported in this study is unique because of its large size (5,228 data points each for APC, EBC, and Salmonella isolate) and temporal breadth (March 2005 to September 2006). Thus, evaluation of the relationship between levels of indicator organisms (i.e., APCs and EBCs) and the presence or absence of Salmonella is appropriate. The logistic regression analysis determined both APC and EBC log values were significantly related (APC, P < 0.0001; EBC, P < 0.0001) to the presence of Salmonella. Furthermore, the odds ratio indicated that, across the three abattoirs, for every log increase of APCs and EBCs, there was a 51 and 56% greater probability of a Salmonella-positive result at that sampling step, respectively. This relationship could be used to establish a protocol for basing prefabrication (postchilling) carcass Salmonella testing frequency on the APC or EBC results obtained for the same group of carcasses before chilling.

The relationship of binary EBC (positive = detected CFU; negative = no CFU) data with the presence or absence of Salmonella was also evaluated. In particular, we focused on the relationship between the absence of Enterobacteriaceae and the absence of Salmonella. For each abattoir, with data pooled for all sample steps, the occurrence of three carcass samples at a given sampling step being EBC negative corresponded with the composite sample testing Salmonella negative >97% of the time (Table 4). A similarly strong link between EBC- and Salmonella-negative results was seen when combined data from the three abattoirs were grouped by year or month (Table 5). When data were analyzed for each sampling step across the three abattoirs (Table 5), EBC-negative results for sampling steps 4, 5, and 6 were linked to Salmonella-negative results 98 to 99% of the time. However, EBC-negative results at sampling steps 2 and 3 corresponded to Salmonella-negative results only about 72 and 79% of the time, respectively. Therefore, our results suggest that for these three abattoirs, EBC results from sampling steps 4, 5, and 6 can be used as an accurate predictor of the absence of Salmonella in a sample. The EBC results for prechill carcasses (sampling step 4) could therefore be used to set the Salmonella testing frequency for that step and possibly also for chilled carcasses (sampling steps 5 and 6). Reducing the frequency of Salmonella testing would result in cost savings for the processor. Further studies will investigate the ramifications of using EBC results at a given sampling step to set the Salmonella testing frequency at the corresponding step or later sampling steps.
The data in this study were obtained from abattoirs with an up-to-date, typical, large-scale beef intervention process over a longer time frame than previous research has been able to evaluate. Many of our results corroborate previous research (3, 4, 23), showing the effectiveness of the “multihurdle approach” of a beef intervention system, as well as demonstrating that many factors, including geographic region, year, month, and intervention treatment, have an influence on the level of both pathogen (i.e., Salmonella) and indicator organism (i.e., APC and EBC) contamination on beef carcasses. However, the magnitude of the data set is unique, which provided a much better understanding of the relationships between indicator organisms (i.e., APCs and EBCs) and the presence of Salmonella in their abattoirs and maximize the cost-effectiveness of qualitatively testing carcasses for Salmonella. Further research is planned to determine parameters that must be met by a given abattoir-specific study to adequately describe the EBC-Salmonella relationship in their operation.

### REFERENCES


