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Use of *Enterobacteriaceae* Analysis Results for Predicting Absence of *Salmonella* Serovars on Beef Carcasses

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ABSTRACT

Previous work using a large data set (no. 1, $n = 5{,}355$) of carcass sponge samples from three large-volume beef abattoirs highlighted the potential use of binary (present or absent) *Enterobacteriaceae* results for predicting the absence of *Salmonella* on carcasses. Specifically, the absence of *Enterobacteriaceae* was associated with the absence of *Salmonella.* We tested the accuracy of this predictive approach by using another large data set (no. 2, $n = 2{,}163$ carcasses sampled before or after interventions) from the same three data set no. 1 abattoirs over a later 7-month period. Similarly, the predictive approach was tested on smaller subsets from data set no. 2 ($n = 1{,}087$, and $n = 405$) and on a much smaller data set (no. 3, $n = 100$ postintervention carcasses) collected at a small-volume abattoir over 4 months. Of *Enterobacteriaceae*-negative data set no. 2 carcasses, >98% were *Salmonella* negative. Similarly accurate predictions were obtained in the two data subsets obtained from data set no. 2 and in data set no. 3. Of final postintervention carcass samples in data set nos. 2 and 3, 9 and 70%, respectively, were *Enterobacteriaceae* positive; mean *Enterobacteriaceae* values for the two data sets were −0.375, and 0.169 log CFU/100 cm^2 (detection limit = −0.204, and *Enterobacteriaceae* negative assigned a value of −0.505 log CFU/100 cm^2). *Salmonella* contamination rates for final postintervention beef carcasses in data set nos. 2 and 3 were 1.1 and 7.0%, respectively. Binary *Enterobacteriaceae* results may be useful in evaluating beef abattoir hygiene and intervention treatment efficacy.

Salmonellosis causes an estimated 400 deaths per year in the United States *(16).* A compilation of 1999 to 2005 foodborne illness outbreaks showed that *Salmonella* was implicated in just over 8% of the 506 outbreaks involving beef products *(4).* In an effort to reduce contamination of beef products with *Salmonella* and other pathogenic bacteria, the U.S. Food Safety and Inspection Service (FSIS) enacted regulations in 1996 that established abattoir microbiological performance standards. These standards were expressed as a maximum number of *Salmonella*-positive results allowed over a series of beef carcass samples. *Salmonella* was chosen as the target pathogen for the performance standards because it is the most frequent bacterial cause of foodborne illness in the range of products regulated by the FSIS. The beef carcass standards were based on national baseline *Salmonella* prevalence values of 1.0% for steer and heifer carcasses, and 2.7% for cow and bull carcasses *(15).* Carcass sampling to determine *Salmonella* spp. prevalence is done by sponging three 100-cm^2 areas, and FSIS personnel perform the sponge sampling and subsequent sample analysis. The FSIS sampling program and performance standard and the requirement that ground beef sold to the U.S. Department of Agriculture's school lunch program be free of *Salmonella,* provide incentives for many beef abattoir operators to maintain their own program of testing beef carcass sponge samples for *Salmonella.* For abattoir operators choosing to test beef carcasses for the presence of *Salmonella,* testing can be a significant expense. In addition, the *Salmonella* analysis can take up to 2 days. One possible approach to lessen the financial and time costs associated with *Salmonella* testing is to instead test samples for an organism or group of organisms whose presence, absence, or concentration provides predictive evidence about the *Salmonella* status of carcasses.

Qualitative analysis of food for index or indicator bacteria may provide useful information for predicting the likelihood of pathogen contamination *(2, 10, 14, 17).* In beef abattoirs, index or indicator bacteria analysis of pre- and postintervention carcasses may be useful for validating and/or verifying the antipathogen efficacy of the abattoir's intervention system. Testing for index or indicator organisms may also provide information useful in evaluating the overall hygienic condition of the abattoir. The FSIS requires each beef abattoir operator to quantitatively analyze carcasses at a specified frequency for *Escherichia coli* biotype I and use results of this indicator bacteria analysis to guide the maintenance of hygienic conditions during slaughter *(15).* The European Union has also set hygienic criteria for beef carcasses: no more than 4% of tested dressed unchilled carcasses can test positive for *Salmonella*, and daily mean *Enterobacteriaceae* levels on these carcasses cannot exceed 2.5 log CFU/cm^2 *(7).* In addition, testing for a very broad indicator group, the aerobic plate count (APC), has already been studied as a tool for evaluating the hygienic control of the beef-slaughter process *(1).* Other researchers have studied the reduction of indicator bacterial groups, as measured by tests such as APC, *E. coli* count, total coliform

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count, and *Enterobacteriaceae* count (EBC), as a measure of postslaughter intervention system efficacy in beef abattoirs *(3, 12).*

This study focused on the potential importance of the family *Enterobacteriaceae* as an index bacterial group. The *Enterobacteriaceae* are a large family of facultative anaerobic, gram-negative bacilli that inhabit the intestines of many animal species. This family includes pathogenic *Escherichia* and *Salmonella* serovars. EBC have already been used to evaluate the efficacy of online beef-slaughter antimicrobial interventions and to identify optimal hygienic practices *(1, 11).* However, the use of *Enterobacteriaceae* as beef-slaughter index organisms, i.e., as an indication of the likelihood of pathogen contamination, has not yet been generally accepted. This slow acceptance may perhaps result from methodological differences in analyses for indicators and pathogens *(5, 6, 9, 10).* However, Ghafir et al. *(8)* reported that *Enterobacteriaceae* levels on pork carcasses were higher when the carcasses were contaminated with *Salmonella.* We believe that EBC analysis can provide important information about how well intervention treatments are working and overcomes the major drawbacks of directly testing for *Salmonella*: the expense and time required, and the usually low prevalence of pathogenic bacteria cells found on beef carcasses and raw products *(2).* Index bacteria analyses cost less than pathogen testing does, may require less time to perform, and may alert processors to situations in which fecal contamination is occurring, regardless of whether pathogens are involved.

In an earlier study *(13),* carcass sponge samples were collected from three commercial high-volume beef abattoirs located in geographically different regions of the United States. Over an 18-month period at each abattoir, samples were collected at various steps throughout the beef carcass intervention system. Samples were analyzed for APC and EBC, and for the presence or absence of *Salmonella.* EBC results expressed qualitatively (presence or absence) were compared with *Salmonella* results. From these results, we learned of the potential usefulness of binary (present or absent) EBC test results for predicting the likelihood of *Salmonella* contamination. Specifically, we found that EBC-negative samples were nearly always also negative for *Salmonella.* Thus, we hypothesized that EBC analysis could be used as a simple, quick way for processors to determine if their intervention systems were likely to produce carcasses with undetectable levels of *Salmonella.* However, the transferability of these results to other abattoirs was necessarily limited by the fact that the results were obtained at only three abattoirs over a specific period of time.

The objective of this study was to examine the general accuracy of EBC-negative results as a predictor of the absence of *Salmonella* contamination on postintervention beef carcasses by examining data sets obtained at the same three large-volume abattoirs during a more recent 7-month period, and at a small-volume abattoir over a 4-month period.

MATERIALS AND METHODS

Data sets. In this study, three independent data sets were collected. Based on analysis of a very large data set (no. 1, n = 5,355) obtained at three large-volume abattoirs, our earlier article *(13)* suggested the possibility of predicting the absence of *Salmonella* on beef carcasses based on the absence of *Enterobacteriaceae.* The accuracy of using a negative-EBC result to predict the absence of *Salmonella* was then tested using data set no. 2, which was obtained from the same three abattoirs (n = 2,163), and data set no. 3 (n = 100), which was obtained from a small-volume abattoir. Data set no. 1 was obtained over an 18-month period beginning the first week of March 2005. Data set no. 2 was collected over a 7-month period beginning January 2007. Identical procedures were used to collect samples for both of these data sets at the same locations (steps) throughout the beef production process in each plant. In an effort to determine if less frequent sampling and smaller data sets could still be used to effectively develop a predictive tool based on using *Enterobacteriaceae* as a negative screen for *Salmonella,* two additional data subsets were randomly generated from each of data sets nos. 1 and 2. Those generated from data set no. 1 were denoted as data subsets A and B, and those generated from data set no. 2 were denoted as data subsets C and D. Subsets A and C were generated by randomly selecting two or three sample collection days, each 1 day apart, from each week during the sampling periods. This procedure resulted in data subsets A and C having n = 2,625 and n = 1,087, respectively. Data subsets B and D were generated by randomly selecting one sample collection day from each week during the sampling periods, resulting in data sets of n = 1,100 and n = 405, respectively.

Data set no. 3 was obtained from a small-volume abattoir during 10 visits in February to May, 2008. The same sampling techniques were used in the small-volume abattoir, but samples were only obtained from postintervention carcasses 14 to 20 h after slaughter.

Sample collection steps and antimicrobial interventions. Data set nos. 1 and 2 were obtained from analysis of carcass sponge samples collected from three commercial beef abattoirs located in geographically different regions of the United States: the East (A), the Midwest (B), and the Southwest (C). Samples were collected at six steps throughout the process, but data for step no. 1 (hide on) were excluded. Steps 2 through 6 were no. 2, after complete hide removal but prior to any whole-carcass antimicrobial intervention (preintervention); no. 3, after evisceration, on-line carcass trimming, and final inspection but before carcass washes (prepasteurization–lactic acid spray); no. 4, after the pasteurization and lactic acid sprays but before carcass chilling (postpasteurization–lactic acid); no. 5, after chilling the carcass 36 to 48 h postmortem (postchill); and no. 6, after chilled carcasses were sprayed with lactic acid before going into fabrication (postchilled–lactic acid).

At each of the three large-volume abattoirs the microbial intervention systems were the same. 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 uneviscerated carcasses were sprayed with 4.0 to 5.0% lactic acid (target lactic acid temperature range of 43 to 54°C), 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, which was followed by a pasteurization water wash (target water temperature of $>$82.2°C) and a 4.0 to 5.0% lactic acid spray (target lactic acid temperature range of 43 to 54°C). 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 (target lactic acid temperature range of 43 to 54°C) was again sprayed on the carcass immediately before fabrication.

At the small-volume abattoir from which data set no. 3 was obtained, the sponge samples were obtained on carcasses 14 to 20 h after slaughter and after all interventions were completed. These interventions consisted of a 4.5 to 4.7% lactic acid spray (solution prepared using 54.4 to 60°C water, but temperature not maintained thereafter) applied after partial skinning, using a handheld pump sprayer, and a final lactic acid spray (concentration and preparation the same as described earlier) applied using a handheld pump sprayer immediately before carcass chilling.

Sample collection procedure. Samples were collected daily for both data set nos. 1 and 2. On each sampling day for data set no. 1, at each sample step three carcasses were randomly selected and sampled. In contrast, on each sampling day for data set no. 2, at each sample step one carcass was randomly selected and sampled. Each carcass was sampled using two Speci-Sponges (Nasco, Fort Atkinson, WI), each moistened with 25 ml of buffered peptone diluent (BPD; Difco, Becton Dickinson, Sparks, MD). Excess diluent was removed from each sponge inside the Speci-Sponge bag before sampling. Sampling was performed as described by Arthur et al. *(1)* and consisted of sampling an area of approximately 8,000 cm^2 on the carcass. In order to sample such a large area on the carcass, two sponges were used: one sponge sampled the inside and outside round (~4,000 cm^2) and the second sponge sampled the navel-plate-brisket-shank area (~4,000 cm^2). Sponge samples were stored in insulated coolers containing ice packs, transported to the laboratory, and stored at 4°C until microbial analysis was performed. Microbiological analyses were done within 24 h of the samples being obtained at the abattoir. The same carcass sampling technique was used to obtain data set no. 3 on 10 carcasses in the chiller at each visit, with the following minor differences in technique: sponges were each premoistened with 10 ml of BPD, both sponges were then placed in a single sample bag, and then 15 ml of BPD per sponge was poured into the sample bag. Sponge samples were stored as described above, shipped to the laboratory, and analyzed within 24 h after being obtained at the abattoir.

Microbiological analysis. For data set nos. 1 and 2, samples from steps 2 through 6 were analyzed for APC, EBC, and *Salmonella.* For data set no. 3, samples were analyzed for EBC and *Salmonella.*

For data set nos. 1 and 2 carcass sponge samples, the two sponges were placed in a single sample bag before stomaching at normal speed for 1 min (Seward Stomacher 400, Fisher Scientific, Itasca, IL). For data set no. 3 carcass sponge samples, the two sponges were already in the same bag and were directly stomached. After stomaching, either a 2-ml aliquot was removed, with 1 ml for APC and 1 ml for EBC analyses (data sets nos. 1 and 2), or only 1 ml was removed for EBC analysis (data set no. 3). Then, either six sponges (two sponges per carcass × three carcasses per step obtained in data set no. 1) or two sponges (data set no. 2) were placed in a single sample bag and enriched for *Salmonella* detection, or enrichment was done in the original sample bag containing two sponges (data set no. 3 samples).

Total EBC were determined by plating appropriate dilutions onto 3M Petrifilm *Enterobacteriaceae* Count Plates (3M Microbiology, St. Paul, MN) and incubating at 37 ± 1°C for 24 ± 2 h. After incubation, enumeration was performed by selecting plates with 10 to 150 isolated colonies and counting all red colonies that produced gas and/or acid (yellow zone surrounding colony). If fewer than 10 colonies were present on the least dilute plate, the number of colonies was still counted. If no colonies were present, a hypothetical value of 0.5 colony was assigned for the least dilute plate. Quantitative data were tested for usefulness in predicting the probability of *Salmonella* presence by using logistic regression analysis and logit transformation (described below). The detection limit of the EBC analysis was 0.625 CFU/100 cm^2 (−0.204 log CFU/100 cm^2).

Salmonella detection was performed by first screening the samples (each of which consisted of liquid expressed from the six or two sponges) by using the automated VIDAS *Salmonella* enzyme-linked fluorescent assay (bioMérieux, Hazelwood, MO). Each sample was enriched with 225 ml of BPD 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 tetrathionate broth supplemented with iodine (Difco, Becton Dickinson), respectively. Inoculated Rappaport-Vassiliadis and tetrathionate broths were incubated at 41 to 42°C for 18 to 24 h. After incubation, 1.0 ml of Rappaport-Vassiliadis and 1.0 ml of tetrathionate broth were each separately 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, allowed to cool, and the automated VIDAS *Salmonella* assay was performed. Samples testing positive on the enzyme-linked fluorescent assay screen were confirmed as *Salmonella* by first streaking onto brilliant green sulfa agar supplemented with 0.1% sodium sulfapyridine (BGS; Difco, Becton Dickinson) and xylose–lysine–tergitol 4 agar (Difco, Becton Dickinson) from the Rappaport-Vassiliadis and tetrathionate enrichments and incubating at 35 ± 2°C for 18 to 24 h. Three typical colonies from each of the incubated BGS and xylose–lysine–tergitol 4 agar plates were selected for biochemical analysis. Typical uncrowded *Salmonella* colonies on the BGS agar are pink and opaque with a smooth appearance and an entire edge surrounded by a red color in the medium. When crowded on BGS plates, *Salmonella* will produce typical colonies that appear tan against the green background. Typical colonies selected from the xylose–lysine–tergitol 4 agar plates appeared black or red, with or without black centers, with a red rim around the colony. Biochemical analysis was performed using triple sugar iron (Difco, Becton Dickinson) and lysine iron agar (Difco, Becton Dickinson) slants. Inoculation of the triple sugar iron and lysine iron 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 slants were subjected to serological analysis using polyvalent (O) antiserum (serogroups A through I; Difco, Becton Dickinson). Isolates were considered confirmed with positive agglutination from the polyvalent (O) antiserum.

Statistical analysis. To determine the accuracy of predicting a negative *Salmonella* result from a negative *Enterobacteriaceae* result, the EBC values were first converted to binary EBC data (positive = detected CFU, negative = no CFU). After the EBC data were converted to binary form, the number of EBC-negative samples was divided by the total number of EBC samples and multiplied by 100 to determine the percentage of samples that were EBC negative. The percent accuracy of predicting a negative *Salmonella* result from a negative *Enterobacteriaceae* result was determined by dividing the total number of negative *Salmonella* results for the EBC-negative samples by the total number of negative *Enterobacteriaceae* samples and multiplying by 100. The mean levels of EBC for each data set and abattoir were also cal-

TABLE 1. *Data set nos. 1 and 2: percentage of carcass sponge samples testing negative for EBC and percentage of* Enterobacteriaceae-*negative samples that tested negative for* Salmonella[a]

Abattoir	Data set no. 1: *n*	Data set no. 1: EBC-negative carcasses (%)	Data set no. 1: *Salmonella*-negative, EBC-negative carcasses (%)	Data set no. 2: *n*	Data set no. 2: EBC-negative carcasses (%)	Data set no. 2: *Salmonella*-negative, EBC-negative carcasses (%)
East (A)	1,432	33.0	99.2	692	52.5	99.5
Midwest (B)	1,797	40.0	97.9	746	42.0	98.7
Southwest (C)	2,126	47.8	97.3	725	72.6	98.1
Total	5,355	41.2	97.9	2,163	55.6	98.7

[a] Data are combined across five sampling steps: 2, preintervention; 3, prepasteurization–lactic acid spray; 4, postpasteurization–lactic acid; 5, postchill; and 6, postchilled–lactic acid.

culated. Logistic regression analysis (SAS PROC LOGISTIC, SAS version 9.1, SAS Institute, Cary, NC) was performed with data set no. 1 to describe the relationship between the probability of a positive-*Salmonella* test result and a given log CFU per 100 cm^2 level of *Enterobacteriaceae.* The continuous explanatory variable, log CFU per 100 cm^2 of EBC, was related to the dichotomous dependent variable, presence or absence of *Salmonella*, at incremental increases of $x = 0.01$ log CFU/100 cm^2. Logit (P) values for the probability of a *Salmonella*-positive result were calculated using the intercept (a) = -2.0563 and slope (b) = 0.79 parameters determined by logistic regression analysis: logit (P) = $a + bx$. The logit equation was then transformed: $P = (e^{a+bx})/(1 + e^{a+bx})$. The estimated probability of a *Salmonella*-positive result could then be calculated for any x value (EBC level).

RESULTS AND DISCUSSION

Based on the previously published results from data set no. 1 *(13),* we chose to predict the absence of *Salmonella* based on EBC-negative results, rather than the presence of *Salmonella* based on EBC-positive results. The proportion of samples in data set no. 1 that were EBC positive and *Salmonella* negative was too high (43.3%; *(13)*) to support decision-making based on EBC-positive results, i.e., the rate of incorrectly concluding that carcasses were contaminated with *Salmonella* would be unacceptably high. Instead, we chose to develop a predictive tool that had a low likelihood of incorrectly concluding that *Salmonella* was absent from carcasses. Therefore, the key criterion was the proportion of EBC-negative samples that were also *Salmonella* negative. If this proportion was high, the likelihood of carcasses being EBC negative and *Salmonella* positive would be low.

For the three large-volume abattoirs, we validated the concept of predicting a *Salmonella*-negative carcass based on an EBC-negative result with data set no. 2. When EBC-negative results from all five sampling steps in data set no. 2 were pooled, the accuracy of predicting a *Salmonella*-negative result was 99.5, 98.7, and 98.1% for abattoirs located in the East (A), the Midwest (B), and the Southwest (C), respectively (Table 1). When EBC-negative results were pooled across abattoirs and sorted by sampling step (Table 2), the accuracy of predicting a *Salmonella*-negative result at each data set no. 2 sampling step was 94.4, 95.4, 100, 100, and 99.2% at steps 2 (preintervention), 3 (prepasteurization–lactic acid spray), 4 (postpasteurization–lactic acid), 5 (postchill), and 6 (postchilled–lactic acid), respectively.

An important aspect of developing a predictive tool is to understand the limits of the tool's effectiveness. The tool for determining the presence or absence of *Enterobacteriaceae* was initially developed and validated using data set nos. 1 and 2, consisting of $n = 5{,}355$ and $n = 2{,}163$ samples, respectively. However, we wanted to determine whether such large data sets are necessary to establish the relationship between EBC-negative and *Salmonella*-negative results. When data subset A results from sampling steps 2 through 6 in data set no. 1 were pooled and sorted by ab-

TABLE 2. *Data set nos. 1 and 2: percentage of carcass sponge samples testing negative for EBC and percentage of* Enterobacteriaceae-*negative samples that tested negative for* Salmonella[a]

Sampling step no.	Data set no. 1: *n*	Data set no. 1: EBC-negative carcasses (%)	Data set no. 1: *Salmonella*-negative, EBC-negative carcasses (%)	Data set no. 2: *n*	Data set no. 2: EBC-negative carcasses (%)	Data set no. 2: *Salmonella*-negative, EBC-negative carcasses (%)
2	1,074	3.4	72.2	432	24.8	94.4
3	1,080	8.3	78.9	433	24.9	95.4
4	1,079	80.2	98.3	433	88.0	100
5	1,061	35.9	99.7	433	49.0	100
6	1,061	78.7	99.6	432	91.2	99.2

[a] Data are combined across the three abattoirs, East (A), Midwest (B), and Southwest (C), and are shown for five sampling steps: 2, preintervention; 3, prepasteurization–lactic acid spray; 4, postpasteurization–lactic acid; 5, postchill; and 6, postchilled–lactic acid.

TABLE 3. *Data subsets A and B of data set no. 1 and data subsets C and D of data set no. 2: percentage of carcass sponge samples testing negative for EBC and percentage of* Enterobacteriaceae*-negative samples that tested negative for* Salmonella[a]

	Data set no. 1				Data set no. 2			
Abattoir	Data subset	*n*	EBC-negative carcasses (%)	*Salmonella*-negative, EBC-negative carcasses (%)	Data subset	*n*	EBC-negative carcasses (%)	*Salmonella*-negative, EBC-negative carcasses (%)
East (A)	A	717	33.6	99.2	C	350	53.7	100
	B	340	33.2	99.1	D	140	56.4	100
Midwest (B)	A	847	42.9	98.6	C	372	42.5	98.7
	B	359	40.0	97.9	D	140	42.0	98.7
Southwest (C)	A	1,061	49.1	97.7	C	365	73.2	97.4
	B	401	48.9	96.4	D	125	77.6	97.9
All abattoirs	A	2,625	42.9	98.3	C	1,087	56.4	98.5
	B	1,100	41.2	97.6	D	405	58.0	98.7

[a] Data comprising subsets A and C were obtained 2 to 3 times per week, and data comprising subsets B and D were obtained weekly. Data are combined across five sampling steps: 2, preintervention; 3, prepasteurization–lactic acid spray; 4, postpasteurization–lactic acid; 5, postchill; and 6, postchilled–lactic acid.

attoir (n = 2,625, Table 3) the percentages of EBC-negative carcasses that were negative for *Salmonella* were 99.2, 98.6, and 97.7% for abattoirs located in the East (A), the Midwest (B), and the Southwest (C), respectively. The corresponding rates in data subset C (derived from data set no. 2, n = 1087) were 100, 98.7, and 97.4% for abattoirs located in the East (A), the Midwest (B), and the Southwest (C), respectively (Table 3). When data subset A (n = 2,625) results were pooled across all three abattoirs and sorted by step (Table 4), the percentages of *Enterobacteriaceae*-negative carcasses that were negative for *Salmonella* were 84.6, 82.0, 98.4, 99.5, and 100% at steps 2 through 6, respectively. The analysis of data subset C (n = 1,087) by step, across all three abattoirs, yielded accuracy rates of 92.5, 93.2, 100, 100, and 99.5% for steps 2 through 6, respectively (Table 4).

The intent of generating data subsets B and D was to determine if a predictive tool could be developed and validated with samples obtained weekly. Analysis of data subset B results pooled from all six sampling steps and sorted by abattoir (derived from data set no. 1, n = 1,100) showed that the percentages of *Enterobacteriaceae*-negative carcasses negative for *Salmonella* were 99.1, 97.9, and 96.4% at abattoirs in the East (A), the Midwest (B), and the Southwest (C), respectively (Table 3). Data subset D (derived from data set no. 2, n = 405) yielded accuracy rates of 100, 98.7, and 97.9% in the East (A), Midwest (B), and Southwest (C) abattoirs, respectively (Table 3). When subset B results were pooled across all three abattoirs and sorted by step (Table 4), the percentages of EBC-negative samples that tested negative for *Salmonella* result were 84.5, 76.0, 98.9, 100, 100%, respectively, at steps 2 through 6. When the data subset D results were pooled across the three abattoirs and sorted by sampling step, the accuracy rates were

TABLE 4. *Data subsets A and B of data set no. 1 and data subsets C and D of data set no. 2: percentage of carcass sponge samples testing negative for EBC and percentage of* Enterobacteriaceae*-negative samples that tested negative for* Salmonella[a]

	Data set no. 1				Data set no. 2			
Sampling step	Data subset	*n*	EBC-negative carcasses (%)	*Salmonella*-negative, EBC-negative carcasses (%)	Data subset	*n*	EBC-negative carcasses (%)	*Salmonella*-negative, EBC-negative carcasses (%)
2	A	525	2.5	84.6	C	217	53.7	92.5
	B	220	3.6	84.5	D	81	56.4	95.2
3	A	526	9.5	82.0	C	217	42.5	93.2
	B	221	11.3	76.0	D	81	42.0	90.9
4	A	528	82.2	98.4	C	217	73.2	100
	B	221	80.5	98.9	D	125	77.6	100
5	A	524	38.6	99.5	C	218	73.2	100
	B	219	37.9	100	D	125	77.6	100
6	A	522	81.4	100	C	218	73.2	99.5
	B	219	78.1	100	D	125	77.6	100

[a] Data comprising subsets A and C were obtained 2 to 3 times per week, and data comprising subsets B and D were obtained weekly. Data are combined across three abattoirs, East (A), Midwest (B), and Southwest (C), and are shown for five sampling steps: 2, preintervention; 3, prepasteurization–lactic acid spray; 4, postpasteurization–lactic acid; 5, postchill; and 6, postchilled–lactic acid.

95.2, 90.9, 100, 100, and 100% at steps 2 through 6 (Table 4). These results conclusively demonstrate that when the production process is consistent at a large-volume abattoir, a relatively infrequent, e.g., weekly, sampling interval can be used to produce data sets for the development and validation of a *Salmonella*-absence prediction tool. The utility of this prediction tool is based on the relative sensitivities of the analytical methods used. Because *Salmonella* is a subset of the family *Enterobacteriaceae,* the predictive tool will work well if the methods have comparable sensitivity. If the sensitivity of the *Salmonella* method were increased, or the sensitivity of the *Enterobacteriaceae* method were decreased, a negative *Enterobacteriaceae* result would less often correspond to a negative *Salmonella* test result.

Because the basic premise of this prediction tool is the fact that *Salmonella* is a member of the family *Enterobacteriaceae,* it is possible that the prediction tool might be used at a range of abattoirs, not just at the large-volume abattoirs providing the samples in data set nos. 1 and 2. When we tested this idea using data set no. 3 ($n = 100$ carcasses sampled 14 to 20 h after slaughter, i.e., postintervention), an accuracy rate of 100% was obtained (of the 30% of EBC-negative carcasses). Although, the prediction tool should be tested at a wider variety of beef abattoirs, this result suggests potential industry-wide usefulness.

There are two important benefits of using EBC-negative results to predict absence of *Salmonella* on beef carcasses: (i) the EBC analysis is rapid and inexpensive compared with the *Salmonella* analysis, and (ii) the predictions appear to be very unlikely to be fail-dangerous, i.e., *Salmonella* is seldom detected on an EBC-negative carcass. Ideally, to effectively implement this tool at beef abattoirs, personnel at each individual facility should collect separate data sets to first determine if the same *Enterobacteriaceae*-*Salmonella* relationship exists, and then to determine whether the prediction tool can be validated over time. A critical factor in determining the size of these data sets is the consistency of the intervention system. Extremely consistent abattoir operation is likely to support implementation of an accurate predictive tool with a much smaller data set and a reduced sampling frequency.

Some abattoir operators may wish to make inferences about slaughter process hygiene or intervention system efficacy based on the proportion of carcass samples that are EBC positive. In this study, there was a general but imprecise relationship between the percentage of samples yielding EBC-positive results, the mean EBC levels, and the percentage of samples yielding *Salmonella*-positive results. The percentages of chilled postintervention carcasses yielding EBC-positive results in data set nos. 1, 2, and 3 were 21, 9, and 70%, respectively. The corresponding chilled-carcass mean values for *Enterobacteriaceae* for the three data sets were −0.382, −0.375, and 0.169 log CFU/100 cm^2 (detection limit = −0.204 log CFU/100 cm^2). Percentages of *Salmonella*-positive chilled postintervention carcasses for the three data sets were 0.5, 1.1, and 7.0%. These trends suggest that abattoir operators could also use baseline data to set maximum EBC-positive prevalence or EBC log CFU/100 cm^2 levels in an effort to reduce *Salmonella* prevalence.

We used logistic regression analysis and the logit transformation in investigating the potential usefulness of the latter approach, i.e., predicting *Salmonella* status of a carcass based on quantitative EBC results. Logistic regression analysis of data set no. 1 showed that EBC log CFU/100 cm^2 results were significantly related to the presence of *Salmonella* ($P < 0.0001$). The logit transformation allows the setting of a quantitative criterion for EBC that corresponds to a probability of *Salmonella* presence. This criterion can be set at any level equal to or greater than the detection limit of the EBC method. As an example, an EBC level of −0.18 log/100 cm^2 (equivalent to 0.66 CFU/100 cm^2, or 2.1 colonies on the least dilute plate) corresponds to a 0.10 probability of *Salmonella* presence. We applied this criterion to data set no. 2, and evaluated each quantitative EBC result based on the corresponding probability of *Salmonella* presence. Each EBC result was classified as "safe" (corresponding to a probability of *Salmonella* presence of <0.10), or "dangerous" (corresponding to a probability of *Salmonella* presence of ≥ 0.10). For data set no. 2, the criterion was accurate, i.e., the sample was *Salmonella* positive when the EBC result corresponded to dangerous or was *Salmonella* negative when the EBC result corresponded to safe for 91.8% of the samples. The criterion was false positive, i.e., it incorrectly predicted dangerous when the sample was actually *Salmonella* negative for 7.0% of samples, and was false negative, i.e., it falsely predicted safe when the sample was actually *Salmonella* positive for 1.2% of samples. When the same criterion was applied to data subsets C and D from data set no. 2, the accuracy rates were 92.0 and 92.1%, respectively, and the false-positive and false-negative rates were 6.7 and 1.3% and 6.9 and 1.0%, respectively. Application of this criterion to results obtained at a different abattoir (data set no. 3) did not yield satisfactory results, with accurate, false-positive and false-negative rates of 58.0, 41.0, and 1.0%, respectively. These results suggest that although it may be possible to obtain useful predictions on *Salmonella* status based on qualitative EBC results across abattoirs using different intervention systems, quantitative EBC criteria for predicting *Salmonella* status must be developed separately for each abattoir intervention system.

In summary, the results of our study suggest that there is a general relationship on beef carcasses between the absence of *Enterobacteriaceae* and the absence of *Salmonella*. The absence of *Enterobacteriaceae* might be a useful criterion for predicting absence of *Salmonella* on postintervention carcasses across a broad range of abattoir intervention systems. The accuracy of such predictions is dependent on the relative sensitivities of the microbiological methods used. A relationship between quantitative *Enterobacteriaceae* results and *Salmonella* presence or absence also appears to exist and may be useful, for the same abattoir intervention systems for which the mathematical relationship was identified, in predicting carcass *Salmonella* status based on *Enterobacteriaceae* levels.

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