Survey of Antimicrobial Effects of Beef Carcass Intervention Treatments in Very Small State-Inspected Slaughter Plants

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ABSTRACT: U.S. beef slaughter facilities are required to use a carcass intervention treatment to reduce contamination by *Escherichia coli* O157:H7. Very small beef slaughter operators generally are unable to carry out challenge studies to validate intervention treatment effectiveness, and in-plant pathogen challenge studies are not permitted. The objective of this study was to evaluate the effectiveness, measured by decreases in generic *E. coli*, coliforms, Enterobacteriaceae, and aerobic plate count, of intervention treatments used at very small beef slaughter facilities in Wisconsin. Over a 9-mo period, 265 head of beef were sampled at 22 very small beef slaughter facilities before and after the intervention treatment. The interventions studied were dry-aging, low-pressure hot-water spray, high-pressure hot-water spray, 2.5% acetic acid spray, and Fresh Bloom™ (a mix of citric acid, ascorbic acid, and erythorbic acid) spray. Sprays were applied using a hand-held nozzle (hot water) or a pump-type sprayer (acid). There was no significant difference ($P > 0.10$) between intervention treatments and all treatments caused significant reductions ($P < 0.10$) in indicator organisms. Ranges in average reductions for generic *E. coli*, coliforms, and Enterobacteriaceae among the treatments were 0.6 to 2.0 log CFU/cm^2, 0.7 to 2.2 log CFU/cm^2, and 0.4 to 2.2 log CFU/ cm^2, respectively. For all treatments, rapid decreases in cooler temperature and relative humidity significantly affected indicator reduction, and for hot-water washing, increasing spray time led to significantly greater reductions. Further studies using actual or simulated very-small-plant intervention treatments directly against *E. coli* O157:H7 would provide additional validation of treatment efficacy.

Keywords: beef slaughter interventions, *Escherichia coli*, indicator bacteria

Introduction

From 1990 to 2003, 438 outbreaks of foodborne illness, with 12702 cases, were linked to beef and beef-containing products. Of these outbreaks, 43% involved the fecal pathogens *Escherichia coli* O157:H7 and *Salmonella* serovars (DeWaal and others 2006). *E. coli* O157:H7 causes an estimated 73480 illnesses per year in the United States, which lead to an estimated 2168 hospitalizations and 61 deaths. Foodborne transmission has been implicated in 52% of all *E. coli* O157:H7 infection outbreaks, ground beef accounting for 41% of foodborne outbreaks (Rangel and others 2005). The 1st documented outbreak of foodborne illness due to *E. coli* O157:H7, in 1982, was attributed to contaminated hamburger patties (Riley and others 1983). Since then, *E. coli* O157:H7 steadily gained notoriety as a deadly pathogen, with 1993 becoming the year in which it gained national attention in association with a large, multistate outbreak connected to undercooked ground beef patties from a fast-food restaurant chain.

In 1996 the U.S. Dept. of Agriculture/Food Safety and Inspection Service (USDA/FSIS) established requirements designed to reduce the occurrence and levels of pathogenic microorganisms on meat and poultry products and thereby reduce the incidence of foodborne illness associated with the consumption of these products. These requirements were set forth in the "Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems; Final Rule" ("Final Rule") (Food Safety and Inspection Service, U.S. Dept. of Agriculture 1996), which required meat processors to implement HACCP as their primary food safety program.

In 2002 FSIS required all raw beef processors to reassess their HACCP plans to determine whether *E. coli* O157:H7 contamination was a hazard reasonably likely to occur in their process. If the reassessment led to the conclusion that contamination was reasonably likely to occur, then this hazard was to be addressed in the processor's HACCP plan. This requirement was imposed following studies published by Smith and others (2001) and Elder and others (2000), which suggested that *E. coli* O157:H7 was more prevalent than originally believed and may be a hazard that is reasonably likely to occur at all stages of raw beef handling (Food Safety and Inspection Service, U.S. Dept. of Agriculture 2002). The mandated reassessment effectively resulted in all beef slaughter facilities implementing at least 1 carcass intervention treatment to serve as a critical control point to reduce the hazard of *E. coli* O157:H7 to an acceptable level. The implementation and validation of an effective intervention treatment have been especially challenging for very small facilities, those defined under the final rule as having less than 10 employees or less than \$2.5 million in annual sales. By this definition, 2892 federally inspected facilities were defined as very small. Operators of very small plants cannot afford the mechanized intervention systems used at large facilities and lack the resources needed to carry out challenge studies to validate the intervention treatment they are using. In-plant challenge studies using pathogens are not permitted, so an alternative in-plant approach

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would be to evaluate the effectiveness of intervention treatments against potential indicator bacteria. In this study, we define indicator bacteria as groups of bacteria whose presence in given numbers points to inadequate processing for safety (Mossel and others 1995). The objective of this study was to evaluate the effectiveness, using several bacterial indicator groups, of intervention treatments used at very small beef slaughter facilities in Wisconsin.

Materials and Methods

Experimental design

Twenty-two very small state-inspected beef processing facilities located throughout Wisconsin were visited from January through September of 2006 and a total of 265 head of beef were sampled. Each plant was visited 1 to 3 times, as described in Table 1. The interventions studied included dry aging (multiday refrigeration without water spray-chilling), acid spray, Fresh Bloom™ spray, and 2 types of hot water washing. The acid spray intervention consisted of the application of a 1:1 dilution of commercially available vinegar (5% acetic acid) to the carcass with a hand-held spray wand prior to chilling. The Fresh Bloom intervention consisted of the application of a 59.9 g/L (8 oz per gallon) dilution of Fresh Bloom, a mix of citric acid, ascorbic acid, and erythorbic acid (Excalibur Seasonings, Pekin, Ill., U.S.A.) to the carcass with a hand-held spray wand prior to chilling. The hot water intervention consisted of either washing the carcass with $\geq$ 65.56°C (150°F) water using a low-pressure spray nozzle or spraying $\geq$ 48.89°C (120°F) water at $\geq$ 6894.76 kPa (1000 psi) using a pressure washer prior to chilling. Acid spray, Fresh Bloom, and both hot water treatments all were followed by storing the carcass halves in a cooler for 24 h prior to fabrication. One half of each carcass was sampled just prior to the intervention step and the other half was sampled after the intervention on the day of fabrication. Sampled halves were alternated for sequential carcasses (right, left, right, and so on) and the half not sampled on the slaughter day was sampled on the fabrication day. In addition to microbiological samples, carcass storage conditions (cooler temperature and relative humidity) were monitored using data loggers (TP 120; Dickson, Addison, Ill., U.S.A.). The weight and age ($>$ 30 mo opposed to $\leq$ 30 mo) were recorded for every carcass sampled, along with pH and weight of acid solution applied (calculated from weight of sprayer before and after spraying) or temperature and duration of hot-water wash, when applicable.

Sampling protocol

Carcass surface sampling was done using sponge-sampling kits obtained from 2 suppliers (Nasco, Fort Atkinson, Wis., U.S.A.; Intl. BioProducts Inc., Redmond, Wash., U.S.A.). The sampling procedure involved swabbing 100 cm^2 areas at 3 carcass sites (flank, brisket, and rump) as described by the USDA/FSIS for conducting generic *E. coli* testing as part of the final rule (Food Safety and Inspection Service, U.S. Dept. of Agriculture 1996). The 3.5 × 7.5 cm sponge was moistened with approximately 10 mL of supplied buffered peptone water (BPW). The sponge was then used to swab 10 times back-and-forth horizontally and 10 times up-and-down vertically over a 10 × 10 cm area on each of the 3 designated surfaces of the carcass. The same side of the sponge was used for sequential swabbing of the flank and brisket. The other side of the sponge was then used for swabbing the rump. Following swabbing, the sponge was returned to the sampling bag and approximately 15 mL of BPW was added. Samples were transported in coolers containing ice packs to the laboratory and immediately refrigerated for analysis within 24 h. At the laboratory, the sample bag containing the sponge was stomached for 2 min at 230 rpm using a Stomacher 400 (Seward, Fisher Scientific, Itasca, Ill., U.S.A.) and as much diluent as possible was then expressed from the sponge by manually squeezing the bag. From the expressed diluent, serial dilutions were prepared using Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, Wis., U.S.A.). Using the expressed diluent and serial dilutions, 3M Petrifilm *E. coli*/Coliform Count plates (PF-EC; 3M Microbiology Products, St. Paul, Minn., U.S.A.), 3M Petrifilm Enterobacteriaceae Count plates (PF-EB; 3M), and 3M Petrifilm Aerobic Count Plates (PF-AC; 3M) were used for enumeration of indicator bacteria, with 1 plate prepared per dilution for each analysis. The PF-EC and PF-EB plates were incubated at 35°C for 24 h and the PF-AC plates were incubated at 35°C for 48 h.

Table 1 — Summary of facilities visited and interventions employed

Intervention	Nr of visits	Facility nr(s)
Dry aging – 4 d	1	15
Dry aging – 6 d	1	14, 16, 17, 21, 22
	3	1
Dry aging – 7 d	1	10, 18, 19
Acetic acid spray	1	6, 7, 8, 13
	2	4
Fresh Bloom spray	1	5
	2	2
Low-pressure hot water	1	9, 11, 12, 20
High-pressure hot water	3	3

Enumeration

Counted *E. coli* colonies on PF-EC plates were blue and associated with entrapped gas, within approximately 1 colony diameter. Coliforms enumerated on PF-EC plates included *E. coli* colonies as well as red colonies, which were associated with entrapped gas, within approximately 1 colony diameter. Counted Enterobacteriaceae colonies on PF-EB plates were red and fell into 1 of 3 categories: colonies associated with gas bubbles and no yellow zones of clearing, colonies with yellow zones of clearing (indicative of acid production) but no gas production, or colonies producing both gas and acid. All red colonies were counted on PF-AC plates, regardless of their size or the intensity of their color, to determine the aerobic plate count (APC). The log CFU/cm^2 was calculated from the colony totals. When no cells were detected on the lowest dilution plate, a log value of −1.38, which is equivalent to 0.5 colony-forming units, was used. This value was used because a value of zero colony-forming units cannot be converted to a log value.

Handling and disposal

Due to the possible presence of pathogenic microorganisms, all of the procedures stated above were performed using gloved hands and all materials used were autoclaved prior to disposal.

Statistical analysis

For each type of bacteria enumerated, a mixed-effects model was used to evaluate effectiveness of the intervention at reducing the number of recoverable cells. In this model, the response variable was the difference in bacterial counts (log CFU/cm^2) between pre- and post-intervention samples, the fixed-effect treatment factor was the intervention, and the random-effect blocking factors were the slaughter facilities and months. If the treatment factor was found to have a statistically significant effect on the response variable, a pairwise comparison was performed to determine which interventions were significantly different. In addition, a linear regression model was used to examine the relation between the changes in bacterial counts and the various explanatory variables: whether or not the carcass was over 30 mo of age, the cooler temperature (°C) and relative

humidity at 12 a.m. (midnight after slaughter) and 5 a.m. on the day following slaughter, and the weight of the carcass (in kilograms). An individual regression was performed for each explanatory variable, and then a backward elimination was performed to reach a regression model that had the best set of significant explanatory variables. For carcasses that received an acetic acid or Fresh Bloom spray, 3 additional explanatory variables, acid weight (kg), acid pH, and ratio of acid weight to carcass weight, were used in a regression model similar to that described above. For carcasses that received a hot-water wash or pressure washing, 2 additional explanatory variables, water temperature and wash time(s), were used in a regression model similar to that described above. These models were fitted using PROC MIXED and PROC REG in SAS 9.2 statistical software (SAS Inst. Inc., Cary, N.C., U.S.A.) with the significance level for hypothesis testing fixed at 0.10.

Results and Discussion

In the present study 10 of the 22 plants employed dry-aging, 5 used acetic acid, 2 used Fresh Bloom, 4 used low-pressure hot water, and 1 used high-pressure hot water. Comparable proportions were seen in a 2005 survey of Wisconsin meat inspectors, which indicated that roughly half of the inspected facilities used a dry-aging treatment as the beef carcass intervention step and 1/3 of the plants sprayed carcasses with an acetic acid solution. The majority of the remaining plants sprayed the carcasses with either lactic acid or Fresh Bloom and only a few plants used a hot water wash (data not shown). It should be noted that it is not uncommon for small and very small Wisconsin processors to change their intervention treatment. In contrast, large-scale beef slaughter operations typically employ multiple-hurdle decontamination procedures with steps such as mechanized lactic acid spraying, hot water washing, steam pasteurization, and spot decontamination by manual steam vacuuming.

Measured values for the intervention treatments (Table 2) ranged from 93.4 to 550.7 kg (206 to 1214 lb) for carcass weight, 0.085 to 1.895 kg for weight of acid spray applied, 48.9 to 85.0°C (120 to 185°F) for hot water temperatures, and 70 to 380 s for hot water wash times. In addition, cooler conditions (Table 2) ranged from −0.3 to 15.7°C (31.4 to 60.2°F) for temperature at 5 a.m. the day after slaughter and 38.8% to 93.5% for relative humidity. Variation in intervention efficacy between plants using the same intervention step may be attributed to these sizeable differences.

A strong correlation exists between the presence of indicator organisms on beef carcasses, specifically *E. coli*, and the presence of enteric pathogens and fecal contamination (Food Safety and Inspection Service, U.S. Dept. of Agriculture 1996). Table 3 depicts the prevalence of the indicators *E. coli*, coliforms, and Enterobacteriaceae both pre- and postintervention. Of these indicators, *E. coli* was the least prevalent both pre- and postintervention, whereas Enterobacteriaceae was the most prevalent for both pre- and postintervention. The comparison between pre- and postintervention indicator levels was studied as a measure of the efficacy of an intervention treatment.

All of the interventions decreased the prevalence of indicators. The 4-d dry aging treatment was the most effective at reducing the prevalence of *E. coli*, although only 9 carcasses were treated this way. If all dry aging treatments were considered collectively, high-pressure hot water was the most effective in reducing the prevalence of *E. coli*, with low-pressure hot water, the combined dry-aging treatments, and Fresh Bloom and acetic acid spray following. Dry-aging resulted in the largest decrease of coliform prevalence followed by low-pressure hot water, high-pressure hot water, acetic acid, and Fresh Bloom sprays. This result may be due to the poor

Table 2 – Description of beef carcass intervention treatment conditions[a]

Intervention	Carcass weight		Cooler conditions[b]				Acid spray conditions				Hot water wash conditions			
	Min kg	Max kg	Min temp °C	Max temp °C	Min percent RH	Max percent RH	Min pH	Max pH	Min weight (kg)	Max weight (kg)	Min temp °C	Max temp °C	Min time (s)	Max time (s)
DA-4	264.0	442.3	1.4	1.4	83.1	83.1	–	–	–	–	–	–	–	–
DA-6	100.7	550.7	0.8	2.7	70.0	82.7	–	–	–	–	–	–	–	–
DA-7	230.9	420.9	2.6	15.7	66.1	93.5	–	–	–	–	–	–	–	–
Ac	93.4	452.7	0.3	9.0	54.9	89.4	2.68	3.02	.085	1.895	–	–	–	–
FB	113.1	418.2	2.8	5.4	59.7	86.1	4.17	4.18	0.495	1.215	–	–	–	–
LPHW	126.1	468.1	−0.3	4.2	68.2	82.7	–	–	–	–	65.6	85.0	70	380
HPHW	155.6	475.8	3.4	7.6	38.8	87.4	–	–	–	–	48.9	50.0	75	150

[a]DA-4, DA-6, DA-7, Ac, FB, LPHW, and HPHW refer to 4-d dry-aging, 6-d dry-aging, 7-d dry-aging, acetic acid spray, Fresh Bloom spray, low-pressure hot water wash, and high-pressure hot water wash, respectively.
[b]Measured at 5 a.m. the day after slaughter.
– = not applicable.

Table 3 — Prevalence and mean levels of *Escherichia coli*, coliforms, and Enterobacteriaceae on beef carcasses pre- and post-intervention[a]

		Prevalence / mean[b]					
		E. coli		Coliforms		Enterobacteriaceae	
Treatment	Carcasses	Pre	Post	Pre	Post	Pre	Post
DA-4	9	9[b] 0.64[c]	0 −1.38[d]	9 0.76	0 −1.38[d]	9 0.96	3 −1.21[d]
DA-6	92	61 −0.76	3 −1.35[d]	70 −0.48	6 −1.34[d]	84 −0.08	33 −0.87[d]
DA-7	26	21 0.10	11 −0.66[d]	25 0.33	16 −0.37[d]	26 0.78	16 0.37
Ac	61	47 −0.34	13 −1.05[d]	50 −0.22	15 −1.03[d]	58 0.15	30 −0.84[d]
FB	30	24 −0.33	7 −1.23[d]	26 −0.19	13 −1.02[d]	28 0.04	22 −0.57[d]
LPHW	28	24 0.13	3 −1.24[d]	26 0.51	9 −1.08[d]	27 0.57	12 −0.86[d]
HPHW	19	18 −0.13	3 −1.29[d]	19 0.04	8 −1.17[d]	19 0.22	15 −0.66[d]

[a]DA-4, DA-6, DA-7, Ac, FB, LPHW, and HPHW refer to 4-d dry-aging, 6-d dry-aging, 7-d dry-aging, acetic acid spray, Fresh Bloom spray, low-pressure hot water wash, and high-pressure hot water wash, respectively.
[b]Number of carcasses.
[c]Mean (log CFU per cm^2) for given n (a value of −1.38 was assigned when no cells detected).
[d]Statistically significant decrease ($P < 0.10$).

cold-tolerance of coliforms since dry aging exposes the bacteria to cold temperatures for a longer period of time prior to follow-up sampling than any other intervention. For example, Ingham and Tautorus (1991) showed a decrease in coliforms of −0.28 to −1.28 log (CFU) on cooked uncured turkey loaf stored under vacuum at 3°C after 6 d. As for Enterobacteriaceae, dry-aging resulted in the largest decrease in prevalence, followed by low-pressure hot water, acetic acid, high-pressure hot water, and Fresh Bloom. The change observed in APC was quite variable across all intervention treatments (Table 4). Overall, acid sprays and hot water washing were the most likely to result in a decrease in APC, whereas 6-d or 7-d dry-aging treatments were most likely to result in increased APC. The APC analysis detects not only bacteria that contaminate the carcass via the slaughter process, but also airborne contaminants. In addition, the APC on dry-aged carcasses reflected any growth that occurred over the 4 to 7 d of storage. Therefore, it is not surprising that APC increased on a large proportion of dry-aged carcasses. There were no obvious differences between facilities at which dry-aging treatments resulted in increased APC and those at which dry-aging resulted in decreased APC.

All of the intervention treatments caused significant ($P < 0.10$) decreases in mean levels of *E. coli*, coliforms, and Enterobacteriaceae (Table 3). In addition, each treatment resulted in a much greater proportion of carcasses on which *E. coli* was not detected (Table 5). The same trend was generally observed for coliform (Table 6) and Enterobacteriaceae (Table 7) counts. Acid spray, hot-water washing, and 4 or 6-d dry-aging treatments appeared to cause the greatest change in frequency distribution for each indicator group, while a notable number of carcasses still had high concentrations of indicators after the 7-d dry-aging treatment. Similar shifts in APC frequency distribution were also observed (Table 8). This anomalous result likely reflects the cooler conditions for half of the carcasses slaughtered at plant number 19. This facility slaughtered at a location that was separate from their retail facility. Normally, all carcasses would be held in a cooler at the slaughter facility until the end of the slaughter day (when all animals had been slaughtered) and then transported to the cooler at the retail facility. When we sampled, one-half of each carcass was kept at the slaughter facility cooler for county fair judging and the other half was transported to the retail facility cooler. Because of our sampling procedure of alternating carcass halves, the

Table 4 — Change in aerobic plate count on beef carcasses after each intervention

		Degree of change in APC (log CFU/cm^2) Number of carcasses		
Treatment	Carcasses per treatment	Decrease >0.5	−0.5 to 0.5	Increase >0.5
Dry aging – 4 d	9	7	2	0
Dry aging – 6 d	92	34	34	24
Dry aging – 7 d	26	4	9	13
Acetic acid	61	31	22	8
Fresh Bloom	30	18	10	2
Hot water	28	17	10	1
High pressure hot water	19	4	13	2

follow-up sampling consisted of 7 carcasses stored at the slaughter facility cooler and 7 stored at the retail facility cooler. While the carcasses at the retail facility showed decreases in the levels of indicators, all 7 carcasses at the slaughter cooler showed increases in indicator levels. These results paralleled the 5 a.m. cooler conditions at each facility with the retail facility cooler being 3.8°C (38.8°F) and 69.1% RH as compared to 15.7°C (60.2°F) and 93.5% RH at the slaughter facility cooler. In addition, increased traffic and handling due to the county fair judging may have been a further contributing factor to these increases in indicator levels.

The intervention treatments did not significantly differ in terms of changing indicator bacteria levels ($P > 0.10$); that is, the treatments were equally effective. In some cases, the age of the animal did have a significant effect on the change in indicator levels. If the animal was over 30 mo of age, significantly greater reductions in *E.* coli and APC were seen. This result was not linked to older animals having a significantly higher initial bacterial load on the carcass or to any differences in carcass weight, weight of acid spray applied, the ratio of acid applied to carcass weight, or hot water spray time; thus, it is unclear why this difference was observed. The temperature at midnight on the day of slaughter had a significant effect on the changes in levels of *E. coli*, coliforms, and APC. A lower temperature at midnight resulted in a greater decrease in these indicators. The same was true for the cooler temperature at 5 a.m. the day after slaughter for APC. The cause of this may be that at lower temperatures,

Table 5—Frequency distribution for *Escherichia coli* levels on beef carcasses before and after intervention treatments

		Percent of carcass halves with *Escherichia coli* levels at indicated CFU/cm^2			
	n	None detected (%)	0.083 to <0.84 (%)	0.84 to <8.4 (%)	≥8.4 (%)
Before intervention	265 (all)	23.0	41.5	25.3	10.2
After 4-d dry aging	9	100.0	0.0	0.0	0.0
After 6-d dry aging	92	96.7	2.2	1.1	0.0
After 7-d dry aging	26	57.7	26.9	0.0	15.4
After acetic acid	61	78.7	11.5	6.6	3.3
After Fresh Bloom	30	76.7	20.0	3.3	0.0
After low-pressure hot water	28	82.1	14.3	3.6	0.0
After high-pressure hot water	19	84.2	15.8	0.0	0.0

Table 6—Frequency distribution for coliform levels on beef carcasses before and after intervention treatments

		Percent of carcass halves with coliform levels at indicated CFU/cm^2			
	n	None detected (%)	0.083 to <0.84 (%)	0.84 to <8.4 (%)	≥8.4 (%)
Before intervention	265 (all)	15.1	40.8	29.1	15.1
After 4-d dry aging	9	100.0	0.0	0.0	0.0
After 6-d dry aging	92	93.5	5.4	1.1	0.0
After 7-d dry aging	26	38.5	23.1	23.1	15.4
After acetic acid	61	75.4	14.8	6.6	3.3
After Fresh Bloom	30	56.7	26.7	16.7	0.0
After low-pressure hot water	28	67.9	28.6	3.6	0.0
After high-pressure hot water	19	57.9	42.1	0.0	0.0

Table 7—Frequency distribution for Enterobacteriaceae levels on beef carcasses before and after intervention treatments

		Percent of carcass halves with Enterobacteriaceae levels at indicated CFU/cm^2			
	n	None detected (%)	0.083 to <0.84 (%)	0.84 to <8.4 (%)	≥8.4 (%)
Before intervention	265 (all)	5.3	35.1	41.1	18.5
After 4-d dry aging	9	66.7	33.3	0.0	0.0
After 6-d dry aging	92	64.1	17.4	15.2	3.3
After 7-d dry aging	26	38.5	3.8	11.5	46.2
After acetic acid	61	50.8	32.8	13.1	3.3
After Fresh Bloom	30	26.7	46.7	20.0	6.7
After low-pressure hot water	28	57.1	21.4	21.4	0.0
After high-pressure hot water	19	21.1	73.7	5.3	0.0

Table 8—Frequency distribution for aerobic plate count levels on beef carcasses before and after intervention treatments

		Percent of carcass halves with aerobic plate count levels at indicated CFU/cm^2			
	n	<200 (%)	200 to ≤2000 (%)	2000 to ≤20000 (%)	≥20000 (%)
Before intervention	265 (all)	12.5	58.1	24.2	5.3
After 4-d dry aging	9	66.7	33.3	0.0	0.0
After 6-d dry aging	92	37.0	45.7	10.9	6.5
After 7-d dry aging	26	15.4	7.7	23.1	53.8
After acetic acid	61	45.9	34.4	16.4	3.3
After Fresh Bloom	30	53.3	43.3	3.3	0.0
After low-pressure hot water	28	57.1	35.7	7.1	0.0
After high-pressure hot water	19	10.5	84.2	5.3	0.0

bacteria cannot survive as well as at higher temperatures and, as previously mentioned, coliforms have a poor cold tolerance. Another factor that significantly affected decreases of *E. coli* and coliform levels was the relative humidity in the cooler at midnight. A lower humidity resulted in a greater decrease in their levels. This may be because the lower humidity promotes increased surface drying, which would also cause a lower temperature at the surface of the carcass, both of which would therefore lead to lower bacterial counts. The pH of acid spray had a significant effect on the decrease in Enterobacteriaceae, with lower pH acid resulting in greater decreases compared to higher pH. This effect likely occurred because all the acids used (acetic, ascorbic, citric, and erythorbic) are weak acids. When these acids are at a low pH, they will predominantly exist in the undissociated form (X-H). Because the undissociated form does not carry a charge, it can easily diffuse across the hydrophobic cellular membrane. Once this molecule enters the cytosol, which is at a higher pH, the molecule dissociates producing protons (H^+) and acid anions (X^-). Because the dissociated anion cannot readily diffuse back through the cellular membrane, it will begin to accumulate, resulting in an increase in turgor pressure, which may cause cellular lysis. In addition to increased turgor pressure, the proton accumulation can begin to lower the intracellular pH, which, due to the cell increasing the use of ATPase pumps to pump the free protons out of the cell, will inhibit many metabolic functions such as cellular synthesis (Salmond and others 1984; Cherrington and others 1990; Russell 1991; Piper and others 2001).

The amount of time that the carcass was hot-water washed had a significant effect on decreases in levels of *E. coli*, Enterobacteriaceae,

M: Food Microbiology & Safety

and APC, with longer wash times resulting in greater decreases. This may be because a longer wash time leads to greater physical removal of bacteria in addition to a greater surface temperature increase, which would kill a greater amount of bacteria. Wash time did not have a statistically significant effect on decrease in the level of coliforms, although the P value was 0.12.

Sofos and others (1999) performed a similar study in large-scale beef slaughter plants using excision as the sampling method. When compared to the current study, APC levels were similar to values from the Wisconsin plants both pre- and postintervention, whereas *E. coli* and coliform levels were higher preintervention and lower postintervention in very small Wisconsin plants. In addition, an inoculated beef-fat fascia study was performed using hot water, at 74°C (165°F) and 2070 kPa (300 psi), and 2% acetic acid solution as interventions. While a direct comparison in bacterial reductions cannot be performed, due to the higher initial levels in the inoculation study, the authors concluded that the intervention treatment should be performed as soon as possible after evisceration because drying of the fecal material, attachment of the bacteria to the carcass surface, or a combination of both factors led to smaller reductions in pathogen numbers (Cabedo and others 1996). This recommendation is followed in very small Wisconsin slaughter plants with intervention treatments occurring within 30 min of evisceration. Furthermore, the current study was compared to that of Bacon and others (2000). In order to perform a direct comparison between the studies, mean values (log CFU/100cm^2) for all postintervention carcasses at each plant employing acetic acid as the intervention were calculated for *E. coli*, coliforms, and APC. No significant difference (2-sample t-test, $P > 0.05$) between the studies was seen for *E.coli* and coliforms, but a significant difference ($P < 0.05$) was seen between the studies for APC levels, with the current study reporting higher levels.

Reductions of indicator bacteria caused by intervention treatments used in the current study do not entirely prove that these treatments will be effective against *E. coli* O157:H7. There are several reasons why this is so. First, the present study used a nondestructive sampling as mandated by USDA/FSIS. Destructive and nondestructive sampling methods exist for the recovery of bacteria from carcasses. Excision is generally accepted as the method that removes the largest percentage of microorganisms from a carcass surface, but debate still remains as to the significance of differences between this method and swabbing (Ware and others 1999; Gill and Jones 2000; Ransom and others 2002; Pepperell and others 2005). Due to the inconsistencies in these studies, it is unclear whether swabbing, as a method of pre- and postintervention carcass sampling, will lead to an overestimation of intervention efficacy.

Second, there has been some uncertainty whether the survival of indicator bacteria groups is similar enough to that of *E. coli* O157:H7 to warrant their use in validating intervention treatments. Although generic *E. coli* was selected as an indicator organism for control of the slaughter process, FSIS also considered other microbial indicator tests such as the aerobic plate count and enumeration of Enterobacteriaceae. The decision to use generic *E. coli* was based on (1) a strong correlation between its presence and the presence of enteric pathogens, (2) the similarity of its survival and growth characteristics to those of enteric pathogens, and (3) its wide acceptance in the international scientific community as an indicator of the potential presence of enteric pathogens. In addition, the prevalence of *E. coli* O157:H7 is low enough that testing the small mandated number of carcasses (13 per year) for a very small plant would be unlikely to detect *E. coli* O157:H7, even if unhygienic slaughter or dressing procedures were being used by the processor. The Wisconsin meat inspection program routinely tests chilled beef carcasses for *E. coli* O157:H7 as a way of verifying that the beef slaughter/dressing process is adequately controlled by the processor. As of November 8, 2006, 1112 beef carcasses had been sampled under this program and *E. coli* O157:H7 was detected on only 1 carcass (0.09%; Theder C., 2006, Personal communication, Wisconsin Dept. of Agriculture, Trade, and Consumer Protection).

Studies conducted by Castillo determined that log reductions for Enterobacteriaceae, coliforms, and generic *E. coli* on beef carcasses were not significantly different from reductions seen in *E. coli* O157:H7 (Castillo and others 1997). Similarly, Marshall found that generic *E. coli* has the greatest potential for use as a meat processing indicator, based on comparative log reductions (Marshall and others 2005). Furthermore, a relationship between prevalence of *E. coli* O157 and Enterobacteriaceae was observed by Arthur and others (2004) when Enterobacteriaceae levels were grouped into classes with the samples being from higher classes exhibiting a greater probability of being positive for *E. coli* O157. These study results strongly support the use of indicator organisms, such as Enterobacteriaceae, for initial evaluation of the efficacy of intervention treatments against *E. coli* O157:H7.

Mossel and others (1995) generally recommended the use of Enterobacteriaceae, instead of coliforms, as indicators of fecal pathogen contamination or survival because this group is well-defined taxonomically and includes a broader range of organisms. For example, coliform tests will not detect *Salmonella* and *Edwardsiella*, which are fecal pathogens in the family Enterobacteriaceae. Furthermore, testing foods for Enterobacteriaceae has an overall good record for protecting public health. The only situation in which Enterobacteriaceae testing was not recommended by Mossel and others (1995) was when *E. coli* O157:H7 and Enterobacteriaceae were both present in very low numbers. Such a situation does not appear to be likely in very small Wisconsin beef slaughter plants.

Conclusion

The results of our study, combined with the low incidence of detected *E. coli* O157:H7 on postintervention beef carcasses from very small facilities in Wisconsin, indicate that all of the interventions studied are likely to have some efficacy against *E. coli* O157:H7. The USDA/FSIS has not mandated a level of lethality for beef carcass intervention treatments. Rather, the intervention treatment is expected to result in an absence of this pathogen from the treated carcass. Compared against this criterion, very small beef slaughter facilities in Wisconsin appear to be using effective intervention treatments. Processors could increase the level of treatment efficacy by increasing the carcass cooling rate and decreasing cooler relative humidity (all treatments), by increasing hot-water spray time and temperature, or by decreasing acid-spray pH and increasing acid-spray volume. Further studies using actual or simulated very-small-plant intervention treatments directly against *E. coli* O157:H7 would provide additional validation of treatment efficacy.

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