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Effect of Chemical Dehairing on the Prevalence of *Escherichia coli* O157:H7 and the Levels of Aerobic Bacteria and *Enterobacteriaceae* on Carcasses in a Commercial Beef Processing Plant†

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ABSTRACT

The objective of this experiment was to test the hypothesis that cleaning cattle hides by removing hair and extraneous matter before hide removal would result in improved microbiological quality of carcasses in commercial beef processing plants. To test this hypothesis, we examined the effect of chemical dehairing of cattle hides on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and *Enterobacteriaceae* on carcasses. Samples from 240 control (conventionally processed) and 240 treated (chemically dehaired before hide removal) hides (immediately after stunning but before treatment) and preevisceration carcasses (immediately after hide removal) were obtained from four visits to a commercial beef processing plant. Total aerobic plate counts (APC) and *Enterobacteriaceae* counts (EBC) were not ($P > 0.05$) different between cattle designated for chemical dehairing (8.1 and 5.9 log CFU/100 cm^2 for APC and EBC, respectively) and cattle designated for conventional processing (8.0 and 5.7 log CFU/100 cm^2 for APC and EBC, respectively). However, *E. coli* O157:H7 hide prevalence was higher ($P < 0.05$) for the control group than for the treated group (67% versus 88%). In contrast to hides, the bacterial levels were lower ($P < 0.05$) on the treated (3.5 and 1.4 log CFU/100 cm^2 for APC and EBC) than the control (5.5 and 3.2 log CFU/100 cm^2 for APC and EBC) preevisceration carcasses. Prevalence of *E. coli* O157:H7 was lower ($P > 0.05$) on treated than on control preevisceration carcasses (1% versus 50%). These data indicate that chemical dehairing of cattle hides is an effective intervention to reduce the incidence of hide-to-carcass contamination with pathogens. The data also imply that any effective hide intervention process incorporated into beef processing procedures would significantly reduce carcass contamination by *E. coli* O157:H7.

Cattle hides are recognized as a major source of carcass contamination in commercial beef processing facilities *(2, 4, 9, 10, 14)*. We demonstrated that *Escherichia coli* O157:H7 and *Salmonella* prevalence was high on hides of cattle from different processing plants *(2)*. The current hazard analysis and critical control point (HACCP) plans *(16)* implemented in most beef processing plants in the United States focus on decontamination of the carcasses by a combination of intervention strategies, including steam vacuuming, acid rinses, steam, and hot water spray. Such antimicrobial interventions, combined with strict hygiene practices, have significantly improved microbial quality of beef carcasses in the processing plants *(1, 9)*.

Earlier work from this laboratory has shown that a significant percentage of carcasses are contaminated with *E. coli* O157:H7 when tested immediately after hide removal *(2)*. It is important to recognize that at this step in the process, carcasses have been subjected to very few, if any, interventions. Because carcasses are not yet eviscerated, it can be concluded that hide is the major source of *E. coli* O157:H7 on carcasses just before evisceration. If this is true, then interventions to reduce or eliminate pathogens from hides should be recognized as critical control points to reduce the incidence of *E. coli* O157:H7 on beef carcasses.

Chemical dehairing *(5)* is a decontamination process that involves removal of hair and extraneous matter from the hide with a sodium sulfide solution, subsequent neutralization with a hydrogen peroxide solution, and water washing before dehiding. The effectiveness of chemical dehairing as a hide intervention in a commercial operation for preventing carcass contamination has not been adequately studied.

The objective of this study was to test the hypothesis that cleaning cattle hides by removing hair and extraneous matter before dehiding would result in improved microbiological quality of carcasses in commercial beef processing plants. To test this hypothesis, we examined the effect of chemical dehairing of cattle on the prevalence of *E. coli* O157:H7 and the levels of aerobic bacteria and *Enterobacteriaceae* on carcasses.

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MATERIALS AND METHODS

Sampling. Four sampling trips were made in June and July 2002 to a Midwestern commercial beef processing plant that used both chemical dehairing and conventional hide removal procedures. The chemical dehairing was a proprietary process based on modifications to previously published procedures *(5, 15).* On each sampling day, approximately every fourth carcass was sampled during the first 2 to 3 h of processing to obtain 60 conventionally processed (control) and 60 chemically dehaired (treated) samples from both hides and carcasses. The assignment of animals into control and treatment groups was dictated by the lineup in the processing plant. Because of the processing constraints of the plant, it was not possible to assign animals from the same lots into control and treatment groups. Hide samples were taken immediately after stunning, before exsanguination or any antimicrobial intervention. The brisket-neck-shank part of the hide (~2,000 cm^2) was sampled on carcasses that were to be dehaired and on control carcasses that were to be conventionally processed. Preevisceration carcass samples were taken immediately after the hide was removed, before application of any antimicrobial intervention to the carcasses. Although the hide and preevisceration carcass samples were not necessarily from the same carcasses, they were from carcasses from the same lots. Carcasses were sampled in the anal-hock area (~8,000 cm^2) for both treatment and control groups. Both hide and carcass samples were taken with the Whirl-Pak Speci-Sponge (Nasco, Ft. Atkinson, Wis.) hydrated with 15 ml of buffered peptone water (BPW; Difco Laboratories, Becton Dickinson Microbiology Systems, Sparks, Md.) plus 0.1% Tween-20 (Sigma, St. Louis, Mo.). All samples were transported to the laboratory on ice and processed within 24 h.

Aerobic plate and *Enterobacteriaceae* counts. A 1-ml aliquot from each sample was taken for aerobic plate count (APC) and *Enterobacteriaceae* count (EBC) before the sample was subjected to enrichment for *E. coli* O157:H7 isolation. Petrifilm aerobic count plates and Petrifilm *Enterobacteriaceae* count plates (3M Microbiology, St. Paul, Minn.) were used for APC and EBC, respectively, following manufacturer's instructions. Each sample was serially diluted to a predetermined range and plated in duplicate on Petrifilm plates using three consecutive dilutions. Petrifilm plates for APC were incubated for 40 h at 35°C and then counted using a CASBA colony image analyzer (Spiral Biotech, Bethesda, Md.). Petrifilm plates for EBC were incubated for 22 h at 37°C and manually counted.

Isolation of *E. coli* O157:H7. Sample enrichment and *E. coli* O157:H7 isolation were performed as previously described *(3)* with minor modifications. Briefly, 90 ml of tryptic soy broth (Difco) was added to each sample bag. The sample was incubated at 25°C for 3 h, subsequently at 42°C for 6 h, and chilled to 4°C until further processing. *E. coli* O157:H7 was isolated from enrichment culture by immunomagnetic separation with anti-*E. coli* O157 Dynabeads (Dynal, Lake Success, N.Y.) following the manufacturer's instructions. Bacterial cells bound to the beads were plated on sorbitol MacConkey (Difco) plates supplemented with 0.05 mg/liter of cefixime and 2.5 mg/liter of potassium tellurite (ctSMAC; Dynal), and Rainbow agar (Biolog, Hayward, Calif.) plates supplemented with 10 mg/liter of novobiocin (nRainbow; Sigma). For each sample, up to three colonies characteristic of *E. coli* O157:H7 on either ctSMAC or nRainbow plates were further tested for confirmation.

Confirmation of *E. coli* O157:H7. The presence of O157 antigen on presumptive *E. coli* O157:H7 colonies from either ctSMAC or nRainbow plates was determined by latex agglutination using the Oxoid Dry-Spot *E. coli* O157 test kit (Oxoid, Hampshire, UK). The O157-positive colonies were restreaked for isolation on ctSMAC plates and subjected to a multiplex PCR for confirmation of *E. coli* O157:H7 using primers described by Hu et al. *(11).* A 1-μl aliquot of overnight culture in tryptic soy broth from an isolated suspect colony was mixed with 24 μl of PCR master mix *(11),* and the PCR reaction was run on a PTC-100 DNA Engine thermocycler (MJ Research, Watertown, Mass.) with a touchdown amplification program *(13).* The numbers and percentages of *E. coli* O157:H7 reported are for those that were confirmed.

TABLE 1. *Aerobic plate counts (APC) and* Enterobacteriaceae *counts (EBC) on hides before chemical dehairing and the effect of chemical dehairing on carcass APC and EBC*[a]

Sample type	No. of samples	APC (log CFU/100 cm^2)	EBC (log CFU/100 cm^2)
Hides[b]			
Treatment group	240	8.1 A (0.5)	5.9 A (0.7)
Control group	240	8.0 A (0.4)	5.7 A (0.6)
Difference[c]		0.1	0.2
Carcasses[d]			
Treatment group	240	3.5 B (0.5)	1.4 B (0.7)
Control group	240	5.5 A (0.7)	3.2 A (1.0)
Difference		−2.0	−1.8

[a] Control and treated means (SD) within sample type lacking a common letter differ significantly ($P < 0.05$).
[b] Hides were sampled before chemical dehairing treatment.
[c] Treatment group mean value subtracted by control group mean value.
[d] Carcasses were sampled immediately after hide removal, before evisceration.

Statistical analyses. Data for APC and EBC were log transformed and analyzed by one-way analysis of variance using the GLM procedure of SAS (SAS Institute, Inc., Cary, N.C.). Frequency data were analyzed by one-way analysis of variance and by Mantel-Haenszel chi-squared analysis with SAS. The same results were obtained with both analyses of frequency data.

RESULTS

APC and EBC. Both chemical dehairing and conventional hide removal procedures were used in the beef processing plant where this study was conducted. Hides were sampled to document that hides for both control and treated groups had approximately equal bacterial loads. Carcasses were sampled to determine the effect of hide treatment on carcass contamination levels following hide removal. Because hides are a major source of contamination on carcasses, differences in bacterial loads between treated and control preevisceration carcasses would be indicative of the efficacy of chemical dehairing in reducing carcass contamination. Aerobic plate counts and EBC on the hides of cattle from control and treated groups were <0.2 log different ($P > 0.05$), indicating the initial bacterial load on the hides could not account for any differences in APC and EBC on preevisceration carcasses (Table 1). In contrast to hides, the preevisceration carcasses processed after chemical dehair-

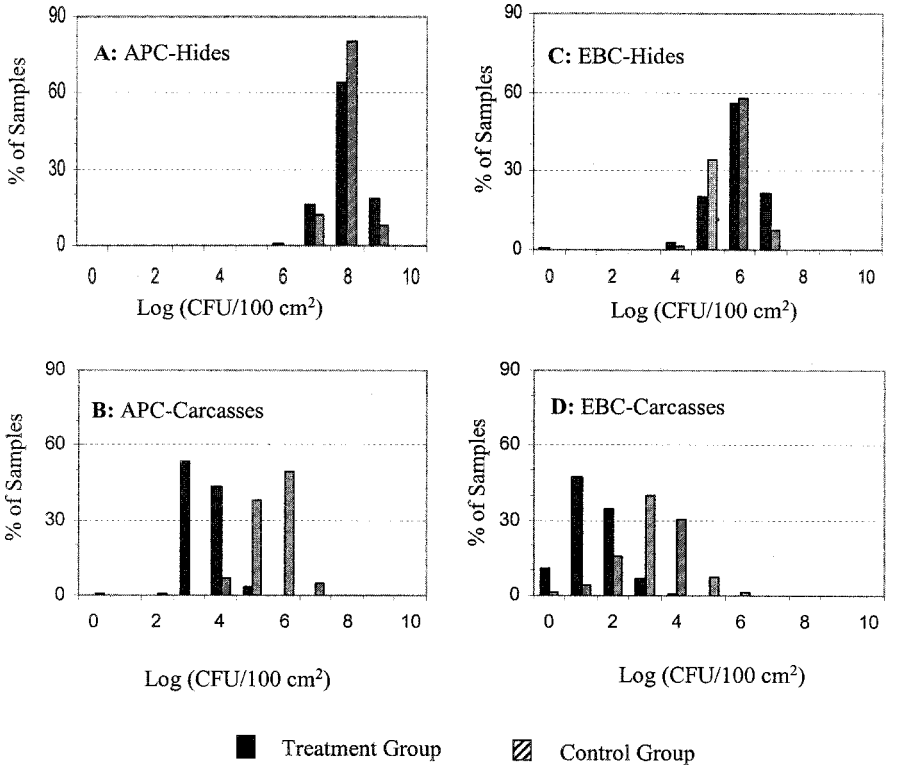

FIGURE 1. *Frequency distribution of aerobic plate counts (APC) and* Enterobacteriaceae *counts (EBC) for hides and carcasses before evisceration. (A) APC of hide samples; (B) APC of carcass samples; (C) EBC of hide samples; (D) EBC of carcass samples.*

ing had lower ($P < 0.0001$) bacterial loads compared with those processed with conventional procedures (~2 logs lower in both APC and EBC). The frequency distributions of APC and EBC for hides and preevisceration carcasses emphasizes both the similarity of initial bacterial loads on the hides and the difference in the bacterial loads on the control and treated preevisceration carcasses (Fig. 1).

***E. coli* O157:H7.** Because of the potentially severe consequences of contamination to meat products by *E. coli* O157:H7 and its regulatory status as an adulterant, isolation of *E. coli* O157:H7 is considered the most direct and relevant assessment of the safety of beef. We determined the prevalence of *E. coli* O157:H7 on hides and carcasses of cattle that were either processed conventionally or by chemically dehairing the hide. *E. coli* O157:H7 was highly prevalent on the hides of both control and treated groups; however, hides destined for chemical dehairing had a lower ($P < 0.05$) prevalence than did control hides (Table 2). Although the difference was statistically significant, the prevalence of *E. coli* O157:H7 for both groups was within the range we had normally observed. Treated preevisceration carcasses had much lower ($P < 0.05$) prevalence of *E. coli* O157:H7 than did control preevisceration carcasses (Table 2). The large reduction in *E. coli* O157:H7 prevalence on preevisceration carcasses resulting from chemically dehairing the hide cannot be attributed to the slightly lower prevalence on the hides. In fact, when carcass *E. coli* O157:H7 prevalence was expressed as a proportion of hide prevalence (to account for the difference between 67 and 88% hide prevalence), then 1.5% (1 of 67) of treated and 56.8% (50 of 88) of control carcasses were positive for *E. coli* O157:H7. These percentages were not different from those in Table 2 calculated using all samples.

TABLE 2. E. coli *O157:H7 prevalence on hides before chemical dehairing and the effect of chemical dehairing on carcass* E. coli *O157:H7 prevalence*

Sample type	No. of samples	*E. coli* O157:H7[a]	
		No. positive	% positive
Hides[b]			
Treatment group	240	161	67 A
Control group	240	212	88 B
Carcasses[c]			
Treatment group	240	3	1 A
Control group	240	120	50 B

[a] Control and treated means within sample type lacking a common letter differ significantly ($P < 0.05$).
[b] Hides were sampled before chemical dehairing treatment.
[c] Carcasses were sampled immediately after hide removal, before evisceration.

DISCUSSION

Hides and viscera have been considered major sources of carcass contamination in beef processing plants *(2, 4, 9, 10, 14).* With advances in detection technology, it has become increasingly evident that the prevalence of *E. coli* O157:H7 and *Salmonella* on cattle hides is higher than previously estimated *(2, 9).* In a recent study, Barkocy-Gallagher et al. *(2)* showed that the prevalence of *E. coli* O157:H7 on hides was approximately 70% during the high-prevalence seasons (spring, summer, and fall) and 30% during the low-prevalence season (winter), whereas the prevalence of *E. coli* O157:H7 in feces was significantly lower (~4% in spring, 13% in summer, 7% in fall, and 0.3% in winter).

As detected in this and previous studies *(2, 9),* the prevalence of *E. coli* O157:H7 on preevisceration carcasses is 30 to 50% in the high-prevalence seasons. Carcass contamination by *E. coli* O157:H7 in the processing plants occurs at a much higher rate than for cattle carrying *E. coli* O157:H7 in their intestinal contents, which suggests that the viscera play a minor role in carcass contamination by *E. coli* O157:H7. Therefore, hides are likely the primary source of *E. coli* O157:H7 contamination of preevisceration carcasses in beef processing plants. This conclusion is consistent with the current results that indicate chemical dehairing is highly effective in preventing carcass contamination by *E. coli* O157:H7. Given the high prevalence of *E. coli* O157:H7 and *Salmonella* on hides, preventing the transfer of pathogens from hides to carcasses represents one of the greatest challenges facing the beef processing industry.

Schnell et al. *(15)* first evaluated the effectiveness of chemical dehairing on the bacterial load and visual cleanliness of beef carcasses. It was observed that the dehairing process resulted in visually cleaner carcasses and reduced the requirement for trimming to meet the zero tolerance policy on fecal contamination. However, they found that the dehairing process did not significantly reduce the bacterial load on carcasses (perhaps because of a low number of observations). In contrast, Castillo et al. *(7)* found that a chemical dehairing process significantly reduced the counts of aerobic bacteria, coliforms, and *E. coli,* as well as artificially inoculated *Salmonella* Typhimurium and *E. coli* O157:H7 strains on hide pieces. However, the study by Castillo et al. *(7)* was conducted in a laboratory using artificially contaminated hide pieces instead of whole animals under commercial processing conditions. It did not address the key question of bacteria, especially pathogens, potentially transferring from the hide to the carcass during the process of hide removal.

In the current study, we evaluated the effectiveness of cleaning the hide before carcass dressing on the reduction of the bacterial load on carcasses under commercial processing conditions. The samples were taken directly from the processing line, which we believe is a better way of evaluating carcass contamination and effectiveness of decontamination interventions in the in-plant environment. The study was conducted in multiple segments spanning a period of 2 months, thus allowing the assessment of the dehairing procedure on cattle from diverse production environments. As did Schnell et al. *(15),* we observed that chemical dehairing prior to hide removal resulted in visually cleaner carcasses and reduced carcass trimming. We also determined that chemical dehairing resulted in a lower bacterial load on carcasses when compared with conventional processing procedures. Our data indicate that following chemical dehairing, *E. coli* O157:H7 were much less likely to be present on the carcasses.

Presumably, successful hide decontamination could be achieved by methods other than chemical dehairing. It has been reported that preslaughter washing was not effective in reducing *E. coli* O157:H7 transfer from hide to carcasses *(6),* although a low number of observations was tested. Recently, McEvoy et al. *(12)* demonstrated that treatment with steam condensing at subatmospheric pressures significantly reduced *E. coli* O157:H7 on bovine hide material in a laboratory test. The applicability of such treatment on a commercial scale needs further evaluation. Application of aqueous solutions such as cetylpyridinium chloride, which has been shown to be effective in reducing contamination on beef carcasses *(8),* might be adaptable for hide decontamination.

It appears that procedures such as chemical dehairing, designed to clean cattle hides before carcass dressing, can be expected to reduce the bacterial load on carcasses immediately after hide removal by about 2 logs and to reduce dramatically the prevalence of *E. coli* O157:H7. Typically, the various antimicrobial interventions in beef processing plants have a combined effectiveness of 3- to 4-log reduction in bacterial load from preevisceration carcasses to carcasses chilling in the cooler *(1, 2).* Combining an effective hide intervention with subsequent carcass interventions should further improve the safety of beef and beef products by virtually eliminating the spikes in pathogen contamination that current carcass interventions alone cannot completely remove.

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