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Effects of a Minimal Hide Wash Cabinet on the Levels and Prevalence of *Escherichia coli* O157:H7 and *Salmonella* on the Hides of Beef Cattle at Slaughter†

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ABSTRACT

Harborage of *Escherichia coli* O157:H7 and *Salmonella* on animal hides at slaughter is the main source of beef carcass contamination during processing. Given this finding, interventions have been designed and implemented to target the hides of cattle following entry into beef processing plants. Previous interventions targeting hides have not been suitable for all beef processing plants because of cost and space restrictions. In this study, a hide wash cabinet was evaluated to determine whether it was more amenable to widespread use in the beef processing industry, especially for small and medium-size plants. Overall, 101 (35.1%) of 288 beef cattle hides sampled before entry into the hide wash cabinet harbored *E. coli* O157:H7 at or above the limit of detection (40 CFU/100 cm^2). After passage through the hide wash cabinet, only 38 (13.2%) of 288 hides had *E. coli* O157:H7 levels ≥40 CFU/100 cm^2. Before the hide wash cabinet, 50 (17%) of 288 hides harbored *E. coli* O157:H7 at levels above 100 CFU/100 cm^2, with one sample as high as 20,000 CFU/100 cm^2. In contrast, only 14 (5%) of 288 hides had *E. coli* O157:H7 levels above 100 CFU/100 cm^2 after hide washing, with the highest being 2,000 CFU/100 cm^2. These same trends also were found for *Salmonella* before and after hide washing. These results indicate that the hide wash cabinet described in this study was effective and should provide small and medium-size processing plants with an affordable hide wash intervention strategy.

Harborage of *Escherichia coli* O157:H7 and *Salmonella* on the hides of animals at slaughter has been identified as the main source of contamination of beef carcasses during processing *(2, 3).* In light of this finding, interventions have been designed and implemented to target the hides of cattle following entry into beef processing plants. Nou et al. *(12)* demonstrated that the use of chemical dehairing as an antimicrobial intervention for hides of beef cattle during processing led to large reductions in *E. coli* O157:H7 prevalence on both hides and carcasses. Bosilevac et al. *(6)* expanded on this work by demonstrating the effectiveness of cattle hide cleansing through the use of a hide wash cabinet. Again, this intervention reduced the prevalence of *E. coli* O157:H7 on both hides and carcasses.

The measure of effectiveness for these hide interventions was the reduction in pathogen prevalence. Most current prevalence assays for *E. coli* O157:H7 are very sensitive for the target organism and will give positive results even when only low numbers of target cells (e.g., 10 to 50 CFU) are present in the sample *(4).* Therefore, hides harboring *E. coli* O157:H7 at 50 and 50,000 CFU/100 cm^2 will both give the same positive results. The interventions described above effectively reduced the prevalence of *E. coli* O157:H7 on beef cattle hides during processing, indicating large reductions in the *E. coli* O157:H7 load on the cattle hides. These interventions, although quite thorough, may not be suitable for all beef processing plants because of cost and space restrictions. In this report, a hide wash cabinet that may be more amenable to widespread use in the beef processing industry was evaluated. Although this cabinet does not dramatically reduce the prevalence of contamination, it does significantly reduce the levels of *E. coli* O157:H7 and *Salmonella* on the hides of beef cattle during processing.

MATERIALS AND METHODS

Cattle hide samples were collected before and after carcasses were washed in a hide wash cabinet at a fed beef processing plant operated by Swift & Co. Samples were collected from 96 animals during each of three plant visits. Immediately before the animals entered the processing plant from the holding pens, the hides were wetted with potable water from a hose.

Hide cabinet. The hide cabinet was constructed with stainless steel walls that partially enclosed the space to minimize spray into the surrounding areas. Potable water was pumped through 18 lines on each side of the cabinet with six to eight ''hog'' nozzles per line to thoroughly saturate the hide. Water flowed at a rate of 231 gal/min (874 liters/min). Carcasses spent 25 to 97 s inside the cabinet. At the end of the cabinet, a chlorine spray (100 to 200 ppm) was applied.

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† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

In-plant sampling. Hide samples were collected after shackling just before the carcass entered the wash cabinet. Samples were collected again from the same carcasses approximately 2 min after exiting the cabinet to allow excess moisture to drip off the carcass. Conditions did not allow for consistent sampling of a particular area of the brisket before cabinet entry. Although the samples collected after the carcasses exited the cabinet were taken from the right side of the brisket, occasional overlap of sampling areas may have occurred. Hide samples were obtained with a sterile sponge (Biotrace International Inc., Bothell, Wash.) moistened with 20 ml of buffered peptone water (Becton Dickinson, Sparks, Md.) by swabbing a brisket area of ≈1,000 cm^2. Samples were transported back to the lab on ice and processed within 4 h.

Enumeration. *E. coli* O157:H7 and *Salmonella* were enumerated from hide samples using a spiral plater (Spiral Biotech, Norwood, Mass.) following previously described methods *(8).* The sponge samples were homogenized by hand massage, and 500 μl of the homogenate was transferred to a microfuge tube. After vortexing and a 3-min holding period to allow the particulates to settle, 50-μl aliquots were spiral plated on plates containing Chromagar O157 (DRG International, Mountainside, N.J.) supplemented with novobiocin (5 mg/liter; Sigma, St. Louis, Mo.) and potassium tellurite (2.5 mg/liter; Sigma) (ntChromagar) and plates containing xylose lysine deoxycholate medium (Remel, St. Louis, Mo.) with 4.6 ml/liter tergitol, 15 mg/liter novobiocin, and 5 mg/liter cefesulodin (Sigma). After incubating the *E. coli* O157:H7 and *Salmonella* plates overnight at 42 and 37°C, respectively, the colonies on the plates were counted, and the identity of suspect colonies was confirmed by PCR assay *(10, 14).* The limit of detection in the enumeration assay for both pathogens was 40 CFU/100 cm^2.

Sample processing for prevalence. Samples were processed according to methods previously described, with slight modifications *(1, 2).* After removing the 500-μl aliquot for enumeration, the sponge samples were enriched with 80 ml of tryptic soy broth (Becton Dickinson) and incubated at 25°C for 2 h, at 42°C for 6 h, and then at 4°C overnight. Following incubation, each enrichment culture was subjected to immunomagnetic separation *(11).* Samples (1 ml) of enrichment culture were each mixed with 20 μl of anti-*Salmonella* immunomagnetic separation beads (Invitrogen, Carlsbad, Calif.). The beads were then extracted from the enrichment samples and washed three times in phosphate-buffered saline plus Tween 20 (Sigma) in an automated magnetic particle processor (KingFisher 96, Thermo Fisher Scientific, Inc., Waltham, Mass.). Anti–*E. coli* O157:H7 beads (Invitrogen) were then added to the same 1-ml enrichment aliquots and similarly extracted and washed. For *E. coli* O157:H7, the final bead-bacteria complexes were spread plated onto ntChromagar and sorbitol MacConkey agar (Becton Dickinson) supplemented with cefixime (0.05 mg/liter; Dynal, Inc.) and potassium tellurite (2.5 mg/liter; Dynal, Inc.). For *Salmonella* enrichment, the beads were transferred to Rappaport-Vassiliadis-soya broth (RVS; Becton Dickinson) and incubated at 42°C overnight. *Salmonella* present in these samples was detected by swabbing the RVS enrichment onto Hektoen Enteric agar (Becton Dickinson) containing novobiocin (5 mg/liter) and brilliant green medium with sulfadiazine (Becton Dickinson). All plates were incubated at 35 to 37°C for 18 to 20 h. Following incubation, up to two suspect colonies were picked for confirmation. A PCR assay was used to confirm that each *E. coli* O157:H7 isolate contained genes for the O157 antigen, H7 flagella, and at least one of the Shiga toxins *(10)* and that each *Salmonella* isolate contained the *invA* gene *(14).*

TABLE 1. E. coli *O157:H7 counts and prevalence on cattle hides before and after processing in a wash cabinet*

Hide processing	Trip 1	Trip 2	Trip 3	Total
No. of cattle hides sampled	96	96	96	288
E. coli enumeration (%)[a]				
Before wash cabinet	27.1	41.2	36.5	35.1
After wash cabinet	3.1[b]	25.0[b]	11.4[b]	13.2[b]
E. coli prevalence (%)[c]				
Before wash cabinet	95.8	96.9	100	97.6
After wash cabinet	82.3[b]	92.7	93.8[b]	89.6[b]

[a] Percentage of total samples that have *E. coli* O157:H7 counts at or above the detection limit of 40 CFU/100 cm^2.

[b] Significant difference between values before and after cabinet processing ($P < 0.05$).

[c] The number of hide samples positive for *E. coli* O157:H7 divided by the total number of hides sampled, expressed as a percentage.

Statistical analysis. Differences in the proportion of positive samples obtained by direct plating and enrichment were calculated using PEPI differ (PEPI software version 2, USD, Inc., Stone Mountain, Ga.) and were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

On each sampling occasion, the number of hide samples yielding detectable levels of *E. coli* O157:H7 by direct plating was significantly lower ($P \leq 0.05$) after the carcasses exited the hide wash cabinet (Table 1). Overall, 101 (35.1%) of 288 samples from unwashed hides contained *E. coli* O157:H7 at ≥40 CFU/100 cm^2. Following passage through the hide wash cabinet, only 38 (13.2%) of the 288 hides had *E. coli* O157:H7 counts of ≥40 CFU/100 cm^2. The prevalence of *E. coli* O157:H7 determined by enrichment also was significantly lower ($P \leq 0.05$) after the carcasses passed through the hide wash cabinet, but the magnitude of the decline was much less than that observed with direct plating (Table 1). Overall, the prevalence of *E. coli* O157:H7 on hides decreased from 97.6% (281 of 288 hides) to 89.6% (258 of 288 hides). Thus, although the frequency of *E. coli* O157:H7 carriage on hides declined only slightly following washing in this type of cabinet, the *E. coli* O157:H7 counts on the individual hides were markedly reduced. This reduction in *E. coli* O157:H7 load is also reflected in the distribution of *E. coli* O157:H7 enumeration data obtained before and after the carcasses passed through the wash cabinet (Table 2). Before the wash cabinet, 50 (17.3%) of the 288 hides harbored *E. coli* O157:H7 at >100 CFU/100 cm^2, with one sample at 20,000 CFU/100 cm^2. In contrast, only 14 (4.9%) of the 288 hides had *E. coli* O157:H7 counts >100 CFU/100 cm^2 after hide washing, with the highest at 2,000 CFU/100 cm^2.

Similar trends also were found for prevalence and counts of *Salmonella.* A significant decrease ($P \leq 0.05$) was seen in the number of hide samples with *Salmonella* detectable by direct plating; 95 (40%) and 21 (7.3%) of the 288 hide samples yielded countable numbers of *Salmonella* before and after processing in the wash cabinet, respective-

TABLE 2. *Enumeration data for* E. coli *O157:H7 and* Salmonella *on cattle hides before and after processing in a wash cabinet*

Count (CFU/100 cm^2)	No. of positive samples[a]	
	Before wash cabinet	After wash cabinet
E. coli O157:H7		
<40	187	250
40–99	51	24
100–999	42	12
1,000–9,999	7	2
10,000–99,999	1	0
Salmonella		
<40	193	267
40–99	58	20
100–999	37	1
1,000–9,999	0	0
10,000–99,999	0	0

[a] No. of samples positive by direct plating.

TABLE 3. Salmonella *counts and prevalence on cattle hides before and after processing in a wash cabinet*

Hide processing	Trip 1	Trip 2	Trip 3	Total
No. of cattle hides sampled	96	96	96	288
Salmonella enumeration (%)[a]				
Before wash cabinet	12.5	7.3	79.2	40.0
After wash cabinet	1.0[b]	1.0	19.8[b]	7.3[b]
Salmonella prevalence (%)[c]				
Before wash cabinet	95.8	88.5	100	94.8
After wash cabinet	47.9[b]	58.3[b]	100	68.8[b]

[a] Percentage of total samples that have *Salmonella* counts at or above the detection limit of 40 CFU/100 cm^2.
[b] Significant difference between values before and after cabinet processing ($P < 0.05$).
[c] The number of hide samples positive for *Salmonella* divided by the total number of hides sampled, expressed as a percentage.

ly (Table 3). *Salmonella* hide prevalence decreased more than 25% during hide washing, with fewer samples yielding countable numbers of *Salmonella* after washing (Table 2).

Multiple postharvest hurdle interventions have been effectively used to reduce cattle carcass contamination *(1, 9, 13).* However, to maintain process control, levels of contamination must be kept below a certain threshold. When contamination exceeds this limit, the intervention strategies can become ineffective, resulting in contaminated carcasses *(1).* Extensive work has shown that *E. coli* O157:H7 contamination on cattle hides entering the processing facility must be reduced to minimize carcass contamination throughout processing *(2, 3, 6, 12).* Recently, postharvest interventions have been targeted at the hide in an attempt to keep contamination levels from overwhelming downstream antimicrobial interventions applied to the carcass *(5–7).* In one report, a hide-on carcass wash cabinet was assessed as a microbial reduction strategy for *E. coli* O157:H7 on hides and the corresponding preevisceration carcasses *(6).* Overall, the prevalence of *E. coli* O157 on hides and preevisceration carcasses decreased from 44 to 17% and from 17 to 2%, respectively, when the cabinet was in use. These results support decontamination of hides as an effective means of reducing pathogen contamination on beef carcasses during processing. In that study, the wash cabinet was much larger and more intricate than the cabinet used in our study, resulting in more thorough washing of the hides. That cabinet utilized a wash containing sodium hydroxide and a proprietary surfactant, a sodium hypochlorite rinse, and a mechanism that rotated the carcasses, thereby allowing the pattern lines to receive direct wash and rinse treatments. In contrast, the cabinet evaluated in the present study utilized a water wash and a chlorine rinse without rotational manipulation of the carcass.

The cabinet studied here is a viable intervention strategy for removing *E. coli* O157:H7 from the hides of cattle at slaughter. However, the efficacy of this cabinet may not have been evident if pathogen prevalence had been the only measure of effectiveness, as most clearly indicated by the *Salmonella* results from the third sampling trip (Table 3). Although prevalence remained constant at 100% before and after processing in the hide wash cabinet, which could be interpreted as no effect, the enumeration data clearly indicate that the hide wash cabinet was effective at removing much of the contaminating bacteria and will provide small and medium-size beef processing plants with an affordable hide wash intervention strategy.

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