Comparison of Antimicrobial Efficacy of Multiple Beef Hide Decontamination Strategies To Reduce Levels of 
Escherichia coli O157:H7 and Salmonella

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ABSTRACT

This study involved a comparison of the antimicrobial efficacy of several beef hide decontamination interventions to identify those that more effectively reduced levels of Escherichia coli O157:H7 and Salmonella. Whole beef hides were inoculated with E. coli O157:H7 and Salmonella and decontaminated with sprays of solutions of acetic acid (AA; 10%, 55°C), lactic acid (LA; 10%, 55°C), sodium hydroxide (SH; 3%, 23°C), sodium metasilicate (SM; 4%, 23°C), or sodium hydroxide (1.5%) followed by high-pressure washing with chlorinated (0.02%) water (SHC; both applied at 23°C) or water (W; 23°C) or by deluging with solutions of potassium cyanate (PC; 2.4%, 30°C) or sodium sulfide (SS; 6.2%, 30°C). All spraying treatments (AA, LA, SH, SM, and SHC) resulted in removal of visual organic material. The PC, SS, and SHC treatments resulted in the greatest reductions of E. coli O157:H7 (P < 0.05), by 5.1, 4.8, and 5.0 log CFU/cm², respectively. The SS and SHC treatments decreased Salmonella by 4.2 and 4.4 log CFU/cm², respectively, compared with the water treatment, which reduced levels by 1.7 log CFU/cm² (P < 0.05). The SH, AA, and LA treatments also lowered both E. coli O157:H7 and Salmonella by at least 2.0 log CFU/cm². The treatments that were effective in this study deserve further consideration for commercial implementation as hide decontamination interventions.

Food safety issues involving beef products contaminated with Escherichia coli O157:H7 surfaced in the early 1980s when an outbreak of human illness characterized by severe abdominal cramps and bloody diarrhea was linked to contaminated ground beef (15). E. coli O157:H7 again was associated with human illness in 1992 and 1993 when contaminated ground beef caused several hundred human infections and subsequently four deaths (18). Recently, consumption of ground beef patties contaminated with E. coli O157:H7 was linked to a foodborne illness outbreak responsible for at least 30 human infections and resulted in the recall of over 21 million pounds (9.5 million kilograms) of ground beef patties (21). Concurrently, Salmonella has become a concern for the beef industry; researchers have suggested that this pathogen is the leading bacterial cause of both hospitalizations (16,430 cases) and deaths (582 deaths) in the United States annually (12). The consumption of beef and poultry pot pies contaminated with Salmonella resulted in a multistate outbreak of salmonellosis subsequently affecting more than 270 people in 35 states (22).

Muscle and fat tissue beneath the hide of healthy animals is essentially sterile (17) and inadvertently becomes contaminated during the normal animal-to-carcass conversion. Many sources can contribute to carcass contamination, such as contact with the hide, gastrointestinal contents, feces, and/or unsanitary workers. Contamination also can originate in the plant environment. Research designed to identify the source of carcass microbial contamination has implicated the hide as the major source of carcass microbial contamination (1–3). An effective means of limiting carcass contamination is to reduce microbial contamination on the hide before its removal (4, 5, 14).

Research has been conducted to evaluate the efficacy of several different chemicals and applications designed to reduce microbial contamination on hides of cattle presented for slaughter. Water alone, when applied for 3 min with a powerhose, can significantly reduce the level of E. coli O157:H7 on beef cattle (6). E. coli O157:H7 prevalence was significantly reduced on hides when they were washed with cetylpyridinium chloride alone or with sodium hydroxide followed by a chlorinated water rinse (4, 5). Solutions of cetylpyridinium chloride, sodium hydroxide, trisodium phosphate, chlorofoam, and phosphoric acid also have been identified as successful interventions with the ability to reduce aerobic plate counts and counts of index organisms (such as Enterobacteriaceae and coliforms) by at least 1.0 log CFU/100 cm² (4, 5). Synergistic effects were observed when treatments were followed by an acidified chlorine rinse at 200 or 500 ppm; reductions increased by 1.0 and 2.0 log CFU/100 cm², respectively (5). Historically, chemical dehairing has been considered the most ef-
ective method for reducing microbial contamination on cattle hides (8, 14). Chemical dehairing lowered both *E. coli* O157:H7 and *Salmonella* by at least 5.1 log CFU/cm² on the surface of treated hides (8), and *E. coli* O157:H7 prevalence was reduced from 67% on the hide to 1% on the corresponding surface of preeviscerated carcasses (14).

The objective of this study was to evaluate the ability of chemical spray applications and chemical dehairing treatments to lower microbial contamination on cattle hides artificially contaminated with *E. coli* O157:H7 and *Salmonella* at levels comparable to those found on hides of incoming slaughter cattle.

**MATERIALS AND METHODS**

**Inoculum preparation.** Five-strain mixtures of both *E. coli* O157:H7 (MRU16, MRU18, MRU19, MRU20 and MRU21; provided by M. Koochmarie, U.S. Meat Animal Research Center, Clay Center, Nebr.) and *Salmonella* (Salmonella Enteritidis phase type 30, Salmonella Heidelberg ATCC 8326, Salmonella Typhi- murium ATCC 14028 and ATCC 13311, and Salmonella Choleraeus ATCC 10708) were used to inoculate fecal slurries that were used as the inoculum for hide samples. All strains were available as frozen stock cultures and were activated by streaking onto tryptic soy agar (TSA; Becton Dickinson, Sparks, Md.) and incubating at 35°C for 24 h. A single isolated colony was transferred into 10 ml of tryptic soy broth (TSB; Becton Dickinson) and incubated for 24 h at 35°C. After incubation, a loopful of activated culture was transferred into fresh TSB (10 ml) and incubated at 35°C for 24 h. Cultures were verified for purity and morphological characteristics by subculturing onto xylose lysine Tergitol agar (XLT4; Becton Dickinson) for *Salmonella* and sorbitol MacConkey agar (Becton Dickinson) supplemented with 0.05 mg/liter cefixime and 2.5 mg/liter potassium tellurite (Dynal BioTech, Oslo, Norway) (SMAC-ct) for *E. coli* O157:H7. Liquid subcultures then were centrifuged (model Straight 8-3k, LW Scientific, Atlanta, Ga.) at 1,000 × g for 10 min at 23°C. Harvested cells were washed with 40 ml of Butterfield’s phosphate buffer (BPB; Oxoid, Lenexa, Kans.) and centrifuged as previously described. After three washings and centrifugations, cells were resuspended in 40 ml of fresh TSB, all of the *E. coli* O157:H7 strains were combined, and all of the *Salmonella* strains were combined to create multistain mixtures of each pathogen. Fecal slurries were prepared by combining 50 g of feces (obtained aseptically from the colon of several gastrointestinal tracts of animals that were harvested each morning) with 50 ml of sterile distilled water and mixed thoroughly. Ten milliliters of slurry were removed and replaced with 10 ml of either the *E. coli* O157:H7 or the *Salmonella* cocktail to create 100 ml of a pathogen-spiked fecal slurry. Each 100-ml spiked fecal slurry sample was kept in an individual sterile bag to allow easy identification for inoculation of hides later that same morning.

**Hide preparation.** Beef hides were obtained immediately after removal from the animal from a commercial beef packing plant and transported to the hide processing area. Upon arrival in the hide processing area, individual hides were stretched out and placed on the concrete floor to facilitate template tracing and pathogen inoculation. A board with four attached 100-cm² templates (a template for each “before” and “after” sample for *E. coli* O157:H7 enumeration and *Salmonella* enumeration) was placed along the midline of the hide, oriented so that the templates were on the left side of the midline (Fig. 1). The area surrounding the templates was outlined with a non-toxic Paintstik livestock marker.

FIGURE 1. Layout of inoculation areas for before-treatment and after-treatment analysis of *E. coli* O157:H7 (E) and *Salmonella* (S) populations. Area inside each pathogen inoculation area was approximately 300 cm² and consisted of two 100-cm² sample areas that permitted collection of samples before and after treatments.
The same procedure was repeated with a different 100-cm² sterile template placed on the equivalent part of the 300-cm² area inoculated with *Salmonella* and swabbed with a new sterile sponge. Sponge samples were placed in a styrofoam cooler with ice packs and then transported to a commercial microbiological testing laboratory for further analysis within 6 h. After the initial microbial samples were collected, each hide (12 per treatment) was subjected to one of the following treatments:

1. water spray (W; 23°C, pH 8.30)
2. 4% sodium metasilicate spray (SM; 23°C, pH 12.94)
3. 3% sodium hydrosulphite spray (SH; 23°C, pH 13.58)
4. 10% acetic acid spray (AA; 55°C, pH 2.04)
5. 10% lactic acid spray (LA; 55°C, pH 1.47)
6. 2.4% potassium cyanate spray (chemical dehairing) (PC; 30°C, pH > 13.00)
7. 6.2% sodium sulfite spray (chemical dehairing) (SS; 30°C, pH 13.29)
8. 1.5% sodium hydrosulphite spray plus chlorinated (0.2% sodium hypochlorite) high-pressure (>34 bar) wash (SHC; both washes applied at 23°C, NaOH [pH 13.15], Cl⁻ [pH 9.22])

Each treatment was applied according to the following procedures.

Treatments 1 through 5 consisted of a 30-s application applied simultaneously to both inoculated areas at 2.04 bar with a stainless steel compressed air sprayer (Chad Co., Olathe, Kans.) that included two 0.3-cm MEG Washjet 2510 nozzle (Spraying Systems Co., Wheaton, Ill.) spaced 40.6 cm apart. After chemical application, hides were allowed to sit for 2 min before they were deluged with water (20°C) from a water hose (22.7 liters/min) for 30 s. Final microbiological levels were evaluated after the water rinse by attaching a 100-cm² sterile template to the board at a point that was equivalent to the half of the 300-cm² area inoculated with *E. coli* O157:H7 that had not been sampled before treatment. To be consistent, the posttreatment sample was always the half of the 300-cm² area that was closest to the original position of the animal's tail (whereas the pretreatment sample was taken from the half of the 300-cm² inoculation area closest to the head). The exposed 100-cm² area within the template was sponge sampled by using 10 horizontal and 10 vertical passes with a premoistened sterile sponge. The same procedure was repeated with a separate sterile template and premoistened sterile sponge to obtain final *Salmonella* levels. Sponge samples were placed in a styrofoam cooler with ice packs and transported to a commercial microbiological testing laboratory for analysis within 6 h.

Before the application of treatments 6 and 7, the A-frame was modified to allow the hide to hang with a 30° slope from the midline so that the treatment would be more likely to penetrate the hide rather than running off of it. Treatments 6 and 7 were both chemical dehairing protocols that were applied in a comparable manner except that the neutralization step for treatment 7 was more extensive. Both treatments 6 and 7 consisted of application of 3 liters of the chemical solution over the inoculated areas (approximately 1,000 cm²) with a garden watering can (model WC81GLZ1, Dynamic Designs, Inc., East Elmhurst, N.Y.). The solution was allowed to act on the hide for 90 s, and then an additional 3 liters of solution was poured over the inoculated areas. This solution was allowed to act on the hide for another 60 s before neutralization was initiated. Treatment 6 was neutralized with a deluging water rinse (22.7 liters/min) for 30 s and removal of liquid and debris with a large sterile squeegee. Treatment 7 was neutralized by removal of excess liquid and debris and then application of 3 liters of a 1% hydrogen peroxide solution over the dehaired area. The dehaired area then received 3 liters of 0.1 M monobasic sodium phosphate (Saratoga Specialties, Elmhurst, Ill.) to further reduce the alkalinity of the hide. Final samples from hides after treatments 6 and 7 were collected for microbiological analysis following the process for final debris and liquid removal using the same sponge-swabbing method described previously.

Treatment 8 consisted of a 30-s application of a 1.5% sodium hydroxide solution that was applied with the same equipment and in the same manner as described above for treatments 1 through 5. After chemical application, hides were allowed to sit for 2 min before being subjected to a 30-s application of chlorinated (0.2% sodium hypochlorite) water applied at 34 bar with a commercial pressure washer (model 4182T, Alkota, Alcetera, S.D.). Chlorinated water was generated by adding a stock solution of 6.25% sodium hypochlorite to the pressure washer and diluting it with tap water through the washer. Final samples for microbiological analysis were obtained after the high-pressure chlorinated water wash.

**Microbiological analysis.** All sample sponges were transported to a commercial microbiological testing laboratory (Foodsafety Net Services, Green Bay, Wis.) in styrofoam coolers containing ice packs and analyzed within 6 h of initial sampling. Sponge samples were hand massaged for 30 s before microbiological analysis. Samples were serially diluted in BPB, and appropriate dilutions were spread plated onto XLT4 for *Salmonella* or SMAC-ct for *E. coli* O157:H7 detection and enumeration. All plates were incubated for 24 h at 35°C, and colonies were enumerated with a Quebec colony counter (Leica, Buffalo, N.Y.). Colonies were manually counted based on typical colony morphology for the media and conditions. Representative colonies were examined for biochemical and serological attributes according to U.S. Department of Agriculture Food Safety and Inspection Service procedures utilizing latex agglutination to verify identity (19, 20). Cell injury was not evaluated because the nonspecific growth of background bacteria on the nonselective medium (TSA) would have created too much interference for accurate enumeration of target pathogens.

**Statistical analysis.** Data were transformed to log CFU per square centimeter and analyzed using the mixed model procedure of SAS (16), with least-squares means generated for each evaluated treatment (n = 12 per treatment). Least squares means were compared at a common initial bacterial level using analysis of covariance techniques. Ultimately, least squares means were separated using a protected paired t test with significant inferences noted when differences between means were detected at the P < 0.05 level.

**RESULTS AND DISCUSSION**

All treatments reduced *E. coli* O157:H7 by at least 1.9 log CFU/cm² and reduced *Salmonella* by at least 0.7 log CFU/cm² (Table 1). The SHC treatment and both of the dehairing treatments resulted in the greatest reductions of *E. coli* O157:H7 (*P* < 0.05), reducing the pathogen by at least 2.5 log CFU/cm² more than did the water control. The SS dehairing treatment and the SHC treatment resulted in the greatest reduction of *Salmonella* by lowering populations by at least 4.2 log CFU/cm². However, the PC dehairing treatment was ineffective for reducing *Salmonella*; it lowered the pathogen by less than 0.7 log CFU/cm². Results of the SS treatment were consistent with those of previous research (8, 14); in both studies the treatment was extremely effective for lowering populations of *E. coli*. 

O157:H7 and Salmonella on hide surfaces. The PC treatment (11) was developed as an environmentally and worker-conscious alternative for hair removal compared with the potentially dangerous SS treatment, but to date the PC treatment has not been evaluated for its antimicrobial efficacy. The PC treatment was effective for removing hair and lowering E. coli O157:H7 counts but was ineffective for reducing levels of Salmonella.

Dehairing treatments are effective antimicrobials because of their caustic properties and because they remove the hair and organic material to which pathogenic bacteria attach. The PC treatment successfully removed the hair and organic material from the hide but was ineffective at lowering populations of Salmonella, possibly because this pathogen can penetrate the hair and attach to the underlying hide. Further research is needed to investigate the ineffectiveness of the PC treatment for reducing counts of Salmonella.

Previous researchers (5) concluded that a combination application of sodium hydroxide followed by a sanitizing high-pressure wash of chlorinated (0.1%) water was effective for lowering bacterial contamination. In the present study, this treatment was extremely efficacious at reducing both E. coli O157:H7 and Salmonella counts. The SHC treatment was the only nondehairing hide treatment that lowered pathogenic bacteria by at least 4.4 log CFU/cm².

Solutions of AA, LA, and SH have been evaluated previously for their efficacy at lowering E. coli O157:H7 and Salmonella on hide surfaces (5, 7, 13). The AA, LA, SH, and SM treatments all reduced E. coli O157:H7 and Salmonella by at least 1.9 and 2.0 log CFU/cm², respectively. When compared with the water treatment, SH, AA, and LA treatments achieved greater reductions of E. coli O157:H7 counts (P < 0.05). Unfortunately, the SH, AA, and LA treatments resulted in inferior reductions of E. coli O157: H7 (P < 0.05) when compared with both the dehairing and SHC treatments. Salmonella was more adversely affected (P < 0.05) by SM, SH, and LA treatments than by water or AA treatments. As reported previously, the most effective treatments numerically for lowering both E. coli O157: H7 and Salmonella counts were the SH and LA treatments. Organic acids have been reported to be effective antimicrobials and currently have industry-wide acceptance as cost-effective carcass antimicrobial interventions (9). Results of the present study confirm that AA, LA, SH, and SM also are very effective antimicrobials that could be utilized in hide decontamination systems.

A review of pertinent scientific literature suggests that the microbiological quality of carcasses can be significantly improved when carcasses are subjected to multiple sequential decontamination interventions (2), known as the multiple-hurdle approach (10). The logic behind multiple-hurdle technology is to subject bacteria to a series of sublethal interventions to create a synergistic bactericidal effect. A hurdle can be added to a series of interventions in a processing plant by including a hide decontamination intervention. Hide decontamination interventions create two bactericidal actions: the physical washing effect and the effect of extreme pH. Many of the treatments investigated in the present study produce very alkaline pH conditions. Bacteria that survive hide decontamination interventions utilizing alkaline solutions could be even more susceptible to subsequent carcass decontamination interventions that employ acidic solutions because of the extreme difference in the pH conditions, which could create a synergistically lethal effect on any remaining bacteria and thus improve the bactericidal efficacy of the plant’s multiple-hurdle intervention system (10).

The SS and the SHC hide decontamination interventions resulted in the greatest reduction of E. coli O157:H7 and Salmonella on the surface of beef hides when compared with single chemical treatments. Applications of AA, LA,
SH, and SM alone also lowered microbial contamination on hide surfaces, and these treatments do not require intensive mechanical application systems. All hide decontamination interventions reduced the amount of microbial contamination on beef hides and therefore deserve consideration for incorporation into a plant’s series of antimicrobial interventions.

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REFERENCES


