The pathogen Escherichia coli O157:H7 has been a concern to the meat processing industry for the last 20 years. In the early 1980s, cases of hemorrhagic colitis caused by E. coli O157:H7 were associated with consumption of undercooked ground beef (25), and a ground beef-related E. coli O157:H7 outbreak caused hundreds of illnesses and four deaths during 1992 and 1993 (28). In response to these events, the U.S. Department of Agriculture Food Safety and Inspection Service declared E. coli O157:H7 to be an adulterant in ground beef and required meat processors to establish hazard analysis and critical control point plans (14). Since then, several interventions that focus on preventing carcass contamination and on decontaminating carcasses have been designed, tested, and put into use. These antimicrobial interventions, combined with strict hygiene practices, have significantly improved microbial quality of beef carcasses and reduced the incidence of E. coli O157:H7 in processing plants (2—4, 12). However, cattle occasionally present for slaughter with a higher level of contamination than can be removed effectively with the current interventions.

The vast majority of E. coli O157:H7 that contaminates beef carcasses during processing originates on the hides of cattle (4, 6, 21). During the hide removal process, E. coli O157:H7 and other pathogens such as Salmonella are transferred from the hide, where they are highly prevalent, to the carcass (2, 4, 6, 21). Processes that effectively clean the hides before hide removal have been effective in lowering carcass microbial concentrations (6, 7, 21). Chemical dehairing of hide-on carcasses was the first intervention to indicate that the prevalence of E. coli O157:H7 on the previsceral carcass was almost eliminated when bacterial contamination of the hide was greatly reduced before hide removal (21). Subsequent studies of an on-line hide washing cabinet revealed that hide washes prior to slaughter were highly effective in reducing carcass contamination during hide removal (7). Chemicals and antimicrobial compounds that have been evaluated for use in hide interventions include cetylpyridinium chloride, NaOH, trisodium phosphate, acidified chlorine, and phosphoric acid (6, 7).

The data reported by Bosilevac et al. (7) formed the basis for effective hide-washing systems now installed in all Cargill Meat Solutions beef processing plants (1). Because hide interventions may be the most effective means to reduce pathogens on beef, we are attempting to provide as many viable alternatives as possible to increase the rate and ease of implementation of these interventions by all processors. Ozonated water and electrolyzed oxidizing (EO) water are generally recognized as safe (GRAS) and can be used inside a processing plant. Ozone has been used since the 1940s to disinfect drinking water at many municipal water

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**Efficacy of Ozonated and Electrolyzed Oxidative Waters To Decontaminate Hides of Cattle before Slaughter**

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**ABSTRACT**

The hides of cattle are the primary source of pathogens such as Escherichia coli O157:H7 that contaminate previsceral carcasses during commercial beef processing. A number of interventions that reduce hide contamination and subsequent carcass contamination are currently being developed. The objective of this study was to determine the efficacy of ozonated and electrolyzed oxidizing (EO) waters to decontaminate beef hides and to compare these treatments with similar washing in water without the active antimicrobial compounds. Cattle hides draped over barrels were used as the model system. Ozonated water (2 ppm) was applied at 4,800 kPa (700 lb in²) and 15°C for 10 s. Alkaline EO water and acidic EO water were sequentially applied at 60°C for 10 s at 4,800 and 1,700 kPa (250 lb in²), respectively. Treatment using ozonated water reduced hide aerobic plate counts by 2.1 log CFU/100 cm² and reduced Enterobacteriaceae counts by 3.4 log CFU/100 cm². EO water treatment reduced aerobic plate counts by 3.5 log CFU/100 cm² and reduced Enterobacteriaceae counts by 4.3 log CFU/100 cm². Water controls that matched the wash conditions of the ozonated and EO treatments reduced aerobic plate counts by only 0.5 and 1.0 log CFU/100 cm², respectively, and each reduced Enterobacteriaceae counts by 0.9 log CFU/100 cm². The prevalence of E. coli O157 on hides was reduced from 89 to 31% following treatment with ozonated water and from 82 to 35% following EO water treatment. Control wash treatments had no significant effect on the prevalence of E. coli O157:H7. These results demonstrate that ozonated and EO waters can be used to decontaminate hides during processing and may be viable treatments for significantly reducing pathogen loads on beef hides, thereby reducing pathogens on beef carcasses.

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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 U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.
treatment plants, and most bottled water has been treated with ozone since the 1980s. In 1997, ozone was granted GRAS status, and its use has been investigated in the processing of fresh produce and red meat. EO water is produced by passing a current of electricity through a dilute saltwater solution. One product of the reaction is NaOH and the other is hypochlorous acid, which has a low pH, contains active chlorine, and has a strong oxidation-reduction potential similar to that of ozone. The efficacy of both ozonated water and EO water has been demonstrated against E. coli O157:H7 and Salmonella Typhimurium (13, 24) and other food-related pathogens such as Listeria monocytogenes (15, 24), the same organisms that are of concern to beef processors. No information exists describing the potential utility of ozonated or EO waters as hide-washing compounds. The objective of these studies was to evaluate the use of ozonated or EO waters in wash steps to reduce hide contamination in experiments using a model hide-washing system.

**MATERIALS AND METHODS**

**Experimental protocol.** At a beef processing plant, hides were selected randomly from the processing line. The selected hides represented a variety of fed cattle breeds (some with long hair and some with short hair). The hides were collected immediately after removal from the carcasses and before any hide processing steps (e.g., trimming, defleshing, or water immersion) occurred. As a matter of standard operating procedure at the participating processing plant, all animals in holding pens and alleyways had been sprayed using low-pressure tap water to remove as much visible contamination as possible before the animals entered the plant. No hide-directed interventions were used during processing.

To evaluate hide decontamination treatments, whole pulled hides were draped over barrels to simulate hide-on carcasses. One side of each pulled hide was used to collect two sets of data. Control samples were obtained before treatment from the anterior and posterior of each hide (Fig. 1). Then, wash treatments were applied to the anterior end of the hide and a sample was collected. The treatment was then applied to the posterior end of the hide and a sample was collected. Ninety-four hides (46 for EO water treatment and 48 for ozonated water treatment) were sampled during two separate sample collection trips. Additional 72 hides were sampled at a later time to determine the potential effects of plain water washing at similar temperatures, durations, and pressures. Thirty-six hides were used for evaluation of EO water treatments and for ozonated water treatments.

**EO water application conditions.** EO water was generated with a P-5000 EO water generator (Electric Aquagenics Unlimited, Inc., Lindon, Utah). Both the alkaline and acidic forms of EO water were used. First, alkaline EO water (NaOH) was applied for 10 s at 4,800 kPa (700 lb in²) with a gasoline-powered pressure sprayer (Briggs & Stratton, Milwaukee, Wis.) that was fitted with a rotating nozzle and an adjustable pressure valve. The alkaline EO water was pH 11.2 and 52°C when applied. A 10-s pause occurred before the 10-s application of the acidic EO water (hypochlorous acid) using a second power sprayer (Electric Aquagenics) at 1,700 kPa (250 lb in²). Acidic EO water was pH 2.4, 70 ppm chlorine, and 60°C. Before each use, the free chlorine content of the acidic EO water was confirmed by titration. Both the alkaline and acidic EO waters were applied with 10 to 15 side-to-side passes of the pressure sprayer. The sprayer nozzle was maintained at a distance of 65 cm from the hide surface during spraying. A 30-s dwell time for antimicrobial activity was allowed after the final spray before samples were collected for microbial analysis.

**Ozonated water application.** Ozonated water was applied using a Mobile Whitewater System (Ozone International, Bainbridge Island, Wash.). This machine generates ozonated water of 2 ± 0.2 ppm and dispenses it at 240 kPa (35 lb in²) in a stream parallel to a high-pressure water stream. Ozone concentration was monitored periodically during use by visual comparison to optical standards. Atmospheric ozone was also monitored throughout applications to ensure that concentrations did not exceed Occupational Safety & Health Administration standards. Ozonated water was applied to the hide at 4,800 kPa and 15°C with 10 to 15 side-to-side passes for 10 s, then the high-pressure stream was turned off and ozonated water alone was applied at the dispensing pressure (240 kPa) for an additional 5 s. Samples were collected 30 s after the final low-pressure ozonated water spray.

**Application of control water washes.** To determine the effects of pressure and temperature of the wash procedures, control washes were performed that mimicked the ozonated and EO water wash conditions in pressure, temperature, and duration. A pressure sprayer (model P14030, Precision Industries, Germantown, Wis.) was used for the control washes. Ozonated water controls were applied for 10 s at 15°C and 4,800 kPa with end-to-end passes of the sprayer. A control for the low-pressure 5-s ozonated water treatment was not included because low-pressure washes such as this have had no effect on hide microbial status (6, 22). Samples were collected after a 30-s dwell time. Control EO water washes
Table 1. Effects of ozonated water treatment on the microbial status of hides

<table>
<thead>
<tr>
<th></th>
<th>APC (log CFU/100 cm²)</th>
<th>EBC (log CFU/100 cm²)</th>
<th>O157 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.4</td>
<td>6.6</td>
<td>89 (85/96)</td>
</tr>
<tr>
<td>Treated</td>
<td>6.3</td>
<td>3.2</td>
<td>31 (30/96)</td>
</tr>
<tr>
<td>Reduction</td>
<td>2.1</td>
<td>3.4</td>
<td>65</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Notes: 

- **a** Values for APC and EBC are means, n = 96. SEM was 0.06 for APC and 0.08 for EBC. Values for *E. coli* O157 prevalence are percentage of positive samples (fraction of positive samples).
- **b** All samples were analyzed directly with a Bactometer, and treated samples were also analyzed using Petrilm.
- **c** Prevalence of *E. coli* O157 was determined by selective enrichment and immunomagnetic separation isolation followed by plating on sorbitol MacConkey agar with cefixime and potassium tellurite on O157 chromogenic media. Positive colonies were confirmed by latex agglutination.
- **d** Treatment was 10 to 15 end-to-end passes (10 s each) of high-pressure (4,800 kPa or 700 lb in²) 15°C ozonated water, followed by 5-s application of low-pressure (240 kPa or 35 lb in²) ozonated water. Samples were collected after a 30-s dwell time.
- **e** Values were determined using general linear model procedures of SAS for APC and EBC. The model included the main effect of treatment, and the least squares means separation was accomplished by the probability of difference (PDIF) option (a pairwise t test). Pairwise comparisons of frequencies of *E. coli* O157 detection were made using PROC FREQ and Mantel-Haenszel chi-square analysis.

**E. coli O157 detection.** The procedure for detection of *E. coli* O157 consisted of enrichment, immunomagnetic separation, and plating as described previously (5, 7) with minor modifications for plating as follows. Bacterial cells bound to the immunomagnetic separation beads were plated on sorbitol MacConkey agar (Difco, Becton Dickinson) plates supplemented with 0.05 mg/liter cefixime and 2.5 mg/liter potassium tellurite (Dynal, Lake Success, N.Y.) and on *E. coli* O157 chromogenic agar plates. Chromogenic media were either CHROMagar O157 agar (CHROMagar, Paris, France) supplemented with 5 mg/liter novobiocin (Sigma, St. Louis, Mo.) and 1 mg/liter potassium tellurite (Sigma) or Rainbow Agar (Biolog, Hayward, Calif.) supplemented with 10 mg/liter novobiocin and 8 mg/liter potassium tellurite. The use of different chromogenic media was based solely on availability from the manufacturers. All plates were incubated at 37°C for 16 h, and suspect colonies (i.e., sorbitol negative on supplemented sorbitol MacConkey agar, characteristic magenta on supplemented CHROMagar, or characteristic blue on supplemented Rainbow agar) were confirmed as *E. coli* O157 using DrySpot O157 latex agglutination tests (Oxoid, Ogdensburg, N.Y.).

**Statistical analyses.** APC and EBC of hides were evaluated by analysis of variance using the general linear model procedures of SAS (SAS Institute, Inc., Cary, N.C.). The model included the main effect of treatment. For significant main effects (*P < 0.05*), least squares mean separation was accomplished by the probability of difference (PDIF) option (a pairwise t test). Data for APC and EBC were log transformed before analysis of variance. Pairwise comparisons of frequencies of *E. coli* O157 detection were made using PROC FREQ and Mantel-Haenszel chi-square analysis (SAS).

**RESULTS AND DISCUSSION**

Recent studies have confirmed that the hide is one of the most significant sites for pathogen intervention in slaughter cattle (6, 7, 21). Not all processors can implement systems such as chemical dehairing (21) or on-line hide wash cabinets (7). We continue to investigate viable alternatives that can be applied in an economical fashion. The latest of these are ozonated water and EO water. Both were evaluated using a hide-washing model system (Fig. 1) to determine their efficacy.

Ozonated water washes of hides resulted in significant reductions (*P < 0.05*) of APC and EBC (Table 1). Before treatment, hide APC were 8.4 log CFU/100 cm² and hide EBC were 6.6 log CFU/100 cm². The ozone washed APC by 2.1 log CFU/100 cm² and reduced EBC by 3.4 log CFU/100 cm². The prevalence of *E. coli* O157 was also reduced (*P < 0.05*) following ozonated water washing. Before treatment, *E. coli* O157 prevalence was 89%, and after treatment the prevalence was 31%, a reduction of 65%.

Cold (15°C) water applied at 4,800 kPa for 10 s, in the...
same as the ozone treatment, reduced APC by 0.5 log CFU/100 cm² and reduced EBC by 0.9 log CFU/100 cm² (Table 2). We concluded that the effect of ozonated water was therefore at least a reduction of 1.5 log CFU/100 cm² in APC between wash conditions was significant (P < 0.05), but there was no difference in the EBC or the prevalence of E. coli O157. In a previous study in which water was applied to hides at 8,300 kPa (1,200 lb in²) for two 20-s periods, APC were reduced by 0.4 log CFU/100 cm² and reduced EBC significantly reduced EBC by 1.6 log CFU/100 cm² and reduced EBC by 0.9 log CFU/100 cm² (Table 2). The conditions of the EO water washes were demonstrably more effective than those of the ozonated water washes, possibly because of the length of time and/or the application pressure. The liquid pressure at which a hide is washed affects the efficacy of the washing treatment (8). The difference of 0.5 log CFU/100 cm² in APC between wash conditions was significant (P < 0.05), but there was no difference in the EBC or the prevalence of E. coli O157.

The observed differences between the EO and ozonated water washes, however, were not solely due to the differences in wash conditions, because when the effect of the hot water washes was taken into account, treatments with EO water still had a greater effect on hide cleanliness than did treatments with ozonated water. Adjusting results

### TABLE 2. Effects of control water treatments on the microbial status of hides

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APC (log CFU/100 cm²)</th>
<th>EBC (log CFU/100 cm²)</th>
<th>O157 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone water control (n = 72)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>7.8</td>
<td>6.8</td>
<td>94 (68/72)</td>
</tr>
<tr>
<td>After</td>
<td>7.3</td>
<td>5.9</td>
<td>99 (71/72)</td>
</tr>
<tr>
<td>Reduction</td>
<td>0.5</td>
<td>0.9</td>
<td>-5</td>
</tr>
<tr>
<td>p&lt;0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.200</td>
</tr>
<tr>
<td>EO water control (n = 72)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>7.8</td>
<td>6.2</td>
<td>100 (72/72)</td>
</tr>
<tr>
<td>After</td>
<td>6.8</td>
<td>5.3</td>
<td>92 (66/72)</td>
</tr>
<tr>
<td>Reduction</td>
<td>1.0</td>
<td>0.9</td>
<td>8</td>
</tr>
<tr>
<td>p&lt;0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Values for APC and EBC are means, SEM ranged from 0.04 to 0.05 for APC and EBC. Values for E. coli O157 prevalence are percentage of positive samples (fraction of positive samples).

### TABLE 3. Effects of electrolyzed water treatment on the microbial status of hides

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APC (log CFU/100 cm²)</th>
<th>EBC (log CFU/100 cm²)</th>
<th>O157 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.5</td>
<td>6.6</td>
<td>82 (75/92)</td>
</tr>
<tr>
<td>Treated</td>
<td>5.0</td>
<td>2.3</td>
<td>35 (32/92)</td>
</tr>
<tr>
<td>Reduction</td>
<td>3.5</td>
<td>4.3</td>
<td>57</td>
</tr>
<tr>
<td>p&lt;0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values for APC and EBC are means, n = 92. SEM was 0.07 for APC and EBC. Values for E. coli O157 prevalence are percentage of positive samples (fraction of positive samples).

Prevalence of E. coli O157 was determined by selective enrichment and immunomagnetic separation isolation followed by plating on sorbitol MacConkey agar with cefixime and potassium tellurite and on O157 chromogenic media. Positive colonies were confirmed by latex agglutination.

Treatment was two 10-s applications of EO water: alkaline EO water applied at 52°C and 4,800 kPa (700 lb in²) and acidic EO water applied at 1,700 kPa (250 lb in²) and 60°C. Samples were collected after a 30-s dwell time.

Values were determined using general linear model procedures of SAS for APC and EBC. The model included the main effect of treatment, and the least squares means separation was accomplished by the probability of difference (PDIF) option (a pairwise t test). Pairwise comparisons of frequencies of E. coli O157 detection were made using PROC FREQ and Mantel-Haenszel chi-square analysis.

The observed differences between the EO and ozonated water washes, however, were not solely due to the differences in wash conditions, because when the effect of the hot water washes was taken into account, treatments with EO water still had a greater effect on hide cleanliness than did treatments with ozonated water. Adjusting results
of treatments using EO water to take into account the hot water effects resulted in APC reductions of 2.5 log CFU/100 cm² and EBC reductions of 3.4 log CFU/100 cm². The hot water wash also had a small (8%) but significant (P < 0.05) effect on the prevalence of E. coli O157. These findings suggest that the temperature and/or duration of the EO water treatments also contributed to some extent to the antimicrobial effect.

We previously examined other hide decontamination methods using the same model system described here (7). Using a similar two-stage application treatment, we evaluated combinations of phosphoric acid, trisodium phosphate, and NaOH as wash and water or acidified chlorine as rinse to reduce hide EBC. The greatest reduction in EBC was observed with the combination of phosphoric acid and acidified chlorine (7), and that reduction was similar to that obtained in the present study for EBC following ozonated and EO water washes.

Ozonated water can instantly reduce populations of E. coli, Salmonella Typhimurium, and other food-related pathogens by 5 to 6 log CFU/ml (24). However, direct applications of ozonated water to various food products have not resulted in such dramatic reductions. When alfalfa seeds were treated with 4 ppm ozone for 2 min to inactivate E. coli O157:H7 (inoculated at 10⁵ CFU/g), no reduction in E. coli O157:H7 numbers was observed (27). Further experiments revealed that reductions of 0.51 to 1.75 log CFU/g required 4 to 21 ppm ozone and exposure times of up to 64 min (27). Treatment of beef carcass surfaces with ozone and water to reduce populations of inoculated E. coli O157: H7 and Salmonella Typhimurium resulted in no significant differences between the two treatments (10). In our system, ozonated water was much more effective than water controls, and concentrations and exposure times were much lower. The efficacy of ozone treatment is increased when competing organic particles are removed and when a mechanical means is used to dislodge bacteria (16). We hypothesize that our results are due in part to the high-pressure application, which satisfies both of these requirements.

In previous studies, acidic EO water reduced E. coli O157 and L. monocytogenes by 8 log CFU/ml when exposed for 30 and 60 s, respectively (15). Salmonella Typhimurium was reduced by 7 log CFU/ml when treated in a similar manner (13). In studies similar to those with ozonated water, direct application to food did not result in reductions of such magnitude. EO water applied to tomatoes reduced populations of E. coli O157:H7, L. monocytogenes, and Salmonella Typhimurium by 4 log CFU/cm² each after exposures of 30 and 60 s (11). Our EO water wash lasted a total of 20 s (10 s for each phase) and was as effective as treatments used on produce products, but not nearly as effective as treatments of pathogens suspended in EO water.

The efficacies of ozonated water, EO water, and other solutions to disinfect lettuce, cucumbers, and strawberries have been compared (17, 18). In each case, electrolyzed water outperformed ozonated water, but not always to a significant extent. APC were reduced about 1 log CFU/g more by EO water than by ozone. This finding is similar to the difference we observed between the two treatments on hides, which may be due to the difference in antimicrobial action rather than the differences in application time and temperature. EO water and ozone both have a strong oxidation-reduction potential, but only EO water contains free chlorine, an additional bactericide. EO water treatments expose organisms to two extremes of pH (alkaline at pH 11.2 and acid at pH 2.4) that can stress and damage cells to a greater extent than can ozonated water, which is only slightly acidic (pH 6).

The presence of foodborne pathogens on cattle hides has been examined, and the hide is considered a likely source of cross-contamination of beef during the hide removal process because the brisket area of the hide is frequently contaminated with pathogens (23). Improved hygienic practices during removal of hides from dirty animals resulted in significant reductions of bacteria on the carcass (19), and a direct relationship between the prevalence of E. coli O157 on hides and the prevalence of this pathogen on previsceration carcasses has been established (1). Several hide interventions have been used with various degrees of success. Washing the hides of cattle with water did not result in significant reductions in hide or carcass contamination (9, 20). The inclusion of an antimicrobial (cetylpyridinium chloride) or a decontamination step (chemical dehairing) was required to obtain improvements in cleanliness of hides and subsequent previsceration carcasses, but use of antimicrobials (6, 20) or chemical dehairing (26) did not have a significant effect. Later use of chemical dehairing on a large sample in an industry setting did clearly demonstrate the efficacy of hide decontamination and improved carcass cleanliness (21). Other successful hide interventions that followed chemical dehairing included treatment with cetylpyridinium chloride (6) and treatment with NaOH and water in an on-line hide wash cabinet (7). These treatments have resulted in effective reduction of hide contamination, and the hide intervention resulted in less contamination of the corresponding carcasses compared with carcasses that were processed conventionally without hide intervention (6, 7, 21). Ozonated and EO waters are not currently validated for use in hide interventions. These experiments were performed in anticipation of potential on-line applications using either a hide wash cabinet or some alternative equipment. The results of the model system reported here suggest that on-line implementation of processes using either ozonated or EO water will be just as successful in controlling pathogens on carcasses as the currently used hide interventions.

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REFERENCES


