Ozone Treatment for Reduction of *Escherichia coli* O157:H7 and *Salmonella* Serotype Typhimurium on Beef Carcass Surfaces

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ABSTRACT

The effectiveness of an aqueous ozone treatment in reducing *Escherichia coli* O157:H7 and *Salmonella* serotype Typhimurium on hot carcass surfaces was determined with the use of a model carcass spray cabinet. Carcass surface regions were removed from carcasses and inoculated with feces containing 10^6 to 10^7 CFU each of *E. coli* O157:H7 and *Salmonella* Typhimurium per g and were then exposed to a water wash or to a water wash followed by a sanitizing ozone treatment. Water washes were applied at 28°C beginning at a pressure of 10 lb/in^2 and gradually increasing to 400 lb/in^2. Ozone treatment was carried out by spraying surfaces with an aqueous ozone solution (80 lb/in^2 at 28°C) containing 95 mg of ozone per liter. Pathogen reductions achieved with ozone treatment were not significantly different from those achieved with a water wash alone. In addition, ozone treatment did not reduce *E. coli* O157:H7 or *Salmonella* Typhimurium contamination that was spread over the carcass surface as a result of the water wash. Under the conditions of this study, the aqueous ozone treatment applied resulted in no significant improvement over a water wash in reducing pathogens on beef carcass surfaces.

The decontamination of carcasses with the use of different sanitizing agents, such as organic acids, hot water, and steam, has been investigated extensively (2, 6, 9, 11). However, alternative treatments, such as sprays of concentrated aqueous ozone solutions, have not been extensively studied. The antimicrobial properties of ozone are well documented (13), and ozone may be suitable for in-plant application. During an evaluation of different carcass interventions, Reagan et al. (12) obtained an aerobic plate count reduction of 1.3 log_{10} CFU/cm^2 and an *Escherichia coli* biotype I reduction of 1.1 log_{10} CFU/cm^2 after the application of ozonated water (0.3 to 2.3 mg of ozone per liter) to beef carcasses contaminated with feces. However, other interventions, such as trimming and hot-water sprays (74 to 87.8°C at the pipe), produced significantly larger reductions. In another evaluation of ozone, Gorman et al. (7) were able to reduce the numbers of a streptomycin-resistant *E. coli* strain on beef briskets by 2.5 to 2.6 log_{10} cycles with a water wash at 16 or 35°C followed by the application of ozone solution (0.5% at 16°C). Reductions obtained with the water wash alone were significantly smaller than those obtained with ozone treatment (1.3 log_{10} CFU/cm^2 at 16°C and 0.9 log_{10} CFU/cm^2 at 35°C).

Ozone treatment for the reduction of bacterial counts on chicken carcasses or chicken meat has also been studied. Factors affecting the stability of ozone solutions as bactericidal agents against chicken microflora were studied by Yang and Chen (15). These authors concluded that the antimicrobial properties of ozone are affected by contact time, temperature, pH, and the presence of inorganic and organic matter in the system. In a study on the effectiveness of ozone in reducing microbial populations on chicken carcasses, Sheldon and Brown (14) could not find significant differences between bacterial counts on chicken carcasses chilled with plain water and counts on carcasses chilled with water containing 0.15 to 15 mg of ozone per liter. However, bacterial counts obtained with nonozonated chill water were significantly higher than bacterial counts obtained with ozonated chill water. The use of ozonated chill water allowed consistent fulfillment of U.S. Department of Agriculture (USDA) requirements for the reuse of chill water. The antimicrobial properties of ozone have also been used in the food industry for the disinfection of fresh produce (1), for the reduction of microbial counts on shrimp (5), for the sanitation of equipment in food plants (8), and even for the reduction of plant pathogens on produce to improve the harvest of carrots by preventing postharvest plant diseases (10).

In this study, a high-concentration ozone solution was applied to various beef carcass surface regions that had been inoculated with a fecal suspension of *E. coli* O157:H7 and *Salmonella* Typhimurium. Counts of these pathogens after ozone treatment were compared with counts after a water wash control treatment to determine the effectiveness of ozone treatment in reducing pathogenic contamination on beef carcasses.

MATERIALS AND METHODS

Objective and overall project design. The objective of this project was to provide a model for the determination of the effectiveness of an ozone treatment in reducing fecal *E. coli* O157:H7 and *Salmonella* Typhimurium contamination on hot carcass surfaces. Carcass surface regions (inside round, outside round,
brisket, and clod) were removed from carcasses at slaughter and transported in insulated containers to facilities located within 5 miles of the slaughter site. Surfaces were inoculated with feces containing $10^7$ CFU each of \textit{E. coli} O157:H7 and \textit{Salmonella} Typhimurium per g and were then subjected to a warm-water (28°C) wash (simulating a typical commercial carcass wash) or to the same warm water wash followed by an aqueous ozone rinse treatment. Counts of each pathogen on the inoculated carcass surfaces were determined before and after treatment.

### Carcass selection
Four steers typical of those entering the U.S. meat supply were selected for use in this study and transported to the Rosenthal Meat Science and Technology Center for slaughtering and dressing by USDA Food Safety and Inspection Service-regulated commercial procedures. Paired beef inside rounds, outside rounds, briskets, and clods were separated from the remainder of the carcass just subsequent to carcass splitting. These particular carcass surface regions were selected for use in this study because they are located in areas in which fecal contamination is likely to occur (4). In addition, it was theorized that differences in the fat surface characteristics of these areas on the carcass might also affect contamination removal. Carcasses were not washed or decontaminated in any way before carcass surface regions were obtained for use in this study.

### Inoculum preparation
On the night before slaughter, feces were collected from dairy cattle at the Dairy Research Center immediately after defecation and transported to the food microbiology laboratory, located within 1 mile of the Dairy Research Center. Feces then were hand kneaded inside a stomacher bag, dispensed in 10-g portions into sterile stomacher bags, and stored at 4°C until the next day. Each bag containing 10 g of feces then was inoculated with 10 ml of a bacterial suspension containing sufficient levels of rifampicin-resistant \textit{E. coli} O157:H7 and rifampicin-resistant \textit{Salmonella} Typhimurium to deliver a load of $10^6$ to $10^7$ CFU of each pathogen per g of feces. The populations of marker organisms in the fecal inoculum were confirmed by plating appropriate dilutions of the inoculated fecal suspension onto lactose-sulfite-phenol red-rifampicin (LSPR) agar, a selective and differential medium for rifampicin-resistant \textit{E. coli} and \textit{Salmonella} (2).

### Carcass inoculation and microbiological analysis
Before inoculation, three 10-cm$^2$ areas (for a total area of 30 cm$^2$) were excised from each hot-boned inside round, outside round, brisket, and clod. To obtain these samples, a sterile borer was used to cut a 10-cm$^2$ sample (about 2 to 3 mm deep), and then the surface sample was sliced (with ±2 mm of interior tissue being taken) from the cut with a sterile scalpel and forceps. The three 10-cm$^2$ samples were composited into a stomacher bag to which 100 ml of 0.1% buffered peptone water was added. This suspension was pumped into a Stomacher 400 (Tekmar Co., Cincinnati, Ohio) and surface-plate onto LSPR agar to make sure that no rifampicin-resistant organisms were present among the background flora of the carcass surface.

For the inoculation of the carcass surface regions, a 400-cm$^2$ area (20 by 20 cm) was marked on the outside carcass surface region of each hot-boned inside round, outside round, brisket, and clod and then contaminated by removing the fecal inoculum (10 g) from the sterile stomacher bag and evenly spreading it with a sterile spatula over the marked 400-cm$^2$ area. The levels of the marker pathogens or indicator organisms on the inoculated surfaces were determined by excising three 10-cm$^2$ areas from various locations within the inoculated area. These samples were ob-

### Table 1. Effect of carcass surface region on mean populations of \textit{E. coli} O157:H7 and \textit{Salmonella} Typhimurium on beef carcass surfaces after application of water wash or ozone treatment

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Treatmenta</th>
<th>Inside round</th>
<th>Outside round</th>
<th>Brisket</th>
<th>Clod</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{E. coli} O157:H7</td>
<td>No treatment (n = 6)</td>
<td>5.7 A</td>
<td>5.8 A</td>
<td>5.7 A</td>
<td>5.7 A</td>
</tr>
<tr>
<td></td>
<td>Water wash (n = 3)</td>
<td>4.0 A</td>
<td>3.0 B</td>
<td>3.2 B</td>
<td>2.6 B</td>
</tr>
<tr>
<td></td>
<td>Water wash + ozone (n = 3)</td>
<td>3.7 A</td>
<td>2.9 AB</td>
<td>2.7 AB</td>
<td>2.1 B</td>
</tr>
<tr>
<td>\textit{Salmonella} Typhimurium</td>
<td>No treatment (n = 6)</td>
<td>5.2 A</td>
<td>5.4 A</td>
<td>5.4 A</td>
<td>5.3 A</td>
</tr>
<tr>
<td></td>
<td>Water wash (n = 3)</td>
<td>3.4 A</td>
<td>2.6 BC</td>
<td>3.1 AB</td>
<td>2.1 C</td>
</tr>
<tr>
<td></td>
<td>Water wash + ozone (n = 3)</td>
<td>3.3 A</td>
<td>2.4 AB</td>
<td>2.4 AB</td>
<td>1.7 B</td>
</tr>
</tbody>
</table>

- The water wash consisted of a 1.5-liter hand wash (90 s, 10 lb/in$^2$) followed by a 5-liter automated cabinet wash (9 s, 250 to 400 lb/in$^2$) at 35°C. The ozone treatment consisted of a water wash followed by a spray of solution containing 95 mg of ozone per liter for 30 s at 80 lb/in$^2$.
- Means with the same letter in the same row are not significantly different ($P > 0.05$).

### Table 2. Mean log reductions in populations of \textit{E. coli} O157:H7 inoculated onto different beef carcass surface regions after water wash and ozone treatments

<table>
<thead>
<tr>
<th>Carcass surface region</th>
<th>Mean log reduction of \textit{E. coli} O157:H7 for treatment (n = 3)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside round (I)</td>
<td>Water wash</td>
</tr>
<tr>
<td>Outside round (O)</td>
<td>2.8 A</td>
</tr>
<tr>
<td>Brisket (B)</td>
<td>2.5 A</td>
</tr>
<tr>
<td>Clod (C)</td>
<td>3.1 A</td>
</tr>
<tr>
<td>Order of means$^b$</td>
<td>C B O I</td>
</tr>
</tbody>
</table>

- The log reduction was calculated as the count (log$_{10}$ CFU/cm$^2$) before treatment minus the count (log$_{10}$ CFU/cm$^2$) after treatment.
- Means underlined with a common line are not significantly different ($P > 0.05$).
TABLE 3. Mean log reductions in populations of Salmonella Typhimurium inoculated onto different beef carcass surface regions after water wash and ozone treatments

<table>
<thead>
<tr>
<th>Carcass surface region</th>
<th>Water wash</th>
<th>Water wash + ozone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside round (I)</td>
<td>1.8 A</td>
<td>1.9 A</td>
</tr>
<tr>
<td>Outside round (O)</td>
<td>2.8 A</td>
<td>3.0 A</td>
</tr>
<tr>
<td>Brisket (B)</td>
<td>2.3 A</td>
<td>3.0 B</td>
</tr>
<tr>
<td>Clod (C)</td>
<td>3.2 A</td>
<td>3.6 A</td>
</tr>
<tr>
<td>Order of meansb</td>
<td>C O B I</td>
<td>C B O I</td>
</tr>
</tbody>
</table>

*The log reduction was calculated as the count (log10 CFU/cm²) before treatment minus the count (log10 CFU/cm²) after treatment. The water wash consisted of a 1.5-liter hand wash (90 s, 10 lb/in²) followed by a 5-liter automated cabinet wash (9 s, 250 to 400 lb/in²) at 35°C. The ozone treatment consisted of a water wash followed by a spray of solution containing 95 mg of ozone per liter for 30 s at 80 lb/in². Mean log reductions in populations of Salmonella Typhimurium for treated and nontreated carcass surface regions. Populations of both pathogens were significantly reduced (by 1.9 to 3.6 log CFU/cm²) regardless of the carcass surface region. Carcass surface region usually did not affect counts of Salmonella Typhimurium to determine whether contamination from the fecal suspension was spread by the water wash treatments to adjacent areas. These three borer samples were collected from the area surrounding the inoculated tissue by obtaining one 10-cm² area to the left or to the right of the inoculated area and two 10-cm² areas from the area below the inoculated surface. Samples were composited in 100 ml of sterile 0.1% peptone diluent and examined as described above.

**Application of treatment and collection of samples.** After inoculation of the surface with the appropriate fecal suspension as described above, paired carcass surface regions were treated with a water wash or with a water wash followed by ozone treatment. Carcass surfaces were washed with the use of model spray equipment (Chad Company, Lenexa, Kans.) and procedures established in previous studies (3, 9). Briefly, each inoculated carcass surface region was positioned in the spray cabinet to simulate a carcass surface in a normal hanging position. A hand-operated spray wash was applied to remove visible fecal contamination with the use of a handheld noncorrosive polyethylene compressed-air sprayer (10.56 liters; Universal-Gerwin, Saranac, Mich.), which delivered 1.5 liters at 10 lb/in² for 90 s. This initial hand-operated wash was followed by a 5-liter automated cabinet water wash (28°C) for 9 s (simulating commercial carcass wash systems; an initial pressure of 200 lb/in²) followed by a 5-liter automated cabinet wash (9 s, 250 to 400 lb/in²) at 35°C. The ozone treatment consisted of a water wash followed by a spray of solution containing 95 mg of ozone per liter for 30 s at 80 lb/in². Mean log reductions in Salmonella Typhimurium on LSPR agar.

**Collection of samples from neighboring tissue.** The effects of water wash and water wash–ozone treatments on the spread of the inoculated organism to areas outside the 400-cm² contamination area was determined by excising random samples (three 10-cm² portions) from areas closely surrounding each inoculated area (within 10 to 20 cm). All areas bordering the inoculated and treated area were examined for E. coli O157:H7 and Salmonella Typhimurium to determine whether contamination from the fecal suspension was spread by the water wash treatments to adjacent areas. These three borer samples were collected from the area surrounding the inoculated tissue by obtaining one 10-cm² area to the left or to the right of the inoculated area and two 10-cm² areas from the area below the inoculated surface. Samples were composited in 100 ml of sterile 0.1% peptone diluent and examined as described above.

**Data analysis.** Counts for each pathogen were transformed into logarithms. Log10 reduction values were calculated as the difference between the log10 counts for each inoculated carcass surface before and after each treatment. To facilitate the statistical analysis of these data, samples with bacterial counts below the detection limit of 0.5 log10 CFU/cm² were assigned a value of 0.25 log10 CFU/cm², which is the value halfway between 0 and the minimum detection level of the counting method used. Analysis of variance was used to compare the mean values for log10 reduction values, log10 counts obtained from outside the inoculated area, and visual scores for treated carcass surface regions with respect to the treatment applied. When analysis of variance indicated significant differences among means (P < 0.05), mean separation by the Tukey-Kramer test was carried out.

**RESULTS AND DISCUSSION**

Table 1 presents counts of E. coli O157:H7 and Salmonella Typhimurium for treated and nontreated carcass surface regions. Populations of both pathogens were significantly reduced (by 1.9 to 3.6 log10 cycles) regardless of the carcass surface region. Carcass surface region usually did not affect counts of E. coli O157:H7; however, a significantly smaller reduction was observed for the inside round region. This region has been reported to be more difficult to decontaminate than any other anatomic region (2, 9). Similar results were obtained for Salmonella Typhimurium. No definite explanation has been provided for these consistent findings; however, we theorize that this peculiar surface, including lean-fat junctions and a collar of fat surrounding a lean surface, may provide an irregular surface on which bacteria might be protected inside crevices and crypts that are not present in the same sizes and at the same frequency on other cuts of meat.

Log reduction values were calculated for each pathogen as the difference between the log10 counts (CFU/cm²) for each inoculated carcass surface region before and after water wash or ozone treatment. With the exception of Salmonella Typhimurium on the brisket, log reductions in pop-
ulations of marker pathogens were only minimally affected by the aqueous ozone treatment (Tables 2 and 3). While overall numbers indicate that an additional bacterial reduction was obtained by spraying ozone solution onto the carcass surfaces after the water wash, statistical analysis of these data revealed no significant differences. When reductions of \( E. \text{coli} \) O157:H7 were compared as a function of the carcass surface region (Table 2), no differences were observed with respect to the treatment applied, and only the above-mentioned effect for the inside round region was observed. Although no differences with respect to treatment were observed for \( \text{Salmonella} \) Typhimurium on most carcass surface regions, the reduction of this pathogen on the brisket was significantly larger after ozone treatment than after the water wash alone (Table 3). Although the numerical difference of 0.7 log cycles is statistically significant, in laboratory evaluations, bacterial reductions achieved with most interventions have been found to range from 3 to 5 log cycles when these interventions are combined with an initial water wash (2, 3, 9, 11). Likewise, scores for visible fecal contamination were not reduced by ozone treatment relative to those for water wash alone (data not shown in tabular form).

In conclusion, under the conditions of this study, a treatment consisting of a water wash followed by an ozone spray did not significantly reduce pathogenic bacterial contamination on hot beef carcass surface regions.

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REFERENCES


