

## Hot Water Rinses

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### Abstract

Carcass surface regions were removed from carcasses and inoculated with feces containing 10<sup>6</sup>/g each of *Escherichia coli* O157:H7 and *Salmonella typhimurium*. Surfaces were then exposed to various carcass wash and hot water sanitizing treatments. Data collection allowed a determination of the expected reduction of *E. coli* O157:H7 and *S. typhimurium* by treatment of contaminated carcass surfaces with hot water. In addition, an identical number of carcass surface regions were inoculated with feces (not inoculated with *E. coli* O157:H7 and *S. typhimurium*) and exposed to the same treatments to allow a determination of the effect of the treatments on traditional indicator microorganisms (Aerobic plate count, coliforms and thermotolerant coliforms).

### Introduction

In an early report on hot water decontamination, Patterson (1969) reported that beef carcasses treated with a steam and hot water spray (80-96°C) for 2 minutes contained significantly lower bacterial numbers. Some discoloration on the carcass surface occurred initially, but normal color returned after cooling for 24 hours. According to Smith & Graham (1978), pouring hot water (80°C) on beef and lamb samples for 10 seconds destroyed more than 99% of *E. coli* and *Salmonella* inoculated (10<sup>6.5</sup>/cm<sup>2</sup>) onto the samples. The surface tissues of the beef and mutton were not permanently discolored; however, when the surface tissues were treated at 90°C for 120 seconds, discoloration was reported. Kelly et al. (1981) reported that lamb carcasses sprayed with hot water at temperatures >80°C caused significant decreases (>1.0 log<sub>10</sub>/cm<sup>2</sup>) in aerobic plate counts (APCs).

In 1993, Barkate et al. published a report on the possible use of hot water for decontamination of beef carcasses. In this investigation, areas of hot beef carcass surfaces were sprayed with 95°C water at various locations on the slaugh-

ter floor with the objective of raising the carcass surface temperature to 82°C for approximately 10 seconds. Significant reductions in bacterial contamination on the surface of carcasses were obtained. Slight discoloration of carcasses occurred immediately after spraying with hot water, but the discoloration was temporary and normal color returned to the carcass within 24 hours, an observation also reported by Patterson (1969).

Removal of any fecal material from beef carcass surfaces during slaughter/dressing is required by USDA-FSIS regulations. Traditionally, this removal of contamination has been accomplished by trimming the affected surface from the carcass. However, bacteria of fecal origin are not necessarily confined to areas of visible fecal material contamination, and trimming the affected areas has been shown to have little benefit over other procedures in reducing the possible bacterial contamination. Concerns associated with washing and decontamination procedures for removal of feces include a less than complete decontamination and possible spreading of contamination to previously uncontaminated areas through carriage of bacteria in liquid runoff.

### Materials and Methods

#### Carcass Selection, Inoculation and Sampling

Fed steers or heifers typical of those entering the U.S. meat supply were transported to the Texas A&M University Rosenthal Meat Science and Technology Center (RMSTC) and slaughtered/dressed in the university abattoir following USDA-FSIS regulated commercial procedures. Paired beef inside rounds, outside rounds, briskets, flanks 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 since they are located in areas where fecal contamination is likely to occur. In addition, it was theorized that differences in fat surface characteristics from these areas on the carcass might affect contamination removal. Carcasses were not washed or decontaminated in any manner before carcass surface regions were obtained for use in this study.

Each slaughter day, feces was collected from dairy cattle (Texas A&M University Dairy Center) immediately after defecation and mixed with 0.1% peptone water sufficient to

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**TABLE 1. Log reductions<sup>a</sup> (log<sub>10</sub> CFU/cm<sup>2</sup>) in populations of *S. typhimurium* and *E. coli* O157:H7 recovered from within 400-cm<sup>2</sup> contaminated areas of carcass surface regions as affected by treatment and type of surface.**

| Inocuum                | Treatment <sup>b</sup>  | Carcass surface region |                   |                  |                  |                  | Order of means <sup>c</sup> |
|------------------------|-------------------------|------------------------|-------------------|------------------|------------------|------------------|-----------------------------|
|                        |                         | Inside Round (I)       | Outside Round (O) | Brisket (B)      | Flank (F)        | Clod (C)         |                             |
| <i>S. typhimurium</i>  | Water wash              | 2.0 <sup>d</sup>       | 2.6 <sup>d</sup>  | 2.3 <sup>d</sup> | 2.0 <sup>d</sup> | 1.9 <sup>d</sup> | OBFIC                       |
|                        | Water wash + hot water  | 2.7 <sup>e</sup>       | 4.3 <sup>e</sup>  | 3.8 <sup>e</sup> | 3.9 <sup>e</sup> | 4.1 <sup>e</sup> | OCFB I                      |
|                        | Difference <sup>f</sup> | 0.7                    | 1.7               | 1.5              | 1.9              | 2.2              |                             |
| <i>E. coli</i> O157:H7 | Water wash              | 2.1 <sup>d</sup>       | 2.7 <sup>d</sup>  | 1.7 <sup>d</sup> | 1.9 <sup>d</sup> | 2.0 <sup>d</sup> | O ICFB                      |
|                        | Water wash + hot water  | 2.9 <sup>e</sup>       | 4.0 <sup>e</sup>  | 3.9 <sup>e</sup> | 3.8 <sup>e</sup> | 4.1 <sup>e</sup> | COBFI                       |
|                        | Difference <sup>f</sup> | 0.8                    | 1.3               | 2.2              | 1.9              | 2.1              |                             |

<sup>a</sup> Log reduction = (log<sub>10</sub> CFU/cm<sup>2</sup> before treatment) - (log<sub>10</sub> CFU/cm<sup>2</sup> after treatment).

<sup>b</sup> Water: 1.5-L hand wash (90 s, 10 psi) followed by 5-L automated cabinet wash (9 s, 250 to 400 psi), 35°C.

Hot water: 95°C water at 24 psi for 5 seconds using a flat spray nozzle (Spraying Systems, H<sup>1</sup>/<sub>4</sub>USS5050) from a distance of 12.5 cm.

<sup>c</sup> Means within rows underlined by a common line are not significantly different.

<sup>d,e</sup> Numbers in columns with same letter are not significantly different.

<sup>f</sup> Difference = (log reduction by water wash+hot water) - (log reduction by water wash).

prepare a thick paste. For treatments requiring inoculation of the carcass surface with pathogens, the paste was inoculated with sufficient rifampicin-resistant *S. typhimurium* and rifampicin-resistant *E. coli* O157:H7 to deliver a load of ca.10<sup>6</sup> CFU of each per g of feces. Additional fecal suspensions were prepared without rifampicin-resistant *E. coli* O157:H7 and *S. typhimurium* for use in treatments where indicator organisms were enumerated. 10-g portions of the fecal suspensions were then weighed and placed in sterile bags for subsequent contamination of carcass surfaces.

A 400-cm<sup>2</sup> area (20 cm x 20 cm) was marked on the outside carcass surface region of each hot-boned inside round, outside round, brisket, flank and clod. Three areas outside (30 cm<sup>2</sup> total area), but surrounding each marked area were composited and sampled for *E. coli* O157:H7 and *S. typhimurium* marker organisms, or coliforms (35 and 44.5°C) and APC (as appropriate to the inoculum) to determine the background count already present on the carcass cut. After obtaining background count data, inoculation of the marked area on each cut with the previously prepared fecal suspension (with or without marker pathogens) proceeded by spreading the fecal suspension (10 g) onto the marked area on the surface of the cuts with a sterile spatula.

To obtain data regarding the level of the marker pathogens or indicator organisms on the inoculated surfaces, three 10-cm<sup>2</sup> areas were obtained from within the inoculated area. These samples were composited in 100 ml of sterile diluent (0.1% peptone) and then enumerated for *E. coli* O157:H7 and *S. typhimurium* marker pathogens or coliforms (35 and 44.5°C) and APC, as appropriate to the fecal suspension applied.

#### Application of Treatment and Collection of Samples

Twelve cattle were slaughtered over 6 slaughter days (2 cattle per day) using normal commercial slaughter/dressing procedures. As described earlier, inside rounds, outside rounds, briskets, flanks and clods were removed from carcasses just subsequent to carcass splitting and the hot carcass surface regions were placed in insulated coolers for transport to the food microbiology laboratory for treatment. Carcass surface regions were positioned during treatment to simulate a normal hanging carcass position. After inoculation of the surface with the appropriate fecal suspension, as described above, paired cuts were assigned to be treated immediately (within 5 min) after inoculation or to be treated after a 20-30 min delay (to simulate possible commercial delays). Treatments included (1) immediate water washing of the contaminated area, (2) delayed water washing (3) immediate water wash followed by a hot water spray (95°C) and (4) delayed water wash followed by a hot water spray. Washing and decontamination of carcass surfaces were conducted using model spray equipment (designed and constructed by Chad Company) and procedures established in previous organic acid treatment studies (Hardin et al. 1995; Prasai et al. 1991). Hot water treatment was conducted by spraying water at 95°C at 24 psi for 5 seconds using a flat spray nozzle (Spraying Systems, H<sup>1</sup>/<sub>4</sub> USS5050) from a distance of 12.5 cm. After treatment, three 10-cm<sup>2</sup> samples were obtained from each washed/decontaminated inoculated area and composited in an appropriate amount of sterile diluent before examining for marker pathogens or coliforms and APC, as appropriate.

**TABLE 2. Mean populations ( $\log_{10}$  CFU/cm<sup>2</sup>) of *S. typhimurium* recovered from outside 400-cm<sup>2</sup> contaminated areas of carcass surface regions as affected by treatment and type of surface.**

| Treatment <sup>a</sup>         | Carcass surface region |               |           |           |          |
|--------------------------------|------------------------|---------------|-----------|-----------|----------|
|                                | Inside Round           | Outside Round | Brisket   | Flank     | Clod     |
| Immediate water wash           | 1.3 (0) <sup>b</sup>   | 0.8 (67)      | 1.3 (0)   | 1.5 (0)   | 1.3 (0)  |
| Immediate water wash+hot water | <0.5 (100)             | <0.5 (67)     | <0.5 (67) | 0.7 (33)  | 0.6 (33) |
| Delayed water wash             | 1.4 (0)                | 0.6 (0)       | 1.6 (0)   | 1.5 (0)   | 1.8 (0)  |
| Delayed water wash+hot water   | <0.5 (100)             | <0.5 (100)    | <0.5 (67) | <0.5 (67) | 0.6 (67) |

<sup>a</sup> Water: 1.5-L hand wash (90 s, 10 psi) followed by 5-L automated cabinet wash (9 s, 250 to 400 psi), 35°C. Hot water: 95°C water at 24 psi for 5 seconds using a flat spray nozzle (Spraying Systems, H<sup>1</sup>/<sub>4</sub>USS5050) from a distance of 12.5 cm.

<sup>b</sup> Values contained in parentheses denote the percentage of samples below the minimum detection level of 0.5  $\log_{10}$  CFU/cm<sup>2</sup>.

Areas bordering the inoculated and treated area were examined for indicator pathogens or APC and coliforms (35 and 44.5°C), as appropriate, to determine if contamination from the fecal suspension was spread by the water wash treatments to adjacent areas. Three borer samples were obtained from the area surrounding the inoculated tissue by obtaining one 10-cm<sup>2</sup> area to the left or right of the inoculated area and two 10-cm<sup>2</sup> areas from the area below the inoculated surface.

## Results and Discussion

All treatments evaluated in this investigation significantly reduced levels of pathogens from fecal contamination on carcass surface regions compared to the initial inoculation level (ca. 5.0  $\log_{10}$  CFU/ml). In all but one instance, no differences were observed in the effects of immediate and delayed (up to 30 minutes) treatment on bacterial reduction. A 1.9 to 2.7- $\log_{10}$ /cm<sup>2</sup> reduction in counts of the marker pathogens was obtained by a 250-400 psi water wash and an additional 0.7 to 2.2- $\log_{10}$ /cm<sup>2</sup> reduction was observed after spraying with hot water. Overall, treatments which included hot water sprays reduced the numbers of *S. typhimurium* and *E. coli* O157:H7 from 2.7 to 4.3  $\log_{10}$ /cm<sup>2</sup> on various carcass surface regions. (Table 1). The measured temperature of carcass surface regions was raised to approximately 82°C during the hot water treatment.

Reduction of marker pathogens by hot water treatment was consistent on all carcass surface regions with the exception of the inside round region. This region displayed consistently smaller reduction of pathogens, and was significantly ( $p < 0.05$ ) smaller in the reduction of *S. typhimurium* (Table 1). This difficulty in decontamination of the inside

**TABLE 3. Mean populations ( $\log_{10}$  CFU/cm<sup>2</sup>) of *E. coli* O157:H7 recovered from outside 400-cm<sup>2</sup> contaminated areas of carcass surface regions as affected by treatment and type of surface.**

| Treatment <sup>a</sup>         | Carcass surface region |               |           |           |           |
|--------------------------------|------------------------|---------------|-----------|-----------|-----------|
|                                | Inside Round           | Outside Round | Brisket   | Flank     | Clod      |
| Immediate water wash           | 1.4 (0) <sup>b</sup>   | 1.1 (33)      | 1.7 (0)   | 1.9 (0)   | 1.2 (33)  |
| Immediate water wash+hot water | <0.5 (67)              | <0.5 (67)     | <0.5 (67) | 0.9 (33)  | <0.5 (33) |
| Delayed water wash             | 1.8 (0)                | 1.0 (0)       | 1.9 (0)   | 1.8 (0)   | 2.2 (0)   |
| Delayed water wash+hot water   | <0.5 (67)              | <0.5 (67)     | 0.7 (67)  | <0.5 (67) | 0.7 (67)  |

<sup>a</sup> Water: 1.5-L hand wash (90 s, 10 psi) followed by 5-L automated cabinet wash (9 s, 250 to 400 psi), 35°C. Hot water: 95°C water at 24 psi for 5 seconds using a flat spray nozzle (Spraying Systems, H<sup>1</sup>/<sub>4</sub>USS5050) from a distance of 12.5 cm.

<sup>b</sup> Values contained in parentheses denote the percentage of samples below the minimum detection level of 0.5  $\log_{10}$  CFU/cm<sup>2</sup>.

round area was also noted by Hardin et al. (1995) when treating carcass surface regions with organic acids. These investigators reported that the inside round region contained a substantial amount of exposed lean tissue and a pronounced collar of fat at the edge of the lean, possibly allowing for fecal material (and bacteria) to become imbedded in the fat/lean juncture and between muscle bundles in the lean surface.

After cleaning the inoculated fecal contamination from carcass surface regions using water only, counts of *S. typhimurium* and *E. coli* O157:H7 were consistently recovered in the range of 0.6 to 1.9  $\log_{10}$ /cm<sup>2</sup> on surface areas adjacent to, but outside the 400-cm<sup>2</sup> inoculation area. This data confirmed that some spreading of bacterial contamination is probable when areas of visible fecal contamination are washed with water. However, after treatment of the surfaces with hot water, counts of both pathogens were consistently reduced to levels at or below the minimum detection level (Tables 2 and 3). It is apparent, therefore, that a water wash of fecal contamination on carcass surfaces may spread any pathogens present over the surface of the carcass; however, sanitizing with hot water is capable of eliminating most of that contamination, even when initial levels were as high as used in this study (5.0  $\log_{10}$ /cm<sup>2</sup>). Similar results were also reported by Hardin et al. (1995) when sanitizing carcass surfaces with organic acids.

As with the pathogens, all treatments significantly reduced levels of indicator organisms from fecal contamination on carcass surface regions (Table 4). No significant differences were observed in the effect of the treatments on individual types of indicators or in the effects of immediate and delayed (up to 30 minutes) treatment on the reduction in APC, total coliforms or thermotolerant coliforms. Over-

**TABLE 4. Log reductions<sup>a</sup> (log<sub>10</sub> CFU/cm<sup>2</sup>) in APC, coliforms and thermotolerant coliforms recovered from within 400-cm<sup>2</sup> contaminated areas of carcass surface regions as affected by treatment and type of surface.**

| Microorganism            | Treatment <sup>b</sup>  | Carcass surface region |                   |                  |                  |                  | Order of means <sup>c</sup> |
|--------------------------|-------------------------|------------------------|-------------------|------------------|------------------|------------------|-----------------------------|
|                          |                         | Inside Round (I)       | Outside Round (O) | Brisket (B)      | Flank (F)        | Clod (C)         |                             |
| APC                      | Water wash              | 1.8 <sup>d</sup>       | 1.8 <sup>d</sup>  | 1.3 <sup>d</sup> | 2.1 <sup>d</sup> | 1.8 <sup>d</sup> | FCIOB                       |
|                          | Water wash +hot water   | 2.3 <sup>d</sup>       | 3.4 <sup>e</sup>  | 2.9 <sup>e</sup> | 3.3 <sup>e</sup> | 2.4 <sup>e</sup> | OFBCI                       |
|                          | Difference <sup>f</sup> | 0.5                    | 1.6               | 1.6              | 1.2              | 0.6              |                             |
| Total coliforms          | Water wash              | 2.0 <sup>d</sup>       | 1.6 <sup>d</sup>  | 1.4 <sup>d</sup> | 2.2 <sup>d</sup> | 1.8 <sup>d</sup> | FICOB                       |
|                          | Water wash +hot water   | 2.6 <sup>d</sup>       | 3.8 <sup>e</sup>  | 3.4 <sup>e</sup> | 4.0 <sup>e</sup> | 2.6 <sup>d</sup> | FOBCI                       |
|                          | Difference <sup>f</sup> | 0.6                    | 2.2               | 2.0              | 1.8              | 0.8              |                             |
| Thermotolerant coliforms | Water wash              | 2.0 <sup>d</sup>       | 1.6 <sup>d</sup>  | 1.3 <sup>d</sup> | 2.3 <sup>d</sup> | 1.9 <sup>d</sup> | FICOB                       |
|                          | Water wash +hot water   | 2.7 <sup>d</sup>       | 3.9 <sup>e</sup>  | 3.2 <sup>e</sup> | 4.0 <sup>e</sup> | 2.7 <sup>d</sup> | FOBCI                       |
|                          | Difference <sup>f</sup> | 0.7                    | 2.3               | 1.9              | 1.7              | 0.8              |                             |

<sup>a</sup> Log reduction = (log<sub>10</sub> CFU/cm<sup>2</sup> before treatment) - (log<sub>10</sub> CFU/cm<sup>2</sup> after treatment).

<sup>b</sup> Water: 1.5-L hand wash (90 s, 10 psi) followed by 5-L automated cabinet wash (9 s, 250 to 400 psi), 35°C.

Hot water: 95°C water at 24 psi for 5 seconds using a flat spray nozzle (Spraying Systems, H<sup>1</sup>/<sub>4</sub>USS5050) from a distance of 12.5 cm.

<sup>c</sup> Means within rows underlined by a common line are not significantly different.

<sup>d,e</sup> Numbers in columns with same letter are not significantly different.

<sup>f</sup> Difference = (log reduction by water wash+hot water) - (log reduction by water wash).

all, treatments which included hot water sprays reduced indicator organisms from 2.3 to 4.0 log<sub>10</sub>/cm<sup>2</sup> on various carcass surface regions. Similar to the reduction pattern observed with the inoculated pathogens, the inside round carcass region displayed consistently smaller reductions.

In a similar pattern to that displayed by the inoculated pathogens, APCs, coliforms and thermotolerant coliforms were consistently recovered from surface areas adjacent to, but outside the 400-cm<sup>2</sup> inoculation area after cleaning the inoculated fecal contamination from carcass surface regions using water only (data not shown). This data confirmed that a similar pattern of spreading bacterial contamination also occurs with the indicator organisms when areas of visible fecal contamination are washed with water. Following treatment of the surfaces with hot water, APCs were usually lowered, but only slightly. However, after treatment of the surfaces with hot water, counts of both coliforms and thermotolerant coliforms were consistently reduced to levels at or below the minimum detection level, a pattern consistent with that demonstrated by both *S. typhimurium* and *E. coli* O157:H7. It is likely, therefore, that verification of the ability of a hot water carcass treatment CCP to reduce these pathogens could be accomplished by obtaining coliform counts before and after the treatment. Log<sub>10</sub> reductions of coliforms by application of hot water to carcass surfaces should approximate the expected log<sub>10</sub> reduction in *S. typhimurium* or *E. coli* O157:H7, if present.

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