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Microbial Decontamination of Beef and Sheep Carcasses by Steam, Hot Water Spray Washes, and a Steam-Vacuum Sanitizer

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ABSTRACT

Three separate studies were conducted to determine the effectiveness of various temperature water spray washes (W_t), wash and steam combinations (W_tS), and vacuum and wash combinations (VW_t) for reducing fecal bacteria on sheep and beef carcasses. W_t of 15.6, 54.4, and 82.2°C were administered to sheep carcasses contaminated with feces, using a hand-held spray nozzle. Initial carcass bacterial populations of approximately 2.5, 4, and 6 log CFU/cm^2 were subjected to all wash combinations. $W_{82.2}$ and $W_{82.2}S$ reduced 6 log CFU/cm^2 bacterial populations as much as 4.0 log cycles. When carcasses were subjected to W_tS and $W_{82.2}$, the initial contamination levels (4 and 6 log CFU/cm^2) had little effect on final bacterial levels (2.7 to 3.3 log CFU/cm^2). However, uninoculated carcasses with initial bacterial populations of 2.5 log CFU/cm^2 experienced a 1.5-log-cycle reduction when subjected to W_tS and $W_{82.2}$. It is possible that hydration of a carcass before and during interventions affords some protection to bacteria. The next study used a commercial carcass washer to apply a hot water (72°C), low pressure (20 psi) wash in combination with a high pressure (125 psi), warm water (30°C) wash ($W_{72/30}$). Reductions on beef of 2.7, 3.3, and 3.4 log cycles for aerobic plate count (APC), coliforms, and *E. coli* populations, respectively, were observed. When a commercial steam-vacuum was used in conjunction with $W_{72/30}$, reductions of 3.1, 4.2, and 4.3 log cycles for APC, coliforms, and *E. coli* populations, respectively, were achieved. Implementation of these interventions could reduce the amount of trimming needed on carcass-processing lines and would increase the microbial safety of beef carcasses.

Key words: Beef, steam, steam-vacuum, washes, interventions

The general hygiene of animal carcasses has long been a concern to the meat-processing industry and recent developments have heightened interest. Fatal cases of disease caused by foodborne *Escherichia coli* O157:H7 have catalyzed this increased concern. Red meat processors are actively looking for reasonable interventions that minimize the risk of introducing bacterial pathogens to processed meats from contaminated raw carcasses. Decontamination of carcasses with organic acids and other chemical sanitizers has been extensively investigated *(2, 3, 4, 8)*. Decontamination with hot water washes (80 to 96°C) shows promise as an effective intervention *(6, 7, 11, 13)*. In all of these studies, bacterial levels on beef or mutton carcasses were significantly reduced using hot water washes and carcass appearance was not permanently or adversely affected.

Though Patterson *(11)* showed a hot water and steam wash supplied through a mixer device (80 to 96°C) for 2 min reduced aerobic plate counts (APCs) on beef carcasses, the reduction was less than 1 log CFU/cm^2. Subsequent studies by Barkate et al. *(6)* showed hot water washes of 95°C reduced APCs on beef carcasses by 1.3 log CFU/cm^2. Carcass surface temperature achieved during their study was 82°C. Davey and Smith *(7)* observed 2.2-log CFU/cm^2 reductions of *Escherichia coli* by cascading 83.5°C water down inoculated beef carcasses for 10 s. Smith and Graham *(13)* were able to achieve greater than 3-log CFU/cm^2 reductions of *E. coli* and salmonellae on inoculated sheep carcasses by submersion in 80°C water for 10 s. In the same study, populations of coliforms, initially as high as 100 cells per cm^2, were reduced to nondetectable levels (<1 cell per cm^2) and aerobic bacteria by 1.5 log CFU/cm^2.

While studies have shown the effectiveness of hot water in significantly reducing aerobic bacterial counts, none have addressed the effects of various contamination levels found on carcasses or efficacy of commercial spray cabinet application methods. Fecal contamination, routinely experienced during normal processing of beef carcasses, contributes high levels of bacterial contamination (>4 log CFU/cm^2) *(1)*. Previous studies have not addressed intervention effects on high levels of fecal contamination on carcasses.

Three separate studies were conducted in an attempt to identify an optimum nonchemical intervention strategy for bacteria on beef carcasses. The first study utilized a multihurdle approach of various temperature water washes and wash-air dry-steam combinations to remove from sheep carcasses both naturally occurring bacteria and those resulting from bovine fecal contamination. The second study used a commercial spray washer for delivery of hot water under low pressure and warm water under higher pressure, sepa-

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rately and in combination, to remove fecal contamination from beef short plates. In the third study, the effectiveness was evaluated of a commercially available steam-vacuum sanitizer system in combination with a water wash to remove fecal contamination from beef short plates.

MATERIALS AND METHODS

Description of steam cabinet

The cabinet was constructed of ¾-in. (1.9-cm) thick uncoated plywood and fastened together with brass screws. Exterior dimensions of the cabinet were approximately 1.2 by 1.2 by 2.4 m. A door capable of being securely fastened was cut and placed in the front wall of the cabinet. A crank-armed iron hook that could be manually turned from the exterior of the cabinet was placed in the top center of the cabinet. Carcasses were hung from this hook inside the cabinet and manually turned during steam treatments.

Steam was piped in from the bottom of the cabinet and distributed up one of the two walls adjacent to the front of the cabinet using a 2.1-cm outside diameter (o.d.) copper line, held with metal fasteners 12 cm from the wall. This line was drilled from the bottom to the top with increasing sized holes of diameters 3 to 6 mm. These holes were placed along the copper line every 15 cm, in duplicate, to facilitate an instant and even distribution of steam throughout the cabinet. Parallel to the steam line, an additional 2.1-cm O.D. copper line was piped into the cabinet for an ambient temperature water (16.6°C) spray wash after steam treatments of the carcass. Four copper spray nozzles were placed in this line at 37-cm intervals. These nozzles were fabricated by squeezing the cut end of 2.1-cm O.D. copper line so as to produce an overlapping flat spray when water at 70 lb per in^2 (psi) was passed through. Ball valves were placed in both copper lines prior to entrance of the cabinet and used to turn steam and water on and off.

To facilitate rapid release of cabinet pressure, a 2-in. (5.1-cm) ball valve was placed through the cabinet top. Between steam and ambient water spray washes, the 2-in. pressure-release valve at the top of the cabinet was opened and the door was slightly released at the top. These precautions adequately relieved the implosive forces which occurred due to rapid temperature changes in the cabinet.

Internal temperature (°F) of the cabinet was monitored with a thermocoupled mercury thermometer (H. O. Trerice Co., Detroit, MI) placed through the cabinet wall. Additionally, for monitoring temperature in °C, a dial-type thermometer (Germonow Simon, Co., Rochester, NY) was placed adjacent to the mercury thermometer. Placement of both thermometers was opposite the steam line and midway up the cabinet. For measurement of internal cabinet pressure, a standard pressure gauge (calibrated in inches of water) was placed in the same area as the thermometers.

Description of spray wash cabinet

The spray wash cabinet used for the second study contained the top half, 8 spray lines per side, of a standard 3.05-m-long commercial carcass washer (W. J. Cary Engineering, Inc., Springfield, MO) mounted into a 4.27 by 1.22 by 1.55 m polypropylene cabinet. Only the top 4 spray lines, separated by a distance of 10.2 cm and positioned 10.2 cm from the center of the cabinet at an angle of 30°, were activated for the study. These lines contained a total of 24 elliptical orifice-designed, medium-capacity spray nozzles (Spraying Systems Co., Wheaton, IL) designed to deliver 1 gallon (3.8 l) of water per min (gpm) as a 25°-angle flat spray at 40 psi. In-line water pressure was monitored using dial-type pressure gauges (Marshall Town, Inc., Hastings, NE) placed at the head line feeder to the cabinet and at the end of 2 of the 4 utilized spray lines. Calculations, as per manufacturer's instructions, for 20 and 125 psi of water, indicate that these nozzles produced an 18° and a 34° flat spray, delivering 0.71 and 1.77 gallons per min (gpm), respectively. The nozzles were positioned to spray at a 30° angle to the sample rail and in a manner so that the axis of the flat spray was horizontal to the floor of the wash cabinet. Spray lines pivoted in a manner to produce an up-and-down sweeping motion of the flat spray over the carcass sample present on the rail. Distance from nozzle to meat was 10.2 cm for the top line and 20.3 cm for the bottom line, yielding a water coverage at these distances of 3.3 and 6.1 cm per nozzle at 20 psi and 6.4 and 12.7 cm at 125 psi, respectively. Droplet sizes at 20 and 125 psi were calculated to have a volume median diameter of 1,200 to 1,400 μm and 700 to 800 μm, with an impact of 0.038 and 0.135 psi, respectively.

Water temperature delivered to the cabinet was adjustable and monitored. A Model 40605 Automatic 10 point temperature scanner with type J Teflon-coated pipe fitting thermocouples (Davis Instruments, Inc., Baltimore, MD) was used: (i) at the mixing valves located outside the cabinet (TP_1), (ii) at the feeder line just prior to entering the cabinet (TP_2), (iii) in the spray line at a point that water had first left the feeder line (TP_3), and (iv) behind the last spray nozzle for the same spray line as TP_3 (TP_4). A fifth thermocouple was positioned in the center of the cabinet to detect chamber temperature (TP_5). Water temperature delivered to the surface of carcass samples (TP_6) in the wash cabinet was monitored using an OM-160 portable dataloger with type T Teflon-coated thermocouples (OMEGA Engineering, Inc., Stanford, CT) attached to the midsection of the samples.

Description of steam-vacuum

The Kentmaster steam-vacuum, trade name Vac-San® (Kentmaster, Mfg., Monrovia, CA), uses a stainless-steel vacuum head to remove bacterial and visible fecal contamination by delivering a continuous stream of 7 to 10 psi water at 88 to 94°C to a 1.5 by 6.5 cm area while simultaneously vacuuming the area around the stream of hot water. The water temperature delivered to the carcass surface was continuously monitored using a thermocouple inserted into the water line inside the vacuum head. Static vacuum for the system was 7 in. of Hg and when contact was made with the meat surface, the vacuum was 10 in. of Hg. A stainless-steel jacket surrounding the vacuum nozzle delivered steam at approximately 45 psi to continuously sanitize the equipment while in use.

Preparation of inoculum

For all studies, fecal material was obtained immediately after defecation by cattle that were fed a corn silage diet. Fecal samples were screened for high levels of bacteria using a rapid mATP test *(12)*. A relative light unit (RLU) value of >15,000, read directly from a Model 3550 Microluminometer (New Horizons Diagnostics, Columbia, MD and ILC Dover, Inc., Frederica, DE), was required for feces to be used in a composite fecal sample. A composite of several fecal samples was used in appropriate amounts to assure a consistent inoculation level. A fecal slurry was made using the composite feces and sterile deionized water (1:2) in ultraviolet (UV) sterilized rectangular plastic containers. The slurry was hand mixed using a sterile metal spatula for 2 min. For the first study only, an additional 1:10 dilution slurry was made from the 1:2 slurry and similarly mixed.

Inoculation of carcasses

For the various temperature water wash and wash-air dry-steam intervention experiment (Study 1), culled ram and/or sheep carcass sides with intact fell membranes were hung in the steam

cabinet immediately after slaughter and marked in duplicate at three general areas using a sterile 5 by 5 cm stainless-steel template, a sterile cotton swab, and edible ink. Areas sampled were the sirloin, rib, and breast. The sirloin area was left uninoculated, the rib area was inoculated with the 1:10 fecal slurry, and the breast area was inoculated with a 1:2 fecal slurry. An even inoculum was applied over the marked areas using either an autoclaved 7.6-cm paint roller or 5.1-cm paint brush. Carcasses were inoculated and allowed to stand undisturbed for 15 min to allow bacterial attachment before being subjected to any intervention.

For the commercial washer and steam-vacuum sanitizer experiments (Studies 2 and 3), an adequate number of short plates from a cow and bull slaughter facility were acquired less than 15 min after slaughter. Beef carcass short plates were taken from the 5th to the 13th rib and about 10 in. (25.4 cm) from the vertebrae to within 4 in. (ca. 10.2 cm) of the midline. Short plates were placed into individual plastic bags upon removal and transported at ambient temperature to the laboratory within 1 h. Prior to the interventions, 4 areas were marked on the short plates with the 5 by 5 cm template as described above. Because it was observed in Study 1 that the hydration of carcass surfaces resulting from fecal contamination might produce some protective effect to fecal bacteria, control areas were inoculated with sterile distilled water. Other areas were inoculated with a 1:2 fecal slurry as described previously. All sampled short plates were sampled within 3 h of slaughter.

Experimental design

Various water washes and steam sanitation experiment: Study 1. After inoculation, each of the six different intervention treatments was replicated eight times on as many carcass sides. Three wash (W_t, t = °C) interventions at three different temperatures and three wash-air dry-steam (W_tS) interventions were conducted. The W_t interventions involved spray washing of each carcass side with either 82.2, 54.4, or 15.6°C water at 75 psi with a Strahman carcass wash nozzle (Packers Engineering & Equipment Co., Inc., Omaha, NE). The spray nozzle was manually passed over the carcass half 12 times for a total exposure time of 10 s, at approximately 30 cm from the surface. Immediately after washing (<2 s), the carcass surface temperature was measured using an infrared noncontact thermometer (Omega, Stamford, CT). The thermometer was held approximately 5 cm from the carcass surface for each reading and in a time frame of approximately 2 s, five random locations were sampled in a pattern that represented a majority of the carcass area.

For W_tS interventions, carcass halves were hung inside the steam cabinet and washed as described above. Carcass halves were air dried by passing an air nozzle approximately 5 cm from the meat surface for 1 min, using bottled compressed air delivered at ambient temperature at 120 psi through a 9.5-mm, 200-psi line and dispersion head air nozzle. The door to the steam cabinet was then sealed and pressurized steam was allowed to enter the chamber for 30 s as the carcass was rotated at about 18 rpm. The cabinet internal pressure, when filled with steam, was 1.5 inches of water. Steam was then shut off, cabinet pressure released, and carcasses immediately sprayed for 10 s with an ambient temperature (ca. 15.6°C) water spray. Carcass surface temperature was then read at five random locations as described previously.

Commercial washer using hot water: Study 2. After inoculation with the fecal slurry, each of four different intervention treatments were replicated 30 times on as many beef carcass short plates. Treatments were performed using the commercial spray washer described previously. Rail speed through the spray washer (exposure time) for each sample was 12 s. This exposure time was selected because it is equivalent to the carcass rail speed in a 300 head per hour commercial slaughter facility. The four intervention treatments used were (i) hot water with a surface contact temperature of 72°C and sprayed at 20 psi (W_{72}), (ii) warm water at 30°C and sprayed at 125 psi (W_{30}), (iii) W_{72}, followed by W_{30} ($W_{72/30}$), and (iv) W_{30}, followed by W_{72} ($W_{30/70}$).

Steam-vacuum followed by a double water wash: Study 3. After inoculation, each of three different intervention treatments was replicated 30 times on as many beef carcass short plates. For the first treatment, inoculated carcass short plate samples were hung on the wash cabinet rail where it exited the cabinet and marked sample sites were vacuumed (V) using the Kentmaster steam-vacuum sanitizer. The second treatment was wash regimen $W_{72/30}$ described previously for study 2. This wash treatment regimen was selected because it proved the most effective for reduction of bacteria from beef carcasses of the four washes tested in study 2. Treatment three ($VW_{72/30}$) was a combination of the first treatment (V) followed by the second treatment ($W_{72/30}$).

Carcass sampling and bacterial enumeration

Samples were taken by excising premarked 5 by 5 cm sections (25 cm^2 by 1 mm). One marked 25-cm^2 area of each inoculation level was sampled prior to any intervention treatments. Adjacent 25-cm^2 areas were sampled after the carcass was treated. Excised samples were placed into stomacher bags and 25 ml of buffered peptone water (BPW) (BBL, Cockeysville, MD) with 0.1% Tween 20 was added. Samples were pummeled for 2 min with a Model 400 Stomacher (Tekmar, Inc., Cincinnati, OH).

For all three studies, appropriate sample dilutions were made in BPW and spiral plated in duplicate using a Model D spiral plater (Spiral Systems Instruments, Bethesda, MD) on Trypticase soy agar (BBL). Plates were incubated aerobically at 35°C for 36 h and enumerated using a CASBA III optical colony-counting system (Spiral Biotech, Inc. Bethesda, MD).

For studies 2 and 3, coliforms and *E. coli* were enumerated using 3M Petrifilm® *E. coli* Count Plates (3M, Inc. St. Paul, MN), from the same samples used for the APCs. Petrifilms were incubated aerobically at 35°C for 24 h and enumerated according to the manufacturer's instructions.

Data analysis

Aerobic plate count, coliform, and *E. coli* data were converted to log CFU/cm^2. To facilitate log analysis of APCs, coliforms, and *E. coli,* any 0-count plate was assigned a value of 20, 0.5, and 0.5, respectively, one-half of the lower limit of detection for the respective count methods *(9).* Differences between untreated samples and those subjected to interventions were calculated as a log reduction factor (LRF) by taking the arithmetic difference of the logs. Least squared means (LSM) of the log CFU/cm^2 values were calculated from the 8, 30, and 30 experimental replications per treatment for studies 1, 2, and 3, respectively. Population and log reduction data were analyzed using the general linear model procedure (GLM) of SAS (SAS Institute, Cary, NC) with a probability level of <0.05 used as the level of significance.

RESULTS AND DISCUSSION

Study 1: Various water washes and steam sanitation experiment

Average internal steam cabinet temperatures at 15 and 30 s of steam treatment were 72.5 and 83.9°C, respectively (data not presented). After 15 s of a 15.6°C water spray to

the carcass in the cabinet, the internal cabinet temperature dropped to 55.4°C (data not presented). Carcass surface temperatures taken immediately before wash, after wash, and after air dry-steam-15.6°C water wash for all treatments are reported in Table 1. No attempt was made to quantify the effects of various heat treatments on carcass appearance. However, a carcass that had been subjected to the most severe heat treatment applied during this study (82.2°C wash and steam for 30 s) and then placed in a forced-air chilling room with an untreated carcass was determined by an experienced carcass judge to show no detrimental alteration of general appearance after 12 h (data not presented). Other studies have described similar effects for both beef and mutton carcasses *(6, 7, 13)*.

Carcasses subjected to $W_{82.2}$ treatments experienced significant reductions of both natural and fecally inoculated bacterial populations (Figure 1). Reductions of bacteria resulting from fecal inoculations of approximately 6 log CFU/cm^2 were 3.3 and 4.0 log CFU/cm^2 for carcasses subjected to treatments $W_{82.2}$ and $W_{82.2}S$, respectively. Davey and Smith *(7)* reported a similar effectiveness of hot water on beef carcasses inoculated with 6.8 log CFU/cm^2 *Escherichia coli* SF. They observed a 3.0 log reduction of bacterial populations when carcasses were treated with cascading 83.5°C water for 20 s.

The two treatments, $W_{82.2}$ and $W_{82.2}S$, exhibited no significant difference of effectiveness for reducing fecal bacteria populations on carcasses (Figure 1). At lower inoculation levels of 4 log CFU/cm^2, reductions of bacteria were 1.8 and 1.7 log cycles for $W_{82.2}$ and $W_{82.2}S$ carcasses, respectively. As with the 6 log CFU/cm^2 inoculum, there was no significant difference in bacterial population reduction between treatments. Fecally inoculated bacterial contamination on carcasses was reduced to approximately 3 log CFU/cm^2 regardless of initial levels or treatment type (Figure 1). It is not clear why bacterial numbers from uninoculated samples were reduced to a lower level than inoculated samples. It is possible the presence of additional moisture provided by the fecal inoculations affects the collagen, lipids, and proteins on the carcass surface, providing a limited level of protection for a given number of bacteria. At high inoculation levels, only bacterial numbers exceeding this saturation level appear to be accessible to heat treatments, regardless of the delivery system.

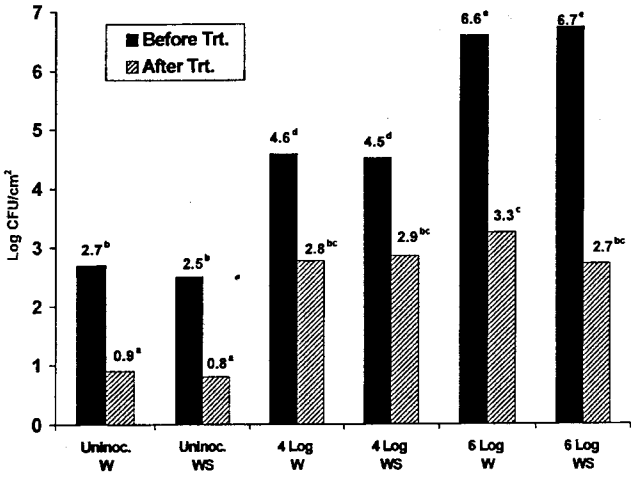

FIGURE 1. *Effect of 82.2°C wash ($W_{82.2}$) and 82.2°C wash followed by steam ($W_{82.2}S$) on aerobic plate counts from varying inoculation levels of bovine fecal contamination on sheep carcasses. Means lacking common superscripts are statistically different ($P < 0.05$) (study 1).*

TABLE 1. *Sheep carcass surface temperatures immediately before and after various temperature washes or wash and/or steam combination treatments (study 1)*

Wash or steam treatment	Carcass surface temperature (°C)		
	Before wash or steam	After wash or steam	After wash + air/dry/steam/ 15.6°C wash
82.2°C ($W_{82.2}$)	27.8	57.1	—
82.2°C + steam ($W_{82.2}S$)	27.1	55.8	39.5
54.4°C ($W_{54.4}$)	27.3	45.2	—
54.4°C + steam ($W_{54.4}S$)	27.9	48.0	39.1
15.6°C ($W_{15.6}$)	27.2	18.4	—
15.6°C + steam ($W_{15.6}S$)	26.0	18.2	35.3

Thomas and McMeekin *(14)* demonstrated a bacterial protective effect resulting from hydrated collagen. They observed that the flat surface of chicken muscle fascia becomes covered by a dense mat of collagen fibers, originally contained within the fascia, when the tissue is exposed to water. They also determined that the degree of cover depended upon the time of exposure and that bacteria became attached and entrapped as the collagen expansion occurred with water absorption. Light *(10)* noted that collagen is distributed within the bovine epimysium (fascia) or muscle sheath *(5)*. A single treatment from the present study, including inoculation time, would have exposed the cutaneous trunci fascia to over 20 min of excess moisture. During this time it is possible that a given population of bacteria became protected by the expanding collagen mat forming over the surface of the tissue, subsequently impeding heat penetration; however, this hypothesis was not tested in the present study.

A protective effect of collagen expansion resulting from cutaneous trunci fascia hydration may also explain the effectiveness of $W_{82.2}$ and $W_{82.2}S$ treatments on uninoculated carcasses. With bacterial populations of about 2.5 log CFU/cm^2, that did not result from a liquid inoculum, $W_{82.2}$ and $W_{82.2}S$ both reduced bacterial loads by 1.7 log CFU/cm^2 (Figure 1). Barkate et al. *(6)* observed 1.3-log CFU/cm^2 reductions in APCs on uninoculated beef carcasses washed with 95°C water. They noted that this was only the case when hot water sprays were applied before hydrating the carcass with an ambient temperature water carcass spray. If the carcass was hydrated with a carcass spray wash first, then washed with hot water, the reduction in APC was only 0.8 log CFU/cm^2. This observation lends additional support to the hypothesis that carcass surface hydration affords some degree of thermal protection to bacterial populations. Defin-

ing the mechanisms involved in this hypothesis goes beyond the limits of the present study, however, and will require additional research.

$W_{54.4}$ did not reduce bacteria as effectively on fecally inoculated carcasses as the $W_{82.2}$ treatment (Figure 2). Regardless of inoculation levels, the $W_{82.2}$ treatment reduced bacterial populations 1 log unit more than the $W_{54.4}$ treatment (Figure 1 and 2). The diminishing effect of hot water washes with decreasing temperature was also observed by Davey and Smith *(7)*. In their study, reductions of *E. coli* ranged from 3.0 to 0.1 log CFU/cm^2 when spray temperatures ranged from 83.5 to 44.5°C, respectively.

The $W_{54.4}S$ treatment was significantly more effective for sanitizing carcasses than $W_{54.4}$ at both fecal inoculation levels (Figure 2). However, as with the hot water spray wash ($W_{82.2}$), when moist heat in the form of steam was applied to a carcass along with a water wash of 54.4°C ($W_{54.4}S$), the effects of the heat treatment, regardless of inoculation levels, were similar to that of both $W_{82.2}$ and $W_{82.2}S$ treatments. The $W_{54.4}$ treatment was not effective in reducing bacterial populations of uninoculated carcasses, as was the case with the $W_{82.2}$ treatment (Figure 2). However, on uninoculated areas, the $W_{54.4}S$ treatment produced a significant bacterial reduction of 1.2 log CFU/cm^2.

The $W_{15.6}$ treatment significantly reduced bacterial populations derived from 4 and 6 log CFU/cm^2 fecal inoculations (Figure 3). As observed with the $W_{54.4}$ treatment, which produced 3.6 to 4.2 log CFU/cm^2 end-point populations, the $W_{15.6}$ yielded a final bacterial population level of 3.8 to 4.5 $\log_{10}$ CFU/cm^2. An end-point bacterial population of about 3.5 to 4.0 log CFU/cm^2 was achieved regardless of the initial contamination level.

The $W_{15.6}S$ treatment was effective for reducing bacterial levels of carcasses at all levels of fecal inoculations in this study (Figure 3). When the $W_{15.6}S$ treatment was applied, a final population level of 2.8 to 3.2 log CFU/cm^2, was observed. This 3-log CFU/cm^2 level was statistically similar to that observed for $W_{82.2,54.4}S$.

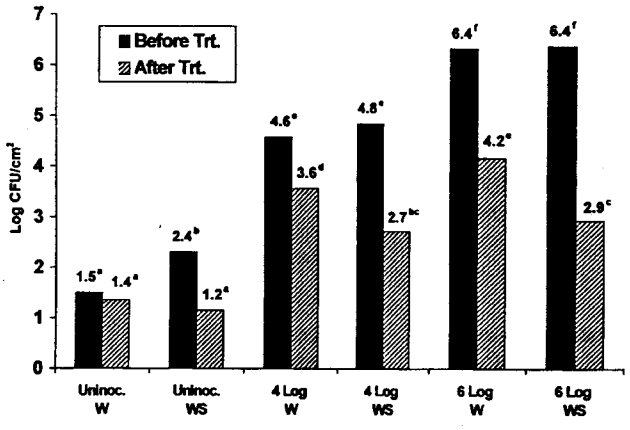

FIGURE 2. *Effect of 54.4°C wash ($W_{54.4}$) and 54.4°C wash followed by steam ($W_{54.4}S$) on aerobic plate counts from varying inoculation levels of bovine fecal contamination on sheep carcasses. Means lacking common superscripts are statistically different (P < 0.05) (study 1).*

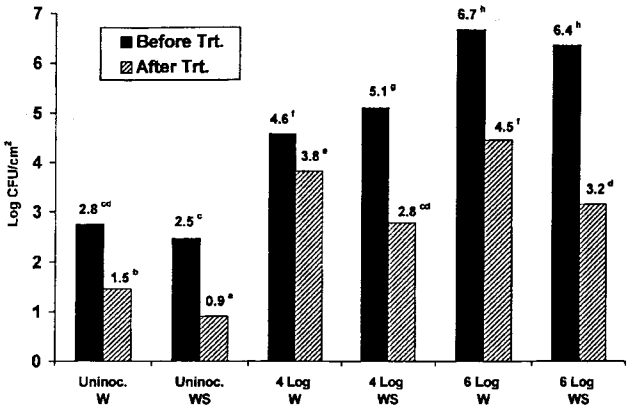

FIGURE 3. *Effect of 15.6°C wash ($W_{15.6}$) and 15.6°C wash followed by steam ($W_{15.6}S$) on aerobic plate counts from varying inoculation levels of bovine fecal contamination on sheep carcasses. Means lacking common superscripts are statistically different (P < 0.05) (study 1).*

Regardless of the wash water temperature, if an air dry-steam treatment was subsequently used, the resulting bacterial flora of inoculated carcasses ranged from 2.7 to 3.2 log CFU/cm^2, which was not statistically different between treatments (Figures 1, 2, and 3). The achievable bacterial level for carcasses inoculated with 4 to 6 log CFU/cm^2 and subjected to all W_tS treatments and the $W_{82.2}$ treatment were observed to be in the range of 2.7 to 3.3 log CFU/cm^2. It appears that when moist heat is used for the reduction of bacteria on carcasses, there is a bacterial population that cannot be further reduced, regardless of the initial bacterial contamination level.

When the naturally occurring, initial carcass bacterial population was approximately 2.5 log CFU/cm^2, all W_tS treatments produced approximately 1.5-log CFU/cm^2 reductions (Figures 1, 2, and 3). Consequently, it might appear that a successive moist heat intervention, applied to highly contaminated carcasses, would be successful in reducing populations below levels achieved by the initial moist heat intervention. However, this was not the case with the $W_{82.2}S$ intervention when applied to inoculated carcasses. The $W_{82.2}S$ intervention could not reduce the higher levels of fecal bacteria populations below the 2.5 to 3.0-log CFU/cm^2 level, even though it employed two separate and distinctly different moist heat treatments. It might be possible for an intervention that does not expose the carcass fascia to additional moisture, used prior to the introduction of moist heat, to improve the overall effectiveness of a moist heat intervention and reduce bacterial populations to below the 3.0-log CFU/cm^2 level.

Regardless of the source, moist heat is effective for reducing bacterial populations on carcasses. As mentioned previously, the effectiveness of moist heat is not without limitations. These limitations are likely due to one or several mechanisms that protect bacteria present on the surface of a carcass. If delivering consistent hot water of 82.2°C is not practical or possible, a wash of 15.6 to 54.4°C water, followed by application of air drying-steam-15.6°C wash, will produce significant reductions of bacterial populations.

Study 2: Commercial washer using hot water

The average water temperatures achieved at various locations along the wash line and at the carcass are given in Table 2. At 31°C, there was little difficulty delivering water to the carcass surface at a temperature equivalent to that at the nozzle. However, in order to deliver 72°C water to a carcass surface located ca. 15 cm from the wash nozzle tip, 80 to 82°C water was required. It was observed that any increase in water pressure above 20 psi would induce additional atomization of the water, resulting in a carcass surface temperature below 72°C (data not published). The hand-held spray nozzle that produced noticeably larger droplets in study 1 did not affect a drop in water temperature delivered to the carcass. Additionally, any increase in distance between the nozzle and the carcass had similar effects. It is apparent that nozzle type, water pressure, water temperature at the nozzle, and distance from the nozzle to the carcass must be considered when designing a hot-water sanitizing system.

Results of bacterial reduction for all four wash treatments are given in Tables 3 and 4. Statistical differences in reductions were observed among treatments when samples were inoculated with feces. Both combination washes, $W_{72/30}$ and $W_{30/72}$, produced about 2.5-log CFU/cm^2 reductions, significantly larger reductions than either wash, W_{30} or W_{72}, accomplished independently (Table 3). The range of residual APCs for the four treatments (data not shown) indicated the combination wash $W_{72/30}$ effected the reduction of bacterial populations more consistently than the other treatments. $W_{72/30}$ ranged from 2.1 to 4.0 log CFU/cm^2 while $W_{30/72}$, W_{72}, and W_{30} residual ranges were 2.3 to 4.5, 2.9 to 4.6, and 2.7 to 5.3 log CFU/cm^2, respectively. The ability of an intervention to produce consistently repeatable reduction results is important when addressing beef carcass microbial safety. While both combination wash interventions produced similar reductions, $W_{30/72}$ allowed 0.5 log CFU/cm^2 higher residual bacteria populations than $W_{72/30}$ did.

The 4 wash treatments reduced bacterial populations from uninoculated samples ranging from 0 to 0.8 log CFU/cm^2 (Table 4). Smaller reductions observed for uninoculated samples were consistent with the inoculation effects observed and discussed previously in study 1. However, reductions were even smaller for uninoculated samples in study 2 than in study 1, even though initial levels of bacteria were the same. This could have resulted from the water inoculation used in study 2, that was not used in study 1, and supports the hypothesis that hydration of the carcass surface somehow protects bacteria. It is again apparent that initial inoculation level significantly affects a treatment's ability to exhibit reductions of bacterial populations from beef carcasses. While the $W_{30/72}$ treatment showed a statistically larger reduction of 0.8 $\log_{10}$ CFU/cm^2 when compared to the other wash treatments, it is important to note that the initial bacterial population level for this treatment was also significantly higher than that of the W_{72} and $W_{72/30}$, and numerically higher than the W_{30} treatment (Table 4).

Reductions of coliforms and *E. coli* from fecally inoculated beef samples are reported in Tables 5 and 6. As with aerobic bacterial populations, both combination treatments produced significantly larger reductions of coliform and *E. coli* populations from beef carcass when compared to either single wash treatment. However, $W_{72/30}$ treatment was significantly more effective than $W_{30/72}$ treatment at reducing both coliforms and *E. coli*. This reduction, along with the range of residual APCs reported above, indicates the $W_{72/30}$ treatment would be best in a beef slaughter facility to assure decontamination of beef carcasses.

Uninoculated samples were not analyzed for coliforms

TABLE 2. *Mean water and cabinet temperatures at various locations of the wash cabinet for all water washes and resulting sheep carcass surface contact temperature (studies 2 and 3)*

Carcass temperature (°C)	Water and cabinet temperatures (°C)				
	TP_1[a]	TP_2	TP_3	TP_4	TP_5
~30	NA	31.2	31.1	31.1	30.7
~72	99.0	81.7	81.8	80.6	58.4

[a] TP_x denotes thermocouple locations: TP_1, in the mixing valves located outside the cabinet; TP_2, in the feeder line just prior to entering the cabinet; TP_3, in the spray line at a point that water has first left the feeder line; TP_4, behind the last spray nozzle of the same spray line as TP_3; and TP_5, positioned in the center of the cabinet to detect chamber temperature.

TABLE 3. *Effect of using a commercial carcass washer on removing bacterial contamination from fecally contaminated beef carcass short plates (study 2)*

Treatment (water temp. at carcass surface)	n	Aerobic plate count (log CFU/cm^2), mean ± SE		
		Before wash	After wash	Reduction
72°C @ 20 psi (W_{72})	30	5.7 ± .08	3.7 ± .10	2.0 ± .09A[a]
30°C @ 125 psi (W_{30})	30	5.8 ± .08	3.8 ± .10	2.1 ± .09A
72 + 30°C ($W_{72/30}$)	30	5.7 ± .08	3.2 ± .10	2.5 ± .09B
30 + 72°C ($W_{30/72}$)	30	5.8 ± .08	3.4 ± .10	2.4 ± .09B

[a] Values in a column followed by different letters are statistically different ($P < 0.05$).

TABLE 4. *Effect of using a commercial carcass washer on removing bacterial contamination from uninoculated beef carcass short plates (study 2)*

Treatment (water temp. at carcass surface)	n	Aerobic plate count (log CFU/cm), mean ± SE		
		Before wash	After wash	Reduction
72°C @ 20 psi (W_{72})	30	2.2 ± .16A[a]	1.9 ± .13	0.3 ± .18C
30°C @ 125 psi (W_{30})	30	2.5 ± .16AB	2.5 ± .13	0.0 ± .18C
72 + 30°C ($W_{72/30}$)	30	2.4 ± .16A	2.3 ± .13	0.2 ± .18C
30 + 72°C ($W_{30/72}$)	30	2.9 ± .16B	2.1 ± .13	0.8 ± .18D

[a] Values in a column followed by different letters are statistically different ($P < 0.05$).

TABLE 5. *Effect of using a commercial carcass washer on removing coliform contamination from fecally contaminated beef carcass short plates (study 2)*

Treatment (water temp. at carcass surface)	*n*	Coliform count (log CFU/cm²), mean ± SE: Before wash	After wash	Reduction
72°C @ 20 psi (W_{72})	30	5.2 ± 1.0	2.5 ± 1.3	2.7 ± .10C[a]
30°C @ 125 psi (W_{30})	30	5.1 ± 1.0	2.6 ± 1.3	2.5 ± .10C
72 + 30°C ($W_{72/30}$)	30	5.2 ± 1.0	1.9 ± 1.3	3.3 ± .10A
30 + 72°C ($W_{30/72}$)	30	5.2 ± 1.0	2.2 ± 1.3	3.0 ± .10B

[a] Values in a column followed by different letters are statistically different ($P < 0.05$).

and *E. coli* reductions because of variations in initial population levels for both coliforms and *E. coli* along with an inordinate amount of undetectable numbers for both the before and after treatment samples. When coliforms or *E. coli* were present in samples prior to receiving any intervention, reductions were observed.

Davey and Smith *(7)* allowed 83.5°C water to flow down carcasses for 10 s, resulting in a 2.2-log CFU/cm² reduction of initial *E. coli* inoculations of 6.8 log CFU/cm². The present study showed that a combination wash, which both sanitizes with hot water and physically removes bacteria using pressure, can be a more effective means to reduce *E. coli* even at a lower water temperature than washes alone. Our initial inoculation levels of *E. coli* were 5.1 log CFU/cm², with reductions of 3.4 log CFU/cm² observed. This reduction was greater than Davey and Smith's *(7)* observations, despite a higher initial inoculum in their study. Barkate et al. *(6)* determined that decontamination of beef carcasses with hot water at a carcass surface of 82°C was effective in reducing bacterial numbers. The present study has shown that similar conclusions can be drawn when lower temperature (72°C) water is placed in sequence with a higher pressure wash.

Previous studies *(6, 7)* have shown that carcass discoloration due to 10-s exposures to ≥80°C water washes is temporary, with normal carcass color returning within 24 h. While no attempt was made in the present study to quantify color changes of the beef samples, casual observation did not indicate permanent discoloration of samples. It is important to note that the combination hot/warm wash ($W_{72/30}$) used was able to produce significant population reductions of APCs, coliforms, and *E. coli* while exposing the carcasses to a heat treatment not exceeding 72°C for 12 s.

The major obstacle preventing the use of hot water washes, as noted by Barkate et al. *(6)*, is producing satisfactory bacterial reductions in a commercially applicable manner. The present study has shown efficacy of a hot water wash plus high pressure wash combination treatment ($W_{72/30}$) for reducing bacteria on beef carcasses using a commercially available wash system.

Study 3: Steam-vacuum followed by a double water wash

Study 1 demonstrated that 82.2°C water delivered to a carcass surface would reduce high levels of bacterial contamination to near 3.0 log CFU/cm². Study 2 indicated that a double wash combination ($W_{72/30}$) was also capable of significantly reducing high levels of bacterial contamination. However, since it is possible that washing large amounts of fecal bacterial contamination on beef carcasses might redistribute some of the contamination over additional areas of a carcass, it would be desirable to physically remove as much feces as possible prior to a wash treatment. The steam-vacuum system is designed to deliver >82.2°C water plus steam directly to the carcass surface, while physically removing the contamination through a vacuum. Consequently, study 3 was designed to evaluate the ability of this system to reduce aerobic bacteria from beef carcasses and determine if an additive effect would be realized when used in combination with a wash treatment.

Bacterial reductions on uninoculated short plates were approximately 0.2 log CFU/cm² for all treatments (data not shown). For reduction of aerobic bacteria from fecally inoculated beef short plates, the steam-vacuum plus wash combination sequence ($VW_{72/30}$) produced 0.4 log CFU/cm² greater reductions than the $W_{72/30}$ wash treatment alone (Table 7). While the reductions indicated no significant difference between treatments, the range of residual APCs indicate that the steam-vacuum treatments are more consis-

TABLE 6. *Effect of using a commercial carcass washer on removing* E. coli *contamination from fecally contaminated beef carcass short plates (study 2)*

Treatment (water temp. at carcass surface)	*n*	*E. coli* count (log CFU/cm²), mean ± SE: Before wash	After wash	Reduction
72°C @ 20 psi (W_{72})	30	5.0 ± .09	2.3 ± .12	2.7 ± .11C[a]
30°C @ 125 psi (W_{30})	30	5.0 ± .09	2.4 ± .12	2.6 ± .11C
72 + 30°C ($W_{72/30}$)	30	5.1 ± .09	1.7 ± .12	3.4 ± .11A
30 + 72°C ($W_{30/72}$)	30	5.1 ± .09	2.0 ± .12	3.0 ± .11B

[a] Values in a column followed by different letters are statistically different ($P < 0.05$).

TABLE 7. *Effect of steam-vacuum sanitizing and washing on removing bacterial contamination from fecally contaminated beef carcass short plates (study 3)*

Treatment	*n*	Aerobic plate count (log CFU/cm²), LSM ± SE: Before	After	Reduction
Steam-vac (V)	32	6.2 ± .14	3.2 ± .08	3.0 ± .14A[b]
Wash[a] ($W_{72/30}$)	29	6.1 ± .14	3.4 ± .08	2.7 ± .14A
Steam-vac + Wash ($VW_{72/30}$)	30	6.1 ± .14	3.0 ± .08	3.1 ± .14A

[a] Washed with 72°C water @ 20 psi + 30°C water @ 125 psi reaching the carcass in a commercial washer.

[b] Not statistically different ($P > 0.05$).

TABLE 8. *Effect of steam-vacuum sanitizing and washing on removing coliform contamination from fecally contaminated beef carcass short plates (study 3)*

Treatment	*n*	Coliform count (log CFU/cm²), LSM ± SE		
		Before	After	Reduction
Steam-vac (V)	31	5.0 ± .09	1.0 ± .13	4.0 ± .12A[b]
Wash[a] ($W_{72/30}$)	29	5.0 ± .09	1.7 ± .14	3.4 ± .13B
Steam-vac + Wash ($VW_{72/30}$)	30	5.0 ± .09	0.8 ± .13	4.2 ± .13A

[a] Washed with 72°C water @ 20 psi + 30°C water @ 125 psi reaching the carcass in a commercial washer.
[b] Statistically different ($P > 0.05$).

tently effective than water washes alone. The range of residual APCs for the V and $VW_{72/30}$ were 2.3 to 4.0 and 2.4 to 3.6 log CFU/cm², respectively. The range for residual APCs of $W_{72/30}$ was much higher, 2.6 to 5.1 log CFU/cm². All but 1 of the short plates inoculated and treated with the steam-vacuum treatments were reduced to 3.6 log CFU/cm² or below, while 21% of the short plates treated with $W_{72/30}$ were above 3.6 log CFU/cm².

When the intervention $VW_{72/30}$ was used, coliform and *E. coli* population reductions of 4.2 and 4.3 log CFU/cm² yield additional indications that both treatment combinations with the steam-vacuum were significantly more effective than the $W_{72/30}$ combination wash (Table 8 and 9). While no attempt was made to isolate *E. coli* O157:H7 during the present study, these results indicate that $VW_{72/30}$ or V treatments administered to beef carcasses in a slaughter facility, prior to entering the chill boxes, has the potential to reduce the risk of *E. coli* O157:H7 contamination.

Some bleaching of the carcass surface was noticeable immediately post treatment, but was not permanent. Also an additional study conducted using the protocols described for the present study ($n = 10$), but using short plates with distinctive lean and adipose areas, demonstrated no significant difference of treatment ($VW_{72/30}$ or V) effectiveness between the two tissue types (data not shown).

TABLE 9. *Effect of steam-vacuum sanitizing and washing on removing* E. coli *contamination from fecally contaminated beef carcass short plates (study 3)*

Treatment	*n*	*E. coli* count (log CFU/cm²), LSM ± SE		
		Before	After	Reduction
Steam-vac (V)	31	4.8 ± .08	0.8 ± .12	4.0 ± .12A[b]
Wash[a] ($W_{72/30}$)	29	4.9 ± .08	1.5 ± .12	3.4 ± .12B
Steam-vac + Wash ($VW_{72/30}$)	30	4.9 ± .08	0.6 ± .12	4.3 ± .12A

[a] Washed with 72°C water @ 20 psi + 30°C water @ 125 psi reaching the carcass in a commercial washer.
[b] Statistically different ($P > 0.05$).

CONCLUSION

These studies indicate that moist heat interventions were effective for reducing bacterial populations on beef and sheep carcasses regardless of the administration procedure. Also, the eventual reduction resulting from a moist heat intervention on beef or sheep carcasses was influenced by the initial inoculation level. In general, the higher the initial starting inoculum, the more effective the intervention was. Additionally, when moist heat was used for reduction of bacteria on fecally contaminated carcasses, there was a bacterial population that, once achieved, could not be reduced further. Readministration of moist heat to the same area, regardless of method, did not have any additional effect. Apparently, extended hydration of a carcass before and during moist heat interventions protects a limited bacterial population. Thus, the development of a nonhydrating intervention, administered prior to moist heat, might prove successful in reducing bacterial populations to very low levels on red meat carcasses.

A hot water (72°C) wash at low pressure (20 psi) used in combination with a high pressure (125 psi) warm water (30°C) wash, administered through a commercial carcass washer, was very effective for reducing bacterial populations from fecally contaminated beef carcass surfaces. However, residual bacterial populations as high as 5.1 log CFU/cm² on carcass surfaces after wash treatments indicate that washing alone was not adequate for the complete removal of fecal contamination. When a commercially available steam-vacuum was added to the best wash treatment, greater bacterial reductions were obtained. Implementation of these interventions could reduce the amount of trimming needed on carcass processing lines and would increase the microbial safety of beef carcasses.

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