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Comparison of Steam Pasteurization and Other Methods for Reduction of Pathogens on Surfaces of Freshly Slaughtered Beef

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ABSTRACT

The effectiveness of a recently invented "steam pasteurization" (S) process in reducing pathogenic bacterial populations on surfaces of freshly slaughtered beef was determined and compared with that of other standard commercial methods including knife trimming (T), water washing (35°C; W), hot water/steam vacuum spot cleaning (V), and spraying with 2% vol/vol lactic acid (54°C, pH 2.25; L). These decontamination treatments were tested individually and in combinations. Cutaneus trunci muscles from freshly slaughtered steers were inoculated with feces containing *Listeria monocytogenes* Scott A, *Escherichia coli* O157:H7, and *Salmonella typhimurium* over a predesignated meat surface area, resulting in initial populations of ca. 5 log CFU/cm^2 of each pathogen. Tissue samples were excised from each portion before and after decontamination treatments, and mean population reductions were determined. Treatment combinations evaluated were the following (treatment designations within the abbreviations indicate the order of application): TW, TWS, WS, VW, VWS, TWLS, and VWLS. These combinations resulted in reductions ranging from 3.5 to 5.3 log CFU/cm^2 in all three pathogen populations. The TW, TWS, WS, TWLS, and VWLS combinations were equally effective ($P > 0.05$), resulting in reductions ranging from 4.2 to 5.3 log CFU/cm^2. When used individually, T, V, and S resulted in pathogen reductions ranging from 2.5 to 3.7 log CFU/cm^2. Steam pasteurization consistently provided numerically greater pathogen reductions than T or V. Treatments T, V, and S were all more effective than W (which gave a reduction on the order of 1.0 log CFU/cm^2). Steam pasteurization is an effective method for reducing pathogenic bacterial populations on surfaces of freshly slaughtered beef, with multiple decontamination procedures providing greatest overall reductions.

Key words: Steam pasteurization, beef, pathogens, meat safety

Bacterial contamination of beef carcass surfaces is an unavoidable consequence of processing cattle into meat for human consumption. During beef slaughter the carcass surfaces may become contaminated with bacteria from many sources including processing equipment, workers, and the environment; however, the predominant source of bacterial contamination is the animal itself *(12)*. The hide, hooves, intestinal contents, and milk have the potential to harbor not only large numbers of bacteria but also pathogenic bacteria. For this reason, current USDA-FSIS regulations require that all visible feces, hair, ingesta, and milk be removed from the surface of beef carcasses during slaughter *(7)*, often referred to as "zero tolerance." According to the USDA-FSIS, the investigation of carcass processing procedures that effectively eliminate physical and microbial contamination is a high priority *(15)*. Researchers have studied many decontamination or antimicrobial processes for beef processing including knife trimming, water washing, hot water/steam spot vacuuming, and applying various antimicrobial compounds.

Knife trimming of a defined area on beef carcasses was shown to reduce aerobic bacterial populations from an initial level of 3.5 log CFU/cm^2 to a level of 0.5 log CFU/cm^2 *(23)*. Other researchers, using artificially contaminated beef adipose tissue and sterile utensils, demonstrated a reduction of 2 log CFU/cm^2 in aerobic plate counts (APCs) from an initial level of 7.1 log CFU/cm^2 *(17)*. Reductions of 2.9 to 3.9 log cycles in pathogenic populations (from initial levels of 5.0 log CFU/cm^2) have been demonstrated using knife trimming *(18)*.

Both cold and hot water washes have been used as decontamination treatments *(2, 3, 12, 14, 17, 18, 21, 23)*. By applying hot (76 to 80°C at meat surface) or cold (15.6°C) water sprays to frozen and thawed beef tissue, Anderson et al. *(2)* reduced APCs by up to almost 1 log cycle (from an initial level of 5.3 log CFU/cm^2). Other researchers have shown that cold or ambient-temperature washes generally reduce APCs by up to about 1 log cycle *(14, 23)*. Barkate et al. *(3)* used hot water (95°C reduced to 82°C at carcass surface) to treat freshly slaughtered carcasses either before or after the final carcass wash and demonstrated reductions of 1.3 and 0.8 log CFU/cm^2, respectively, when initial populations were 2.4 log CFU/cm^2. A hot water/steam mixture (80 to 96°C) sprayed onto freshly slaughtered beef carcasses for 2 min just before chilling reduced APCs by up to about 1 log cycle from initial populations of ca. 4.5 log CFU/cm^2 *(21)*. Researchers using inoculated beef tissue have shown that warm (35°C) water washes can reduce pathogenic bacterial populations from 6 log CFU/cm^2 to 3.3 log CFU/cm^2 *(14)*.

Spot cleaning systems using hot water/steam vacuum or steam vacuum only are designed to remove visible spots of contamination from small, localized areas on the carcass and are used to reduce or augment commercial knife trimming procedures. The effectiveness of a hot water/steam vacuum spot cleaning system for reducing naturally occurring and artificially inoculated bacterial populations on beef carcasses has been evaluated *(14)*. APCs on uninoculated tissue were reduced by approximately 0.2 log CFU/cm^2, and inoculated bacterial populations were reduced from ca. 6.2 log CFU/cm^2 to 3.2 log CFU/cm^2.

Organic acids *(2, 4, 6, 8, 11, 12, 13, 16, 18, 22)* have been studied extensively for reducing bacterial populations on meat animal tissues. Their effectiveness depends on concentration, temperature, exposure time, mode of application, type of meat tissue evaluated, and sensitivity of specific bacterial populations *(1)*. In addition to the antimicrobial effectiveness of organic acids, considerations affecting their commercial applicability include equipment requirements, employee safety, waste disposal, corrosiveness, and costs. Varied effectiveness of organic acids against specific target bacterial populations has been reported *(2, 4, 8, 16, 22)*.

Researchers have reported various degrees of effectiveness for use of steam to decontaminate meat surfaces. Carpenter *(6)* showed that live steam was effective at eliminating *Salmonella enteriditis* populations on pork carcasses; however, significant distortion of the pigskin occurred. Use of a jacketed chamber to expose meat surfaces to steam at atmospheric pressure for 5 to 20 s was effective at reducing bacterial populations *(26)*. Anderson et al. *(2)* applied steam onto frozen and thawed beef tissue from a distance of 10 cm using a specially fabricated nozzle. Under the conditions of their study, steam was fairly ineffective at reducing bacterial contamination. Davidson et al. *(10)* used a specially built steam chamber to decontaminate whole chicken carcasses and chicken pieces by exposing them to a continuous flow of steam for 20 s at atmospheric pressure. The effectiveness of the process apparently was related to the geometry of the samples. The researchers noted a slightly cooked appearance of the skin. Cygnarowicz-Provost et al. *(9)* used a specially designed steam chamber to "surface pasteurize" beef frankfurters inoculated with *Listeria innocua* (at a dose of 7 log cycles). A 4 log cycle reduction in *L. innocua* was reported. Dorsa et al. *(14)* used a wash–air dry–steam treatment to decontaminate uninoculated and fecally inoculated areas of freshly slaughtered sheep carcasses. Carcasses were exposed to low pressure steam for 30 s in a custom-built plywood cabinet, resulting in reductions of 1.5 log cycles in APCs on uninoculated surfaces (with initial APCs of ca. 2.5 log CFU/cm^2). The APCs on inoculated tissue were reduced by ca. 3 log CFU/cm^2, regardless of initial levels (4 or 6 log CFU/cm^2).

The objectives of this research were to determine and compare the effectiveness of (1) various combinations of steam pasteurization (S), knife trimming (T), warm water washing (W), hot water/steam vacuum spot cleaning (V), and spraying with 2% lactic acid (L) for reducing high levels of pathogenic bacteria on the surfaces of unchilled freshly slaughtered beef and (2) these treatments when used individually for reducing high levels of pathogenic bacteria on unchilled freshly slaughtered beef.

MATERIALS AND METHODS

Bacterial cultures

Listeria monocytogenes Scott A (Kansas State University food microbiology culture collection, Call Hall), *Escherichia coli* O157:H7 (rifampicin-resistant strain developed by Gary Acuff, Texas A&M University, College Station, TX, and obtained through James Dickson, Iowa State University, Ames, IA), and *Salmonella typhimurium* (nalidixic acid-resistant strain, obtained from Stan Bailey, USDA-ARS, Athens, GA) were used for inoculation studies. Cultures were maintained on tryptic soy agar (TSA; Difco, Detroit, MI) slants at 4°C. For combination decontamination treatments (phase I), one loopful of each culture was transferred to 7 ml brain heart infusion broth (BHI; Difco) and incubated for 12 to 15 h at 35°C (*L. monocytogenes*) or 37°C (*E. coli* O157:H7 and *S. typhimurium*) for use as stationary phase inocula.

For individual decontamination treatments and combined treatments using short exposure times to steam pasteurization (phase II), one loopful of each bacterial culture was transferred to 7 ml BHI containing 1% glucose and incubated for 12 to 15 h as previously described. The BHI broth was supplemented with 1% glucose to achieve a greater reduction in culture pH (the glucose supplemented broth reached pH 5.0, whereas a pH of ca. 6.0 was reached in 14 h without glucose addition). This acid habituation selected for acid resistance in the inocula *(5, 25)* to provide a more conservative estimate of the effectiveness of lactic acid (2%) in combination treatments.

Prior to the decontamination studies, growth of the antibiotic-resistant strains of *E. coli* O157:H7 and *S. typhimurium* was evaluated on TSA containing 100 ppm rifampicin (Sigma, St. Louis, MO) (Rif-TSA) and MacConkey Agar (Difco) containing 200 ppm nalidixic acid (Sigma) (NA-MAC), respectively, and compared to the respective media containing no antibiotic. No significant differences ($P > 0.05$) in bacterial populations were observed in this comparison study. *L. monocytogenes* was confirmed on Listeria Selective Agar Base (Oxford formulation, MOX; Unipath LTD, Oxoid Division, Basingstoke, UK) containing colistin methanesulfonate (10 mg/l; Sigma) and moxalactam (15 mg/l; Sigma), added to the tempered agar *(20)*. All cultures were confirmed biochemically and/or serologically.

Inoculum preparation

Fecal material (ca. 500 g) from the overnight holding pen of the animals to be slaughtered for this study was collected in a plastic bag and was transported to the microbiology laboratory within 30 min of collection for inoculum preparation. One portion (200 g) of feces was weighed into a stomacher bag to be inoculated. A second portion (10 g) was weighed into a separate filter stomacher bag and analyzed for background populations of each pathogen by homogenizing in 90 ml 0.1% peptone water (PW) diluent and spread plating 0.25 ml onto each of four (total of 1 ml) Rif-TSA, NA-MAC, and MOX plates for *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes*, respectively.

To prepare the fecal inoculum, 2 ml of each pathogen in BHI (or BHI + 1% glucose) broth (12- to 15-h stationary phase

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cultures; approximately 10^8 CFU/ml) was transferred to a single test tube containing 18 ml of sterile PW and agitated on a Vortex mixer for 30 s. The 24 ml PW suspension then was added to the 200 g of feces (providing approximately 10^6 CFU/g), and the bag was hand kneaded for 2 min to ensure even distribution of inocula. Portions (7.5 g for phase I or 5.0 g for phase II) of the inoculated feces were weighed into medium-sized plastic weigh boats and used to inoculate a predesignated meat surface area (300 cm^2 for phase I; 200 cm^2 for phase II). In both phases, the same amount of inoculated feces was used per unit area (0.025 g/cm^2) to provide approximately 10^5 CFU/cm^2 of each pathogen. Ten grams of inoculated feces was diluted serially with PW and spiral plated onto specified selective media to enumerate pathogen populations. For all treatments within each replication, the meat surfaces were inoculated within 2 h of preparation of the fecal inoculum to ensure that appropriate levels of each pathogen were maintained.

Preparation of meat samples

For combination decontamination treatments (phase I), four market-weight steers were purchased from a local producer. Two steers were slaughtered on each of two successive days in the Kansas State University meat science abattoir using USDA-FSIS approved slaughter procedures. On each day of slaughter, the second animal was slaughtered approximately 4 h after the first. The paired cutaneus trunci (rose meat) muscles were removed from each carcass after hide removal and evisceration without any washing. These muscles were subdivided into four segments per side (approximately 30 by 30 by 4 cm), providing a total of eight meat samples per carcass (carcass = replication). These portions were placed into a plastic bag inside a Styrofoam vessel for holding and transportation to the microbiology laboratory (<30 min elapsed time).

For the individual decontamination and short-time steam pasteurization treatments (phase II), two market weight steers were processed. The cutaneus trunci muscle from each carcass side was divided into six portions of approximately equal dimensions (25 by 25 by 4 cm) and transported as previously described (side = replication). All experiments were replicated four times.

Inoculation of meat surfaces

Meat samples were removed randomly from the insulated container at approximately 15-min intervals. Each sample was placed onto clean polyethylene-coated freezer paper, and a stainless steel bacon comb was inserted through one edge of the sample, ca. 3 cm outside of the fecal inoculation area, to suspend the sample during treatments. A sterile foil template was used to specify the appropriate area for inoculation (25 by 12 cm for 300-cm^2 areas, 20 by 10 cm for 200-cm^2 areas). After the appropriate area had been marked with sterile straight pins, the template was removed and inoculated feces were spread uniformly over the designated area using a small flame-sterilized stainless steel spatula as described by Hardin et al. *(18)*. The fecal inoculum on the meat surface was left undisturbed for 20 min at room temperature to allow pathogen attachment. After sampling to determine pathogen populations on the inoculated surface, the inoculated meat sample received the specified decontamination treatments. An uninoculated meat sample was analyzed microbiologically to estimate background levels of the three pathogens.

Steam pasteurization (S) treatments

Because the effects of the recently developed steam pasteurization process (Frigoscandia Food Process Systems, Bellevue, WA, and Cargill, Inc., Minneapolis, MN) *(27)* on beef tissue were unknown, preliminary experiments were performed to determine appropriate process parameters. Based on microbiological data and tissue color evaluations (data not presented), exposure times of 5 to 20 s were found to be acceptable. A 15-s exposure time was chosen for use in phase I. In phase II, exposure times were 15 s for the individual steam pasteurization treatment and 5 and 10 s for the two combination treatments.

An experimental steam pasteurization chamber was constructed (Fig. 1) and consisted of an insulated stainless steel cabinet (91.4 by 76.2 by 68.6 cm) with two internal compartments separated by a removable (sliding) barrier. One compartment (61.0 by 76.2 by 68.6 cm) was the steam reservoir, and the other (30.5 by 76.2 by 68.6 cm) contained the meat sample to be treated. The steam reservoir was filled with steam through an inlet valve at the bottom of the compartment, samples were suspended in the sealed sample compartment, and the barrier between the two compartments was removed quickly for the specified exposure time. At the end of the exposure time, the barrier was replaced quickly and the sample was removed from the chamber and immediately cooled with a cold (1°C) water spray for 20 s. A manometer was used to measure the pressure in the steam reservoir compartment. During steam treatment, the reservoir pressure was above atmospheric pressure (ca. 2.5 cm water). When the sliding door was opened to allow the sample compartment to fill with steam, the pressure remained above atmospheric (ca. 0.6 cm water). The surface temperature of the meat sample during treatment was measured using type K thermocouples (Omega Engineering Inc., Stamford, CT) attached to the meat surface with sterile straight pins. Thermocouples (C01-T; 1/5000 in. [ca. 5 μm] thick) were placed at six positions on the meat sample and a Hydrologger (Fluke, Everett, WA) recorded the meat surface temperature every 0.5 s.

Hot water/steam vacuum spot cleaning (V) treatments

A commercially available hot water/steam vacuum spot cleaning system (Vac-San, Kentmaster, Mfg., Monrovia, CA) was used to remove all visible fecal material from the meat surface. Temperatures were recorded as described for the steam pasteurization system. Further description of this unit was given by Dorsa et al. *(14)*.

Other decontamination treatments

For knife trimming treatments (T), a boning knife sterilized in a hot water (82.2°C) knife sterilizer was used with a sterilized meat hook to cut away all visible fecal contamination. The initial incision was made ca. 1 cm outside of the visible contamination at one corner of the fecally inoculated area and the entire inoculated

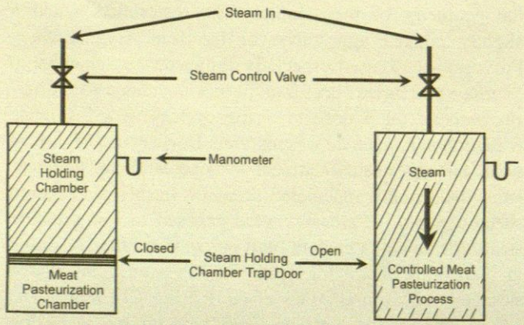

FIGURE 1. *Schematic diagram (top view) of the prototype steam pasteurization chamber prior to treatment (left) and during treatment (right).*

surface area was removed as a unit (0.5 cm depth) *(18)* to meet USDA zero tolerance criteria for visible contamination *(19)*. The knife was sterilized once per sample.

For warm water wash (W) treatments, meat samples were treated in a model carcass wash system. A steel pipe (2.5 cm diameter, 2.0 m length) was inserted through 3.8 cm holes that had been drilled through opposite ends of an oval-shaped plastic tub (124.5 cm length by 71.1 cm width by 61.0 cm height) approximately 5 cm from the top. The pipe was free to slide in the holes. Inside the tub, a series of 3.8-cm water pipes was fixed against one long wall to form two horizontal rows with four spray nozzles (0.25-in. S10 BAX nozzle brass, Bete Fog Nozzle, Inc., Sumner, WA) each. During wash treatments, meat samples suspended from bacon combs were hung on the steel pipe at the top of the tub, which resulted in a 20.3-cm distance between the meat surface and the spray nozzles. Warm tap water (35°C) was sprayed onto the inoculated meat surface at a pressure of 38 to 40 psi for 23 s. Throughout this treatment, the meat sample was moved horizontally back and forth by sliding the pipe from which it was suspended to simulate an oscillating wash action.

For lactic acid decontamination (L) treatments, a 2% vol/vol solution was prepared from an 88% lactic acid solution (Purac America, Lincolnshire, IL) with a final pH of 2.25. The acid solution (54°C) was applied to the meat surface at 25 lb/in^2 using a pressurized hand-held stainless steel sprayer. The meat surface was drenched with 200 ml of the lactic acid solution for 22 s and then was allowed to drip for 30 s.

Meat surface decontamination

In phase I, five decontamination treatments incorporating T, W, S, V, and L were used in seven combinations to determine their effectiveness in reducing levels of pathogens on the surface of the meat samples. Inoculated meat samples were assigned randomly to treatments: TW, TWS, WS, VW, VWS, TWLS, or VWLS (treatment letter designations indicate the order of application to the meat sample and represent typical commercial beef slaughter operations). After treatments, the meat sample was placed immediately on clean freezer paper and sampled to enumerate the residual marker pathogenic populations.

In phase II, each of six inoculated meat samples was assigned randomly to decontamination treatments: T, W, V, S, VWLS*5 (steam exposure time of 5 s), or VWLS*10 (steam exposure time of 10 s). After treatment, the meat sample was placed immediately on clean freezer paper and sampled for enumeration of residual marker pathogenic populations.

Sample collection and microbiological analyses

After inoculation with feces and before decontamination treatments, three 11.4-cm^2 core samples (ca. 2 mm in depth; a total of 34.2 cm^2 external surface area) were obtained from the leftmost portion of the inoculated area to establish initial marker pathogen levels before treatment. A sterile steel coring device was used to initially penetrate the meat surface. The core then was excised from the meat portion using sterile forceps and a scalpel. The core samples excised before treatments were removed from the left side of the inoculated area so the resulting void spaces and uneven surfaces would not influence the effectiveness of treatments by entrapping fecal material and bacteria in areas to be sampled after treatments. The three cores were combined into a composite sample in a filter stomacher bag for microbiological analyses. After treatments, three additional cores were excised from the rightmost portion of the inoculated area and combined into a composite sample for analyses. The effectiveness of each treatment was determined by the mean log CFU/cm^2 difference in pathogen populations before and after treatment.

Composite core samples were pummeled for 1 min in 50 ml PW in a Stomacher 400 (Tekmar, Cincinnati, OH). For before-treatment composite samples, serial dilutions in PW were enumerated by spiral plating on Rif-TSA, NA-MAC, and MOX for *E. coli* O157:H7, *S. typhimurium,* and *L. monocytogenes,* respectively. For after-treatment composites, 1 ml of sample homogenate was spread plated onto each agar (four 0.25 ml aliquots on each of four plates; detection limit of 0.7 log CFU/cm^2). Additionally, a 1:100 dilution of each after-treatment sample was spiral plated. All platings were performed in duplicate. Plates were incubated for 24 h at 35 and 37°C for *L. monocytogenes* and *E. coli* O157:H7/*S. typhimurium,* respectively. Two representative colonies from Rif-TSA were confirmed in each replication as *E. coli* O157 by latex agglutination assay (Oxoid). Two representative colonies on NA-MAC were confirmed per replication as *Salmonella* by slide agglutination assay using Salmonella polyvalent O antisera (Difco). All colonies demonstrating typical dark color with a darkened halo surrounding the colony on MOX agar were enumerated as *L. monocytogenes*.

Statistical analysis

Average pathogen counts from duplicate plates for each treatment were calculated. Log CFU/cm^2 differences in population levels between before and after treatments were calculated for each pathogen per treatment. The mean differences from four replications were analyzed using analysis of variance (ANOVA) procedures, and means were separated using the least significant difference (LSD) procedure *(24)*.

RESULTS AND DISCUSSION

Dickson and Anderson *(12)* provided an extensive review of the application aspects and effectiveness of various washing and sanitizing protocols on animal carcasses. Recent work by Hardin et al. *(18)* demonstrated the antimicrobial effectiveness of trimming, water washing, and organic acid applications to beef carcasses at various anatomical locations. The present study was designed to simultaneously evaluate and compare the effectiveness of current commercial decontamination methods versus two newly developed carcass decontamination processes (hot water/steam spot vacuuming and steam pasteurization) for removal of gross fecal contamination and high levels of pathogenic bacterial contamination from freshly slaughtered beef tissue.

Temperature profile of the steam pasteurization process

Temperatures were monitored extensively at different locations within the prototype steam pasteurization unit during all experiments (Fig. 2). The atmospheric temperature within the steam reservoir remained stable at 99 to 101°C throughout the entire process. The atmospheric temperature in the adjacent sample compartment remained constant at 69 to 70°C until the sliding door separating the two chambers was lifted. The temperature within the sample compartment quickly rose to 91 to 93°C, where it remained steady for the specified steam exposure period. The surface temperature of the meat sample equilibrated with the atmospheric temperature of the compartment within ca. 1 s.

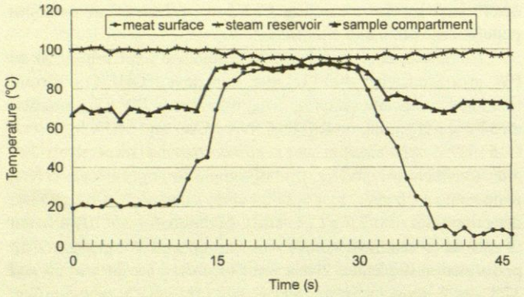

FIGURE 2. *Typical temperature profiles of meat surfaces and atmospheres of the sample compartment and the steam reservoir during steam pasteurization treatments in the prototype unit.*

After the door was replaced, the meat sample was cooled with a chilled water spray and reached 6 to 8°C in ca. 8 s.

Combination decontamination treatments for pathogen removal (phase I)

All combination treatments were effective in reducing the three marker pathogen populations attached to raw meat surfaces, demonstrating reductions of 3.5 to 5.3 log CFU/cm^2 from initial levels of ca. 5 log CFU/cm^2 (Table 1). For *E. coli* O157:H7 populations, no significant differences ($P > 0.05$) were detected among the treatments (Table 1). However, TW and VWLS treatments reduced populations by 0.9 to 1.2 log CFU/cm^2 more than treatments VWS and VW. The TWS, WS, and TWLS treatments were intermediate in their bactericidal effects, demonstrating reductions of 0.3 to 0.6 log CFU/cm^2 lower than the TW and VWLS treatments. All treatments effectively reduced *S. typhimurium* populations (Table 1). The TWLS process was more effective ($P \leq 0.05$) than VWS and VW, demonstrating reductions 1.1 and 1.7 log CFU/cm^2 greater, respectively. Additionally, VWLS, TW, and WS were superior ($P \leq 0.05$) to VW in eliminating *S. typhimurium*. The VW procedure was less effective ($P \leq 0.05$) than the other treatments involving two decontamination procedures (TW and WS), though VW did reduce populations by 3.6 log CFU/cm^2. Reductions in *L. monocytogenes* populations followed those observed for *S. typhimurium* (Table 1) with TWLS, VWLS, and TW treatments being most effective.

The order in which decontamination procedures were applied in combination treatments was chosen to represent the process flow that likely would be found in a commercial processing situation. Treatment combinations that do not include trimming or hot water/steam vacuum spot cleaning presently would not be applicable to commercial settings because USDA-FSIS regulations require all visible contamination be removed from carcass surfaces during slaughter *(7)*. A combination water wash and steam pasteurization (WS) system was evaluated, however, for comparative purposes.

Currently, T and V treatments are used in commercial facilities to meet zero tolerance regulations. Carcasses are knife trimmed and/or hot water/steam vacuum spot cleaned by line workers, followed by a water wash after USDA inspection. In this laboratory study, the TW combination was highly effective in removing gross fecal contamination and high levels (≥4.7 log units) of the three target pathogens. However, the laboratory trimming procedure was extensive, using freshly sanitized instruments and great control to prevent cross contamination of underlying beef tissue. This type of control would be difficult to achieve on an actual processing line. Hardin et al. *(18)* performed a similar study with fecally inoculated carcasses and pointed out that, in the laboratory, the trimmer has the advantage of knowing the exact boundaries of the inoculated area, so that cutting through inoculated tissue, and thereby, transferring bacteria to underlying surfaces can be avoided. Typical commercial trimmers must subjectively identify naturally contaminated areas quickly and estimate contamination borders (which are not always visible). The current study was not designed specifically to evaluate the spreading of microorganisms through knife trimming; however, other researchers *(17, 18)* have shown that adjacent areas can become contaminated. Prasai et al. *(23)* pointed out that the effectiveness of knife trimming is dependent upon the skill and training of the employee and the sanitary status of the trimming instruments. Additionally, certain areas of the carcass, particularly

TABLE 1. *Effectiveness of combination decontamination treatments in reducing pathogens on surfaces of freshly slaughtered beef*

	E. coli O 157:H7		*S. typhimurium*		*L. monocytogenes*	
Treatment[a]	Initial[b]	Mean red.[c]	Initial	Mean red.	Initial	Mean red.
TW	5.19 ± 0.11	4.71 ± 0.53^{d}	5.26 ± 0.13	4.95 ± 0.34^{de}	5.52 ± 0.22	4.96 ± 0.34^{d}
TWS	5.24 ± 0.07	4.44 ± 0.53^{d}	5.31 ± 0.17	4.38 ± 0.34^{def}	5.57 ± 0.15	4.56 ± 0.34^{de}
WS	5.18 ± 0.08	4.22 ± 0.53^{d}	5.24 ± 0.08	4.85 ± 0.34^{de}	5.46 ± 0.03	4.40 ± 0.34^{def}
VW	5.27 ± 0.08	3.53 ± 0.53^{d}	5.35 ± 0.02	3.64 ± 0.34^{f}	5.56 ± 0.12	3.49 ± 0.34^{f}
VWS	5.20 ± 0.15	3.79 ± 0.53^{d}	5.24 ± 0.08	4.15 ± 0.34^{ef}	5.49 ± 0.04	3.84 ± 0.34^{ef}
TWLS	5.05 ± 0.15	4.14 ± 0.53^{d}	5.31 ± 0.08	5.31 ± 0.34^{d}	5.51 ± 0.19	5.07 ± 0.34^{d}
VWLS	5.20 ± 0.05	4.65 ± 0.53^{d}	5.27 ± 0.11	5.08 ± 0.34^{de}	5.51 ± 0.13	5.01 ± 0.34^{d}

[a] Order of treatment within abbreviation indicates order of application. T = trim; W = 35°C water wash; S = 15 s steam pasteurization; V = hot water/steam vacuum spot cleaning; L = 2% lactic acid spray.
[b] Mean initial pathogen population (log CFU/cm^2) from four replications ± standard error of mean.
[c] Mean reduction in pathogen population (log CFU/cm^2) from four replications ± standard error of mean.
[d,e,f] Means having the same superscript within columns are not significantly different ($P < 0.05$).

the inside round area, are difficult to trim effectively, and microbiological reductions in these areas are less pronounced *(18)*. Prasai et al. *(23)* demonstrated that aseptic trimming alone in a commercial slaughter facility, performed in a manner comparable to the current laboratory trimming method, decreased native aerobic bacterial populations (APCs) by 3 log CFU/cm^2 (initial contamination level of 3.5 log CFU/cm^2). However, the APCs in the same trimmed area rose to 2.6 log CFU/cm^2 after the carcass went through a commercial water wash cabinet. Hardin et al. *(18)* demonstrated slight spreading of microbiological contamination during water washing; however, Gorman et al. *(17)* suggested that water washing did not translocate contamination to adjacent areas of the carcass.

The addition of low-pressure steam (S) to the conventional TW treatment of inoculated samples provided no significant increases ($P > 0.05$) in pathogen reductions in the trimmed area (Table 1). However, the laboratory TW procedure removed virtually all contamination in the defined surface area and likely masked the antimicrobial effect of subsequent steam application. In actual processing situations only small surface areas of a carcass where visible contamination exists are trimmed routinely. Significant levels of bacterial contamination are often present on visibly clean tissue that would not receive trimming. However, steam above atmospheric pressure condenses uniformly over all surfaces of an irregularly shaped carcass and theoretically should uniformly decontaminate all carcass surfaces.

No differences ($P > 0.05$) in pathogen reductions were observed for the WS treatment, which removed 4.2 to 4.8 log CFU/cm^2, when compared to the TW and TWS treatments. As will be discussed in the following section, washing alone at 35°C (ca. 40 lb/in^2) was ineffective compared to other treatments in reducing pathogen levels on the inoculated meat surfaces (reductions of 0.7 to 1.3 log cycles) (Table 2). This implies that the steam pasteurization step in the combination WS treatment was very effective in destroying attached target pathogens. Dorsa et al. *(14)* found that water washing at 82.2°C followed by low pressure steam application resulted in APC reductions of 1.7, 1.6, and 4.0 log CFU/cm^2 when initial inoculation levels were 2.5, 4.5, and 6.7 log CFU/cm^2, respectively. However, these reductions were not different ($P > 0.05$) from those with washing only at 82.2°C. Water washing at temperatures of 54.4 and 15.6°C combined with steam remained as effective as the combination at 82.2°C. However, with the wash-only treatment at these lower temperatures, APC reductions were markedly less.

The addition of a 2% lactic acid solution (54°C) to the TWS combination treatment immediately preceding the steam pasteurization step provided slightly greater reductions for *L. monocytogenes* and *S. typhimurium,* although not different ($P > 0.05$) from those with TW, TWS, or WS (Table 1). The lactic acid treatment did not increase ($P > 0.05$) *E. coli* O157:H7 reductions among the four replications. However, a review of reductions in *E. coli* O157:H7 counts in individual replications showed that one replication with a reduction of only 1.5 log cycles influenced the statistical analyses for this treatment. That lack of reduction might have resulted from inadequate washing, inadequate residual water removal prior to steam pasteurization, or recontamination of the sampling site after steam application during transport to the analytical laboratory. Recoveries from the remaining three replications were always below the experimental detection level.

The Vac-San unit is designed to remove small areas of visible physical contamination from carcass surfaces and reduce the amount of knife trimming required to meet zero tolerance standards. It is supposed to deliver water plus steam at >82.2°C directly to the carcass surface, simultaneously vacuuming away physical contamination *(14)*.

The VW and VWS combinations were the least effective for reducing populations of all three pathogens tested (Table 1), removing 3.5 to 4.2 log CFU/cm^2. Additionally, reductions tended to be more varied. During this test, fairly small meat samples (approximately 900 cm^2) were inoculated with a large amount of feces over an area of 300 cm^2. Although the V treatments removed a large percentage of the pathogens present, they still were less effective than the other treatments. This could have been due to a number of

TABLE 2. *Effectiveness of individual decontamination treatments and short-time steam pasteurization for reducing pathogens on surfaces of freshly slaughtered beef*

	E. coli O157:H7		*S. typhimurium*		*L. monocytogenes*	
Treatment[a]	Initial[b]	Mean red.[c]	Initial	Mean red.	Initial	Mean red.
T	5.14 ± 0.12	3.10 ± 0.49^{d}	5.27 ± 0.52	2.72 ± 0.42^{e}	5.26 ± 0.33	2.54 ± 0.33^{f}
W	5.17 ± 0.07	0.75 ± 0.49^{e}	5.19 ± 0.20	1.23 ± 0.42^{f}	5.27 ± 0.35	1.28 ± 0.33^{g}
V	5.07 ± 0.05	3.11 ± 0.49^{d}	5.20 ± 0.07	3.37 ± 0.42^{de}	5.37 ± 0.20	3.33 ± 0.33^{ef}
S	5.05 ± 0.05	3.53 ± 0.49^{d}	5.15 ± 0.08	3.74 ± 0.42^{de}	5.38 ± 0.16	3.44 ± 0.33^{ef}
VWLS * 5	5.08 ± 0.19	3.37 ± 0.49^{d}	5.02 ± 0.30	4.54 ± 0.42^{d}	5.18 ± 0.35	4.51 ± 0.33^{d}
VWLS * 10	5.11 ± 0.21	3.57 ± 0.49^{d}	5.12 ± 0.21	3.95 ± 0.42^{de}	5.21 ± 0.33	4.23 ± 0.33^{de}

[a] Order of treatment within abbreviation indicates order of application. T = trim; W = 35°C water wash; S = 15 s stream pasteurization; V = hot water/steam vacuum spot cleaning; L = 2% lactic acid spray; S * 5 = 5 s exposure time for steam pasteurization; S * 10 = 10 s exposure time for steam pasteurization.
[b] Mean initial pathogen population (log CFU/cm^2) from four replications ± standard error of mean.
[c] Mean reduction in pathogen population (log CFU/cm^2) from four replications ± standard error of mean.
[d,e,f,g] Means having the same superscript within columns are not significantly different ($P > 0.05$).

contributing factors. Because the actual temperature of the meat surface during application of the hot water reached only 34 to 49°C, thermal destruction of bacterial populations was not appreciable. The nozzle head design of the Vac-San was such that the suction generated by the vacuum cooled the water temperature prior to reaching the surface. More recent head designs may eliminate this problem. During experimental vacuuming of the heavily contaminated meat surfaces, several back and forth passes were required to remove all visible fecal contamination. This physical action, which slightly alters the surface characteristics of the meat, possibly could "embed" bacteria, making them more difficult to remove by vacuuming/rinsing. This embedding would further protect the organisms from the effects of steam condensing on the surface in the VWS treatment. The USDA allows the use of steam vacuuming equipment for the removal of visible contamination smaller than 1 in. in any dimension, which would reduce the need for the significant back and forth action. Dorsa et al. *(14)* found the Vac-San hot water/steam vacuum followed by consecutive hot and cold water wash treatments to be effective in reducing APC, coliform, and generic *E. coli* levels on inoculated meat tissue (reductions of 2.7, 4.2, and 4.3 log cycles, respectively, from an initial contamination level of 5 to 6 log units). However, they hypothesized that collagen expansion resulting from hydration of the fascia during washing treatments entrapped bacteria, making microbial reductions with subsequent moist heat treatments more difficult and variable. This hypothesis was supported by Barkate et al. *(3)* and could provide insight into the slightly less effective pathogen reductions in the VWS treatment in this study. The application of lactic acid (2% vol/vol) at 54°C to the inoculated meat surface immediately prior to steam pasteurization (VWLS) provided increased bactericidal effectiveness compared to the VW and VWS treatments (Table 1).

Individual decontamination treatments for pathogen removal (phase II)

To better understand the antimicrobial contribution and capability of each individual processing step, each step (T, W, V, and S) was evaluated independently. All treatments except W effectively reduced ($P \leq 0.05$) pathogen populations (Table 2) and were equivalent ($P > 0.05$) in their abilities to remove *E. coli* O157:H7 attached to meat surfaces, reducing counts by 3.1 to 3.6 log CFU/cm^2. These treatments reduced *S. typhimurium* and *L. monocytogenes* populations by 2.7 to 3.7 log cycles. The W treatment removed only 0.7 to 1.3 log cycles of these pathogens. The heavy fecal presence within the 200 cm^2 area prior to washing remained noticeable as significant staining of the area after washing. Of these individual treatments, steam pasteurization (S) for 15 s consistently provided the largest reductions (reductions of 3.4 to 3.7 log CFU/cm^2 from initial levels of 5.0 to 5.4 log CFU/cm^2) for all pathogens, although not statistically different ($P > 0.05$) from reductions with the T and V treatments. This degree of effectiveness in microbial reductions was not expected for S alone, because a very heavy presence of fecal material remained on the meat surface with the absence of a W, T, or V preparatory treatment. Steam pasteurization was developed only for the purpose of augmenting approved physical contaminant removal processes by providing final microbiological cleanliness to the entire carcass after other processes have been applied. Obviously, from regulatory, safety, and aesthetic standpoints, physical contaminants must be removed from carcasses prior to steam pasteurization.

In this phase of experimentation, V provided slightly higher reductions compared to T (Table 2). Reductions with V ranged from 3.1 to 3.4 log cycles (from initial levels of 5.1 to 5.4 log CFU/cm^2), whereas T reductions ranged from 2.5 to 3.1 log cycles (from initial levels of 5.1 to 5.3 log CFU/cm^2). Both treatments were very effective in providing visually clean tissue. Water washing at 35°C alone was ineffective ($P > 0.05$) in reducing pathogenic populations, removing only 0.7 to 1.3 log cycles (from initial levels of 5.2 to 5.3 log CFU/cm^2). In this experimental situation, washing was incapable of removing the large amounts of organic matter applied to the tissue surface. Dorsa et al. *(14)* also demonstrated marginal effectiveness of warm and cold water washes for reducing aerobic bacterial populations on meat tissue. Given the effectiveness of T and V for removing physical contamination, along with the demonstrated antimicrobial effectiveness of S over the entire carcass surface, future studies should address the actual need for water washing of carcasses in plants utilizing the other three decontamination methods. As previously discussed, the moist heat effect of S might be enhanced if surface hydration of the collagen fibers is kept to a minimum *(3, 14)* before pasteurization. To minimize this effect, the S process includes a cold water spray chill step (drenching) immediately after the carcass exits the steam chamber. Perhaps this step would provide sufficient carcass washing.

Because the four-way combination treatments in phase I of the studies were so effective in eliminating all three pathogens (Table 1), the VWLS combination was reevaluated using shorter (5 and 10 s) steam application times (Table 2). Both treatments provided pathogen reductions from 3.4 to 4.5 log cycles (initial levels of 5.0 to 5.1 log CFU/cm^2). With the VWLS 15 s application in phase I, reductions ranged from 4.7 to 5.1 log cycles. The slight reduction in effectiveness for the 5 and 10 s treatments in phase II could have been due to the initial inoculum being grown in media with reduced pH that allowed adaptation to the lactic acid component of the treatment. Thippareddi et al. *(25)* found that acid-habituated cultures were more resistant to subsequent acid exposure but were more susceptible to thermal treatments. Using the order of application in the current study (VWLS), it is probable that cultures contaminating the meat surface in phase II were better able to survive the lactic acid drenching but were more susceptible to the thermal steam application immediately following. No difference ($P > 0.05$) occurred between the 5 and 10 s treatments. The *E. coli* O157:H7 culture was more resistant to the VWLS and TWLS treatments compared to *S. typhimurium* and *L. monocytogenes* in both experimental phases.

CONCLUSIONS

The primary goal of all decontamination treatments is to reduce the risk to consumers of pathogenic organisms in meat and meat products. Additionally, these treatments may increase shelf life and provide higher quality products. When choosing the best decontamination methods for pathogen reduction, advantages and disadvantages of using the treatments in commercial settings must be taken into account. Pathogenic organisms are not visible to the naked eye; therefore, the practical effectiveness of interventions that rely on contamination being visible, such as knife trimming and steam vacuum spot cleaning, automatically decreases. The actual effectiveness of these treatments also relies on the human operator using the equipment properly. Knives that are not sterilized properly or steam vacuum units used incorrectly actually may spread bacterial contamination to previously uncontaminated areas of a carcass. Also, these methods will decontaminate only small defined areas of a carcass. Conditions in commercial facilities do not permit the treatment of an entire carcass using these methods. Sprays or dips that utilize antimicrobial compounds, such as organic acids, chlorine, or trisodium phosphate can corrode processing equipment, provide unfavorable working conditions, and lead to difficulty in treating waste water. Some bacterial populations may be resistant to these compounds. Also, the issue still remains of effectively treating an irregularly shaped surface.

The steam pasteurization process used in this study has several potential advantages over the other decontamination methods. It uses a condensable gas, steam, to uniformly heat the entire surface of a carcass and kill microorganisms. One of the advantages of steam vapor is that it can uniformly cover irregularly shaped surfaces. Also, the process causes no waste water treatment issues because no chemicals are used. A commercial steam pasteurization unit should not be subject to operator misuse, because the entire system could be automated. However, like all of the decontamination treatments discussed, steam pasteurization would be applied at a fixed point in processing and would have no ability to protect a product from recontamination by processing line workers, distributors, retailers, food service workers, or consumers. Data from this study indicate that steam pasteurization can be an effective intervention in an overall system of pathogen reduction on beef carcasses. Knife trimming and/or steam vacuum spot cleaning to remove visible contamination should be performed prior to steam pasteurization to achieve optimal microbial reductions. The greatest effectiveness in reducing bacteria on beef carcasses is achieved through using combinations of decontamination treatments.

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