VALIDATION OF HOT WATER AND LACTIC ACID SPRAYS FOR THE REDUCTION OF ENTERIC PATHOGENS ON THE SURFACE OF BEEF CARCASSES

A Thesis

by

KYLE DENE WRIGHT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2009

Major Subject: Food Science and Technology

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Approved by:

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ABSTRACT

Validation of Hot Water and Lactic Acid Sprays for the Reduction of Enteric Pathogens on the Surface of Beef Carcasses.

(December 2009)

Kyle Dene Wright, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Gary R. Acuff

Escherichia coli O157:H7 and *Salmonella* have emerged as the most common foodborne enteric pathogens causing human illness from the consumption of beef. By mandate of the U.S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS), the industry has implemented a Hazard Analysis and Critical Control Points (HACCP) system that utilize intervention technologies for controlling, preventing, and/or reducing enteric pathogens. In addition, USDA-FSIS has mandated that each facility must validate, monitor, and verify the effectiveness of each intervention implemented to eliminate *E. coli* O157:H7 and *Salmonella*. For this study, microbial decontamination interventions at two beef slaughter facilities were validated to demonstrate effectiveness in eliminating or reducing enteric pathogens. The facilities selected utilized either a lactic acid spray treatment or a combination of hot water followed by a lactic acid treatment. At both facilities, mesophilic plate counts (MPC) were significantly ($P < 0.05$) reduced, and *E. coli* and coliforms were eliminated below detectable limits at both facilities. No *Salmonella* positive samples were detected after

either facility's intervention sequence. The framework used in this research to validate interventions can also be utilized in the future for yearly verification of the effectiveness of each intervention.

DEDICATION

This study is dedicated to my best friend, my soul mate, my wife, Mary Beth, for her undying love, friendship, support, and patience. I would also like to dedicate it to my daughter, Claire Mackenzie, as a reminder to never give up and always keep striving to make your dreams reality. Finally, to my parents and friends who have always been there to support me and provide encouragement, God bless.

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INTRODUCTION

Escherichia coli O157:H7 and *Salmonella* have emerged as the most common foodborne enteric pathogens resulting in human illness from the consumption of beef. Due to the increased trend of foodborne disease outbreaks in recent years associated with *E. coli* O157:H7 and *Salmonella*, the U.S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) has directed the industry to implement a system for controlling, preventing, and/or reducing enteric pathogens called Hazard Analysis and Critical Control Point (HACCP) systems. Under HACCP, facilities recognize hazards reasonably likely to occur, whether chemical, physical, or biological, and implement measures to control the hazards called Critical Control Points (CCPs).

During the process of slaughtering cattle, the initially sterile beef carcass tissue becomes contaminated with bacteria from the hides, exposed viscera, plant workers, and the plant environment. With biological hazards such as *E. coli* O157:H7 and *Salmonella* being common to the beef industry, USDA-FSIS has approved a variety of scientifically validated physical, chemical, and thermal decontamination interventions to be utilized at various points through the processing of beef to eliminate enteric pathogens. Interventions most commonly used in the industry are knife trimming to remove visible fecal contamination, hot water washes and organic acid rinse treatments.

This thesis follows the style of the *Journal of Food Protection.*

Due to the recent increases in foodborne outbreaks, and as processing conditions differ from facility to facility, USDA-FSIS has mandated that each facility must validate each intervention implemented to assure effectiveness, monitor the intervention to assure consistent control, and verify to confirm continued operation according to the HACCP plan. Unfortunately, research presented to USDA-FSIS has shown that many facilities have not yet begun to validate the interventions within their HACCP plan. This could be due in part to a misunderstanding of what it actually means to validate or verify, or it could also be due to a lack of knowledge or direction in carrying out a scientifically valid study. Nonetheless, to assure the safety of the food produced, each facility must reassess their HACCP plan.

For the scope of this study, beef carcass decontamination interventions at two beef slaughter facilities were validated to demonstrate the effectiveness for eliminating enteric pathogens by monitoring reductions of key indicator microorganisms. In the first phase, Facility A utilized a post-evisceration hot water spray wash immediately followed by a lactic acid spray treatment. In the second phase, Facility B utilized only a post-evisceration lactic acid spray treatment. Many research studies have shown enteric pathogens such as *E. coli* O157:H7 and *Salmonella* are only present on carcasses at extremely low levels, preventing usefulness for validation of intervention effectiveness. Therefore, the goal of this study was to lay a foundation by which beef slaughter facilities can utilize key bacterial indicator organisms to validate and verify interventions when designing and executing scientific studies while reassessing their HACCP plan.

LITERATURE REVIEW

Human infection associated with consumption of beef

From 2003-2007, the consumption of beef in the United States has increased significantly from 27.0 billion pounds to 28.1 billion pounds followed by a slight decrease in 2008-2009 (65). Foodborne infections related to the consumption of beef are also on the rise, as the Centers for Disease Control and Prevention (CDC) estimate there are > 6.5 million reported and non-reported cases of foodborne illness resulting in 9,000 deaths in the United States each year (18). Of reported bacterial related foodborne outbreaks that occurred from 1973-1987, beef was most common vehicle accounting for 159 outbreaks, and *Salmonella* accounted for 77 (48%) of the total outbreaks (7). Although the number of *Salmonella* cases associated with beef has declined in recent years, beef contaminated with *Salmonella* still remains a human threat (16). In 2007, the CDC reported Salmonella outbreaks resulted in 47,995 cases, while *E. coli* (STEC) resulted in 4,847 cases in the United States (15). *Salmonella* is estimated to account for 800,000-4,000,000 cases, while *E. coli* O157:H7 is estimated between 10,000-20,000 total cases each year (25). More than 95% of cases of *Salmonella* infections are foodborne, and also account for ~30% of deaths resulting from foodborne illness in the United States (43). The USDA-FSIS estimates that foodborne illness costs $9.3-$12.9 billion annually, and that the treatment of salmonellosis costs $0.6-$3.5 billion annually (25). In 2005, the CDC (17) compiled a list of the 30 most frequently reported *Salmonella* serotypes associated with human infection.

Salmonella

Salmonella was named after the pathologist Daniel E. Salmon who discovered the first strain in 1885 (71). The genus *Salmonella* is included in the family Enterobactereaceae along with *E. coli* and *Enterobacter* (35). The organism is described as a small, Gram-negative, non-sporeforming aerobic/facultative anaerobic, mesophilic rod (0.7-1.5 μm x 2-5 μm) that is indistinguishable from *E. coli* microscopically. *Salmonella* species are biochemically characterized by the following reactions: oxidase negative, catalase positive, indole and Voges-Proskauer negative, methyl red and Simmons citrate positive, lysine and ornithine decarboxylase positive and a variable arginine dehydrolase reaction. Hydrogen sulfide (H_2S) is produced and urea is not hydrolyzed. The classification of *Salmonella* species has changed greatly over time, and currently is divided into two species, *S. bongori* and *S. enterica*. *S. enterica* is divided into six subspecies (I. *S. enterica* subsp. *enterica*; II. *S. enterica* subsp. *salamae*; IIIa. *S. enterica* subsp. *arizonae*; IIIb. *S. enterica* subsp. *diarizonae*; IV. *S. enterica* subsp. *indica*), and nearly 2,500 serovars are distributed among them (12). The serovars are classified based on the somatic (O) antigen (A, B, C, etc.), and by the flagellar (H) antigen (phase 1 and phase 2) (39).

Illness related to *Salmonella* occurs from the ingestion of food contaminated with the organism. The Food and Drug Administrations (FDA) Center for Food Safety & Applied Nutrition (CFSAN) estimates that ingesting as few as 15-20 viable cells is required for infection; however, this can vary due to condition of the host and strain ingested (26). The onset of illness usually takes between 6-72 h, averaging between 12-

36 h (20). Symptoms related to non-typhoidal salmonellosis include nausea, vomiting, mild abdominal pain, headache, chills, and diarrhea (usually non-bloody) and illness is often accompanied by prostration, muscular weakness, faintness, moderate fever, restlessness, and drowsiness (39). Symptoms usually persist for 2-3 days, and up to 5% of cases can then become asymptomatic carriers. Death is highly unlikely among otherwise healthy individuals, resulting in a 0.1% death rate (25). Secondary disease syndromes and death from gastroenteritis, bacteremia, and subsequent focal infection are more likely to occur in immunocompromised patients such as those of extreme ages or with HIV (41).

Escherichia coli

E. coli was discovered in 1885 by a German bacteriologist named Theodore von Escherich and, like *Salmonella*, is a member of the family Enterobacteriaceae (40). *E. coli* is described as a Gram-negative, straight rod (1.1-1.5 μm x 2.0-6.0 μm) that is a mesophlic, facultative anaerobe that sometimes possess peritrichous flagella (42). Biochemically, *E. coli* is characterized by the ability to catabolize D-glucose and other carbohydrates with the formation of gas. It is oxidase negative, catalase positive, methyl red positive, Voges-Proskauer negative, and usually citrate negative. *E. coli* is negative for H_2S production, urea hydrolysis, and lipases. Optimum growth has been found 37°C, while growth is halted at 44.5°C (53, 70). Further classification of *E. coli* is based on antigenic differences (serotyping) and virulence factors (verotyping) (44). Serology

is described similar to other Enterobacteriaceae by classification of the O and H antigens.

E. coli is commonly found in the intestinal tract of humans and animals, and although most strains do not cause gastrointestinal illness, some pathogenic groups have been identified. Pathogenic *E. coli* has been divided into 5 groups based on virulence: enteroaggregative (EAggEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and enterotoxigenic (ETEC) (62). Although many strains exist as pathogens, enterohemorrhagic *E. coli* O157:H7, discovered in 1982, has gained a great deal of attention as the causative agent in a 1993 outbreak in the northwestern United States from undercooked contaminated ground beef at a fast-food chain (54). *E. coli* O157:H7 differs from other pathogenic *E. coli* in that it only affects the large intestine and it is distinguished by its inability to ferment sorbitol within 24 h and does not produce β-glucuronidase (50).

The importance of acid tolerance in *E. coli* O157:H7 became apparent in an outbreak associated with contaminated apple cider in 1993 (9). The pH of the freshly pressed apple cider was between 3.7-3.9. Additional studies by Zhao et al. (72) reported survival of *E. coli* O157:H7 in acidic conditions was as long as 31 days. Glass et al. (30) demonstrated the tolerance *E. coli* O157:H7 exhibited in salt up to 8.5%. Despite resistance to salt and acid, *E. coli* O157:H7 appears to be more heat sensitive than most salmonellae (2). However, the resistance to heat has been shown to increase as the fat content of the food increases (41).

Gastrointestinal illness caused by *E. coli* O157:H7 may occur from ingesting food contaminated with as few as 10 cells (38). The mean incubation period for illness is approximately 3-4 days, and symptoms typically last approximately 1 week (19). Symptoms from *E. coli* O157:H7 may range from mild and non-bloody diarrhea to a stool almost entirely consisting of blood. The illness is differentiated from shigellosis by a lack of fever. Several clinical manifestations can result from infection including hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). Approximately 2-7% of *E. coli* O157:H7 infections will result in HUS (32). Sequellae are usually a result of the patient's medical condition prior to infection. Those who are of extreme age or are immuno-suppressed become more likely to develop these conditions.

Beef slaughter process

Prior to slaughter, most commercial beef cattle production occurs in three phases: the cow-calf, stocker-yearling, and feedlot operations (63). During the first two phases, cattle are fed forage and high-roughage feeds in large pastures. However, in the feedlot, cattle are held and fed in small pens where the spread of enteric pathogens can occur rapidly. Most cattle are marketed between the ages of 15 and 24 months.

At the slaughter facility, the cattle are most often rendered unconscious by mechanical stunning. Following stunning, the cattle are shackled by the back legs, suspended upside down, and the jugular vein is then cut to drain the blood. Once the blood has drained, the head and shanks are removed. At this point in the process, the

hide is removed, which exposes the carcass surface to foreign contaminants such as dust, fecal matter, and contamination from the workers. The viscera are then removed by first cutting and tying the bung, or the posterior end of the gastrointestinal tract, followed by the removal of the gastrointestinal tract from the body cavity. Special care is taken not to release any of the stomach or intestinal contents because this material can be a large source of bacteria, including enteric pathogens. After evisceration, the carcass is split in half, followed by trimming, washing, and chilling. Dehiding and evisceration are the steps along the process where contamination is most likely to occur.

Hazard Analysis and Critical Control Point (HACCP) system

Since enteric pathogens are considered to be a hazard that is reasonably likely to occur in foods processed for human consumption, a system for controlling, preventing, and/or reducing enteric pathogens has become a necessity in the food industry in recent years. In 1959, the first HACCP system was developed by the Pillsbury Company, the U.S. Army Natick Laboratories, and the National Aeronautics and Space Administration (NASA) to try and produce a food safe from pathogens and biological toxins for the astronauts on space missions, and to assure that crumbs or liquid droplets would not interfere with electrical equipment (61). It was recognized that sampling of the food for pathogens would require significantly large quantities of food and would be unable to assure safety. Therefore, it was determined that the best plan of action would be to identify possible hazards and how they might occur. Based on this information, it would be possible to recognize and monitor the points in the process capable of preventing,

reducing, or eliminating a food safety problem. These points were called critical control points (CCPs).

Throughout the 1970's, the FDA (66) began implementing a HACCP-based program focused on three principles for controlling *Clostridium botulinum* in low-acid and acidified canned foods. The three principles are (1) identify any safety-related problems associated with the ingredients, products and process; (2) determine the specific factors that need to be controlled to prevent these problems from occurring; and (3) establish systems that can measure and document whether or not these factors are being controlled. The new regulations from the FDA were only required for low-acid and acidified foods, and therefore, HACCP failed to be adopted by most of the remainder of the food industry.

In 1985, a report issued by a Subcommittee of the Food Protection Committee of the National Academy of Sciences (NAS) (45) titled, "An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients," made strong recommendations to government agencies for the use of a HACCP system in the food industry. This led to the formation of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) in 1988. The mission of the NACMCF was to encourage the adoption of HACCP and to help develop a consensus on the HACCP process. In 1989, the NACMCF (48) issued "HACCP Principles for Food Production," outlining the seven HACCP principles and a systematic approach for the application of HACCP to food production. Their HACCP document was revised in 1992 (47), and again in 1997 (46). The new document titled, "Hazard Analysis and

Critical Control Point Principles and Application Guidelines," described the current seven principles currently used in the industry. They are as follows: (1) conduct a hazard analysis; (2) determine the CCPs; (3) establish critical limits; (4) establish monitoring procedures; (5) establish corrective action; (6) establish verification procedures; and (7) establish record-keeping and documentation procedures.

In 1996, the USDA-FSIS (69) issued a regulation requiring establishments to develop and implement written sanitation standard operating procedures (SSOPs) in federally inspected meat and poultry plants. It required regular microbial testing to verify the adequacy of the establishment's process controls for the prevention and removal of fecal contamination and associated bacteria. Performance Criteria were established for *E. coli* (Table 1) and Performance Standards for *Salmonella* (Table 2) in an effort to verify effectiveness of these plans, and all meat and poultry establishments were required to develop and implement a HACCP program.

In summary, modern HACCP systems are designed to identify physical, chemical, and biological hazards likely to occur in a process. CCPs are established to eliminate, prevent or reduce each hazard through the implementation of process interventions. CCPs are validated to assure effectiveness, monitored to assure consistent control, and verified to confirm continued operation according to the HACCP plan.

Determination of CCPs. Stevens and Bernard (61) have provided an illustrated example of a decision tree to aid food production facilities in the determination of appropriate CCPs for a HACCP plan (Figure 1). In the case of a beef slaughter process,

TABLE 1. Escherichia coli *performance criteria (69)*

Slaughter Class	**Acceptable Range**	**Marginal Range**	**Unacceptable Range**
Cattle	Negative	< 100 CFU/cm^2	> 100 CFU/cm^2
Poultry	≤ 100 CFU/ml	100-1000 CFU/ml	> 1000 CFU/ml
Swine	≥ 10 CFU/cm^2	10-10,000 CFU/cm^2	> 10,000 CFU/cm^2

TABLE 2. Salmonella *performance standards (69)*

Slaughter Class	% Positive	# of Samples	Max. # of Positives
Steers/Heifers	1.0	82	1
Cows/Bulls	2.7	58	2
Ground Beef	7.5	53	5
Hogs	8.7	55	6
Broilers	20.0	51	12
Ground Chicken	44.6	53	26
Ground Turkey	49.9	53	29

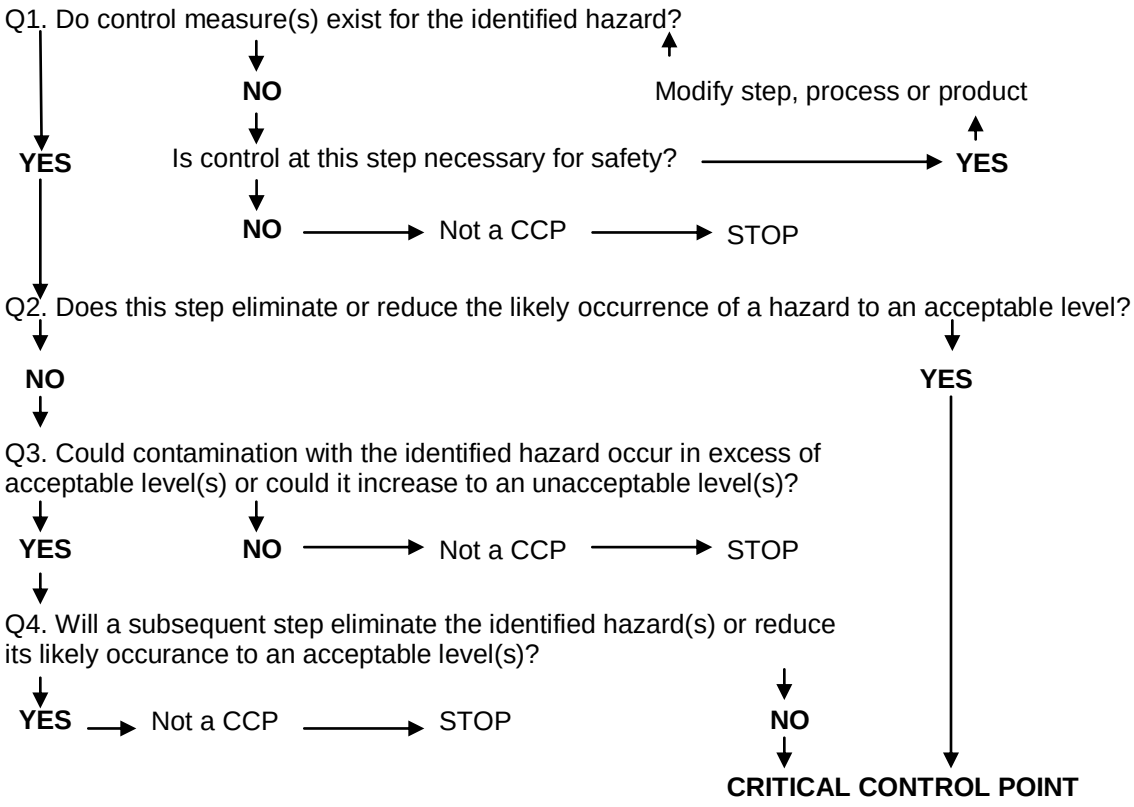

FIGURE 1. *Critical Control Point (CCP) decision tree example. (61)*

the decision tree could be use to evaluate the appropriateness of assigning a CCP to evisceration. Control measures do exist for the identified biological hazard (Q1), but this step does not eliminate or reduce the likely occurrence of biological hazard to an acceptable level (Q2). Although, contamination with biological hazards can occur in excess of acceptable levels (Q3), a subsequent intervention step will eliminate or reduce its likely occurrence to an acceptable level (Q4). Therefore, evisceration is not an effective CCP for biological hazards. If a beef slaughter facility has implemented a carcass decontamination treatment then it could be classified as a CCP because this control measure exists for the biological hazard (Q1), and the step does eliminate or reduce the likely occurrence of a hazard to an acceptable level (Q2).

Beef carcass decontamination

Much research has been published on the development and implementation of carcass decontamination treatments for the removal of visible fecal contamination as well as enteric pathogens associated with feces. USDA-FSIS states that all visible feces, ingesta, and milk must be physically removed by knife trimming, although under new policy USDA-FSIS will permit the use of vacuuming beef carcasses with hot water or steam as an alternative (68). Therefore, a variety of physical, chemical, and thermal decontamination interventions are listed and approved by USDA-FSIS to be used in conjunction with knife trimming or hot water/steam vacuuming for removal of feces and bacterial contamination (64). Approved additional interventions are as follows: (1) a pre-evisceration system which consists of a water rinse, followed by a rinse with an

organic acid rinse; (2) organic acid treatment; (3) chlorinated water; (4) trisodium phosphate; (5) hot water or steam; or (6) air or steam. According to a survey conducted by Boleman et al. (10), knife trimming (93.3%), water washes (77.6%), and organic rinses (46.1%) were the most commonly used methods in 233 slaughter plants surveyed. Bacteria of fecal origin are not necessarily confined to areas of visible fecal material contamination (1).

Carcass water rinses. The effectiveness of using water washes at various temperatures as a means of reducing bacteria on beef carcass surfaces has been reported in numerous studies (5, 13, 14, 28, 29, 36). Important factors to consider for implementing an effective water wash include water temperature, volume or application time, pressure, nozzle type, and distance of the nozzle to the carcass surface. Several studies have demonstrated that a significant reduction in bacterial numbers does not occur from the application of cold or warm water as a decontamination method (8, 29, 42, 56). They also reported that instead of destroying vegetative cells or removing them from the carcass, bacteria were redistributed to other regions of the carcass. Patterson et al. (49) conducted an early study where cattle carcasses were washed with water at 80-96°C for 2 min, significantly reducing total bacterial contamination on the carcass surface. Smith and Graham (58) inoculated beef and mutton carcasses and destroyed 99% of *E. coli* and *Salmonella* ($10^{6.5}/cm^2$) by pouring hot water (80°C) onto the surface for 10 s. In a similar study by Castillo et al. (14), beef carcass surface regions were inoculated with rifampicin-resistant strains of *Salmonella* Typhimurium and *E. coli*

O157:H7 and treated with a hot water (95°C) spray. The carcass regions were treated at various times after inoculation (5 min and 20-30 min), and significant reductions were documented for pathogens, aerobic plant count (APC), and coliforms for all treatments with little difference in reduction between each treatment. Barkate et al. (5) determined that applying hot water (95°C) to beef carcasses for 40 s resulted in significant reductions in bacterial numbers. In this study, the surface of the carcass increased to 82°C in the first 30 s and remained at that temperature for 10 s. Hot water used in these studies was found to have a bactericidal effect by destroying and inactivating the bacteria, but also served as a wash to physically remove some bacteria prior to attachment to the carcass surface. Advantages of using hot water as a carcass surface treatment include a lower cost compared to chemical interventions, and water is not as destructive to the equipment as some chemicals are. Hot water was documented in these studies to cause some initial discoloration of the carcass surface, but the appearance returned to normal within 24 h.

Carcass treatment with organic acid. Various studies have demonstrated the efficacy of organic acid application for red meat (3, 13, 33, 51, 59, 60). Castillo et al. (13) demonstrated that warm (55˚C) 2% lactic acid alone or in combination with hot water (95˚C) effectively reduced *S.* Typhimurium, *E. coli* O157:H7, APCs, *Enterobacteriacea*, total coliforms, thermotolerant coliforms, and generic *E. coli* on inoculated hot carcasses. Results from treatment with lactic acid demonstrated a reduction in mean log reduction ranges of 4.6 to > 4.9 log CFU/cm^2. A study by

Dormedy et al. (23) using a 2% lactic acid solution demonstrated significant reductions in APC, coliform, and generic *E. coli* counts by1 log. Gill and Badoni (27) conducted an in-plant evaluation using various organic acid interventions. The 4% lactic acid resulted in reductions of all bacterial counts ≥ 2 log units at distal surfaces, but ≤ 2 log units at medial surfaces.

Multi-hurdle interventions. Additional studies have demonstrated the added effect of combined treatments of water washes and organic acids (4, 13, 31, 33, 50, 60). Samelis et al. (55) found that rinsing carcass with non-acid water washings at 10°C sensitizes *E. coli* O157:H7 to organic acids.

HACCP reassessment

Research conducted by Smith et al. (57) and Elder et al. (24) reported that *E. coli* O157:H7 was likely more prevalent on beef carcasses than initially reported, due to new tests that provided greater sensitivity. In light of this newly collected data, in the last quarter of 2002, USDA-FSIS issued a notice, "Instructions for Verifying *E. coli* O157:H7 Reassessment," reminding slaughter establishments that all CCPs we required to be validated to ensure successful prevention, elimination or reduction of *E. coli* O157:H7 to below detectable levels (67). The regulatory agency indicated that until establishments collected data to demonstrate that CCPs functioned properly under actual in-plant conditions, the effectiveness of the CCP would be considered theoretical and not validated. USDA-FSIS also noted that many establishments had not validated CCPs

based upon actual in-plant conditions. The beef industry as well as federal and state regulators are in agreement regarding the need to properly validate and verify carcass decontamination critical control points, but confusion exists regarding the most effective and economical method for validation (67).

In 2007, USDA-FSIS issued notice for reassessment of *E. coli* O157:H7 control (66). Due to developments involving *E. coli* O157:H7 in beef products, questions were raised regarding interventions and controls that beef operations were employing. The notice stated that operations were required to reassess their HACCP plan to determine necessary changes and complete an online checklist on how each establishment addresses *E. coli* O157:H7. Included in the reassessment, every establishment was required to validate their HACCP plan's adequacy in controlling the hazard identified during the hazard analysis, and provide verification that the plan is being effectively implemented. Reassessment of the HACCP plan's accuracy was required to occur at least annually, and whenever any changes in beef operations occur.

In its decision, USDA-FSIS cited the following developments to support a need for establishments to reassess HACCP plans. From 2002-2006, the percent positive rate for *E. coli* O157:H7 steadily decreased from 0.787% to < .200%. In 2007, however, the first increase in percent positive rate was recorded at 0.208%. During an atypical short period of time in July 2007, 5 positive samples occurred within 3 days. Furthermore, through October 6, 2007, the total number of recalls associated with *E. coli* O157:H7 increased significantly compared to the prior year. A total of 29 million lbs of product was recalled from 13 incidents, compared to 8 total recalls effecting 200,000 lbs of

product in 2006. The final point of emphasis leading to the decision by USDA-FSIS is that certain source materials used in the production of ground beef have repetitively tested positive for *E. coli* O157:H7.

After the plant's completion of the reassessment checklist attachment, USDA-FSIS is planning to (1) identify those beef operations that are not employing certain interrelated practices; (2) capture production practices used by the establishment to control *E. coli* O157:H7, and to identify vulnerabilities; (3) help prioritize whether and when a Food Safety Assessment (FSA) should be conducted; and, (4) ascertain which establishments to target for more frequent testing.

MATERIALS AND METHODS

Description of selected plants

Samples were collected at two separate slaughter facilities (Facility A and Facility B) in Texas. Facility A was a large federally inspected commercial beef slaughter facility (≥ 500 employees), and Facility B was a small state inspected commercial beef slaughter facility (≥ 10 but ≤ 500 employees). Facility A utilized a hot water wash (> 70°C at the nozzle), subsequently followed by a warm 3.5% L-lactic acid spray (> 45°C at the nozzle). Both interventions at Facility A utilized a fully automated system for mixing, heating, and spraying the carcasses. Facility B manually mixed and applied only a warm (55°C) 2% L-lactic acid solution for the decontamination of its carcasses.

Custom-made insulated sprayer

Facility B utilized a generic garden chemical sprayer in their facility to apply the lactic acid solution, but the one used was not insulated and did not maintain the temperature of the warm lactic acid solution at 55°C. Therefore, an insulated chemical sprayer was constructed to help maintain the temperature of the lactic acid solution at 55°C for an extended period of time. All supplies for insulating the hand sprayer were purchased at a local hardware store. The insulation was constructed from DAPtex® brand canned latex-based multi-purpose insulating foam sealant (DAP® Inc., Baltimore, MD). The foam was applied to the surface of a 6-liter garden hand-sprayer (RL

Flomaster, Wal-Mart Stores, Inc., Bentonville, AR) layer by layer to a total diameter of approximately 2 ft. Using a knife, the foam was trimmed until the sprayer would fit tightly into a 5-gal plastic paint bucket. A hole was cut in the lid of the paint bucket just large enough to fit over the top of the sprayer. In order to prevent liquids from entering the paint bucket, DAP® silicone based aquarium sealant was used to seal the gap between the lid and the top of the sprayer (DAP® Inc.).

Comparison of different insulated sprayers

Tests were conducted to compare the effectiveness of the insulated sprayer to maintain the temperature of the 2% L-lactic acid for an extended period of time versus a non-insulated sprayer and another sprayer insulated by an insulation blanket held on by tape. Three 6-liter batches of 2% L-lactic acid were heated to approximately 60°C and the temperature of each sprayer was monitored every 15 min for 4 h using a Traceable® Total-Range Thermometer (VWR International, West Chester, PA) until the final sprayer temperature was below 55°C. The solution temperatures in the foam-insulated, blanket-insulated, and non-insulated sprayers fell below 55°C after 3.50, 1.75, and 0.50 h, respectively. Therefore, the foam-insulated sprayer with was selected for this study.

Inoculum preparation

Fresh feces samples were collected from the animal holding pens upon arrival at the slaughter facility, using a spatula to transfer feces into a 1-gal Ziplock® bag. 10 g of feces were transferred from the Ziplock® bag into each of 12 sterile (177 x 304 mm)

stomacher bags (Seward Limited, London, UK). 10 ml of sterile buffered peptone water (BPW) (International BioProducts, Redmond, WA) was added and the inoculum was hand-kneaded for 1 min to mix.

Carcass selection and surface inoculation

Ten carcass sides were selected randomly to serve as non-inoculated controls. At Facility A, in order to minimize the risk of cross-contamination from the inoculated carcass sides, the final 10 carcass sides of the day were inoculated with a fecal slurry on the neck region. On each sampling day at Facility B, 5-10 head of cattle were slaughtered, complicating any random selection of carcass sides. Therefore, the first 10 sides available on each sampling day were used for both inoculating the neck region and for sponge sampling the non-inoculated briskets. The neck region was chosen as the inoculated area to minimize the likelihood of contamination to the rest of the carcass during treatment. Inoculation was carried out by turning the stomacher bag containing the fecal slurry inside-out, allowing the fecal slurry to be transferred onto the surface of the carcass. The stomacher bag was then used as a glove to spread the feces across a 400-cm^2 surface area. Immediately after inoculation, the neck region was rinsed for approximately 10 s (200 ml) using a multipurpose hand sprayer filled with room temperature water (~24°C) to remove the gross excess fecal material. A large plastic collection bucket was placed under the inoculated neck region to collect run-off during inoculation and rinsing, as well as during application of treatment, to prevent splash contamination of the plant floor.

Carcass surface treatments

Facility A reported parameters of the hot water spray cabinet to include a 10-s application of approximately 88°C water at a minimum pressure of 20 lb in^2. The hot water was subsequently followed by a warm 3.5% L-lactic acid spray applied for 6 s at 55°C with a minimum pressure of 20 lb in^2. Both the hot water and lactic acid at Facility A were applied to each carcass side individually utilizing a custom-made automated spray cabinet. The carcass sides passed along a rail system where visible fecal matter was trimmed from the carcass, followed by a brief high pressure spray wash applied by hand using a water hose with an attached high pressure nozzle. The carcass sides then passed through a cabinet that contained multiple sequences of nozzles which drenched the carcass on both sides with a hot water spray (88°C) at 20 lb in^2. The 3.5% L-lactic acid was applied immediately following the hot water treatment and prior to chilling. The lactic acid cabinet differed from the hot water cabinet in that a single pair of nozzles sprayed the acid onto the carcass from both sides. Hot water spray temperatures were recorded at the nozzle, as well as the temperature of the carcass surface before and after treatment using a Traceable® Total-Range Thermometer (VWR International). Data was also collected on the temperature and pH of the 3.5% L-lactic acid spray, as well as the carcass surface pH using the above-mentioned thermometer and a Markson Model 612 portable pH meter (Markson LabSales, Honolulu, HI).

Only one carcass contamination treatment was utilized by Facility B. Six liters of a plant-specified 2% L-lactic acid solution was prepared by diluting 88% L-lactic acid with tap water obtained from the plant's water system. The plant hot water source was

consistently in excess of 70°C; therefore, cold water was added to adjust the temperature to 55-60°C before addition of lactic acid. The solution was then transferred into the custom-made insulated chemical sprayer previously described. The 2% L-lactic acid was applied to each carcass side for 20 s, at a volume of 500 ml/carcass side and at 20 lb in^2 (10 s for necks, at 200 ml). Data was collected on the temperature and the pH of the solution, the spray at the nozzle, and the carcass surface using the same thermometer and pH meter previously mentioned.

Microbiological sampling

Sponge samples were collected from 400 cm^2 of the brisket region of 10 randomly selected non-inoculated carcasses at Facility A before the hot water wash treatment. Post-hot water wash and post-3.5% L-lactic acid spray sponge samples were collected from the same 10 non-inoculated carcasses. With the low volume of production for Facility B, the same 10 carcass sides were sampled both before and after the 2% L-lactic acid spray treatment with special care taken not to sample the same area twice.

A BioPro Sampling System (International BioProducts) was used to sample a 400-cm^2 surface area from the brisket of each carcass side. The BioPro Sampling System consisted of a sterile dehydrated sponge inside a sterile re-sealable 18-oz Whirl-Pak® sampling bag, and attached to each sampling bag was a removable compartment containing 2 sterile polyethylene gloves. Before entering the plant, 25 ml of Butterfields buffer diluent (International BioProducts) was added to each bag containing a sponge in

order to pre-moisten it, and immediately prior to sampling the excess diluent was squeezed from the sponge. Utilizing the provided sterile gloves, the sponge was carefully removed from its bag and was rubbed over a 400-cm^2 surface area of the brisket 10 times horizontally and 10 times vertically. The sponge was flipped over and rubbed 10 times diagonally over the same 400-cm^2 surface area and then returned and sealed in its original bag. An additional 25 ml of Butterfields buffer diluent (International BioProducts) was then added to each sponge sample to make a total volume of 50 ml for each sample.

At Facility A, pre-hot water wash carcass surface samples (10 cm^2 x 2 mm) were excised from the inoculated area with a flame sterilized scalpel (Becton Dickinson Labware, Franklin Lakes, NJ) and forceps and then placed in 24-oz NASCO Whirl-Pak® bags (International BioProducts). For the inoculated samples, a method of excision was utilized to improve accuracy of recovering higher levels of inoculated bacteria. After the carcasses received the hot water spray treatment, the slaughter line was halted and post-hot water/pre-lactic acid samples were collected. Next, the carcasses were treated with a 3.5% lactic acid spray, and the final sample was excised as described before. Before the inoculated carcasses entered the cooler, the inoculated neck tissue was trimmed and discarded to prevent contamination of the cooler or any other carcasses.

The inoculated neck samples from Facility B were collected in the same manner as in Facility A; however, since Facility B only utilized a 2% L-lactic acid spray treatment, samples were only obtained twice, before and after treatment.

Sample bags containing sponge samples were placed in a Ziplock® bag, and Whirl-Pak® bags containing the inoculated tissue samples were placed into a separate Ziplock® bag. Samples were packed in a Freezsafe® insulated cooler (Polyfoam Packers Corp., Wheeling, IL) containing UTEK® +30°F frozen refrigerant packs (360 ml and 1500 ml) (VWR Scientific, Suwanee, GA). The Freezsafe® insulated cooler was packed with 1 layer of refrigerant packs on the bottom of the cooler and a layer of cardboard on top. The samples were wrapped in newspaper and placed on top of the cardboard layer. Preliminary tests indicated that the newspaper would prevent samples from freezing, which could affect the accuracy of the microbiological samples. A second layer of cardboard was placed on top of the samples followed by more refrigerant packs. The Freezsafe® insulated cooler containing the samples was then transported to the food microbiology laboratory (Texas A&M University, College Station, TX) where samples were removed for analysis.

Microbiological analysis

The samples were stored for 24 h at 4°C prior to analysis. Each non-inoculated sponge sample was hand massaged for 1 min. Ninety-nine ml of 0.1% sterile peptone water (Difco Laboratories, Detroit, MI) was added to each excised sample; then, samples were stomached for 1 min using a Tekmar® Stomacher Lab-Blender 400 (Tekmar Co., Cincinnati, OH). The additional 25 ml of Butterfield's buffer diluent (International BioProducts) previously added to each sponge sample was then separated and dispensed into Falcon Blue Max™ 50 ml polystyrene conical tubes (Becton Dickinson Labware).

The tubes were placed at 4°C to be used for confirmation following any samples that were found to be presumptively positive for *Salmonella*. For completion of the validation process, the samples were analyzed using various methods.

Mesophilic plate counts (MPC) were obtained from the non-inoculated sponge samples by plating on Aerobic Count Plate (APC) Petrifilm® (3M Microbiology Products, St. Paul, MN). APC Petrifilm® consisted of a polyethylene coated paper card with a yellow grid (~10 x 8 cm^2) printed on the surface and standard methods nutrients held together with an adhesive. Attached to the top of each card was a peelable propylene protective film with an indicator dye and cold water soluble gels adhered to it. The indicator dye in the APC Petrifilm® was 2,3,5-triphenyltetrazolium chloride (TTC) which gave the colonies a red color for visualization. A 1-ml portion was drawn out of the sponge sample using a sterile pipette as the 0 dilution. The propylene film was lifted, the inoculum was dispensed onto the middle of the first APC Petrifilm®, and the film was gently rolled down to cover the inoculum. A spreader provided by 3M was placed over the film with the inoculum centered, and was gently pressed onto the card to spread the sample on the plate to cover an area of 20 cm^2. An additional 1-ml portion of the sponge sample was transferred into 9 ml of 0.1% sterile peptone water (Difco Laboratories). Serial dilutions 1-5 were then performed following the same technique. Prior to incubation, the Petrifilm® were allowed to sit for a few minutes to allow the re-hydrated cold water soluble gels set and solidify. The APC Petrifilm® were placed in the incubator face up. Following 24 h of incubation at 37°C, the plates were removed from

the incubator, and all red colonies on each APC Petrifilm® were counted using a Darkfield Quebec® colony counter (AO, American Optical, Keene, NH).

E. coli/coliform Petrifilm® (3M Microbiology Products) were utilized to achieve counts for both the non-inoculated sponge samples and the inoculated excise samples. *E. coli*/coliform Petrifilm® consisted of a polyethylene coated paper card with violet red bile (VRB) nutrients held together by an adhesive. The crystal violet and bile salts from the VRB nutrients inhibit Gram-positive growth, the neutral red was used as a pH indicator, and the lactose was utilized for fermentation, resulting in acid and gas production. Attached to the top of each card was propylene protective film with indicator dyes and cold soluble gels adhered to it. There were 2 indicator dyes present, 5-bromo-4-chloro-3-indolyl-β-D-glucoronide and TTC. The 5-bromo-4-chloro-3-indolyl-β-D-glucoronide was an indicator of the β-glucuronidase activity of *E. coli* that gave positive colonies a blue color. The coliforms only utilized the TTC to exhibit a red color. Dilutions 0-5 on the *E. coli*/coliform Petrifilm® were prepared for the non-inoculated sponge samples, and dilutions 1-6 were prepared for the inoculated excised samples. For each plate, the film was lifted from the plate, 1 ml of inoculum was transferred to the plate, just above center, and the film was gently rolled down over the inoculum with special care taken not to create any bubbles under the film. Dilutions were prepared the same manner as described above for both sets of samples by transferring 1 ml into 9 ml of 0.1% sterile peptone water, followed by inoculation of each plate. The flat side of the provided spreader was gently pressed on the card to evenly spread the inoculum across an area of 20 cm^2. Each plate was allowed to sit for a

few min to let the re-hydrated cold water soluble gels solidify before incubation. The Petrifilm® were placed in the incubator face up, and after 24 h of incubation at 37°C, the *E. coli*/coliform Petrifilm® were counted. *E. coli* colonies were identified by their characteristic blue colonies with associated gas bubbles, and the coliform colonies were identified by their characteristic red colonies with associated gas bubbles. Any colonies of either color lacking a gas bubble were ignored in the counting.

Salmonella **screening (non-inoculated samples)**

The non-inoculated sponge samples were screened for the presence of *Salmonella*. USDA-FSIS outlined a procedure for screening beef samples for *Salmonella* by using a 3-step enrichment process: pre-enrichment, selective enrichment, and post-enrichment (21). 225 ml of 1.0% sterile peptone water (Difco) was added to the remaining 20-ml sponge sample as a pre-enrichment for *Salmonella*. After 24 h of incubation at 37°C, 0.5 ml of the pre-enriched sample was transferred into 10 ml of Tetrathionate Broth (TT, Difco), and an additional 0.1 ml of the pre-enriched sample was transferred into 10 ml of modified Rappaport Vassiliadis Broth (mRV, Difco). Both sets of selective enrichment media were incubated in a Magni Whirl constant temperature water bath (Blue M, Blue Island, IL) at 42°C for 24 h. Following incubation, 0.5 ml from the TT Broth was transferred into 10 ml of M Broth (Difco), and 0.5 ml from the mRV Broth was transferred into another M Broth tube. Both tubes were incubated at 42°C for 6 h. After the final enrichment step, the samples were screened using the TECRA® *Salmonella* Visual Immunoassay (International BioProducts). This

enzyme-linked immunosorbant assay (ELISA) kit contained all solutions and reagents needed for performing the test. 0.5 ml of each of the post-enrichment M Broth tubes (TT and mRV) were combined into 5-ml Falcon polystyrene round-bottom tubes (12 x 75 mm) (Becton Dickinson Labware). The samples were boiled for 15 min to inactivate any *Salmonella* present. After boiling, 200 µl of each sample was transferred using an Eppendorf® *Reference* 200-µl micropipette (VWR International) and sterile micropipette tips into corresponding ELISA Removawells® containing the primary polyclonal antibody specific for *Salmonella*. A new sterile micropipette tip was used for each sample to prevent cross contamination. The wells were then covered by a piece of Parafilm® (American National Can™, Chicago, IL) to reduce evaporation and incubated at 37°C for 30 min. This incubation sped up the reaction of the antibody to bind to the specific epitope of the *Salmonella* antigen. Each well was individually rinsed with a sterile buffered rinse solution 3 times by filling each well with the rinse solution and then expelling the solution from the well with special care given to removing all remaining bubbles inside the wells. 200 µl of an enzyme-labeled secondary antibody (conjugate) was added to all sample wells, re-covered with Parafilm®, and incubated at 37°C for another 30 min to allow attachment of the antigen to the antibody. A final rinse step was used to remove any excess conjugate by rinsing 4 times with the same solution. Finally, 200 µl of the substrate was added to each well and incubated at room temperature (~24°C) for 10 min. Any *Salmonella* positive samples resulted in a color change to green from the enzyme-substrate reaction and results were recorded.

Confirmation of positive *Salmonella* isolates

All presumptive positive *Salmonella* isolates that were detected from the immunoassay were confirmed by using the method outlined in the Bacteriological Analytical Manual (BAM) for the isolation of *Salmonella* (19). A sample from each corresponding positive M Broth tube was transferred using a Puritan® sterile cotton tipped applicator (Hardwood Products Company LLC, Guilford, ME) onto Xylose-Lysine-Deoxycholate Agar (XLD), Hektoen Enteric Agar (HE), and Bismuth Sulfite Agar (BS, Difco). The transfer was followed by streaking individual colonies for isolation using a flame sterilized inoculating loop, and the plates were incubated for 24 h at 37°C. Isolated typical and atypical colonies for *Salmonella* on each plate were selected and streaked onto Tryptic Soy Agar (TSA) slants (Difco) and incubated at 37°C for 24 h. Typical colonies for each agar are as follows; (1) XLD: pink colonies with or without black centers, (2) HE: blue-green to blue colonies with or without black centers, (3) BS: brown, gray, or black colonies, sometimes having a metallic sheen or a brown or black halo. Atypical colonies for each agar are as follows; (1) XLD: yellow colonies with or without black centers, (2) HE: yellow colonies with or without black centers, (3) BS: green colonies with little or no darkening of the surrounding medium.

Following incubation of the TSA slants, each isolate was tested for Gram reaction, oxidase, and catalase. A smear was prepared for each isolate by placing a small drop of distilled water onto a microscope slide. Using a sterile inoculating loop, a small amount of the culture was transferred to the drop of water, and smeared across the slide. The smear was allowed to air dry and then heat fixed to the slide by passing it

through a flame 3 times. The Gram staining procedure was conducted by drenching with crystal violet (Fisher HealthCare, Houston, TX) for 45 sec followed by a distilled water rinse; addition of Gram iodine (Fisher HealthCare) for 45 sec and rinsed again with distilled water; decolorized by flooding the stained smear with a decolorizer (Fisher HealthCare) for 2-3 sec; counter stained with safranin for 45 sec and then rinsed with distilled water. After each slide dried, the stained smears were microscopically examined for its Gram reaction (Gram-positive = purple cells, negative = red or pink cells). Pathotec® Cytochrome Oxidase strips (Remel, Lenexa, KS) were used to test for the oxidase characteristic of the presumptive *Salmonella* isolates by inoculating each test strip and a color change to deep blue was recorded as a positive reaction. Catalase reactions were carried out by placing a drop of 3% hydrogen peroxide (EM Science, Gibbstown, NJ) to a microscope slide with a small amount of each culture and any bubble formation observed was recorded as a positive reaction.

All Gram-negative, oxidase negative, catalase positive, rods were then streaked onto Triple Sugar Iron Agar (TSIA), Lysine Iron Agar (LIA), and Urea Agar slants (Difco) and incubated at 37°C for 24 h. In TSIA, *Salmonella* produces alkaline (red) slants with acid (yellow) butts, with or without blacking of agar from hydrogen sulfide production. In LIA, *Salmonella* typically produces alkaline (purple) throughout the tube, and most produce H_2S. On Urea Agar, *Salmonella* results in no color change (a positive result would be to pink). The isolates characteristically positive for *Salmonella* were then confirmed by biochemical tests using an industrial VITEK® automated *in vitro* testing system (BioMérieux, Hazelwood, MO). For VITEK® confirmation, each isolate

was streaked onto TSA + 5% sheep blood plates (Difco), and incubated for 24 h at 37°C. For each sample, 2 ml of a 0.45% sodium chloride inhalation solution (Allegiance Health Care Corporation, McGaw Park, IL) were added to 5-ml Falcon polystyrene round-bottom tubes (Becton Dickinson Labware). Sterile cotton tipped applicators (Hardwood Products Company LLC) were used to transfer 2-3 colonies from each sample into each corresponding tube to reach a McFarland turbidity standard of 1.0. The turbidity was determined by placing each tube into a calibrated VITEK® electronic colorimeter (BioMérieux) which measures the amount of light that passes through the sample. Light transmittance of 67-77% (blue region) is the equivalence of 1.0 McFarland standard. VITEK® Gram-Negative Identification (GNI+) cards (BioMérieux) were labeled with each corresponding sample number. Each card and associated sample tube was placed into a provided holder, and a transfer tube was used to link the card to the sample tube that the VITEK® utilized to draw the sample from the tube into the card. Each card was inserted into the VITEK® card reader, and the program was initiated. After approximately 24 h, the results were obtained, and confirmed *Salmonella* isolates were then re-streaked onto TSA slants. Confirmed isolates were then sent to the National Veterinary Services Laboratories (NVSL, Ames, IA) for serological testing.

Enumeration of positive *Salmonella* isolates

Simultaneous to the confirmation of *Salmonella* isolates, the previously separated and stored 25 ml of each positive screened sample held in refrigeration was used to

inoculate a most probable number (MPN) series following the procedure outlined in the USDA-FSIS Microbiology Laboratory Handbook (MLG) (22). Samples were inoculated into the same pre-enrichment, selective enrichment, and post-enrichment media as previously described for the screening process. A 3-tube x 4-dilution MPN procedure was utilized for each enrichment. For the first dilution, 1 ml of the sample was transferred into each of the first 3 test tubes containing 9 ml, 1.0% sterile peptone water for pre-enrichment. For the second dilution, 0.1 ml was transferred from the sample into each of the next 3 test tubes of 1.0% sterile buffered peptone water. Each stored sample was diluted by transferring 10 ml from the sample into 90 ml of 0.1% sterile peptone water, and this 100-ml dilution was used to inoculate the third and fourth dilutions in the same manner as dilutions 1 and 2. After 24 h of incubation at 37°C, each sample was transferred into selective enrichment. A 0.5 ml portion of each dilution of pre-enriched sample was transferred into a corresponding 3 x 4 test tube set containing 10 ml of TT Broth, and an additional 0.1 ml of each pre-enriched sample was transferred into another corresponding 3 x 4 test tube set containing 10 ml of mRV Broth. The samples were incubated for 24 h in a 42°C Magni Whirl constant temperature water bath (Blue M). After incubation, 0.5 ml of each TT Broth tube was transferred into a corresponding tube of the final 3 x 4 test tube set containing 10 ml of M Broth tube and incubated for an additional 6 h in the same 42°C water bath as previously described. The same was done for each mRV Broth tube. After the post-enrichment in the M Broth, each tube was screened using the TECRA® *Salmonella* Visual Immunoassay

using the same techniques described above. The MPN Tables provided in the MLG were compared to the positive samples to obtain quantification data on the *Salmonella*.

Statistical analysis

The analysis of variance (ANOVA) procedure in SAS® (Statistical Analysis Systems Institute, Inc., Cary, NC) was used to identify significant differences in mean reductions of both the non-inoculated (log_{10}/400 cm^2) and inoculated (log_{10}/cm^2) samples. Microbiological count data was recorded in a Microsoft® Excel (Redmond, WA) spreadsheet which was used to transform counts into logarithms, and these logarithmic values were then used to calculate the reduction values by subtracting the log count post-treatment from the log count pre-treatment. When significant differences ($P < 0.05$) among means were indicated, mean separation was accomplished using Duncan's multiple range test, as it is customarily used in agricultural research. To facilitate the statistical analysis of these data, samples with bacterial counts below the minimum detection level were given a value of 1.4 log_{10}/400 cm^2 for non-inoculated sponge samples or a value of 0.7 log_{10}/cm^2 for inoculated neck samples. These were the values between 0 and the minimum detection level (1.7 log_{10}/400 cm^2 for non-inoculated sponge samples, and 1.0 log_{10}/cm^2 for inoculated neck samples) of the counting method.

RESULTS AND DISCUSSION

Validation results at Facility A

The objective of the initial phase of this research was to validate the antimicrobial interventions at a large beef slaughter facility (Facility A). USDA-FSIS classifies large establishments as employing ≥ 500 employees.

The antimicrobial strategy utilized by Facility A included a post-evisceration hot water spray application followed by a pre-chill lactic acid spray treatment. Hot water was applied directly to each carcass side via automated spray cabinet for a 10-s exposure time at a temperature of > 70°C recorded at the nozzles. Immediately following the hot water cabinet, each carcass was then treated with an L-lactic acid solution in an automated spray cabinet. A 3.5% L-lactic acid solution was applied as a warm solution (> 45°C recorded at the nozzles) for a 6-s dwell time. Average carcass surface temperatures during hot water treatment for three replications were 74°C, 78°C, and 71°C, respectively. Mean carcass surface pH values immediately after lactic acid sprays were consistently recorded at 3.0 for all 3 replications. The plant's fast paced processing speed (~150 head per h) did not accommodate for utilizing the same carcass side for both non-inoculated sponge samples (brisket) and inoculated excision samples (neck). Therefore, a separate group of 10 carcasses was selected for inoculated excision samples.

Hot water wash (non-inoculated samples). The hot water carcass spray was found to significantly ($P < 0.05$) reduce MPCs as well as counts of *E. coli* and coliforms for all 3 sample replications. Mean MPC $\log_{10}$/400 cm^2 reductions with the hot water spray for replications 1, 2, and 3 were 1.8 (Table 3). Reductions for the 3rd replication were smallest, possibly due to lower temperature of the hot water applied compared to replications 1 and 2. In a study conducted by Barkate et al. (5), significant reductions in APC were achieved by applying hot water (95°C) to beef carcasses with sufficient dwell time to achieve a surface temperature of 82°C. Although the carcass temperature produced by the hot water wash at Plant A did not reach a similar temperature, the fluctuations in recorded temperature over all three replicates corresponded with the fluctuations in average reductions. (highest temp = greatest reduction, lowest temp = lowest reduction). Mean coliform $\log_{10}$/400 cm^2 reductions were 1.4 (Table 4), while mean *E. coli* $\log_{10}$/400 cm^2 reductions for replications 1, 2, 3 were 1.4 (Table 5).

Lactic acid treatment (non-inoculated samples). The lactic acid carcass spray during replications 1 and 2 provided no significant reductions ($P > 0.05$). However, MPCs and coliforms were significantly ($P < 0.05$) reduced in replication 3. Mean MPCs $\log_{10}$/400 cm^2 reductions for replication 3 with the lactic acid were 2.1 (Table 3). Mean coliform $\log_{10}$/400 cm^2 reductions were 1.4 (Table 4), while *E. coli* $\log_{10}$/400 cm^2 saw similar trends (Table 5). In most cases, the hot water cabinet reduced counts to below the detection level, preventing a subsequent validation of the lactic acid cabinet. Significant ($P < 0.05$) reductions in MPC's and coliforms caused by the lactic acid

TABLE 3. *Facility A: Mean mesophilic plate count populations and mean log reductions from beef carcass brisket regions at various processing locations following intervention treatments.*

	Mean Populations (log CFU/400 cm^2)[a]			**Mean Reductions (log CFU/400 cm^2)[b]**		
	A[d]	**B[d]**	**C[d]**	**HW[e]**	**LA[e]**	**Combined**
Replication 1[c]	3.9A	≤ 2.3B	≤ 1.7C	≥ 1.6	≥ 0.6	≥ 2.4
Replication 2[c]	5.1A	≤ 2.6B	≤ 1.7C	≥ 2.5	≥ 0.9	≥ 3.7
Replication 3[c]	5.1A	3.8B	≤ 1.7C	1.3	≥ 2.1	≥ 3.4

[a]Detection Limit = 1.7 $\log_{10}$ CFU/400 cm^2.

[b]Log reduction = ($\log_{10}$ CFU/400 cm^2 before treatment) - ($\log_{10}$ CFU/400 cm^2 after treatment).

[c]Means with different letters in the same row are significantly different ($P < 0.05$).

[d]A = Pre-Hot Water, B = Post-Hot Water, C = Post-Lactic Acid.

[e]HW = Hot Water, LA = Lactic Acid.

TABLE 4. *Facility A: Mean coliform populations and mean log reductions from beef carcass brisket regions at various processing locations following intervention treatments.*

	Mean Populations (log CFU/400 cm^2)[a]			**Mean Reductions (log CFU/400 cm^2)[b]**		
	A[d]	**B[d]**	**C[d]**	**HW[e]**	**LA[e]**	**Combined**
Replication 1[c]	$\leq 2.9_A$	$\leq 1.7_B$	$< 1.7_B$	≥ 1.4	≥ 0.0	≥ 1.5
Replication 2[c]	3.5_A	$\leq 1.8_B$	$< 1.7_B$	≥ 1.7	≥ 0.1	≥ 2.1
Replication 3[c]	$\leq 4.1_A$	$\leq 2.8_B$	$< 1.7_C$	≥ 1.3	≥ 1.1	≥ 2.7

[a]Detection Limit = 1.7 $\log_{10}$ CFU/400 cm^2.
[b]Log reduction = ($\log_{10}$ CFU/400 cm^2 before treatment) - ($\log_{10}$ CFU/400 cm^2 after treatment).
[c]Means with different letters in the same row are significantly different ($P < 0.05$).
[d]A = Pre-Hot Water, B = Post-Hot Water, C = Post-Lactic Acid.
[e]HW = Hot Water, LA = Lactic Acid.

TABLE 5. *Facility A: Mean* Escherichia coli *populations and mean log reductions from beef carcass brisket regions at various processing locations following intervention treatments*.

	Mean Populations (log CFU/400 cm^2)[a]			**Mean Reductions (log CFU/400 cm^2)[b]**		
	A[d]	**B[d]**	**C[d]**	**HW[d]**	**LA[d]**	**Combined**
Replication 1[c]	$\leq$ 2.3$_A$	< 1.7$_B$	< 1.7$_B$	$\geq$ 0.6	$\geq$ 0.0	$\geq$ 0.9
Replication 2[c]	$\leq$ 2.9$_A$	$\leq$ 1.7$_B$	< 1.7$_B$	$\geq$ 1.2	$\geq$ 0.0	$\geq$ 1.5
Replication 3[c]	$\leq$ 3.7$_A$	$\leq$ 1.7$_B$	< 1.7$_B$	$\geq$ 2.0	$\geq$ 0.0	$\geq$ 2.3

[a]Detection Limit = 1.7 $\log_{10}$ CFU/400 cm^2.

[b]Log reduction = ($\log_{10}$ CFU/400 cm^2 before treatment) - ($\log_{10}$ CFU/400 cm^2 after treatment).

[c]Means with different letters in the same row are significantly different ($P < 0.05$).

[d]A = Pre-Hot Water, B = Post-Hot Water, C = Post-Lactic Acid.

[e]HW = Hot Water, LA = Lactic Acid.

treatment during replication 3 may have been due in part to a lower hot water cabinet temperature allowing higher counts to be present prior to acid treatment. In a study by Gill and Badoni (27), bacterial populations were reduced ≥ 2.0 log units on beef quarters treated with a 4% lactic acid solution, thus validating the reductions achieved by the lactic acid treatment at Plant A.

Salmonella *screening (non-inoculated samples).* No positive *Salmonella* samples, either pre- or post- treatment, in replications 1 and 3 were detected using ELISA. Presumptive positives for *Salmonella* were detected in 4 out of 10 pre-hot water samples tested in replication 2, but none for post-hot water or post-lactic acid carcass sprays (Figure 2). The low prevalence of *Salmonella* detected on the cattle was expected as recent data collected by Bosilevac et al. (11) demonstrated that *Salmonella* prevelance in commercial ground beef is also low (4.2%).

Salmonella *confirmation, serotyping, and enumeration*. All isolates from presumptive positive *Salmonella* samples were confirmed and enumeration of the split sample was conducted by the MPN method. Following isolation and confirmation procedures, all 4 presumptive positive samples exhibited typical colony morphologies on the respective agar. Gram stain, oxidase, and catalase results for all 4 isolates were found typical of *Salmonella*. The isolates also produced typical reactions on TSIA and LIA slant agar tubes. Further biochemical testing via industrial VITEK® confirmed with 98-99% confidence that all 4 isolates were *Salmonella* (Table 6) (BioMérieux). All

FIGURE 2. *ELISA results from replication 2 samples at Facility A. The first two rows on the top left represent the two positive and negative controls. The first row of 10 wells correspond with the pre-hot water samples, the second row of 10 wells correspond with the pre-lactic acid samples, and the final row of 10 wells corresponds with the post-lactic acid samples. Pre-hot water sample 2, 4, 6, and 9 are presumptive positive for* Salmonella.

TABLE 6. *VITEK® confirmation results on presumptive positive isolates from Facility A replication 2.*

	Isolate 1[a]	Isolate 2[b]	Isolate 3[c]	Isolate 4[d]	Control[e]
DP3	-	-	-	-	-
URE	-	-	-	-	-
MLT	+	+	+	+	+
INO	-	-	-	-	-
ARA	+	+	+	+	+
OFG	+	+	+	+	+
CIT	-	+	+	+	-
MAN	+	+	+	+	+
ADO	-	-	-	-	-
GLU	+	+	+	+	+
GC	+	+	+	+	-
MAL	-	-	-	-	-
XYL	+	+	+	+	+
COU	+	+	+	+	+
ARG	-	-	-	-	-
ACE	-	-	-	-	-
TDA	-	-	-	-	-
RAF	-	-	-	-	-
H2S	+	+	+	+	+
LYS	+	+	+	+	+
ESC	-	-	-	-	-
PXB	-	-	-	-	-
SOR	+	+	+	+	+
ONP	-	-	-	-	-
ORN	+	+	+	+	+
PLI	-	-	-	-	-
LAC	-	-	-	-	-
SUC	-	-	-	-	-
RHA	+	+	+	+	+
OXI	-	-	-	-	-

[a]Serotype Montevideo (NVSL, Ames, IA)

[b]Serotype Montevideo (NVSL, Ames, IA)

[c]Serotype Typhimurium (NVSL, Ames, IA)

[d]Serotype Typhimurium (NVSL, Ames, IA)

[e]Serotype Typhimurium (NVSL, Ames, IA)

4 confirmed isolates were shipped to the NVSL for serotyping. Results indicated 2 isolates as Montevideo, 1 Kentucky, and 1 Typhimurium. Of the 30 most common serotypes listed by the CDC, Montevideo was listed at number 7, while Typhimurium was listed at number 1 on the list (17). Enumeration of the original samples determined that *Salmonella* was present in 3 of the 4 positives samples at < 15 MPN/400 cm^2, while the remaining sample had 140 MPN/400 cm^2 (Table 7).

Inoculated samples. Hot water carcass sprays significantly ($P < 0.05$) reduced counts of coliforms on inoculated carcass surfaces in replication 1, 2, and 3 with mean log_{10}/cm^2 reductions of 2.9, 2.5, and 1.6, respectively (Table 8). As with non-inoculated samples, the 3rd replication demonstrated a lower reduction, most likely due to lower temperature of the hot water applied. Counts of *E. coli* followed similar trends (Table 9). Hot water treatment demonstrated such high efficacy in reducing inoculated coliforms and *E. coli* in replication 1 and 2 that insufficient levels remained for evaluation of reduction caused by the lactic acid cabinet. A significant ($P < 0.05$) reduction in *E.coli* and coliform counts was found after the lactic acid spray as noted previously for replication 3.

Validation results at Facility B

The objective of the second phase of this research was to validate antimicrobial interventions at a small beef slaughter facility (Facility B). USDA-FSIS classified a

TABLE 7. *Enumeration of confirmed* Salmonella *isolates collected from Facility A in replication 2.*

Isolate	**MPN/400 cm^2**
1 (Montevideo)	<15
2 (Montevideo)	<15
3 (Kentucky)	<15
4 (Typhimurium)	<140

TABLE 8. *Facility A: Mean coliform populations and mean log reductions for inoculated beef carcass neck regions at various processing locations following intervention treatments.*

	Mean Populations (log CFU/cm^2)[a]			**Mean Reductions (log CFU/cm^2)[b]**		
	A[d]	**B[d]**	**C[d]**	**HW[e]**	**LA[e]**	**Combined**
Replication 1[c]	4.8A	≤ 1.9B	≤ 1.9B	≥ 2.9	≥ 0.0	≥ 2.9
Replication 2[c]	4.9A	2.4B	≤ 2.5B	2.5	≥ 0.0	≥ 2.4
Replication 3[c]	5.0A	≤ 3.4B	≤ 2.5C	≥ 1.6	≥ 0.9	≥ 2.5

[a]Detection Limit = 1.0 $\log_{10}$ CFU/400 cm^2.
[b]Log reduction = ($\log_{10}$ CFU/400 cm^2 before treatment) - ($\log_{10}$ CFU/400 cm^2 after treatment).
[c]Means with different letters in the same row are significantly different ($P < 0.05$).
[d]A = Pre-Hot Water, B = Post-Hot Water, C = Post-Lactic Acid.
[e]HW = Hot Water, LA = Lactic Acid.

TABLE 9. *Facility A: Mean* Escherichia coli *populations and mean log reductions for inoculated beef carcass neck regions at various processing locations following intervention treatments*.

	Mean Populations (log CFU/cm^2)[a]			**Mean Reductions (log CFU/cm^2)[b]**		
	A[d]	**B[d]**	**C[d]**	**HW[e]**	**LA[e]**	**Combined**
Replication 1[c]	4.8A	≤ 1.9B	≤ 1.9B	≥ 2.9	≥ 0.0	≥ 2.9
Replication 2[c]	4.8A	2.3B	≤ 2.4B	2.5	≥ 0.0	≥ 2.4
Replication 3[c]	4.7A	≤ 3.2B	≤ 2.2C	≥ 1.5	≥ 1.0	≥ 2.5

[a]Detection Limit = 1.0 $\log_{10}$ CFU/400 cm^2.
[b]Log reduction = ($\log_{10}$ CFU/400 cm^2 before treatment) - ($\log_{10}$ CFU/400 cm^2 after treatment).
[c]Means with different letters in the same row are significantly different ($P < 0.05$).
[d]A = Pre-Hot Water, B = Post-Hot Water, C = Post-Lactic Acid.
[e]HW = Hot Water, LA = Lactic Acid.

small establishment as one employing ≥ 10 and ≤ 500 employees. Facility B only utilized a pre-chill lactic acid spray treatment as an antimicrobial intervention, and manually mixed 88% L-lactic acid to create a 6-liter, 2.0% L-lactic acid solution in a hand-pump chemical garden sprayer. An initial assessment of Facility B's application method determined the solution was not applied at a warm temperature (8°C). Also, the volume of L-lactic acid sprayed on the carcass (500 ml) was insufficient to bring the initial carcass pH of 6.7 down to a bactericidal level. Instead, the pH was only lowered to 5.6. According to Prost and Rieman (51), in order to have a killing effect for *Salmonella*, the pH is required to be < 4.5. A pre-trial was conducted to demonstrate the efficacy of the lactic acid solution when applied using the facility's parameters. At this low temperature and volume, the lactic acid spray was found largely ineffective, only achieving a 0.5-log reduction in *E. coli* and coliform counts (Table 10). Based on the results from the pre-trial, the parameters for applying the lactic acid solution were optimized. A fresh 2.0% L-lactic acid solution (pH = 2.3) was prepared and warmed to 55°C. Each carcass was then treated with the lactic acid solution using a hand-pump 6-liter garden chemical sprayer (RL Flomaster). The lactic acid solution was applied to each carcass side for a 20 sec dwell time (10 sec for inoculated necks). This dwell time, which resulted in a total dispensed volume of 500 ml per carcass side (200 ml for inoculated necks), lowered the carcass surface pH to 3.0.

Facility B did not process the same high volume of cattle each day as at Facility A. On an average day Facility B harvested between 5-10 head of cattle. To determine the reductions achieved by lactic acid at Facility B, on each of 3 replications the neck

TABLE 10. *Facility B pre-trial* Escherichia coli *and coliform counts treating beef carcass sides using low temperature/low volume lactic acid.*

	Non-Inoculated (log_{10} CFU/400 cm^2)[a]		**Inoculated (log_{10} CFU/cm^2)**[b]	
	E. coli[c]	**Coliform**[c]	***E. coli***[c]	**Coliform**[c]
Pre LA[d]	1.8A	1.7A	5.1A	3.4A
Post LA[d]	1.7A	1.7A	4.5A	3.0A

[a]Detection Limit = 1.7 log_{10} CFU/400 cm^2

[b]Detection Limit = 1.0 log_{10} CFU/cm^2

[c]Means with different letters in the same column are significantly different ($P < 0.05$).

[d]LA = Lactic Acid

regions of 10 carcass sides were inoculated with a fecal slurry and excise samples were collected before and after treatment with lactic acid. The non-inoculated briskets of the same 10 carcass sides were also sampled using sponges both before and after treatment of the entire side with lactic acid.

Lactic acid treatment (non-inoculated samples). The 2% L-lactic acid carcass spray significantly ($P < 0.05$) reduced MPCs in samples collected in all 3 replications. Reductions for replication 1 were the highest, but the initial counts for that replication were also higher. Mean $\log_{10}$/400-cm^2 reductions in MPCs attributed to the lactic acid spray for replications 1, 2, and 3 were 4.4, 3.3, and 3.7, respectively (Table 11). Only in replication 1 were significant ($P < 0.05$) reductions found for *E. coli*. The *E. coli* counts for replications 2 and 3 were not initially high enough to allow the demonstration of a significant ($P < 0.05$) reduction, although *E. coli* counts were eliminated below detectable levels. Significant ($P < 0.05$) reductions were reported for total coliform counts for all 3 replications, although initial counts for replications 2 and 3 were significantly lower than the 1st replication. Mean total coliform $\log_{10}$/400 cm^2 reductions were 2.9, 1.0, and 1.5, respectively (Table 12). Mean *E. coli* $\log_{10}$/400 cm^2 reductions for replications 1, 2, 3 were 2.9, 0.8, and 0.8, respectively (Table 13).

TABLE 11. *Facility B: Mean mesophilic plate count populations and mean log reductions from beef carcass brisket regions at various processing locations following intervention treatments.*

	Mean Populations (log CFU/400 cm^2)[a]		**Mean Reductions (log CFU/400 cm^2)[b]**
	A[d]	**B[e]**	**Lactic Acid**
Replication 1[c]	6.8A	2.4B	4.4
Replication 2[c]	5.1A	≤ 1.8B	≥ 3.3
Replication 3[c]	5.6A	≤ 1.9B	≥ 3.7

[a]Detection Limit = 1.7 $\log_{10}$ cfu/cm^2

[b]Log reduction = ($\log_{10}$ CFU/cm^2 before treatment) - ($\log_{10}$ CFU/cm^2 after treatment)

[c]Means with different letters in the same row are significantly different ($P < 0.05$).

[d]A = Pre-Lactic Acid

[e]B = Post-Lactic Acid

TABLE 12. *Facility B: Mean coliform populations and mean log reductions from beef carcass brisket regions at various processing locations following treatment with lactic acid.*

	Mean Populations (log CFU/400 cm^2)[a]		**Mean Reductions (log CFU/400 cm^2)[b]**
	A[d]	**B[e]**	**Lactic Acid**
Replication 1[c]	4.3A	< 1.7B	> 2.6
Replication 2[c]	2.4A	< 1.7B	> 0.7
Replication 3[c]	2.9A	< 1.7B	> 1.2

[a]Detection Limit = 1.7 $\log_{10}$ CFU/cm^2

[b]Log reduction = ($\log_{10}$ CFU/cm^2 before treatment) - ($\log_{10}$ CFU/cm^2 after treatment)

[c]Means with different letters in the same row are significantly different ($P < 0.05$).

[d]A = Pre-Lactic Acid

[e]B = Post-Lactic Acid

TABLE 13. *Facility B: Mean* Escherichia coli *populations and mean log reductions from beef carcass brisket regions at various processing locations following treatment with lactic acid.*

	Mean Populations (log CFU/400 cm^2)[a]		**Mean Reductions (log CFU/400 cm^2)[b]**
	A[d]	**B[e]**	**Lactic Acid**
Replication 1[c]	4.3A	< 1.7B	> 2.6
Replication 2[c]	2.2A	< 1.7A	> 0.5
Replication 3[c]	2.2A	< 1.7A	> 0.5

[a]Detection Limit = 1.7 $\log_{10}$ CFU/cm^2

[b]Log reduction = ($\log_{10}$ CFU/cm^2 before treatment) - ($\log_{10}$ CFU/cm^2 after treatment)

[c]Means with different letters in the same row are significantly different ($P < 0.05$).

[d]A = Pre-Lactic Acid

[e]B = Post-Lactic Acid

Salmonella *screening (non-inoculated samples).* No samples testing positive for *Salmonella*, either pre- or post- treatment, in replications 1 through 3 were detected using ELISA. The lack of positive cattle could be due in part to the time of season in which the cattle were processed. Studies have demonstrated lower prevalence of *Salmonella* in cattle during colder months of the year (6, 11).

Inoculated samples. The lactic acid carcass spray significantly reduced counts of coliforms on inoculated carcass surfaces in replication 1, 2, and 3 samples with observed mean $\log_{10}/cm^2$ reductions of 3.7, 2.6, and 2.1, respectively (Table 14). Similar trends were seen for *E. coli* with mean $\log_{10}/cm^2$ reductions of 3.6, 2.6, and 2.0, for replications 1, 2, and 3, respectively (Table 15). Results from this study were similar to reductions demonstrated in a study conducted by Prasai et al. (51) in which 500 ml of a lactic acid solution were applied to beef carcasses.

TABLE 14. *Facility B: Mean coliform populations and mean log reductions for inoculated beef carcass neck regions at various processing locations following treatment with lactic acid.*

	Mean Populations (log CFU/cm^2)[a]		**Mean Reductions (log CFU/cm^2)[b]**
	A[d]	**B[e]**	**Lactic Acid**
Replication 1[c]	5.3A	≤ 1.6B	≥ 3.7
Replication 2[c]	4.5A	≤ 1.9B	≥ 2.6
Replication 3[c]	3.0A	≤ 1.0B	≥ 2.0

[a]Detection Limit = 1.0 $\log_{10}$ CFU/cm^2

[b]Log reduction = ($\log_{10}$ CFU/cm^2 before treatment) - ($\log_{10}$ CFU/cm^2 after treatment)

[c]Means with different letters in the same row are significantly different ($P < 0.05$).

[d]A = Pre-Lactic Acid

[e]B = Post-Lactic Acid

TABLE 15. *Facility B: Mean* Escherichia coli *populations and mean log reductions for inoculated beef carcass neck regions at various processing locations following treatment with lactic acid.*

	Mean Populations (log CFU/cm^2)[a]		**Mean Reductions (log CFU/cm^2)[b]**
	A[d]	**B[e]**	**Lactic Acid**
Replication 1[c]	5.1A	≤ 1.5B	≥ 3.6
Replication 2[c]	4.5A	≤ 1.9B	≥ 2.6
Replication 3[c]	2.9A	≤ 1.0B	≥ 2.0

[a]Detection Limit = 1.0 $\log_{10}$ CFU/cm^2

[b]Log reduction = ($\log_{10}$ CFU/cm^2 before treatment) - ($\log_{10}$ CFU/cm^2 after treatment)

[c]Means with different letters in the same row are significantly different ($P < 0.05$).

[d]A = Pre-Lactic Acid

[e]B = Post-Lactic Acid

CONCLUSIONS

During a two-phase experiment, beef carcass decontamination interventions at two beef slaughter facilities were validated to demonstrate effectiveness at reducing or eliminating enteric pathogens by reductions of key bacterial indicator organisms. In the first phase, Facility A utilized a post-evisceration hot water spray wash immediately followed by a lactic acid spray treatment. In the second phase, Facility B utilized only a post-evisceration lactic acid spray treatment.

At Facility A, the hot water spray wash of beef carcasses was proven to significantly ($P < 0.05$) reduce or eliminate MPC, *E. coli,* and coliform populations, whether naturally introduced or artificially inoculated. More specifically, *E. coli* and coliforms were reduced below the detection limit for most samples; therefore, benefit was observed by inoculating the carcasses with a fecal slurry to increase initial bacterial counts. In doing this, the maximum reduction potential for the hot water wash to effectively reduce *E. coli* and coliforms was able to be determined.

Due to the highly effective application of hot water at Facility A, key indicator organisms, whether naturally introduced or artificially inoculated, were not always detectable prior to treatment with lactic acid. Therefore, validation of the total reduction potential of the lactic acid treatment was not possible; however, the combination of the two interventions post-evisceration significantly ($P < 0.05$) reduced and eliminated all key bacterial indicator organisms. To determine the total reduction potential of the lactic acid at this facility, it would be recommended to inoculate a group of cattle separately

with a fecal slurry after the hot water wash to increase the level of detectable bacterial indicator organisms present for the lactic acid treatment.

In USDA-FSIS notices for validation, verification, and reassessment of interventions, justification for the notices was based on information gathered showing evidence that many facilities had not validated or verified interventions utilized in their HACCP plans (66, 67, 68, 69). Facility B was utilizing an intervention that had not been validated or verified to control *E. coli* O157:H7 and *Salmonella*. Upon initial investigation at Facility B, an audit of the lactic acid intervention utilized determined lower than optimal dwell time and temperature resulting in little to no microbial reduction. Following optimization of the intervention parameters, time and temperature, the lactic acid treatment of beef carcasses significantly ($P < 0.05$) reduced MPCs and eliminated *E. coli* and coliform populations, both naturally introduced and artificially inoculated. Similar to Facility A, *E. coli* and coliforms were reduced below the detection limit for most non-inoculated samples at Facility B; therefore, similar benefit was observed by inoculating the carcasses with a fecal slurry. Thus, the total reduction potential for the lactic acid treatment to effectively reduce *E. coli* and coliforms was able to be determined.

Previously conducted research determined coliforms were more resistant than *E. coli*, with *Salmonella* being somewhat less resistant (13, 14, 33). Therefore, since both *E. coli* and coliforms were eliminated below a detectable limit at both Facility A and B, it is very likely that any *Salmonella* present would have also been eliminated. Of the 90 total non-inoculated samples taken at various processing points at Facility A, although 4

samples pre-hot water wash tested positive for *Salmonella*, none tested positive for *Salmonella* after the hot water wash or the lactic acid treatment. At Facility B, no samples collected from any location, whether before or after treatment, tested positive for *Salmonella*.

After a facility has determined the microbiological hazard(s) reasonably likely to occur within their process and selected and implemented the intervention(s) that were most cost effective while maintaining the integrity of their process, the intervention parameters (time, temperature, concentration, pH, volume, pressure) would then need to be optimized per USDA-FSIS regulatory limits and manufacturer validated recommendations. After these objectives are completed, beef slaughter facilities can utilize the research conducted at these two facilities and in this laboratory as a method to reassess their HACCP plans and validate the effectiveness of all interventions for controlling *E. coli* O157:H7 and *Salmonella*. The framework used to validate interventions can be utilized in the future for yearly verification of the effectiveness of each intervention.

In recent years, much attention has been on establishing food safety objectives (FSO). An FSO is defined as, "the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP) (37)." The FSO does not give direction on how to achieve the specific target; thus, allowing the processor flexibility in determining individual control systems or intervention strategies. Three concepts that summarize an FSO are: (1) the initial level (H_0) of that contaminant on the food, (2) the sum total of the

contaminant reductions (Σ R) occurring up to the point of consumption, and (3) the sum total of contaminant increases (Σ I) up to the point of consumption ($H_0 - \Sigma R + \Sigma I \leq$ FSO). Furthermore, after an FSO has been determined for a specific product, the before-and-after sampling validation design conducted in this research can be utilized as a process-flow biomapping tool for determination of initial contamination levels, reduction achieved, and levels of contaminant increases for confirming that a FSO has been met.

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