USE OF FLUORESCENT SURROGATE ORGANISMS FOR ENTERIC PATHOGENS IN VALIDATION OF CARCASS DECONTAMINATION TREATMENTS

A Thesis

by

TIFFANY MARIE MOSELEY

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

May 2007

Major Subject: Food Science and Technology

USE OF FLUORESCENT SURROGATE ORGANISMS FOR ENTERIC PATHOGENS IN VALIDATION OF CARCASS DECONTAMINATION TREATMENTS

A Thesis

by

TIFFANY MARIE MOSELEY

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

MASTER OF SCIENCE

Approved by:

Chair of Committee, Gary R. Acuff
Committee Members, Leon H. Russell
Alejandro Castillo
Head of Department, Gary R. Acuff

May 2007

Major Subject: Food Science and Technology

ABSTRACT

Use of Fluorescent Surrogate Organisms for Enteric Pathogens in Validation of Carcass Decontamination Treatments. (May 2007)

Tiffany Marie Moseley, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Gary R. Acuff

During the harvesting process, meat products can become contaminated with enteric pathogens, such as *Escherichia coli* O157:H7 and *Salmonella* Typhimurium. Surrogates for these pathogens would be beneficial for validating carcass decontamination treatments. Surrogate organisms are organisms that behave similarly to specific pathogens but are non-pathogenic and can be used to determine efficacy of decontamination regimes for pathogens. The surrogates proposed are non-pathogenic, ampicillin-resistant *E. coli* biotype I strains that were previously isolated from beef cattle hides. Each *E. coli* strain was transformed to express a fluorescent protein (red: EcRFP; green: EcGFP; yellow: EcYFP) that is detectable under an ultraviolet light source. Surface areas on hot boned beef carcasses (clod, brisket, outside round) were inoculated with a fecal slurry containing EcRFP, EcGFP, EcYFP and rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium. Surface regions were then treated in a model spray cabinet using an initial water wash (28ºC) followed by treatments using 2% L-lactic acid (55ºC), hot water (95ºC at source) or a combination of the two. Treatments were compared for their effectiveness at reducing populations of inoculated (4.7 to 6.7 log

CFU/cm^2) *E. coli*, *S.* Typhimurium, EcRFP, EcGFP and EcYFP. Log reductions for inoculated organisms were calculated individually and then total and average surrogate cocktail values were calculated.

All decontamination treatments reduced the inoculated numbers of pathogens and surrogates to near or below the detection limit of 0.5 log CFU/cm^2. The combined treatment resulted in the greatest log reductions. The three individual surrogate organisms varied in log reductions according to the different decontamination treatments applied; however, log reductions for the total surrogate cocktail did not differ significantly from that of *E. coli* O157:H7. With the exception of EcYFP, the individual surrogates and average surrogate cocktail were significantly more resistant to microbial interventions including lactic acid than *S.* Typhimurium. Because abattoirs utilize different carcass decontamination treatments, it is difficult for one single fluorescent protein-producing isolate to accurately represent the behavior of *E. coli* O157:H7 or *S.* Typhimurium. Instead, surrogates should be used as a total cocktail to accurately represent the effectiveness of different treatments for reduction of enteric pathogens.

DEDICATION

I would like to dedicate this manuscript to my parents, Ronald and Ana, and my family who have always supported my educational endeavors and stood by my side throughout my life. Thank you for believing in me when I lost all faith in myself. Thank you for your support.

ACKNOWLEDGMENTS

To Dr. Gary Acuff, for his expertise and assistance, as well as for obtaining the funding for this project. Most of all I'd like to thank him for allowing me to pursue graduate studies.

To Mrs. Lisa Lucia, for providing technical assistance, experienced advice and friendship throughout this research.

To Ms. Elisa Cabrera Diaz, for training, technical assistance and at times "manual labor", which were invaluable for the completion of this project.

To Dr. Alejandro Castillo, for providing his expertise and knowledge both before and after the completion of the research.

To Dr. Rhonda Miller, for providing help with the statistical analysis.

To Mr. Ray Riley, for his understanding and cooperation in providing the Rosenthal Meat Science and Technology Center facilities.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

TABLE Page

INTRODUCTION

Escherichia coli O157:H7 and *Salmonella* Typhimurium are both natural inhabitants of the bovine gastrointestinal tract and, as a result of fecal shedding, they are often found on the hides of cattle. During the slaughter process special care must be taken to ensure that the hide and the contents of the gastrointestinal tract do not contaminate the carcass surface. Many efforts have been made to reduce or eliminate the presence of enteric pathogens on the surface of beef carcasses. The United States Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) responded to food safety concerns by mandating the use of the Hazard Analysis and Critical Control Point (HACCP) system in food production areas where biological hazards, such as enteric pathogens, can be prevented, reduced or eliminated. Microbiological testing for enteric pathogens as part of a HACCP system is not recommended because they are usually present in low concentrations and unevenly distributed; therefore, it is difficult to obtain a representative sample. Microbiological testing for other organisms such as indicators or surrogates is beneficial and can be used as a part of a HACCP plan to validate and verify the effectiveness of carcass decontamination treatments and confirm that a specific pathogen is being controlled.

An effective approach to reduce populations of pathogenic bacteria on carcasses would consist of a final decontamination step where a carcass intervention would be applied as part of HACCP plan. Carcass intervention methods have been developed and

---

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

studied for use at Critical Control Points (CCPs) in the slaughter process to eliminate or reduce the microbial population present on the carcass surface. Some of the treatments that have been used on beef carcasses include, but are not limited to, trimming, hot water, steam pasteurization, organic acids, chlorinated water, trisodium phosphate, hydrogen peroxide and combinations of the treatments. Optimal treatment parameters for several carcass decontamination treatments have been outlined in previous research. The treatment parameters that are required for optimal reduction of pathogenic bacteria are not always utilized in commercial slaughter facilities because of lack of knowledge or capabilities.

E. coli biotype I are currently used to determine if the carcasses have been contaminated with fecal matter and if the decontamination treatments applied are effective. These organisms are referred to as indicators for enteric pathogens. Indicator organisms are organisms that are naturally occurring and always present when the pathogen of interest is present. The presence of these indicators is usually indicative of the presence of *E. coli* O157:H7 and *S.* Typhimurium. In this research study, fluorescent surrogate organisms and enteric pathogens were used during carcass decontamination treatments to evaluate the effectiveness of the treatments. The fluorescent surrogates are genetically modified non-pathogenic *E. coli* strains that in previous studies demonstrated similar growth, heat and acid resistance to rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium. The fluorescent surrogate organisms are also ampicillin-resistant which allows them to grow on ampicillin-supplemented media without any interference from other microorganisms. The fluorescent surrogate organisms are beneficial for use

because they are easily identified in ultraviolet light and can be experimentally controlled and evaluated.

The first objective of the current study was to evaluate the effectiveness of the following decontamination treatments: 2% L-lactic acid spray, hot water wash (95°C) and hot water wash + 2% L-lactic acid spray using the fluorescent surrogate organisms, rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium. This portion was conducted under laboratory conditions and may not accurately represent the conditions under which carcass contamination and treatment occurs during normal slaughter operations. Therefore, it was necessary to evaluate the surrogate organisms in a commercial slaughter atmosphere as well. The second objective of this study was to validate the decontamination treatments using the fluorescent surrogate organisms in a small commercial slaughter facility.

LITERATURE REVIEW

E. coli O157:H7 and *S.* Typhimurium are both natural inhabitants of the bovine gastrointestinal tract. These bacteria are often shed in the feces, and as a result of fecal shedding, they can be found on the hides of cattle and rapidly spread among cattle during transportation to slaughter facilities (*24, 30*). This poses a problem during the slaughter process and special care must be taken to ensure that the hide and contents of the gastrointestinal tract do not contaminate the carcass surface. In bovine feces, O157:H7 and O157:H $^{-}$ are the most commonly isolated Shiga toxin-producing *E. coli* (STEC) serotypes (*37*). *S.* Typhimurium is one of the most commonly isolated *Salmonella* serotypes along with *S.* Dublin and *S.* Agona (*33, 50*). The presence of small numbers of *E. coli* O157:H7 or *S.* Typhimurium on the surface of carcasses is significant because the surface cuts and trimmings can be used for ground beef production and the pathogens will be spread throughout (*19, 53, 62*). Small concentrations of these pathogens present on foods is also of public health significance because it is believed that many foodborne illnesses are caused by temperature abusing or mishandling of foods during preparation, which allow the pathogens to grow to concentrations that can cause illness (*70*). The prevalence of *E. coli* O157:H7 and *Salmonella* is higher in the summer months and, as a result, that is usually when more foodborne infections occur (*33, 37*).

E. coli O157:H7 has a wide geographic distribution and can be found in approximately 63% of feedlots in the United States (*3*). Cattle at three feedlots in the U. S. were recently tested for *E. coli* O157:H7 and 86.7% of the cattle pens examined had at

least one positive fecal sample (*25*). Fecal material containing both *E. coli* O157:H7 and *Salmonella* is often found on the hides of animals and contamination is easily spread during transportation. In an experiment conducted by Collis et al. (*24*) 11% of the hides of cattle going to slaughter were inoculated with non-pathogenic bacterial markers (*E. coli* and *Pseudomonas fluorescens*). By the time the cattle were unloaded from the trailer, 100% of the hides contained the bacterial marker and later 88% of the pre-washed carcasses contained the marker. In a recent study conducted by Aslam et al. (*4*) it was confirmed that the *E. coli* which typically contaminates beef carcass surfaces originates from cattle feces, and the populations found in the feces are highly diverse and constantly changing. Bacon et al. (*5*) found that *E. coli* O157:H7 was present on 3.56% of hides and 0.44% of beef carcasses tested in 2000. In another experiment in 2002, *S.* Typhimurium was found on 15.4% of hides and 1.3% of beef carcasses (*6*). Experiments conducted by Barkocy-Gallagher et al. (*8*) in 2003 found *E. coli* O157:H7 present in 5.9% of fecal samples, 60.6% of hides and 26.7% of pre-evisceration carcasses, while *S.* Typhimurium was found on 4.4% of fecal samples, 71% of hides and 12.7% of pre-evisceration carcasses.

Muscle tissues of healthy cattle are considered sterile, but the hide removal process can allow bacteria present on the hide to contaminate the carcass surface (*30, 46*). High levels of contamination have been found at areas on the carcass associated with opening cuts or areas subject to hide contact during the hide removal process (*9*). Removal of the hide with a hide puller creates fewer opportunities for the hide to contact the carcass, but it also creates aerosols that can facilitate contamination of the surface

(*46*). High levels of physical contamination on hides have been shown to correlate to high levels of subsequent contamination on the resulting carcass (*49*). The brisket area has been found to be the area with the highest microbial contamination (*9, 49, 59*). These findings are thought to be due to the cattle laying down on contaminated surfaces at the farm, during transport and in the stunning boxes.

Foodborne Illness

In 1999 it was estimated that foodborne diseases account for 76 million cases of illness, 325,000 hospitalizations and 5,000 deaths in the U. S. each year (*51*). *E. coli* O157:H7 was confirmed as a food-related pathogen in 1982 and again in 1993 when it was determined to be the cause of multi-state outbreaks related to the consumption of undercooked hamburgers (*21, 60*). Several other *E. coli* O157:H7 outbreaks have occurred with different food commodities, such as potatoes in 1988 (*52*), apple cider in 1993 (*10*) and, more recently, vacuum packaged frozen marinated steaks in 2005 (*42*). *E. coli* and *Salmonella* have also been detected on the external surfaces of the packaging of raw meat (*13*). *E. coli* was reported on 4% of the packages and *Salmonella* was found on less than 1%. These findings are significant because the contamination on the exterior surfaces of the packaging could cross contaminate ready-to-eat foods. *E. coli* O157:H7 is now recognized as an important cause of foodborne illness in several countries, including the U. S. and Canada. The illness produced by this microorganism is usually severe and can present itself through three different syndromes: hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura

(TTP) (*29*). TTP is the most severe of the diseases produced since it is a life threatening condition associated with high fatality rates. HUS has been found to be the leading cause of renal failure in children in the U. S. and 1996 it was found to be caused primarily by *E. coli* O157:H7 infections (*3*).

Foods most commonly associated with salmonellosis include beef, pork, eggs, raw milk, fruits, fruit juices and vegetables (*12, 39*). Public health laboratories in the U. S. with culture confirmed *Salmonella* infections identified 25% of the cases to be caused by *S.* Typhimurium in 1996 (*34, 50*). Salmonellosis has been identified as the second leading cause of gastroenteritis (*33*), and 95% of salmonellosis infections involve foodborne transmission (*6*). Symptoms of salmonellosis include diarrhea, fever, headache, nausea, abdominal pain, vomiting, and possibly bloody stools (*39*). Antimicrobial therapy is usually not necessary for these types of self-limiting infections (*6*). In some instances, however, antibiotic therapy is considered essential, especially when the infections occur in immunocompromised patients or when bacteremia develops. Bacteremia is a very serious type of infection that occurs in approximately 3-10% of cases and usually leads to disability or death (*34*). Some strains of *Salmonella* are resistant to multiple antimicrobial agents and have become a worldwide health problem (*34*). The emergence of multi-drug resistant *Salmonella* began to gain attention in 1980 when it was found that 1-16% of isolates examined were resistant to at least one type of antibiotic (*34, 45*). In 1996 this percentage increased to 34% (*34*) and in 2002 the number of antibiotic-resistant *Salmonella* increased to 49.2% (*40*). A specific strain of *S.* Typhimurium called DT 104 has health officials worldwide concerned because it has

been found to be resistant to five or more antimicrobial agents. It is also thought that infections caused by this strain are associated with greater morbidity and mortality (*34, 43, 44*). It is believed by many researchers that the emergence of multi-drug resistant strains of *Salmonella* and *E. coli* are the result of cattle management practices that utilize multiple antibiotics (*34*) at sub-therapeutic doses to treat diseases. It was thought that administering sub-therapeutic doses of antibiotics lead to microorganisms developing a resistance to the antibiotics and therefore resulting in larger numbers of antibiotic-resistant bacteria present in the meat products produced. However, a recent study (*44*) challenged this theory by comparing the microbiological quality of ground beef prepared from cattle raised with and without antimicrobial agents. Microbial contamination with multi-drug resistant *E. coli* and *Salmonella* were found to be similar in both types of samples.

Regulatory Action

The severity of the diseases caused by *S.* Typhimurium and *E. coli* O157:H7 has caused the government to take regulatory action to try to prevent, reduce or eliminate their occurrence in foods. As a result of the high prevalence of these microorganisms on the surface of beef carcasses, the Food Safety and Inspection Service (FSIS), a division of the U. S. Department of Agriculture (USDA), recently published a "Guidance for Minimizing the Risk of *Escherichia coli* O157:H7 and *Salmonella* in Beef Slaughter Operations." This guide was written as an aid for slaughter establishments to help accomplish the following: reduce entry of *E. coli* O157:H7 and *Salmonella*, reduce

transfer of *E. coli* O157:H7 and *Salmonella* (from unskinned cattle to carcasses), reduce contamination by establishing CCPs, apply intervention methods and maintain reduced levels of *E. coli* O157:H7 and *Salmonella* after the final carcass wash (*67*). It is emphasized in this document that although the final carcass wash is a decontamination step, it should not be relied upon to compensate for poor hygienic practices during the slaughter process.

Hazard Analysis and Critical Control Point (HACCP) System

The establishment of CCPs as part of a HACCP system in the slaughter process is especially important in controlling pathogens. HACCP programs are designed to prevent, reduce or eliminate potential microbiological, chemical and physical hazards in foods and ensure the safe production of food products. It is a practical, cost effective approach to ensuring the quality and safety of meat and meat products (*64*). HACCP systems are currently required for all federally inspected beef and poultry producing facilities because inspection procedures and end product testing alone are not able to guarantee the safety of foods for several reasons (*66*). First, pathogens may not be present on the carcass surface or their presence may be in very low concentrations. Also, the pathogens may be unevenly distributed on the carcass and it would be difficult to obtain a representative sample during routine sampling. As part of the HACCP regulation, FSIS also requires routine testing in slaughter facilities. The FSIS regulatory testing program has reported a 43.3% reduction of *E. coli* O157:H7 positive ground beef samples in 2004 compared with the previous year (*23*). In addition, in 2004 there were

only 6 recalls related to *E. coli* O157:H7 as compared to 12 in 2003 (*23*). This decrease is thought to be attributed to specific regulatory actions by the FSIS and subsequent actions implemented by the industry with the goal of reducing *E. coli* O157:H7 adulteration of ground beef (*54*). Although there has been a significant reduction in this pathogen in recent years, the Centers for Disease Control and Prevention (CDC) still identified it as the pathogen responsible for 2,461 illnesses in the year 2005 and 2,544 illnesses in 2004 (*22*).

Beef producers are aware of the risk of *E. coli* O157:H7 contaminating their products and, as a result, many of them have increased testing. The total number of samples collected for pathogen testing in 2005 increased more than 37% over previous years (*68*). To ensure the safe production of meat products, pathogen detection can be used as a part of HACCP to validate the effectiveness of carcass decontamination treatments. However, end-product testing for enteric pathogens is not recommended since they are not typically present on carcass surfaces in sufficient numbers to demonstrate effectiveness of CCPs. Inoculating surfaces with a pathogen such as *E. coli* O157:H7 or *S.* Typhimurium in a slaughter facility would be extremely dangerous and costly. Therefore, an alternative can be made available through the use of surrogate microorganisms. FSIS currently requires slaughter facilities to verify the adequacy of their process controls to prevent fecal contamination by testing for *E. coli* biotype I *(66)*. Instead of using *E. coli* biotype I, surrogates may be a more suitable choice for use because they can be experimentally controlled and evaluated and can be another alternative for predicting the behavior of *E. coli* O157:H7.

Surrogate Organisms

Surrogate organisms are organisms that behave similarly to a specific pathogen but are non-pathogenic. They can be used to determine the efficacy of decontamination regimes for pathogens without the risk of causing foodborne illness; however, a surrogate organism must meet certain criteria to be effectively used to characterize presence and behavior of a pathogen. The Food and Drug Administration (FDA) has listed the following microbial characteristics as desirable for surrogates: non-pathogenic, inactivation characteristics that can be used to predict those of the target organism, behave similar to target organism when exposed to similar processing parameters, stable and consistent growth characteristics, easily prepared to yield high density populations, easily enumerated and differentiated, attachment characteristics mimic those of the target organism, genetically stable so the results can be reproduced independently, will not establish itself as a "spoilage" organism on equipment or in the production area, and susceptibility to injury similar to that of the target pathogen (*71*).

Detection methods for *E. coli* O157:H7 can be costly and time consuming. In a previous study performed by Ajjarapu and Shelef (*1*), fluorescent protein-expressing *E. coli* O157:H7 were used to make the identification process easier and less time consuming by allowing them to easily differentiate the *E. coli* O157:H7 from the other organisms without further biochemical testing. Green fluorescent protein-expressing (EcGFP) *E. coli* O157:H7 and *S.* Typhimurium strains have been used by other researchers as positive controls in laboratory experiments, and there were no major differences seen in growth kinetics when comparing the transformed strains to the

parental strains (*55*). Furthermore, the strains were recovered successfully when used as positive controls in inoculated food samples. Fluorescent protein-expressing organisms are beneficial for use because their colonies can be easily identified and differentiated in the presence of other organisms by fluorescence under a long wave UV light source. Several fluorescent protein-expressing strains have been created in the past with different excitation and emission wavelengths to create red, blue, yellow or green fluorescence. Development of fluorescing strains allows for the monitoring of multiple species of bacteria simultaneously in a complex microbial community (*32*). Since these organisms are ampicillin-resistant, this allows them to selectively grow on ampicillin-supplemented media for organism differentiating purposes. Growth of these organisms on ampicillin-supplemented media helps to ensure that other competing microorganisms are not preventing their growth because it inhibits the growth of background microflora. The parental strains (non-transformed) of the surrogate organisms that are proposed for use were isolated from cattle hides by researchers at Iowa State University (Ames, IA) (*48*). Previous studies revealed that the parental stains behaved similarly to *E. coli* O157:H7 with regard to growth, acid and heat resistance characteristics (*47*). In previous experiments performed in our laboratory, each of the three different non-pathogenic *E. coli* strains were transformed to fluoresce under a UV light source. Each strain fluoresces a different color (red, green, yellow), making them easy to identify and offering a safe predictor of the behavior of *E. coli* O157:H7 in carcass decontamination studies.

E. coli O157:H7 is well known for its unique acid resistance properties, as it has

been found to be able to survive at pH values as low as 2.0 in some foods and can persist for several weeks (*3*). Therefore, confirmation testing of these and several other properties in the fluorescent surrogate microorganisms has been conducted to confirm their use as potential surrogates for *E. coli* O157:H7. Experiments have been conducted in our laboratory to evaluate the growth, heat and acid resistance properties of the three fluorescent surrogate organisms against *E. coli* O157:H7 and *S.* Typhimurium, the findings of this research have not yet been published. Acid resistance was tested at pH values of 2.5, 3.0 and 3.5. The surrogate organisms had greater acid resistance than *S.* Typhimurium and similar acid resistance to *E. coli* O157:H7 at each pH examined. Thermal resistance testing was conducted at 55, 60 and 65°C. The three surrogate organisms were found to be more resistant to heat than *E. coli* O157:H7 and *S.* Typhimurium (*14*). The evaluation of these characteristics prior to use in the laboratory experiments was important to ensure that the surrogate organisms behaved similarly to the enteric pathogens. Similar or more resistant behavior in these types of experiments demonstrated that they could represent the behavior *E. coli* O157:H7 and *S.* Typhimurium during carcass decontamination treatments. Surrogate organisms increased resistance to certain treatments is a desirable characteristic because it ensures that any pathogens that were present are destroyed when the surrogates are no longer detectible.

Decontamination Treatments

Several carcass decontamination treatments have been developed for use at critical points during the slaughter process as part of a HACCP system to minimize

contamination of the carcass surface with foodborne pathogens. The purpose of these treatments is to eliminate or reduce the number of pathogens present on the carcass, including those that may adhere to the surface. The USDA-FSIS has approved spray solutions of acetic, lactic and citric acids at 1.5-2.5% as acceptable interventions for reducing carcass contamination (*65*). In previous studies, a 2% L-lactic acid solution spray was found to be an effective carcass decontamination intervention when applied at a temperature of 55°C (*15, 35*), producing an average 4- to 5-log reduction in numbers of *E. coli* O157:H7 and *S.* Typhimurium. Hardin et al. (*35*) and Brackett et al. (*11*) have compared the effectiveness of solutions of different organic acids for carcass interventions and lactic acid was found to be the most effective acid spray when compared to acetic acid and citric acid because it produced a lower carcass pH. Castillo et al. used solutions of L-lactic acid and hot water sprays (*15, 16, 19*), ozonated water washes (*20*) and acidified sodium chlorite (*18*) solutions for carcass decontamination treatments. In a study performed by Castillo et al. (*16*), it was determined that using a hot water wash at 95ºC and 166 kPa at a distance of approximately 12.5 cm proved most effective at reducing *E. coli* O157:H7. Several researchers have also found that using a combination of treatments will produce greater log reductions of microbial populations (*15, 27, 31, 57*). Chlorinated water (20-50 ppm) and trisodium phosphate (12%) are also approved for use by the USDA-FSIS (*65*). Researchers have tried using solutions of ozonated water (*20, 58*) and hydrogen peroxide (*58*) for carcass decontamination treatments, but they were found to be less effective than organic acid sprays or hot water washes alone. These interventions cannot be used to remove visible fecal or ingesta

contamination, however, they can be used in conjunction with knife trimming or vacuuming with hot water or steam. Fecal and ingesta contamination must be removed prior to the use of interventions (*65*). Research findings have proven that organic acids are effective at reducing microbial populations on beef carcasses when applied properly. Of the organic acids used, lactic acid is the most effective at reducing populations of *E. coli* O157:H7 (*27, 28, 35*) possibly due to the fact that it produces a lower carcass pH (*27, 35*). Lactic acid has also been found to have long-term antimicrobial effects on meat stored at refrigerated temperatures (*28*).

There are many variables that must be taken into consideration when using an organic acid spray. Factors such as the temperature, pH, concentration, application time, application pressure and the volume of solution applied will create differences in the effectiveness of the treatments. Several researchers have examined the effectiveness of different organic acid solutions at varying concentrations and temperatures. Most agree that higher concentrations and temperatures of organic acid solutions applied are more effective. However, higher concentrations and temperatures of organic acids being applied to carcasses can create slight sensory changes: the meat usually has a pale-color (*41*), for example. Under the proper conditions these sensory changes are minimal when compared to their benefits.

Constant concerns regarding meat safety drive the continual research efforts to minimize the amount of enteric pathogens present on beef carcasses. The goal of this research project was to validate carcass decontamination treatments using fluorescent surrogate organisms for *E. coli* O157:H7 and *S.* Typhimurium. To achieve this goal, the

study was designed with two objectives: 1) evaluate the effectiveness of the following decontamination treatments: 2% L-lactic acid spray, hot water wash (95°) and hot water wash + 2% L-lactic acid spray using the fluorescent surrogate organisms, rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium in laboratory conditions, and 2) validate the decontamination treatments using the fluorescent surrogate organisms in a small commercial slaughter facility.

MATERIALS AND METHODS

Laboratory Portion of Study

Sample Collection

Nine fed heifers or steers typical of those entering the U. S. meat supply were used in this study. The cattle used were transported to the Texas A&M University Rosenthal Meat Science and Technology Center (RMSTC, College Station, TX) abattoir where they were harvested and dressed following USDA-FSIS regulated procedures (*69*). Outside round, brisket and clod carcass surface regions similar to institutional meat purchase specification numbers 171, 120 and 114 (*56*) were removed from the carcass just prior to splitting and washing. These specific carcass surface regions were chosen because they are located in areas were fecal contamination is likely to occur (*5, 9, 15, 16*). It has also been hypothesized that differences in surface characteristics and fat content of these surface regions could affect microbial attachment and the effectiveness of the decontamination treatments applied (*15, 31, 35*). The surface regions were then individually wrapped in cotton shrouds to prevent dehydration, placed inside insulated coolers (31.75 x 60.96 cm, Igloo Products Corp., Katy, TX) and transported to the microbiology laboratory located in the adjacent Kleberg Animal and Food Sciences Center (College Station, TX).

Preparation of Inoculum

Fresh cultures of each organism (EcRFP-1, EcGFP-3, EcYFP-66 and rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium) were prepared from parental strains that

had been maintained on tryptic soy agar (TSA, Becton Dickinson, Sparks, MD) slants at 26°C. The cultures were propagated by inoculating separately into 9 ml of tryptic soy broth (TSB, Becton Dickinson) and incubating at 37ºC for 24 h. Rifampicin resistance of *E. coli* O157:H7 and *S.* Typhimurium was confirmed by streaking the TSB cultures onto lactose-sulfite-phenol red-rifampicin (LSPR, 100 µg/ml rifampicin, Sigma-Aldrich, St. Louis, MO) (*15*) plates (see LSPR description below). Ampicillin resistance was also confirmed for the surrogates by streaking those cultures onto TSA plates supplemented with ampicillin (100 µg/ml, Sigma-Aldrich). Following incubation of the plates, characteristic colonies were selected and transferred to separate tubes containing 9 ml of TSB and incubated at 37ºC for 12-18 h to reach stationary phase and obtain a concentration of approximately 10^9 log CFU/ml. Stationary phase cultures were used because they are more resistant to environmental stressors than log phase cultures (*15, 16, 38, 48*). Then 10 ml of each of the five cultures was added to 50 ml of sterile 0.1% peptone water (Becton Dickinson) to make a total volume of 100 ml. The 100-ml mixture of diluted cultures was then mixed by vortexing for approximately 5 s to make an inoculum cocktail.

Fresh bovine feces were obtained from randomly selected cattle pens at the Texas A&M University O. D. Butler, Jr. Animal Science Complex (College Station, TX) the morning of each experiment. The feces was then separated into 10-g portions and placed into sterile stomacher bags (177 x 304 mm, Seward Medical, London, England). 10 ml of the inoculum cocktail was then added to each bag of feces to obtain a concentration of approximately 10^7 log CFU/g. The bags were massaged by hand for 1

min before use as a fecal slurry. All bags of fecal slurry were used within 4 h of preparation and were maintained at room temperature during the experiments (*15*).

Description of LSPR

LSPR is a selective and differential medium that allows for the simultaneous enumeration of rifampicin-resistant *E. coli* and *Salmonella* (*15*). Colonies of rifampicin-resistant *E. coli* O157:H7 appear yellow on the medium, whereas rifampicin-resistant *S.* Typhimurium develop as light pink colonies with a black center (Figure 1). LSPR was prepared using the following ingredients per liter: 40 g TSA, 3 g yeast extract (Becton Dickinson), 3 g beef extract (Becton Dickinson), 5 g lactose (EM Science, Gibbstown, NJ), 2.5 g sodium sulfite (EM Science), 0.3 g ferrous sulfate (Chempure, Houston, TX), 0.1 g cyclohexamide (Sigma-Aldrich), 0.1 g rifampicin (Sigma-Aldrich), and 0.25 g phenol red (Becton Dickinson). Phenol red was dissolved in 2 ml 0.1N NaOH solution and added to the medium prior to autoclaving. The medium was autoclaved without rifampicin for 15 min at 121°C. Rifampicin was dissolved in 5 ml methanol and added to medium after it had cooled to 50°C to achieve a concentration of 100 µg/ml. Plates were then poured and allowed to dry at 26°C for 48 h.

Description of Model Carcass Spray Cabinet and Hot Water Delivery System

The model carcass spray cabinet used was designed and constructed by Chad Company (Lenexa, KS) to simulate a commercial carcass decontamination cabinet and provided for the carcass surface regions to be hung in the same orientation as if they

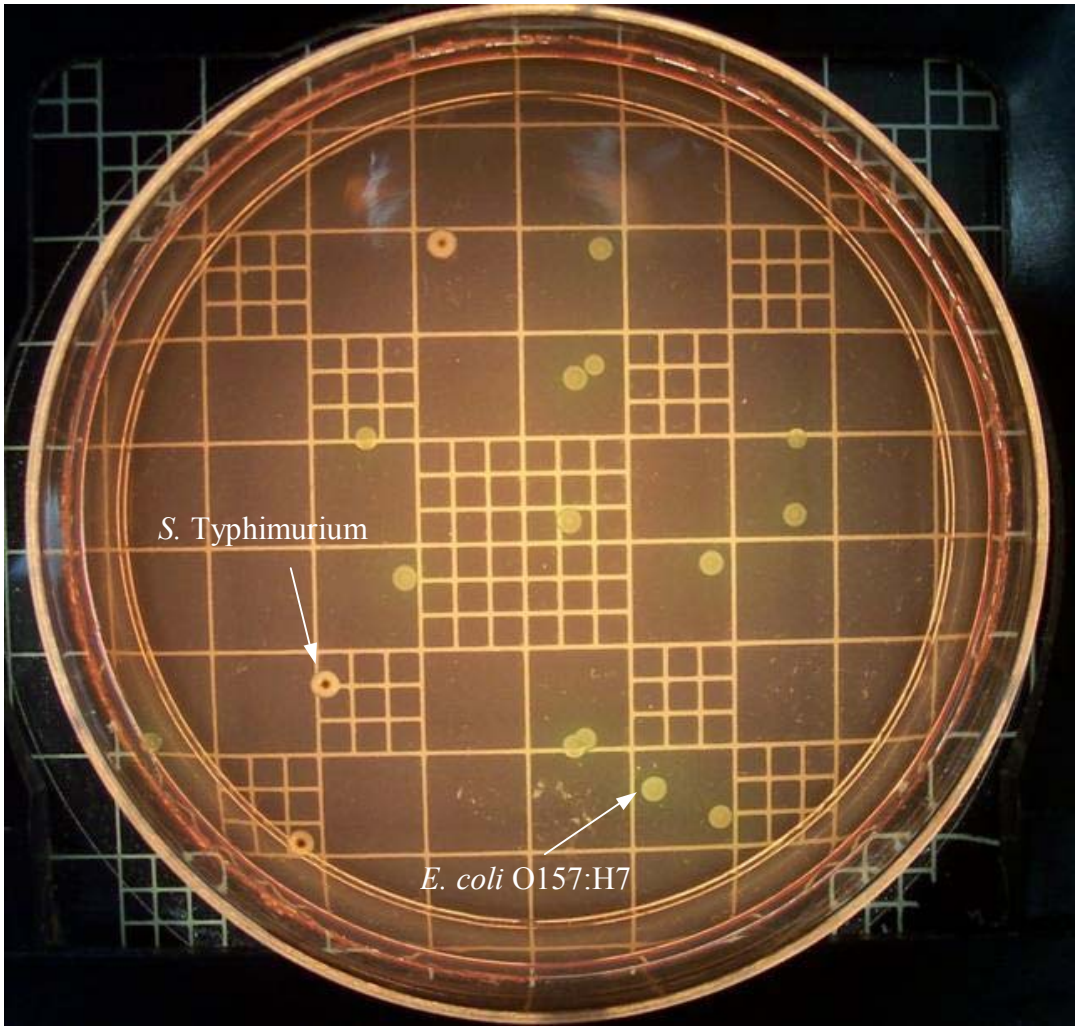

FIGURE 1. Appearance of *E. coli* O157:H7 and *S.* Typhimurium on LSPR agar

were part of an intact carcass (*15, 35*) (Figure 2). Attached to the cabinet was a water reservoir and pumping mechanism that could be turned on/off using a switch. The pumping mechanism delivered the water through a spray nozzle-head with four sprayers inside the cabinet (Figure 3). The spray nozzle-head had a separate motor (located on top of the cabinet) that moved it in an up and down motion while delivering the water. The pressure of the water coming out of the four sprayers could be controlled using a pressure-adjust valve. Inside the cabinet there were adjustable stainless-steel hooks to suspend each carcass surface region in two different directions. Each carcass surface region could be suspended to face the opening of the cabinet to apply treatments with the hand-held sprayer or towards the spray nozzle-head for the automated water wash. The model carcass spray cabinet also had two access panels, which were closed prior to the automated water wash.

The hot water source was a 184-liter capacity stainless-steel water tank (Figure 4) that was also designed and constructed by Chad Company. The walls of the tank were insulated (5.08 cm thick) and it had a removable lid for filling the tank and a drain valve for emptying. The tank contained a heating element that was controlled by a Chromalox On/Off Proportional Temperature Controller with a digital display (Model PDS 3910, Emerson Electric Co., Weigand Industrial Division, LaVergne, TN) that operated on 120 V (*31*). Preliminary testing determined that the water in the tank needed to be heated to 96.6°C in order to obtain the desired water temperature of 95°C at the spray nozzle (*31*). The hot water delivery system had a high-pressure pump (Franklin Electric, Model # 1313470103, Bluffton, IN) that delivered the water to the spray nozzle and could be

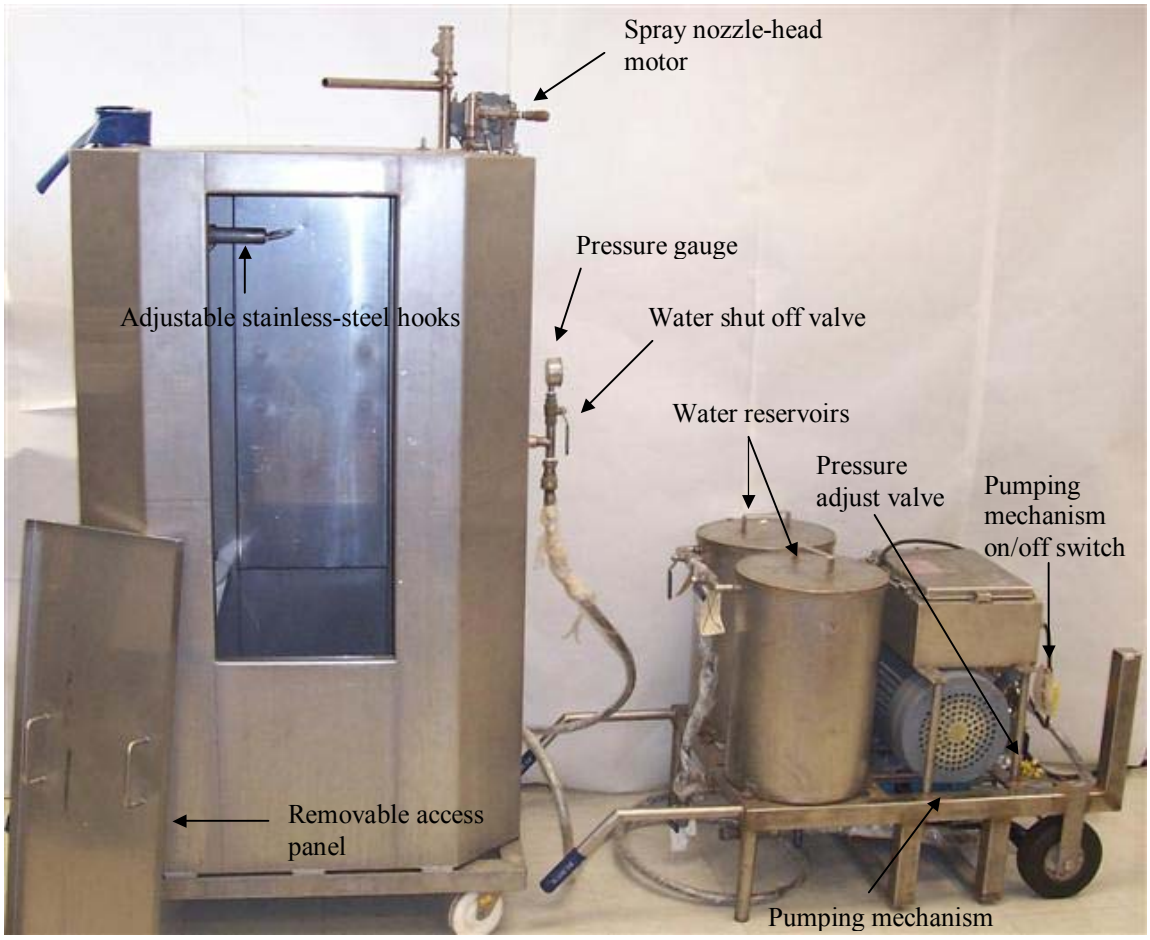

FIGURE 2. Model carcass spray cabinet and attached water system

FIGURE 3. Inside of the model carcass spray cabinet

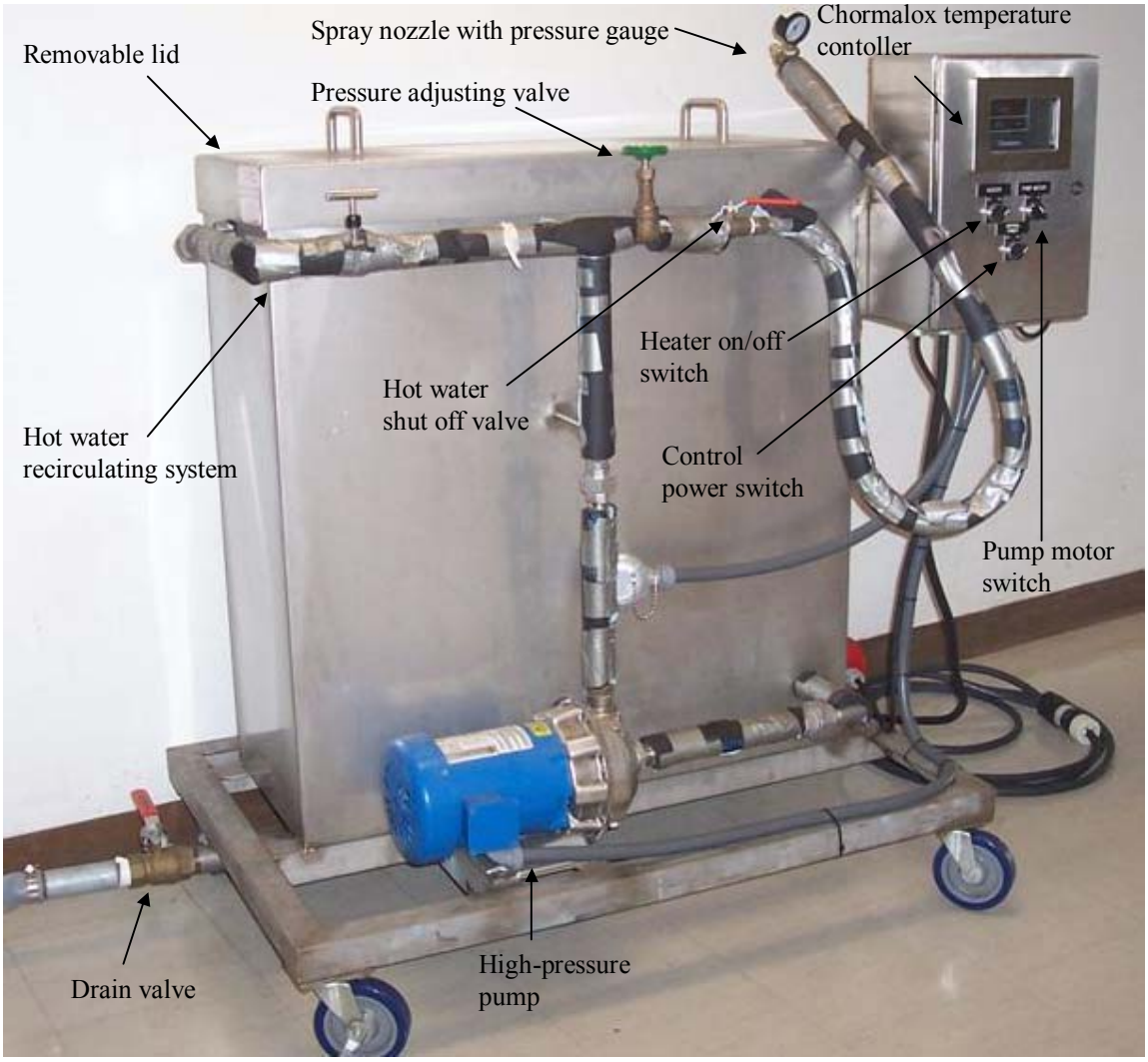

FIGURE 4. Hot water delivery system

turned on and off with a switch located on the control box. The hot water delivery system also had a pressure-adjust valve to control the pressure of the hot water that was delivered through the spray nozzle, and stainless-steel pipes attached to the water reservoir allowed water to recirculate back into the tank (*31*). The amount of water that was allowed to recirculate was controlled by an adjustable bypass needle valve. On the end of the nozzle there was a pressure gauge to verify the set pressure. For this study the pressure was set to 166 kPa, which was found to be the most effective in previous studies (*15, 16, 31*). The hot water tank also contained a shut off valve that could completely turn off the water supply to the spray nozzle (*31*), redirecting water through the recirculation system.

Sample Inoculation

In the laboratory the contents of each bag of fecal slurry were evenly spread over a 400-cm^2 (20 x 20 cm) area on each surface region in a back and forth motion using a sterile stainless-steel spatula. Prior to inoculation, two stainless steel hooks were also inserted at the top of each surface region to facilitate hanging the individual cut in the model carcass spray cabinet (see description below) to apply the treatments.

Treatments Applied

Immediately following inoculation, each surface region was suspended in the model carcass spray cabinet in approximately the same position as it would be found on an intact carcass and subjected to one of three treatments. Individually, all samples were

treated with the same initial water wash, which consisted of 1.5 liters of warm (~ 28ºC) potable water applied using a hand-held, non-corrosive, polyethylene compressed-air sprayer (7.57-liter capacity, Fountainhead Group, Inc, New York Mills, NY) to remove all visible fecal material (*15, 63*) with a low pressure rinse (69 kPa, 90 s). The spray was applied at a distance of approximately 5 cm from the surface region and at a 45° angle pointing the stream of water downward (*15*). In the first 60 s a wide-angled spray pattern was applied beginning at the top of the inoculated surface region and working in a back and forth motion to the bottom of the surface region. The spray nozzle was then adjusted to provide a concentrated stream that was used in the last 30 s to remove any visible particles from the feces that were still clinging to the surface region. An automated water wash was then applied consisting of a spray of 5 liters potable water (~28ºC), beginning at an initial pressure of 1.72 MPa for 4 s and gradually increasing to 2.76 MPa within 2 s, maintaining this pressure for 3 s to complete a total treatment time of 9 s. The temperature of the initial water wash was lower than what has been used in previous studies (*15, 35*) to more closely represent industry parameters.

Treatment 1: In the first treatment, a 2% L-lactic acid (Purac Inc., Arlington Heights, IL) solution followed the water wash. In the cabinet, 200 ml of the 2% L-lactic acid was applied to the carcass surface region at a low pressure (69 kPa) using a hand-held, non-corrosive, polyethylene compressed-air sprayer over an 11-s period of time in an up and down motion. The 2% L-lactic acid solution was maintained at 55ºC (*15*) in a Magni-Whirl water bath (Blue M, Blue Island, IL) set to 70ºC and only removed for enough

time to apply the treatments. The temperature and pH of the 2% L-lactic acid solution was monitored using a Traceable® Long Stem Digital Thermometer (Control Company, Friendswood, TX) and a Thermo Orion Portable pH/ISE Meter (model 250A+, Beverly, MA).

Treatment 2: In the second treatment, a hot water wash followed the initial cabinet water wash. The hot water wash was applied for 5 s at a temperature of 95ºC and 166 kPa. The source for the hot water was a hot water delivery system (Figure 4) that was designed and constructed by Chad Company. When applying the hot water the spray nozzle was kept at an approximate distance of 12.5 cm from the inoculated surface region because this was found to be the most effective distance in previous testing (*15*). During the hot water treatment the temperature of the carcass was monitored using a Traceable® Total Range Thermometer (Control Company) with a type K thermocouple (Control Company) inserted 0.1 mm below the surface. A thin layer of fascia, muscle tissue or fat covering the surface of the meat was lifted and punctured with the end of the thermocouple. The thermocouple was then bent into a U-shape to secure it to the carcass surface region.

Treatment 3: For the third treatment, the same surface regions that were treated with hot water were further treated with 200 ml of 2% L-lactic acid for 11 s. This 2% L-lactic acid solution was applied as described for treatment 1.

Immediately after each treatment, the temperature and pH of the surface region

was measured using a Raynger® MX2 Enhanced Noncontact Thermometer (Raytek, Santa Cruz, CA) and the above mentioned pH meter.

Sampling Technique

Prior to inoculation, three 10 cm^2 x 2-mm thick samples surrounding the area to be inoculated were excised to ensure that there were no naturally occurring ampicillin- or rifampicin-resistant microorganisms (negative control). Following inoculum application but prior to treatment application three 10 cm^2 x 2-mm thick samples from within the inoculated area were collected as positive controls. The inoculum level was determined using the positive control samples and was found to be approximately 10^7 log CFU/cm^2. This is much higher than what the actual inoculum level should be per cm^2 because when the fecal slurry was spread it was impossible to spread it evenly due to the consistency so lumps of the slurry were excised with the positive control samples. Following each water wash and treatment, three randomly selected 10-cm^2 samples were excised from the inoculated area (*15*). All samples were excised using a sterile borer to outline a 10-cm^2 sample region 2-3 mm deep. The 10 cm^2 x 2-mm thick surface sample was then removed using a sterile scalpel and forceps.

Sample Analysis

Each set of three samples (for a total of 30 cm^2) obtained was combined into a stomacher bag and 99 ml of sterile 0.1% peptone water was added to each bag. The samples were then pummeled for 1 min in a Tekmar® Stomacher Lab Blender 400

(Tekmar Company, Cincinnati, OH) to homogenize the samples and then decimal dilutions were made. 0.1-ml aliquots of the samples or appropriate dilutions were then plated on TSA plates supplemented with 100 μg/ml ampicillin to enumerate the fluorescent surrogates and LSPR agar to enumerate the rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium. The samples were spread on the agar using a sterile bent glass rod. All the plates were allowed to incubate for 24 h at 37˚C before being enumerated.

Testing at a Very Small Slaughter Facility

The data collected from the laboratory portion of this experiment indicated that the combined water wash + hot water + 2% L-lactic acid spray treatment was the most effective at reducing all microbial populations tested. An in-plant validation was conducted at a small slaughter facility in Texas using only the fluorescent surrogate organisms to validate and verify effectiveness.

Carcass Selection

The final seven carcass sides produced at RMSTC were used in this study. The cattle that were used were typical of those entering the U. S. meat supply and were slaughtered and dressed following USDA-FSIS regulated procedures (*69*).

Preparation of Inoculum and Sample Inoculation

Fresh cultures of each organism (EcRFP-1, EcGFP-3, EcYFP-66) were prepared

from the parental strains, as previously described. Then 10 ml of each of the three cultures was added to 70 ml of sterile 0.1% peptone water to make a total volume of 100 ml. The 100-ml mixture of diluted cultures was then mixed by vortexing for approximately 5 s to prepare an inoculum cocktail. Fresh feces were collected from the cattle holding pens at the slaughter plant. The feces were separated into 10-g portions and placed into sterile stomacher bags (Seward Medical), and 10 ml of the inoculum cocktail was added to each bag to obtain a concentration of approximately 10^7 log CFU/g. The bags were massaged by hand for 1 min before use as a fecal slurry.

At the slaughter facility the contents of each bag of fecal slurry were evenly spread over a 400-cm^2 (20 x 20 cm) area on the neck region. Only the neck region was used in this portion of the experiment to prevent the water run-off from contaminating other areas of the carcass and because this area could be completely removed from the carcass and discarded as "inedible" at the end of the experiment.

Treatments Applied and Sample Collection

Immediately following inoculation, a hand-held, non-corrosive, polyethylene compressed-air sprayer containing warm water (28ºC) was used to wash away the visible fecal material (1.5 L, 69 kPa, 90 s). The fecal material and water run-off was collected in a large plastic tub that was placed under the suspended carcass side. At the end of the day, a sufficient amount of bleach (~3 L, The Clorox Company, Oakland, CA) was added to the contents of the tub to kill any remaining bacteria and the mixture was poured down the drain.

The treatments applied after the hand-held water wash were hot water followed by 2.4% L-lactic spray. This lactic acid concentration was higher than the one used in the laboratory experiments. At this particular slaughter facility, the concentration of the lactic acid spray was determined at the beginning of each operation day, and an acceptable concentration was 2-2.5%. The hot water treatment was manually applied in an existing decontamination chamber using the hot water that supplied the entire facility. Carcass sides were conveyed through the cabinet and a worker would manually apply the hot water for times ranging from 1.0 – 2.0 min. The mean temperature of the surface of each carcass side during the hot water treatment was 51.4°C and the temperature of the hot water spray was 63.8°C. Following the hot water wash each carcass side was conveyed to another area where the 2.4% L-lactic acid spray was applied using a Hydro·Blend® (Crown Technology Corporation, Boise, ID) system. This system was connected to both a hot water (the same that supplied the rest of the facility) and lactic acid source and had a mixing chamber in which proportions of lactic acid and water were combined to provide the 2.4% solution that was delivered through a spray wand. The temperature of the lactic acid spray was 59°C and it was applied for times ranging from 0.2 – 1.0 min. Surface temperature and pH were also monitored as previously described.

Prior to inoculation, three 10-cm^2 x 2-mm thick samples surrounding the area to be inoculated were excised to ensure that there were no naturally occurring ampicillin-resistant microorganisms (negative control). Following inoculum application but prior to treatment application three 10 cm^2 x 2-mm thick samples from within the inoculated area

were collected as positive controls. The inoculum level was determined using the positive control samples and was found to be approximately 10^7 log CFU/cm^2, the same as in the laboratory setting. Again this is much higher than what the actual inoculum level should be per cm^2 because of the consistency of the fecal slurry. Following each water wash and treatment, three randomly selected, 10-cm^2 samples were excised from the inoculated area (*15*). All samples were excised using a sterile borer to outline a 10-cm^2 sample region 2-3 mm deep. The 10 cm^2 x 2-mm thick surface sample was then removed using a sterile scalpel and forceps.

After the samples were taken, the inoculated neck region was removed from the carcass and discarded as inedible. The samples were placed inside sterile stomacher bags (Seward Medical) and then packed into an insulated cooler (12.5 x 24 in., Igloo Products Corp.) with ice packs (Polyfoam Packers Corp., Wheeling, IL) and transported to the Texas A&M Food Microbiology Laboratory for analysis, which were performed as previously described.

Statistical Analysis

Counts (CFU/cm^2) were transformed into logarithms prior to calculating log reduction values. Log reduction values were calculated by subtracting the log count after each treatment or combination of treatments applied from the log count of inoculated bacteria before applying any treatments. Log reductions for each inoculated organism were calculated and reported individually and then log reductions of the surrogates were averaged and reported as an “average surrogate cocktail”. A “total surrogate cocktail”

was also calculated by adding the counts from the EcRFP, EcGFP and EcYFP organisms together and then transforming the added values into logarithms. The "total surrogate cocktail" log reduction values were calculated by subtracting the log count after each treatment or combination of treatments applied from the additive log count of inoculated bacteria before applying any treatments. Log reductions for the fluorescent surrogate cocktail was calculated as an averaged and additive value to determine which method would better serve as a predictive value for *E. coli* O157:H7 log reductions.

After application of the treatment some samples had counts below the minimum detection limit of 0.5 log CFU/cm^2. For statistical analysis purposes, those samples were assigned a count of 0.2 log CFU/cm^2, which is a number between zero and the minimum detection limit. Mean log reduction values for each treatment were compared using the general linear model (GLM). When significant differences ($P < 0.05$) among means were found, further analyses were performed using least square means (LS Means). All statistical analyses were performed using SAS procedures (*61*).

RESULTS AND DISCUSSION

Fluorescent surrogates for enteric pathogens are beneficial for validating carcass decontamination treatments for several reasons. They can be easily utilized in the laboratory since there are no known naturally occurring fluorescent protein-producing organisms in the environment, and their fluorescent properties make them easy to identify among other background microflora. Fluorescent proteins have been found to be extremely stable in heat and acid environments (*32*). Also, these organisms may be used to represent pathogenic bacteria in commercial food processing facilities without risking foodborne illness. The surrogates used in this study were non-pathogenic *E. coli* strains that were previously isolated from beef cattle hides (*48*). They were later transformed to express a fluorescent protein (red: EcRFP; green: EcGFP; yellow: EcYFP) that is detectable under an ultraviolet light source. Their fluorescent properties and ampicillin-resistance make them easy to identify and enumerate without any interference from other organisms.

Three hot-boned beef carcass surface areas (clod, brisket and outside round) were used in the laboratory portion of this study. These specific carcass surface regions were chosen because they were located in areas where fecal contamination is likely to occur (*5, 9, 15, 16*). It has also been hypothesized that differences in surface characteristics and fat content of these surface regions could affect microbial attachment and the effectiveness of the decontamination treatments applied (*15, 31, 35*). In the commercial slaughter facility, only the neck regions were used as part of the study. The surface regions were inoculated with a fecal slurry that had been contaminated with stationary-

phase cultures of *E. coli* O157:H7, *S.* Typhimurium, EcRFP, EcGFP and EcYFP. Stationary phase cultures were used because they are more resistant to environmental stressors than log phase cultures (*15, 16, 38, 48*). The surface regions were treated in a model spray cabinet using an initial water wash (28ºC). Decontamination treatments, such as 2% L-lactic acid (55ºC), hot water (95ºC) and a combination of the two were then applied. Treatments were compared for their effectiveness in reducing populations of inoculated (4.7 to 6.7 log CFU/cm^2) *E. coli* O157:H7, *S.* Typhimurium, EcRFP, EcGFP and EcYFP.

Laboratory Portion of Study

Use of 2% L-lactic Acid for Beef Carcass Decontamination

All decontamination treatments reduced the inoculated numbers of pathogens and surrogates to near or below the detection limit of 0.5 log CFU/cm^2. For all carcass surface regions, a mean log reduction of 1.9 and 1.5 log CFU/cm^2 (Table 1) was obtained by application of the water wash and an additional 2.3- and >3.7-log CFU/cm^2 mean reduction was observed for *E. coli* O157:H7 and *S.* Typhimurium, respectively after spraying with 2% L-lactic acid. The water wash + 2% L-lactic acid treatment achieved a mean log reduction for *E. coli* O157:H7 of 4.2 log CFU/cm^2 and *S.* Typhimurium of >5.2 log CFU/cm^2 on all carcass surface regions examined; these reductions were significantly greater ($P < 0.05$) than those obtained by the water wash alone or the water wash + hot water treatment.

The mean reduction of fluorescent surrogate organisms on all carcass surface

TABLE 1. *Mean[a] log reductions[b] (log CFU/cm^2) of pathogens and fluorescent protein expressing* E. coli *strains recovered from within 400-cm^2 contaminated areas of three carcass surface regions as affected by decontamination treatments applied*

	Inoculated Organism							
Treatment[c]	*E. coli* O157:H7 (1)	*S.* Typhimurium (2)	EcRFP (3)	EcGFP (4)	EcYFP (5)	Average Surrogate Cocktail[d] (6)	Total Surrogate Cocktail[e] (7)	Order of Means[f]
Water wash	1.9D[g]	1.5C	2.1C	1.9C	2.0C	2.0C	1.8C	3 6 5 4 1 7 2
Water wash + Lactic acid	4.2B	>5.2A	>4.4A	3.2B	>5.6A	>4.4A	>3.4B	5 2 3 6 1 7 4
Water wash + Hot water	3.7C	2.8B	>3.4B	3.2B	3.4B	3.3B	3.1B	1 3 5 6 4 7 2
Water wash + Hot water + Lactic Acid	>4.8A	>5.0A	>4.4A	>4.1A	>5.7A	>4.8A	>4.1A	5 2 1 6 3 7 4

[a] Mean = average obtained from clod, brisket and outside round surface regions during three repetitions.
[b] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).
[c] Water wash: 1.5-liter hand wash followed by 5-liter automated cabinet wash (9s, 1.72 MPa-2.76MPa), 28ºC. Lactic acid: 2% L-lactic acid spray (200 ml, 55ºC for 11s). Hot water: 95ºC water at 166 kPa for 5 s from a distance of 12.5 cm.
[d] Average Surrogate Cocktail = log of the calculated average reduction of EcRFP, EcGFP and EcYFP.
[e] Total Surrogate Cocktail = log of the sum of EcRFP, EcGFP and EcYFP reductions.
[f] Means within rows underlined by a common line are not significantly different (P > 0.05).
[g] Means within columns with the same letter (A, B, C, D) are not significantly different (P > 0.05).

regions was 1.9 to 2.1 log CFU/cm^2 for the water wash. The water wash + 2% L-lactic acid treatment achieved an additional 1.3- to >3.6-log CFU/cm^2 (EcRFP >2.3, EcGFP 1.3 and EcYFP >3.6-log CFU/cm^2) mean reduction. Overall, the water wash + 2% L-lactic acid treatment reduced mean populations of EcRFP by >4.4 log CFU/cm^2, EcGFP by 3.2 log CFU/cm^2 and EcYFP by >5.6 log CFU/cm^2 on all carcass surface regions examined. Log reductions achieved by this treatment were significantly different ($P < 0.05$) than those obtained by the water wash alone. For all organisms except EcGFP, log reductions achieved by the 2% L-lactic acid treatment were significantly different ($P < 0.05$) than those obtained by the water wash + hot water treatment. Water wash + 2% L-lactic acid and water wash + hot water treatments achieved similar ($P > 0.05$) log reductions for EcGFP. Log reductions for *E. coli* O157:H7, EcRFP and the average surrogate cocktail were not significantly different ($P > 0.05$) during the 2% L-lactic acid treatment. Log reductions of *S.* Typhimurium and EcYFP were not significantly different ($P > 0.05$) from one another during this same treatment. EcYFP and *S.* Typhimurium were considerably more sensitive to the 2% L-lactic acid spray treatment than the other inoculated organisms.

In a previous study conducted by Castillo et al. (*15*), mean log reductions achieved for *E. coli* O157:H7 and *S.* Typhimurium were 4.6 log CFU/cm^2 and >4.9 log CFU/cm^2 by water wash + 2% lactic acid, respectively. In a study conducted by Hardin et al. (*35*), similar mean log reductions were achieved for *E. coli* O157:H7 at 4.2 log CFU/cm^2 and *S.* Typhimurium at 4.6 log CFU/cm^2 by water wash + 2% lactic acid. In the present study, the log reductions achieved for the pathogens from the water wash +

2% lactic acid treatment were equivalent or greater than the log reductions achieved in the previous two studies.

Use of Hot Water for Beef Carcass Decontamination

The hot water treatment was less effective than the 2% L-lactic acid treatment at reducing surface bacterial contamination. A 1.8-log CFU/cm^2 mean reduction for *E. coli* O157:H7 and a 1.3-log CFU/cm^2 mean reduction for *S.* Typhimurium was observed after spraying with hot water (Table 1). The water wash + hot water treatment achieved a mean log reduction for *E. coli* O157:H7 of 3.7 log CFU/cm^2 and *S.* Typhimurium of 2.8 log CFU/cm^2 on all carcass surface regions examined, and these reductions were significantly greater ($P < 0.05$) than those obtained by the water wash alone.

The water wash + hot water treatment achieved a 3.2- to >3.4-log CFU/cm^2 mean reduction in fluorescent surrogates. Individually, the water wash + hot water treatment achieved a mean log reduction for EcRFP of >3.4 log CFU/cm^2, EcGFP of 3.2 log CFU/cm^2 and EcYFP of 3.4 log CFU/cm^2 on all carcass surface regions examined, and these reductions were significantly greater ($P < 0.05$) than those obtained by the water wash alone. Log reductions for *E. coli* O157:H7, EcRFP, EcYFP and the average surrogate cocktail were not significantly different ($P > 0.05$) during this treatment. *S.* Typhimurium, EcGFP and the total surrogate cocktail reductions were not significantly different ($P > 0.05$) from one another during this same treatment. Throughout this treatment and the other treatments, the EcGFP was more resistant or had equal log reductions when compared to *E. coli* O157:H7.

In a previous study conducted by Castillo et al. (*15*), mean log reductions achieved for *E. coli* O157:H7 and *S.* Typhimurium were 4.0 log CFU/cm^2 and 4.2 log CFU/cm^2, respectively, by water wash + hot water. Hardin et al. (*35*) did not use hot water; however, their research used an initial water wash that achieved a mean log reduction for *E. coli* O157:H7 of 3.0 log CFU/cm^2 and 2.6 log CFU/cm^2 for *S.* Typhimurium. In the present study, smaller log reductions were achieved and may have been due to several factors. First, differences in log reductions may have been due to differences in the temperature of the initial water wash. In the experiments conducted by Castillo et al. (*15*) and Hardin et al. (*35*), the temperature of the initial water wash was 35°C; however, in this study the temperature of the water wash was 28°C. Secondly, in the previously mentioned study conducted by Castillo et al. (*15*) the carcass treatment parameters allowed a carcass surface temperature of at least 82°C to be reached during the hot water treatment. In this study, however, a lower carcass surface region temperature was achieved during the hot water treatment that may have been due to a lower ambient temperature or the surface regions may have been allowed more time to cool before applying the treatments. The treatment parameters in this study were designed to more closely represent carcass decontamination treatment conditions in the industry which may utilize a lower water temperature.

Use of Combined Hot Water + 2% L-lactic Acid for Beef Carcass Decontamination

In this study, the combined treatment of water wash + hot water + 2% lactic acid spray was determined to be the most effective treatment for reducing all microbial

populations, and these results are consistent with those of previous studies (*15, 35*). The combined treatment resulted in a mean reduction of *E. coli* O157:H7 and *S.* Typhimurium of >4.8 and >5.0 log CFU/cm^2, respectively, for all carcass surface regions examined (Table 1). The log reductions achieved for *E. coli* O157:H7 by the combined treatment were significantly greater ($P < 0.05$) than those obtained by any of the other treatments alone. Log reductions for *S.* Typhimurium achieved by this treatment were not significantly different ($P > 0.05$) from the log reductions achieved by the water wash + lactic acid treatment.

Overall, the combined treatment reduced mean populations of EcRFP by >4.4 log CFU/cm^2, EcGFP by >4.1 log CFU/cm^2 and EcYFP by >5.7 log CFU/cm^2 on all carcass surface regions examined (Table1). Log reductions achieved for *E. coli* O157:H7, EcGFP and the total surrogate cocktail by this treatment were significantly greater ($P < 0.05$) than those obtained by any of the other treatments alone. Log reductions achieved for *S.* Typhimurium, EcRFP, EcYFP and the average surrogate cocktail were not significantly different than those obtained by the water wash + lactic acid treatment. *E. coli* O157:H7 and the calculated average surrogate cocktail demonstrated equivalent log reductions, that were also similar ($P > 0.05$) to the EcRFP log reductions achieved. EcYFP was considerably more sensitive to the combined treatment, similar to the sensitivity detected with the 2% L-lactic acid spray during the previous treatment.

In a previous study conducted by Castillo et al. (*15*), mean log reductions achieved for *E. coli* O157:H7 and *S.* Typhimurium were 4.9 log CFU/cm^2 and >4.5 log CFU/cm^2 by water wash + hot water + 2% lactic acid treatment, respectively. In this

study, the log reductions achieved for the pathogens from this treatment were similar, or in some cases higher, than those achieved in the previous experiments.

Table 1 demonstrates that throughout all four treatments, EcGFP was more resistant or demonstrated equivalent log reductions when compared to *E. coli* O157:H7. Individually, none of the surrogate organisms consistently had similar log reductions when compared to *E. coli* O157:H7 except for EcRFP and the calculated average surrogate cocktail across all treatments. During the first three treatments, the log reductions of EcYFP were similar ($P > 0.05$) to that of *S.* Typhimurium. This could be because *S.* Typhimurium and EcYFP appeared to be more sensitive to treatments that included 2% L-lactic acid spray.

Overall, the EcRFP and the average surrogate cocktail had log reductions that did not vary significantly ($P > 0.05$) from *E. coli* O157:H7 and were more resistant than *S.* Typhimurium during treatments that included a lactic acid spray (Table 1). For all inoculated organisms, the water wash + hot water + 2% L-lactic acid treatment was the most effective at achieving log reductions. The water wash + 2% L-lactic acid treatment was equally effective at reducing populations of *S.* Typhimurium, EcRFP, EcYFP and the average surrogate cocktail. These results were consistent for each of the three different carcass surface regions examined: outside round, brisket and clod.

Mean log reductions achieved on each carcass surface region for each organism by treatment are shown in Tables 2, 3 and 4. Table 2 demonstrates that throughout each treatment, *E. coli* O157:H7, EcRFP and the average surrogate cocktail log reduction values were not significantly different ($P > 0.05$) on the outside round carcass surface

TABLE 2. *Mean[a] log reductions[b] (log CFU/cm^2) of pathogens and fluorescent protein expressing* E. coli *recovered from within 400-cm^2 contaminated outside round carcass surface regions as affected by decontamination treatments applied*

	Inoculated Organism							
Treatment[c]	*E. coli* O157:H7 (1)	*S.* Typhimurium (2)	EcRFP (3)	EcGFP (4)	EcYFP (5)	Average Surrogate Cocktail[d] (6)	Total Surrogate Cocktail[e] (7)	Order of Means[f]
Water wash	1.9C[g]	1.6C	2.4C	2.1B	2.1C	2.2C	1.6C	3 6 4 5 1 2 7
Water wash + Lactic acid	4.2AB	>5.1A	>4.5A	3.4A	>5.6A	>4.5A	>3.2AB	5 2 3 6 1 4 7
Water wash + Hot water	3.7B	2.6B	3.2B	3.0A	3.2B	3.1B	2.6B	1 3 5 6 4 2 7
Water wash + Hot water + Lactic Acid	4.8A	>5.2A	4.6A	3.6A	>6.0A	>4.8A	>3.4A	5 2 1 6 3 4 7

[a] Mean = average obtained from three repetitions.

[b] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).

[c] Water wash: 1.5-liter hand wash followed by 5-liter automated cabinet wash (9 s, 1.72 MPa-2.76MPa), 28ºC. Lactic acid: 2% L-lactic acid spray (200 ml, 55ºC for 11 s). Hot water: 95ºC water at 166 kPa for 5 s from a distance of 12.5 cm.

[d] Average Surrogate Cocktail = log of the calculated average reduction of EcRFP, EcGFP and EcYFP.

[e] Total Surrogate Cocktail = log of the sum of EcRFP, EcGFP and EcYFP reductions.

[f] Means within rows underlined by a common line are not significantly different (P > 0.05).

[g] Means within columns with the same letter (A, B, C, D) are not significantly different (P > 0.05).

TABLE 3. *Mean[a] log reductions[b] (log CFU/cm^2) of pathogens and fluorescent protein expressing* E. coli *recovered from within 400-cm^2 contaminated brisket carcass surface regions as affected by decontamination treatments applied*

	Inoculated Organism							
Treatment[c]	*E. coli* O157:H7 (1)	*S.* Typhimurium (2)	EcRFP (3)	EcGFP (4)	EcYFP (5)	Average Surrogate Cocktail[d] (6)	Total Surrogate Cocktail[e] (7)	Order of Means[f]
Water wash	1.8C[g]	1.3C	2.0C	1.6C	1.7C	1.8C	1.7C	3 1 6 5 7 4 2
Water wash + Lactic acid	4.2AB	>5.1A	4.5A	2.9B	5.4A	>4.2A	>3.3B	5 2 3 6 1 7 4
Water wash + Hot water	3.5B	2.4B	>3.3B	2.8B	2.8B	>3.0B	>2.8B	1 3 6 5 7 4 2
Water wash + Hot water + Lactic Acid	>4.5A	>4.5A	>4.4A	4.1A	>5.1A	>4.5A	>4.2A	5 6 1 2 3 7 4

[a] Mean = average obtained from three repetitions.

[b] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).

[c] Water wash: 1.5-liter hand wash followed by 5-liter automated cabinet wash (9 s, 1.72 MPa-2.76MPa), 28ºC. Lactic acid: 2% L-lactic acid spray (200 ml, 55ºC for 11 s). Hot water: 95ºC water at 166 kPa for 5 s from a distance of 12.5 cm.

[d] Average Surrogate Cocktail = log of the calculated average reduction of EcRFP, EcGFP and EcYFP.

[e] Total Surrogate Cocktail = log of the sum of EcRFP, EcGFP and EcYFP reductions.

[f] Means within rows underlined by a common line are not significantly different (P > 0.05).

[g] Means within columns with the same letter (A, B, C, D) are not significantly different (P > 0.05).

TABLE 4. *Mean[a] log reductions[b] (log CFU/cm^2) of pathogens and fluorescent protein expressing* E. coli *recovered from within 400-cm^2 contaminated clod carcass surface regions as affected by decontamination treatments applied*

	Inoculated Organism							
Treatment[c]	*E. coli* O157:H7 (1)	*S.* Typhimurium (2)	EcRFP (3)	EcGFP (4)	EcYFP (5)	Average Surrogate Cocktail[d] (6)	Total Surrogate Cocktail[e] (7)	Order of Means[f]
Water wash	1.9C[g]	1.6C	1.9B	2.0C	2.1C	2.0C	2.0C	7 5 6 4 1 3 2
Water wash + Lactic acid	4.1B	>5.3A	>4.3A	3.4B	5.7A	>4.4AB	>3.8B	5 2 6 3 1 7 4
Water wash + Hot water	4.0B	3.5B	3.7A	3.8AB	4.1B	3.9B	4.0B	5 1 6 7 4 3 2
Water wash + Hot water + Lactic Acid	5.1A	>5.3A	>4.3A	4.5A	5.9A	>5.0A	>4.8A	5 2 1 6 7 4 3

[a] Mean = average obtained from three repetitions.
[b] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).
[c] Water wash: 1.5-liter hand wash followed by 5-liter automated cabinet wash (9 s, 1.72 MPa-2.76MPa), 28ºC. Lactic acid: 2% L-lactic acid spray (200 ml, 55ºC for 11 s). Hot water: 95ºC water at 166 kPa for 5 s from a distance of 12.5 cm.
[d] Average Surrogate Cocktail = log of the calculated average reduction of EcRFP, EcGFP and EcYFP.
[e] Total Surrogate Cocktail = log of the sum of EcRFP, EcGFP and EcYFP reductions.
[f] Means within rows underlined by a common line are not significantly different (P > 0.05).
[g] Means within columns with the same letter (A, B, C, D) are not significantly different (P > 0.05).

region. The average surrogate cocktail log reductions were numerically higher, but not significantly different ($P > 0.05$) than those achieved for *E. coli* O157:H7 during the first two treatments. The total surrogate cocktail log reductions were significantly lower ($P < 0.05$) than that of *E. coli* O157:H7 for all treatments except the water wash. Table 3 shows the same trend of *E. coli* O157:H7, EcRFP and the average surrogate cocktail log reductions that were not significantly different ($P > 0.05$) during each individual treatment applied to the brisket carcass surface region. This table also shows that log reductions of *S.* Typhimurium are similar to that of EcYFP ($P > 0.05$) across all treatments. On the brisket carcass surface region, the log reductions for the average surrogate cocktail were equivalent to that of *E. coli* O157:H7 during all treatments except the water wash + hot water treatment. The log reductions for the total surrogate cocktail were consistently lower than *E. coli* O157:H7 or the average surrogate cocktail; however, they were only significantly different ($P < 0.05$) during the water wash + 2% L-lactic acid treatment. Similar trends can be seen in the clod surface region data in Table 4. Log reductions of *S.* Typhimurium were similar to that of EcYFP ($P > 0.05$) during all four treatments. Log reductions of *E. coli* O157:H7 were not significantly different ($P > 0.05$) from EcRFP, the average surrogate cocktail and the total surrogate cocktail during all four treatments. The log reductions for the average surrogate cocktail were higher than those achieved for *E. coli* O157:H7 during the first two treatments, but not significantly different ($P > 0.05$). The differences and importance of the average and total surrogate cocktail values will be discussed in the following section.

Results of the present study demonstrate that a water wash alone is not effective

in eliminating fecal contamination of beef carcass surfaces. Removal of visible fecal material from carcass surfaces after harvesting is currently required by USDA-FSIS regulations. The removal of fecal contamination can be accomplished by knife trimming, hot water or steam vacuuming (*65*); however, the effectiveness of steam vacuuming has been questioned (*17*). Since enteric pathogens are not necessarily confined to areas with visible fecal contamination, treating only those carcass surface areas cannot ensure the removal of pathogens. Therefore, it is important to also include a sanitizing step such as hot water or lactic acid spray.

Total Surrogate Cocktail vs. Average Surrogate Cocktail

The calculated total surrogate cocktail reduction was lower than the average surrogate cocktail reduction in all but two instances that occurred with the clod water wash and water wash + hot water treatments (Table 4). These values were calculated differently (see Materials and Methods) to determine which would be a better predictor of the behavior of the enteric pathogens. Many researchers choose to use a cocktail of different strains of an organism when conducting research to account for any differences among the strains (*2, 11, 28, 36*). Usually, they have no means of differentiating the different strains once they are mixed together and, therefore, they are all counted together similar to the total surrogate cocktail in this experiment. However, since these organisms fluoresce different colors, they could be counted separately and those individual values could be averaged together. In this case, the average value obtained was not significantly different from that of *E. coli* O157:H7 ($P > 0.05$) (Tables 1-4).

However, the average surrogate cocktail log reduction value obtained was in 66.7% of cases greater than or equivalent to the *E. coli* O157:H7 log reductions achieved as seen in Tables 2, 3 and 4. The underestimated total surrogate cocktail log reduction value is better to use when trying to determine the efficacy of carcass decontamination treatments because one can be certain of the minimum log reduction achieved. Use of the average surrogate cocktail value might lead to an over prediction of the pathogen reduction. For this reason, the total surrogate cocktail value offers a more reliable prediction of pathogen characteristics.

Effects of Carcass Surface Region

Hardin et al. (*35*) demonstrated that the carcass surface region could affect log reductions achieved by decontamination treatments. It was reported that the inside round consistently had smaller log reductions than the other surface regions examined. Research conducted by Castillo et al. (*15*) eliminated some of the variation in log reductions that were observed in the Hardin et al. (*35*) experiment by not using the inside round surface region. In the present study there were some significant differences ($P < 0.05$) observed in log reductions for each fluorescent protein-expressing organism between different carcass surface regions. The lowest log reductions were consistently observed in the brisket region and these results may be due to several different factors. First, when the hides were removed from the carcasses several knife cuts may have been made on the surface of the meat, which could have created areas for microorganisms that could not be reached by the treatment application. Second, each surface area had varying

degrees of fat present, and it has been suggested by several other researchers that bacterial reductions from microbial intervention methods are greater on adipose tissue than on lean tissue (*26, 48*). Lastly, the brisket tends to be the area with the highest level of contamination (*9, 49, 59*), thought to be due to the cattle laying down on contaminated surfaces at the farm, during transport and in the stunning boxes. Hide cleanliness tends to correlate to contamination of carcass surfaces (*49*). In some instances the inoculated organisms on the clod surface region had significantly higher log reductions than the outside round and brisket. Again, this may be explained by the differences in the surface characteristics of the meat and the number of knife cuts present.

Table 5 shows that inoculated pathogens on all three surface regions examined were affected similarly during each treatment with the exception of the clod. The water wash + hot water treatment resulted in significantly greater *S.* Typhimurium log reductions on the clod surface region when compared to the brisket and outside round surface regions. Treatment of the clod surface region with a water wash + hot water + 2% L-lactic acid treatment caused significantly higher log reductions than observed on the brisket. These differences can most likely be attributed to variations in the surface characteristics of the cuts. During this experiment the clod was observed to be the smoothest surface region and therefore did not have any areas in which microorganisms could be protected from the treatments. Table 6 presents similar data for the fluorescent protein-producing surrogates. Lower log reductions were consistently observed with the brisket surface region (Table 6). The water wash + hot water treatment resulted in

TABLE 5. *Mean*[a] *log reductions*[b] *(log CFU/cm*^{2}*) of pathogens recovered from within 400-cm*^{2} *contaminated areas of three different carcass surface regions as affected by decontamination treatments applied*

Treatment[c]	Inoculated Organism	Outside Round (O)	Brisket (B)	Clod (C)	Order of Means[d]
Water wash	*E. coli* O157:H7	1.9	1.8	1.9	O C B
	S. Typhimurium	1.6	1.3	1.6	O C B
Water wash + Lactic acid	*E. coli* O157:H7	4.2	4.2	4.1	B O C
	S. Typhimurium	>5.1	>5.1	>5.3	C O B
Water wash + Hot water	*E. coli* O157:H7	3.7	3.5	4.0	C O B
	S. Typhimurium	2.6	2.4	3.5	C O B
Water wash + Hot water + Lactic acid	*E. coli* O157:H7	4.8	>4.5	5.1	C O B
	S. Typhimurium	>5.2	>4.5	>5.3	C O B

[a] Mean = average obtained from three repetitions.

[b] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).

[c] Water wash: 1.5-liter hand wash followed by 5-liter automated cabinet wash (9 s, 1.72 MPa-2.76MPa), 28ºC. Lactic acid: 2% L-lactic acid spray (200 ml, 55ºC for 11 s). Hot water: 95ºC water at 166 kPa for 5 s from a distance of 12.5 cm.

[d] Means within rows underlined by a common line are not significantly different (P > 0.05).

TABLE 6. *Mean[a] log reductions[b] (log CFU/cm^2) of fluorescent surrogate* E. coli *recovered from within 400-cm^2 contaminated areas of three different carcass surface regions as affected by decontamination treatments applied*

Treatment[c]	Inoculated Organism	Outside Round (O)	Brisket (B)	Clod (C)	Order of Means[d]
Water wash	EcRFP	2.4	2.0	1.9	O B C
	EcGFP	2.1	1.6	2.0	O C B
	EcYFP	2.1	1.7	2.1	C O B
Water wash + Lactic acid	EcRFP	>4.5	4.5	>4.3	O B C
	EcGFP	3.4	2.9	3.4	C O B
	EcYFP	>5.6	5.4	5.7	C O B
Water wash + Hot water	EcRFP	3.2	>3.3	3.7	C B O
	EcGFP	3.0	2.8	3.8	C O B
	EcYFP	3.2	2.8	4.1	C O B
Water wash + Hot water + Lactic acid	EcRFP	4.6	>4.4	>4.3	O B C
	EcGFP	3.6	4.1	4.5	C B O
	EcYFP	>6.0	>5.1	5.9	O C B

[a] Mean = average obtained from three repetitions.

[b] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).

[c] Water wash: 1.5-liter hand wash followed by 5-liter automated cabinet wash (9 s, 1.72 MPa-2.76MPa), 28ºC. Lactic acid: 2% L-lactic acid spray (200 ml, 55ºC for 11 s). Hot water: 95ºC water at 166 kPa for 5 s from a distance of 12.5 cm.

[d] Means within rows underlined by a common line are not significantly different ($P > 0.05$).

significantly higher EcYFP and EcGFP mean log reductions on the clod surface region (Table 6).

Temperature and pH of Carcass Surface Regions

The average initial carcass surface region temperature, prior to treatment application, was 25.4°C with a range of 24 - 27°C. During the hot water treatments the average carcass surface temperature reached was 77°C, which was lower than expected, given that the temperature of the hot water spray was 95°C. Previous experiments (*15, 16, 31*) have demonstrated that a 95°C hot water spray applied at a distance of 12.5 cm and pressure of 166 kPa should raise the carcass surface temperature to at least 82°C. That was not observed in this study and likely resulted in lower than expected log reductions, discussed later.

The average initial carcass surface region pH was 7.9, and the lactic acid treatment reduced the carcass surface pH to an average of 2.7 with a range of 2.5-3.0. The average pH of the 2% L-lactic acid solution was 2.1. These findings are consistent with those reported by others (*27, 31, 35*).

Surrogate Behavior

Each fluorescent surrogate had distinctive characteristics and responded uniquely to the decontamination treatments applied. No single isolate had the ability to exactly predict the behavior of *E. coli* O157:H7 or *S.* Typhimurium throughout each of the different treatments. The data set in this study had a large standard deviation and the

total surrogate cocktail value obtained offered a better representation of the pathogen behavior. The total surrogate cocktail value tended to underestimate the effects of the interventions applied, which is a desirable characteristic when attempting to predict log reductions from carcass decontamination treatments. For this reason, it is recommended that the surrogates be used collectively as a total surrogate cocktail to more accurately predict the behavior of enteric pathogens. This procedure was also suggested by Marshall et al. (*48*) in a similar study attempting to identify indicators for fresh beef. Data has been reformatted in Table 7 to compare the log reductions of the enteric pathogens and the total surrogate cocktail.

Effect of Treatment Day

Three repetitions of this experiment were conducted over three consecutive days. Although all treatments were applied identically over the different days, statistical analysis revealed that there were significant day effects ($P < 0.05$) over the three days of the experiment. The data for the individual experimental days are presented in Tables 8-13. The differences observed were very small and did not affect the outcome of the experiment.

Testing at a Very Small Slaughter Facility

This portion of the project was conducted at Texas A&M University Rosenthal Meat Science and Technology Center (RMSTC), which is considered a very small slaughter facility with less than 10 employees. Seven carcass sides were utilized per day

TABLE 7. *Mean[a] log reductions[b] (log CFU/cm^2) of pathogens and fluorescent surrogate* E. coli *recovered from within 400-cm^2 contaminated areas of different carcass surface regions as affected by decontamination treatments applied*

Treatment[c]	Organism	Outside Round (O)	Brisket (B)	Clod (C)	Order of Means[d]
Water wash	*E. coli* O157:H7	1.9	1.8	1.9	O C B
	S. Typhimurium	1.6	1.3	1.6	O C B
	Total Surrogate Cocktail[e]	1.6	1.7	2.0	C B O
Water wash + Lactic acid	*E. coli* O157:H7	4.2	4.2	4.1	B O C
	S. Typhimurium	>5.1	>5.1	>5.3	C O B
	Total Surrogate Cocktail	>3.2	>3.3	>3.8	C B O
Water wash + Hot water	*E. coli* O157:H7	3.7	3.5	4.0	C O B
	S. Typhimurium	2.6	2.4	3.5	C O B
	Total Surrogate Cocktail	2.6	>2.8	4.0	C B O
Water wash + Hot water + Lactic acid	*E. coli* O157:H7	4.8	>4.5	5.1	C O B
	S. Typhimurium	>5.2	>4.5	>5.3	C O B
	Total Surrogate Cocktail	>3.4	>4.2	>4.8	C B O

[a] Mean = average obtained from three repetitions.

[b] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).

[c] Water wash: 1.5-liter hand wash followed by 5-liter automated cabinet wash (9 s, 1.72 MPa-2.76MPa), 28ºC. Lactic acid: 2% L-lactic acid spray (200 ml, 55ºC for 11 s). Hot water: 95ºC water at 166 kPa for 5 s from a distance of 12.5 cm.

[d] Means within rows underlined by a common line are not significantly different ($P > 0.05$).

[e] Total Surrogate Cocktail = calculated average log reduction of EcRFP, EcGFP and EcYFP.

TABLE 8. *Mean[a] log reductions[b] (log CFU/cm^2) of pathogens and fluorescent protein expressing* E. coli *recovered from within 400-cm^2 contaminated areas of three carcass surface regions during repetition one as affected by decontamination treatments applied*

	Inoculated Organism							
Treatment[c]	*E. coli* O157:H7 (1)	*S.* Typhimurium (2)	EcRFP[d] (3)	EcGFP (4)	EcYFP (5)	Average Surrogate Cocktail[e] (6)	Total Surrogate Cocktail[f] (7)	Order of Means[g]
Water wash	1.7C[h]	1.4D	.	1.9C	1.9C	1.9D	1.4C	6 4 5 1 2 7
Water wash + Lactic acid	3.9AB	>5.5A	.	3.0B	5.1A	>4.2B	>2.9B	2 5 6 1 4 7
Water wash + Hot water	3.6B	3.2C	.	3.6A	3.6B	3.6C	3.1B	1 6 4 5 2 7
Water wash + Hot water + Lactic Acid	4.5A	>4.9B	.	4.1A	>5.2A	>4.8A	>3.8A	5 2 6 1 4 7

[a] Mean = average obtained from clod, brisket and outside round surface regions during repetition one.
[b] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).
[c] Water wash: 1.5-liter hand wash followed by 5-liter automated cabinet wash (9 s, 1.72 MPa-2.76MPa), 28ºC. Lactic acid: 2% L-lactic acid spray (200 ml, 55ºC for 11 s). Hot water: 95ºC water at 166 kPa for 5 s from a distance of 12.5 cm.
[d] Log reductions for EcRFP are missing because they were not included in the cocktail.
[e] Average Surrogate Cocktail = log of the calculated average reduction of EcGFP and EcYFP.
[f] Total Surrogate Cocktail = log of the sum of EcGFP and EcYFP reductions.
[g] Means within rows underlined by a common line are not significantly different (P > 0.05).
[h] Means within columns with the same letter (A, B, C, D) are not significantly different (P > 0.05).

TABLE 9. *Log reductions*[a] *(log CFU/cm*^{2}*) of pathogens and fluorescent surrogate* E. coli *recovered from within 400-cm*^{2} *contaminated areas of different carcass surface regions as affected by decontamination treatments applied during repetition one*

Treatment[b]	Organism	Outside Round (O)	Brisket (B)	Clod (C)	Order of Means[c]
Water wash	*E. coli* O157:H7	2.0	1.3	1.8	O C B
	S. Typhimurium	1.4	0.8	2.1	C O B
	Total Surrogate Cocktail[d]	0.4	1.2	2.5	C B O
Water wash + Lactic acid	*E. coli* O157:H7	4.4	3.5	3.9	O C B
	S. Typhimurium	>5.3	>5.3	>5.8	C O B
	Total Surrogate Cocktail	1.9	2.9	3.8	C B O
Water wash + Hot water	*E. coli* O157:H7	4.0	2.3	4.6	C O B
	S. Typhimurium	3.3	2.1	4.3	C O B
	Total Surrogate Cocktail	2.4	2.2	4.6	C O B
Water wash + Hot water + Lactic acid	*E. coli* O157:H7	4.7	3.2	5.5	C O B
	S. Typhimurium	>5.3	3.5	>5.8	C O B
	Total Surrogate Cocktail	2.9	3.5	5.1	C B O

[a] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).

[b] Water wash: 1.5-liter hand wash followed by 5-liter automated cabinet wash (9 s, 1.72 MPa-2.76MPa), 28ºC. Lactic acid: 2% L-lactic acid spray (200 ml, 55ºC for 11 s). Hot water: 95ºC water at 166 kPa for 5 s from a distance of 12.5 cm.

[c] Means within rows underlined by a common line are not significantly different (P > 0.05).

[d] Total Surrogate Cocktail = log of the sum of EcGFP and EcYFP reductions.

TABLE 10. *Mean[a] log reductions[b] (log CFU/cm^2) of pathogens and fluorescent protein expressing* E. coli *recovered from within 400-cm^2 contaminated areas of three carcass surface regions during repetition two as affected by decontamination treatments applied*

	Inoculated Organism							
Treatment[c]	*E. coli* O157:H7 (1)	*S.* Typhimurium (2)	EcRFP (3)	EcGFP (4)	EcYFP (5)	Average Surrogate Cocktail[d] (6)	Total Surrogate Cocktail[e] (7)	Order of Means[f]
Water wash	1.9B[g]	2.3B	2.6B	2.2B	2.2B	2.3B	2.2B	3 6 2 5 7 4 1
Water wash + Lactic acid	4.0A	>5.6A	>4.5A	3.5A	>6.1A	>4.7A	>3.8A	5 2 6 3 1 7 4
Water wash + Hot water	3.6A	2.9B	3.3B	2.8AB	3.0B	3.1B	2.9B	1 3 6 5 7 2 4
Water wash + Hot water + Lactic Acid	4.2A	>5.7A	>4.5A	3.6A	>5.9A	>4.7A	>3.9A	5 2 6 3 1 7 4

[a] Mean = average obtained from clod, brisket and outside round surface regions during repetition one.
[b] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).
[c] Water wash: 1.5-liter hand wash followed by 5-liter automated cabinet wash (9 s, 1.72 MPa-2.76MPa), 28ºC. Lactic acid: 2% L-lactic acid spray (200 ml, 55ºC for 11 s). Hot water: 95ºC water at 166 kPa for 5 s from a distance of 12.5 cm.
[d] Average Surrogate Cocktail = log of the calculated average reduction of EcRFP, EcGFP and EcYFP.
[e] Total Surrogate Cocktail = log of the sum of EcRFP, EcGFP and EcYFP reductions.
[f] Means within rows underlined by a common line are not significantly different (P > 0.05).
[g] Means within columns with the same letter (A, B, C, D) are not significantly different (P > 0.05).

TABLE 11. *Log reductions[a] (log CFU/cm^2) of pathogens and fluorescent surrogate* E. coli *recovered from within 400-cm^2 contaminated areas of different carcass surface regions as affected by decontamination treatments applied during repetition two*

Treatment[b]	Inoculated Organism	Outside Round (O)	Brisket (B)	Clod (C)	Order of Means[c]
Water wash	*E. coli* O157:H7	1.6	2.0	2.3	C B O
	S. Typhimurium	2.1	2.4	2.5	C B O
	Total Surrogate Cocktail[d]	2.2	2.3	2.3	B C O
Water wash + Lactic acid	*E. coli* O157:H7	3.5	4.3	4.2	B C O
	S. Typhimurium	>5.0	>5.8	>6.0	C B O
	Total Surrogate Cocktail	>3.6	>3.8	>4.0	C B O
Water wash + Hot water	*E. coli* O157:H7	3.3	3.6	3.8	C B O
	S. Typhimurium	2.3	2.4	4.0	C B O
	Total Surrogate Cocktail	2.5	2.4	3.8	C O B
Water wash + Hot water + Lactic acid	*E. coli* O157:H7	4.5	3.8	4.4	O C B
	S. Typhimurium	5.3	>5.8	>6.0	C B O
	Total Surrogate Cocktail	3.9	>3.8	>4.1	C O B

[a] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).

[b] Water wash: 1.5-liter hand wash followed by 5-liter automated cabinet wash (9 s, 1.72 MPa-2.76MPa), 28ºC. Lactic acid: 2% L-lactic acid spray (200 ml, 55ºC for 11 s). Hot water: 95ºC water at 166 kPa for 5 s from a distance of 12.5 cm.

[c] Means within rows underlined by a common line are not significantly different (P > 0.05).

[d] Total Surrogate Cocktail = log of the sum of EcRFP, EcGFP and EcYFP reductions.

TABLE 12. *Mean[a] log reductions[b] (log CFU/cm^2) of pathogens and fluorescent protein expressing* E. coli *recovered from within 400-cm^2 contaminated areas of three carcass surface regions during repetition three as affected by decontamination treatments applied*

	Inoculated Organism							
Treatment[c]	*E. coli* O157:H7 (1)	*S.* Typhimurium (2)	EcRFP (3)	EcGFP (4)	EcYFP (5)	Average Surrogate Cocktail[d] (6)	Total Surrogate Cocktail[e] (7)	Order of Means[f]
Water wash	2.0C[g]	0.8C	1.7C	1.6C	1.8C	1.7C	1.7C	1 5 3 6 7 4 2
Water wash + Lactic acid	4.5B	>4.5A	>4.0A	3.2B	>5.5A	>4.2A	>3.6B	5 1 2 6 3 7 4
Water wash + Hot water	4.0B	2.4B	>3.2B	3.2B	3.5B	3.3B	3.4B	1 5 7 6 3 4 2
Water wash + Hot water + Lactic Acid	>5.7A	>4.5A	>4.0A	4.5A	>5.9A	>4.8A	>4.6A	5 1 6 7 2 4 3

[a] Mean = average obtained from clod, brisket and outside round surface regions during repetition one.
[b] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).
[c] Water wash: 1.5-liter hand wash followed by 5-liter automated cabinet wash (9 s, 1.72 MPa-2.76MPa), 28ºC. Lactic acid: 2% L-lactic acid spray (200 ml, 55ºC for 11 s). Hot water: 95ºC water at 166 kPa for 5 s from a distance of 12.5 cm.
[d] Average Surrogate Cocktail = log of the calculated average reduction of EcRFP, EcGFP and EcYFP.
[e] Total Surrogate Cocktail = log of the sum of EcRFP, EcGFP and EcYFP reductions.
[f] Means within rows underlined by a common line are not significantly different (P > 0.05).
[g] Means within columns with the same letter (A, B, C, D) are not significantly different (P > 0.05).

Table 13. *Log reductions[a] (log CFU/cm^2) of pathogens and fluorescent surrogate* E. coli *recovered from within 400-cm^2 contaminated areas of different carcass surface regions as affected by decontamination treatments applied during repetition three*

Treatment[b]	Organism	Outside Round (O)	Brisket (B)	Clod (C)	Order of Means[c]
Water wash	*E. coli* O157:H7	2.2	2.2	1.6	O B C
	S. Typhimurium	1.4	0.7	0.3	O B C
	Total Surrogate Cocktail[d]	2.2	1.5	1.4	O B C
Water wash + Lactic acid	*E. coli* O157:H7	4.6	4.8	4.2	B O C
	S. Typhimurium	>5.1	>4.2	>4.1	O B C
	Total Surrogate Cocktail	>4.0	>3.1	>3.7	O C B
Water wash + Hot water	*E. coli* O157:H7	3.7	4.6	3.6	B O C
	S. Typhimurium	2.2	2.6	2.3	B C O
	Total Surrogate Cocktail	2.9	3.8	3.5	B C O
Water wash + Hot water + Lactic acid	*E. coli* O157:H7	5.1	>6.5	5.4	B C O
	S. Typhimurium	>5.1	>4.2	>4.1	O B C
	Total Surrogate Cocktail	>3.5	>5.3	>5.1	B O C

[a] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).

[b] Water wash: 1.5-liter hand wash followed by 5-liter automated cabinet wash (9 s, 1.72 MPa-2.76MPa), 28ºC. Lactic acid: 2% L-lactic acid spray (200 ml, 55ºC for 11 s). Hot water: 95ºC water at 166 kPa for 5 s from a distance of 12.5 cm.

[c] Means within rows underlined by a common line are not significantly different (P > 0.05).

[d] Total Surrogate Cocktail = log of the sum of EcRFP, EcGFP and EcYFP reductions.

in this study. Cattle were harvested and processed on the line following USDA-FSIS regulated procedures (*69*). Following carcass splitting and just prior to washing, the neck region of each carcass side was inoculated with a fecal slurry containing stationary-phase cultures of the three fluorescent surrogate organisms (EcRFP, EcGFP, EcYFP). A water wash consisting of 1.5 liters of warm (~ 28ºC) potable water was applied using a hand-held polyethylene compressed-air sprayer (7.57-liter capacity, Fountainhead Group, Inc) to remove all visible fecal material (*15, 63*) with a low pressure rinse (69 kPa, 90 s). The carcass side was then subjected to the normal treatment procedures of that facility. At the RMSTC, the decontamination treatments consisted of a hot water (63.8°C) wash followed by a 2.4% L-lactic acid spray. The hot water wash was applied to the entire carcass side for varying times ranging from 1.0-2.0 min. The L-lactic acid spray applied was at a concentration of 2.4%. The pH of the 2.4% L-lactic acid solution was measured at 2.0 and was applied at an average temperature of 59°C to each carcass side for varying times ranging from 0.2-1.0 minutes. 237 ml of lactic acid spray were applied per 10 s of application time; therefore, only 474-1422 ml were applied to the entire carcass side. This is a smaller volume applied per cm^2 than the volume applied during the laboratory portion of this study. After each treatment, samples were taken and the treatments were compared for their effectiveness at reducing populations of inoculated EcRFP, EcGFP and EcYFP.

Log Reductions

The log reductions achieved for the total surrogate cocktail were: 1.8 log

CFU/cm^2 by water wash, 2.4 CFU/cm^2 by water wash + hot water and 2.9 CFU/cm^2 by water wash + hot water + L-lactic acid spray (Table 14). These log reductions were lower than expected based upon reductions achieved in the laboratory setting for similar treatments. Several differing factors (temperature, volume, application time) were likely responsible for the smaller log reductions achieved, as discussed below.

Temperature and pH of the Carcass

The initial temperature of the carcass in the RMSTC was 26.6°C, and the temperature of the carcass side was raised to 51.4°C during the hot water wash. This was much lower than the temperature achieved in laboratory studies and was likely due to the low source temperature of the RMSTC hot water spray. The average initial pH of the carcass side was 7.7, which decreased to 3.5 after the lactic acid spray. The reduction in pH was less than expected, and was likely due to the small volume of lactic acid applied. As stated previously, there are many factors that can alter the effectiveness of carcass decontamination treatments. In this study, the lower source temperature of the hot water and smaller volume of lactic acid applied more than likely resulted in significantly smaller log reductions than were observed in the laboratory tests. This information was important because it accurately represented treatment parameters used in industry. Recommendations for this plant would include installation of a hot water system that is capable of reaching higher temperatures and applying a larger volume of lactic acid solution evenly over the carcass.

TABLE 14. *Mean[a] log reductions[b] (log CFU/cm^2) of fluorescent protein expressing organisms recovered from within 400-cm^2 contaminated areas of carcass neck regions as affected by decontamination treatments applied*

	Inoculated Organism					
Treatment[c]	EcRFP (1)	EcGFP (2)	EcYFP (3)	Average Surrogate Cocktail[d] (4)	Total Surrogate Cocktail[e] (5)	Order of Means[f]
Water wash	1.7C[g]	1.8C	1.8C	1.8C	1.8C	2 3 5 4 1
Water wash + Hot water	2.2B	2.4B	2.4B	2.3B	2.4B	2 5 3 4 1
Water wash + Hot water + Lactic Acid	2.9A	2.8A	3.1A	2.9A	2.9A	3 4 1 5 2

[a] Mean = average obtained from seven carcass neck regions.

[b] Log reduction = (log CFU/cm^2 before treatment) - (log CFU/cm^2 after treatment).

[c] Water wash: 1.5-liter hand wash, 28ºC. Hot water: 63.8ºC water for 1.0 - 2.0 min. Lactic acid: 2.4% L-lactic acid spray (0.5 - 1.4 L, 59ºC for 0.2 - 1.0 min.

[d] Average Surrogate Cocktail = log of the calculated average reduction of EcGFP and EcYFP.

[e] Total Surrogate Cocktail = log of the sum of EcGFP and EcYFP reductions.

[f] Means within rows underlined by a common line are not significantly different (P > 0.05).

[g] Means within columns with the same letter (A, B, C) are not significantly different (P > 0.05).

Comparison of Studies

There were several differences observed in the current study, compared to previous studies involving hot water carcass decontamination treatments. Differences in the laboratory experiments included the initial carcass surface region temperatures and the temperature of the initial water wash. Variations in the slaughter facility studies include temperature of the hot water source and spray, application time, distance and pressure, and the initial carcass temperature. These factors likely contributed to the variation in reported log reductions observed in each study. This study was designed to more closely represent the treatment parameters that are used in industry, which is why the initial water wash temperature was lower than in previous studies. In the laboratory experiments conducted in this study, the lower temperature of the initial hand-held water wash was likely a major cause of the smaller than expected log reductions when compared to previous studies conducted in this laboratory. In Castillo et al. (*15*), a 35°C water wash was used prior to the hot water spray. This raised the temperature of the carcass region before the actual hot water treatment was applied, allowing the surface region to reach a higher temperature faster than in the current study, which utilized a 28°C water wash. It is apparent from the data presented in Table 15 that a hot water treatment is more effective when a surface region temperature of at least 82°C is reached. The small difference in only 5ºC between the carcass surface region temperature reached in the laboratory portion of this study compared to previous studies obviously makes a large difference in log reductions achieved.

This study presents important information regarding carcass decontamination

TABLE 15. *Comparison of current and previous hot water treatment experimental designs*

	Moseley et al.		Castillo et al. (*15*)	Barkate et al. (*7*)
	Laboratory Experiment	Slaughter Facility	Laboratory Experiment	Slaughter Facility
Temperature of initial water wash	28°C	None	35°C	None
Temperature of hot water source	96.6°C	-	97°C	-
Temperature of hot water spray	95°C	63.8°C	95°C	95°C
Application time	5 s	1.0-2.0 min	5 s	40 s
Application distance	12.5 cm	≥30.5 cm	12.5 cm	-
Pressure of hot water spray	166 kPa	-	166 kPa	-
Carcass surface temperature during application	77°C	51.4°C	82°C	82°C
Initial carcass temperature	25.5°C	26.6°C	-	-
E. coli O157:H7 log reduction	3.7 log CFU/cm^2	2.2 log CFU/cm^2	4.0 log CFU/cm^2	-
Aerobic plate count log reduction	-	-	3.2 log CFU/cm^2	1.1 log CFU/cm^2

treatments utilizing 2% L-lactic spray and a hot water wash. These treatments, when applied as described, are more effective in combination than when used individually. It is important that several parameters (time, temperature, volume, pressure) are carefully controlled to maximize the effectiveness of the treatments applied. Minor differences in application of these parameters can result in ineffective enteric pathogen reduction. Therefore, it is of utmost importance that slaughter establishments validate carcass interventions to ensure they are effective.

This research was performed using fluorescent surrogate organisms, which have been shown to be helpful in similar studies. The fluorescent surrogates are non-pathogenic and, therefore, can be used in a laboratory setting or in the processing environment without risking foodborne illness. However, even in light of the non-pathogenic nature of the surrogate organisms used in this study, removal of all treated carcass surface areas for non-edible disposal and thorough sanitation of the testing area is recommended. This research suggests that it is better to use the total surrogate cocktail value instead of the individual or average surrogate cocktail values to predict the behavior of enteric pathogens in carcass decontamination studies because the average surrogate cocktail value tends to overestimate the effectiveness of interventions.

CONCLUSIONS

The results of the present study indicate that 2% L-lactic acid and hot water are effective carcass decontamination treatments when applied as described. The treatments produce the greatest microbial log reductions when used in combination rather than separately. If a slaughter establishment found it necessary to choose only one intervention, then the 2% L-lactic acid spray was shown to be more effective than the hot water wash.

Fluorescent surrogate organisms were used in this study to validate carcass decontamination treatments. Individually, none of the fluorescent surrogate organisms had log reductions that consistently predicted reductions of *E. coli* O157:H7 or *S.* Typhimurium after application of interventions. However, methods of combining the surrogate cocktail count data were evaluated and the total count of the surrogate cocktail was determined to be a more consistent and accurate prediction of pathogen reduction. The average surrogate cocktail value tended to overestimate the effectiveness of the carcass interventions that were applied.

REFERENCES

1. Ajjarapu, S., and L. A. Shelef. 1999. Fate of pGFP-bearing *Escherichia coli* O157:H7 in ground beef at 2 and 10ºC and effects of lactate, diacetate and citrate. *Appl. Environ. Microbiol.* 65:5394-5397.

2. Al-Holy, M., H. Al-Qadiri, M. Lin, and B. Rasco. 2006. Inhibition of *Listeria innocua* in hummus by a combination of nisin and citric acid. *J. Food Prot.* 69:1322-1327.

3. Armstrong, G. L., J. Hollingsworth, and J. G. Morris Jr. 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.* 18:29-51.

4. Aslam, M., F. Nattress, G. Greer, C. Yost, C. Gill, and L. McMullen. 2003. Origin of contamination and genetic diversity of *Escherichia coli* in beef cattle. *Appl. Environ. Microbiol.* 69:2794-2799.

5. Bacon, R. T., K. E. Belk, J. N. Sofos, and G. C. Smith. 2000. Incidence of *Escherichia coli* O157:H7 on hide, carcass and beef trimmings samples collected from United States packing plants. Available at: http://www.amif.org/AMIF%20Executive%20Summary%20carcass%20Sampling%202000.pdf. Accessed January 2006.

6. Bacon, R. T., J. N. Sofos, K. E. Belk, D. R. Hyatt, and G. C. Smith. 2002. Prevalence and antibiotic susceptibility of *Salmonella* isolated from beef animal hides and carcasses. *J. Food Prot.* 65:284-290.

7. Barkate, M. L., G. R. Acuff, L. M. Lucia, and D. S. Hale. 1993. Hot water decontamination of beef carcasses for reduction of initial bacterial numbers. *Meat Sci.* 35:397-401.

8. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978-1986.

9. Bell, R. G. 1997. Distribution and sources of microbial contamination on beef carcasses. *J. Appl. Microbiol.* 82:292-300.

10. Besser, R. E., S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barrett, J. G. Wells, and P. M. Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *JAMA* 269:2217-2220.

11. Brackett, R. E., Y. -Y. Hao, and M. P. Doyle. 1994. Ineffectiveness of hot acid sprays to decontaminate *Escherichia coli* O157:H7 on beef. *J. Food Prot.* 57:198-203.

12. Bryan, F. L. 1983. Epidemiology of milk-borne diseases. *J. Food Prot.* 46:637-649.

13. Burgess, F., C. L. Little, G. Allen, K. Williamson, and R. T. Mitchell. 2005. Prevalence of *Campylobacter*, *Salmonella*, and *Escherichia coli* on the external packaging of raw meat. *J. Food Prot.* 68:469-475.

14. Cabrera-Diaz, E., T. M. Musquiz, L. M. Lucia, J. S. Dickson, and G. R. Acuff. 2006. Evaluation of acid and thermal resistance properties of fluorescent-marked nonpathogenic *Escherichia coli* strains for use as surrogates for enteric pathogens, P2-56. International Association of Food Protection annual meeting, Calgary, Alberta, Canada.

15. Castillo, A., L. M. Lucia, K. J. Goodson, J. W. Savell, and G. R. Acuff. 1998. Comparison of water wash, trimming and combined hot water and lactic acid treatments for reducing bacteria of fecal origin on beef carcasses. *J. Food Prot.* 61:823-828.

16. Castillo, A., L. M. Lucia, K. J. Goodson, J. W. Savell, and G. R. Acuff. 1998. Use of hot water for beef carcass decontamination. *J. Food Prot.* 61:19-25.

17. Castillo, A., L. M. Lucia, K. J. Goodson, J. W. Savell, and G. R. Acuff. 1999. Decontamination of beef carcass surface tissue by steam vacuuming alone and combined with hot water and lactic acid sprays. *J. Food Prot.* 62:146-151.

18. Castillo, A., L. M. Lucia, G. K. Kemp, and G. R. Acuff. 1999. Reduction of *Escherichia coli* O157:H7 and *Salmonella* typhimurium on beef carcass surfaces using acidified sodium chlorite. *J. Food Prot.* 62:580-584.

19. Castillo, A., L. M. Lucia, D. B. Roberson, T. H. Stevenson, I. Mercado, and G. R. Acuff. 2001. Lactic acid sprays reduce bacterial pathogens on cold beef carcass surfaces and in subsequently produced ground beef. *J. Food Prot.* 64:58-62.

20. Castillo, A., K. S. McKenzie, L. M. Lucia, and G. R. Acuff. 2003. Ozone treatment for reduction of *Escherichia coli* O157:H7 and *Salmonella* serotype Typhimurium on beef carcass surfaces. *J. Food Prot.* 66:775-779.

21. Centers for Disease Control and Prevention, CDC. 1993. Preliminary report: Foodborne outbreak of *Escherichia coli* O157:H7 infections from hamburgers-Western United States. *Morbid. Mortal. Wkly. Rep.* 42:85-86.

22. Centers for Disease Control and Prevention, CDC. 2006. Table II (part 2) Provisional cases of selected notifiable diseases, United States, week ending December 31, 2005. *Morbid. Mortal. Wkly. Rep.* 54:1320-1330.

23. Cohen, S. 2005. FSIS ground beef sampling shows a substantial *E. coli* O157:H7 decline in 2004. Available at: http://www.fsis.usda.gov/News_&_Events/NR_022805_01/index.asp. Accessed April 2006.

24. Collis, V. J., C. -A. Reid, M. L. Hutchison, M. H. Davies, K. P. A. Wheeler, A. Small, and S. Buncic. 2004. Spread of marker bacteria from the hides of cattle in a simulated livestock market and at an abattoir. *J. Food Prot.* 67:2397-2402.

25. Dewell, G. A., J. R. Ransom, R. D. Dewell, K. McCurdy, I. A. Gardner, A. E. Hill, J. N. Sofos, K. E. Belk, G. C. Smith, and M. D. Salman. 2005. Prevalence of and risk factors for *Escherichia coli* O157 in market-ready beef cattle from 12 U.S. feedlots. *Foodborne Pathog. Dis.* 2:70-76.

26. Dickson, J. S., C. N. Cutter, and G. R. Siragusa. 1994. Antimicrobial effects of trisodium phosphate against bacteria attached to beef tissue. *J. Food Prot.* 57:952-955.

27. Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1997. Effects of acetic acid, lactic acid and trisodium phosphate on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua* and *Clostridium sporogenes*. *J. Food Prot.* 60:619-624.

28. Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1998. Long-term effect of alkaline, organic acid, or hot water washes on the microbial profile of refrigerated beef contaminated with bacterial pathogens after washing. *J. Food Prot.* 61:300-306.

29. Doyle, M. P. 1991. *Escherichia coli* O157:H7 and its significance in foods. *Int. J. Food Microbiol.* 12:289-301.

30. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci.* 97:2999-3003.

31. Ellebracht, E. A. May 1999. Comparison of decontamination methods to reduce microbial levels in fresh beef trimmings [MS Thesis]. College Station, TX: Texas A&M University. 93 p.

32. Errampalli, D., K. Leung, M. B. Cassidy, M. Kostrzynska, M. Blears, H. Lee, and J. T. Trevors. 1999. Applications of the green fluorescent protein as a molecular marker in environmental microorganisms. *J. Microbiol. Meth.* 35:187-199.

33. Fegan, N., P. Vanderlinde, G. Higgs, and P. Desmarchelier. 2004. Quantification and prevalence of *Salmonella* in beef cattle presenting at slaughter. *J. Appl. Microbiol.* 97:892-898.

34. Glynn, M. K., C. Bopp, W. Dewitt, P. Dabney, M. Mokhtar, and F. J. Angulo. 1998. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. *N. Engl. J. Med.* 338:1333-1339.

35. Hardin, M. D., G. R. Acuff, L. M. Lucia, J. S. Oman, and J. W. Savell. 1995. Comparison of methods for decontamination from beef carcass surfaces. *J. Food Prot.* 58:368-374.

36. Hew, C. M., M. N. Hajmeer, T. B. Farver, J. M. Glover, and D. O. Cliver. 2005. Survival of *Listeria monocytogenes* in experimental chorizos. *J. Food Prot.* 68:324-330.

37. Hussein, H. S., and L. M. Bollinger. 2005. Prevalence of shiga toxin-producing *Escherichia coli* in beef cattle. *J. Food Prot.* 68:2224-2241.

38. Jackson, T. C., M. D. Hardin, and G. R. Acuff. 1996. Heat resistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperatures. *J. Food Prot.* 59:230-237.

39. Jay, J. M. 2000. Modern Food Microbiology. p. 511-515. 6th edition. Aspen Publishers, Inc., Gaithersburg, M. D.

40. Kiessling, C. R., J. H. Cutting, M. Loftis, W. M. Kiessling, A. R. Datta, and J. N. Sofos. 2002. Antimicrobial resistance of food-related *Salmonella* isolates, 1999-2000. *J. Food Prot.* 65:603-608.

41. Kotula, K. L., and R. Thelappurate. 1994. Microbiological and sensory attributes of retail cuts of beef treated with acetic and lactic acid solutions. *J. Food Prot.* 57:665-670.

42. Laine, E. S., J. M. Scheftel, D. J. Boxrud, K. J. Vought, R. N. Danila, K. M. Elfering, and K. E. Smith. 2005. Outbreak of *Escherichia coli* O157:H7 infections associated with nonintact blade-tenderized frozen steaks sold by door-to-door vendors. *J. Food Prot.* 68:1198-1202.

43. Larkin, C., C. Poppe, B. McNab, B. McEwen, A. Mahdi, and J. Odumeru. 2004. Antibiotic resistance of *Salmonella* isolated from hog, beef, and chicken carcass samples from provincially inspected abattoirs in Ontario. *J. Food Prot.* 67:448-455.

44. LeJune, J. T., and N. P. Christie. 2004. Microbiological quality of ground beef from conventionally-reared cattle and "raised without antibiotics" label claims. *J. Food Prot.* 67:1433-1437.

45. MacDonald, K. L., M. L. Cohen, N. T. Hargrett-Bean, J. G. Wells, N. D. Puhr, S. F. Collin, and P. A. Blake. 1987. Changes in antimicrobial resistance of Salmonella isolated from humans in the United States. *JAMA* 258:1496-1499.

46. Madden, R. H., K. A. Murray, and A. Gilmour. 2004. Determination of the principal points of product contamination during beef carcass dressing processes in northern Ireland. *J. Food Prot.* 67:1494-1496.

47. Magana-Yepez, M. B. August 2004. Indicator bacteria to be used for validation of *Escherichia coli* O157:H7 [MS Thesis]. College Station, TX: Texas A&M University. 81 p.

48. Marshall, K. M., S. E. Niebuhr, G. R. Acuff, L. M. Lucia, and J. S. Dickson. 2005. Identification of *Escherichia coli* O157:H7 meat processing indicators for fresh meat through comparison of the effects of selected antimicrobial interventions. *J. Food Prot.* 68:2580-2586.

49. McEvoy, J. M., A. M. Doherty, M. Finnerty, J. J. Sheridan, L. McGuire, I. S. Blair, D. A. McDowell, and D. Harrington. 2000. The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Lett. Appl. Microbiol.* 30:390-395.

50. McEvoy, J. M., A. M. Doherty, J. J. Sheridan, I. S. Blair, and D. A. McDowell. 2003. The prevalence of *Salmonella* spp. in bovine faecal, rumen and carcass samples at a commercial abattoir. *J. Appl. Microbiol.* 94:693-700.

51. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607-625.

52. Morgan, G. M., C. Newman, and S. R. Palmer. 1988. First recognized community outbreak of haemorrhagic colitis due to verotoxin-producing *Escherichia coli* O157:H7 in the UK. *Epidemiol. Infect.* 101:83-91.

53. Murphy, R. Y., and R. A. Seward. 2004. Process control and sampling for *Escherichia coli* O157:H7 in beef trimmings. *J. Food Prot.* 67:1755-1759.

54. Naugle, A. L., K. G. Holt, P. Levine, and R. Eckel. 2005. Food safety and inspection service regulatory testing program for *Escherichia coli* O157:H7 in raw ground beef. *J. Food Prot.* 68:462-468.

55. Noah, C. W., C. I. Shaw, J. S. Ikeda, K. S. Kreuzer, and J. N. Sofos. 2005. Development of green fluorescent protein-expressing bacterial strains and evaluation for potential use as positive controls in sample analyses. *J. Food Prot.* 68:680-686.

56. North American Meat Processors Association, NAMP. 2003. The meat buyers guide. NAMP, Reston, VA.

57. Phebus, R. K., A. L. Nutsch, D. E. Schafer, R. C. Wilson, M. J. Riemann, J. D. Leising, C. L. Kastner, J. R. Wolf, and R. K. Prasai. 1997. Comparison of steam pasteurization and other methods for reduction of pathogens on surfaces of freshly slaughtered beef. *J. Food Prot.* 60:476-484.

58. Reagan, J. O., G. R. Acuff, D. R. Buege, M. J. Buyck, J. S. Dickson, C. L. Kastner, J. L. Marsden, J. B. Morgan, R. Nickelson II, G. C. Smith, and J. N. Sofos. 1996. Trimming and washing of beef carcasses as a method of improving the microbiological quality of meat. *J. Food Prot.* 59:751-756.

59. Reid, C. -A., A. Small, S. M. Avery, and S. Buncic. 2001. Presence of food-borne pathogens on cattle hides. *Food Control* 13:411-415.

60. Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* 308:681-685.

61. SAS Institute, Inc. SAS 9.1.3 for Windows. SAS Institute, Inc. Cary, N. C.

62. Scanga, J. A., A. D. Grona, K. E. Belk, J. N. Sofos, G. R. Bellinger, and G. C. Smith. 2000. Microbiological contamination of raw beef trimmings and ground beef. *Meat Sci.* 56:145-152.

63. Shellenberger, A. K. May 2000. Ecological superiority and inhibition of growth of pathogens in ground beef chubs produced from decontaminated beef carcasses [MS Thesis]. College Station, TX: Texas A&M University. 61 p.

64. Sheridan, J. J. 1995. The role of indicator systems in HACCP operations. *J. Food Saf.* 15:157-180.

65. United States Department of Agriculture-Food Safety and Inspection Service, USDA-FSIS. 1996. Achieving the zero tolerance performance standard for beef carcasses by knife trimming and vacuuming with hot water and steam; Use of acceptable carcass interventions for reducing carcass contamination without prior agency approval: Notice of policy change. *Fed. Regist.* 61:15024-15027.

66. United States Department of Agriculture-Food Safety and Inspection Service, USDA-FSIS. 1996. Pathogen reduction; Hazard Analysis and Critical Control Point (HACCP) systems; Final rule. *Fed. Regist.* 61:38805-38989.

67. United States Department of Agriculture-Food Safety and Inspection Service, USDA-FSIS. 2002. Guidance for Minimizing the Risk of *Escherichia coli* O157:H7 and *Salmonella* in Beef Slaughter Operations. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/00-022N/BeefSlauterGuide.pdf. Accessed August 2005.

68. United States Department of Agriculture-Food Safety and Inspection Service, USDA-FSIS. 2006. Microbiological results of raw ground beef products

analyzed for *Escherichia coli* O157:H7, summarized by calendar year. Available at: www.fsis.usda.gov/Science/Ecoli_O157_Summary_Tables/index.asp. Accessed June 2006.

69. United States Department of Agriculture-Food Safety and Inspection Service, USDA-FSIS. 2006 (Rev.). Code of Federal Regulations, title 9, §310 and § 313. Available at: http://www.gpoaccess.gov/cfr/index.html. Accessed January 2006.

70. United States Food and Drug Administration-Center for Food Safety and Applied Nutrition, USFDA-CFAN. 2004. FDA Report on the Occurrence of Foodborne Illness Risk Factors in Selected Institutional Foodservice, Restaurant, and Retail Food Store Facility Types. Available at: http://www.cfsan.fda.gov/~dms/retrs2d.html. Accessed March 2006.

71. United States Food and Drug Administration/Center for Food Safety and Applied Nutrition, USDA/CFAN. 2001. Analysis and evaluation of preventive control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce; The use of indicators and surrogate organisms for the evaluation of pathogens in fresh and fresh-cut produce. Available at: http://www.cfsan.fda.gov/~comm/ift3-toc.html. Accessed September 2005.

VITA

Name:	Tiffany Marie Moseley
University Address:	2471 TAMU
	College Station, TX 77843
E-mail address:	tmm7505@yahoo.com
Educational Background:	Texas A&M University
	Bachelor of Science
	Biomedical Science, 2004
	Texas A&M University
	Master of Science
	Food Science and Technology, 2007
Major Field of Specialization:	Food Microbiology