VALIDATION OF VARIOUS ANTIMICROBIAL SOLUTIONS ON THE MICROBIAL PRESENCE OF *E.COLI* O157:H7 ON BLADE TENDERIZED BEEF SUBPRIMALS

By

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Bachelor of Science in Agricultural Communication and Leadership Development

University of Florida

Gainesville, Florida

2009

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2011

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ACKNOWLEDGMENTS

First, I would like to thank my major adviser, Dr. J. Brad Morgan, for the opportunity to attend Oklahoma State University. I would like to thank him for giving me a complex project that allowed me to learn multitudes and thus fully benefit from my masters research experience. He allowed me to gain a lot of hands on knowledge that I greatly appreciate. Also, thank you Dr. Muriana, Dr. VanOverbeke, and Dr. Hilton for serving on my committee and guiding me through the final aspects of my graduate program. I owe an enormous thanks to all of Dr. Muriana's lab for teaching and guiding me through the micro processes. Kalpana and Denish were a tremendous help along with the entire lab. Thank you to Jake, Kyle, and J.D.for allowing us to take over part of the abattoir for processing. I would like to thank Wayne Spillner and Dennis Smithyman for their technical guidance as well. I must also recognize Kris Novotony for her counseling throughout this entire project and my stay at OSU.

Secondly, I would like to thank all of my mentors from Florida, Mr. Robert Philpot, Dr. Chad Carr, Dr. Dwain Johnson, and Mr. Larry Eubanks. Without their support I never would have become as involved with meat science, as I did. They have been true mentors who I will always look up to and who originally inspired me to pursue graduate school. Thank you also to the entire 2009 University of Florida meat judging team for being wonderful teammates and lifelong friends.

Thank you to Brent for working with me on this project and remaining to be my friend though the stress. I also would like to thank all of the current and previous graduate students for their help with this project. Lastly, I would like to thank my friends and family for continuously supporting my educational endeavors.

This work was supported by Ross Industries and each of the cooperating antimicrobial companies: Enviro Tech Chemical Services, Inc., A & B Ingredients, Safe Foods Corporation, Advanced Food Technologies, Archer Daniel Midland Company, Solution Bio Sciences, Inc. and Danisco.

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CHAPTER I

INTRODUCTION

Escherichia coli O157:H7 is a foodborne pathogen of major concern in today's global food industry. More than 200 known diseases are transmitted through food, with *E. coli* O157:H7 being one of the most dominant bacterial pathogens found in animal products such as red meat, eggs, poultry, seafood, and dairy products. With the growing public concern of food safety, the food animal industry is receiving extra scrutiny to ensure the wholesomeness of their products.

E. coli O157:H7 belongs to the Enterobacteriacea family as part of the enterohemorrhagic *E. coli* (EHEC) class. The EHEC class is a Shiga toxin producing *E. coli* due to its ability to produce potent cytotoxins. The serotype O157:H7 of *E. coli* is associated with gastrointestinal diseases such as hemorrhagic colitis (HC) and can result in life threatening complications as in hemolytic uremic syndrome (HUS; renal failure). The percentage of cases that progress to HUS ranges from 3-7% *(3, 5)* in sporadic cases and up to 20% or more in some outbreaks *(7, 55).* Studies have concluded that *E. coli* O157:H7 alone costs $405 million annually in the United States *(16)*.

Several outbreaks have been linked to red meat with the primary vehicle of contamination being ground beef. Several recent outbreaks were linked to the consumption of non-intact meat products contaminated with *E. coli* O157:H7. According to United States Department of Agriculture - Food Safety Inspection Service (USDA-FSIS), a non-intact product is defined as "ground beef; beef that has been injected with solutions; beef that has been mechanically

tenderized by needling, cubing, Frenching, or pounding devices; and beef that has been reconstructed into formed entrees" *(53).*

Beef tenderness is ranked as the most important attribute by consumers; this is shown by the premium cost for more tender cuts. Tenderness varies greatly among species, anatomically different muscles, and is influenced by both pre-harvest and post-harvest factors. To combat this variation the National Cattlemen's Beef Association (NCBA), estimated that approximately 94% of beef processing plants utilize mechanical tenderization of lower value cuts *(34)*. Blade tenderization is a type of mechanical tenderization that uses sharp blades to penetrate and physically disrupt muscle fibers and connective tissue.

After an investigation by the Centers for Disease Control and Prevention (CDC), three different outbreaks between 2000 and 2004 were linked to the consumption of *E. coli* O157:H7 in non-intact products *(28, 53)*. Following this, the USDA-FSIS published notice that establishments producing non-intact beef products were required to reassess their hazard analysis and critical control points (HACCP) plan because the recent outbreaks indicated *E. coli* O157:H7 was a hazard likely to occur *(53, 55)* due to pathogen translocation from the external surface of the meat to the internal deep tissue muscles. The internalization can occur by direct translocation via contaminated blades or needles, recycled injection fluid, or by combining pieces into reconstructed forms (30, 47, 48).

The use of antimicrobial interventions have been proven effective in reducing the pathogen load on carcass surfaces, trim and ground products. However, they have not been studied to examine pathogen reduction in conjunction with blade tenderization. Therefore, the objective of this study was to validate the use of seven proven antimicrobial intervention sprays when applied in conjunction with a blade tenderizer to control and reduce the presence *E. coli* O157:H7 in fresh beef cuts.

CHAPTER II

REVIEW OF LITERATURE

Foodborne illness is a major concern to consumers and government agencies across the world. More than 200 known diseases are transmitted through food (*6*). The causes of these diseases include: viruses, bacteria, parasites, toxins, metals, and prions. The Centers for Disease Control and Prevention (CDC) estimates that approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths are caused by foodborne diseases in the United States, annually (32). This estimation does not take into account the large number of cases that are not reported or those that cannot be diagnosed due to lack of identification. The most common sources of foodborne disease outbreaks in humans are associated with animal products such as red meat, eggs, poultry, seafood, and dairy products (*51*). The cost of the six most dominant bacterial pathogens found in animal products – *Salmonella, Campylobacter jejuni, Escherichia coli* 0157:H7, *Listeria monocytogenes, Staphylococcus aureus,* and *Colostridium perfingens* – cost between $9.3 billion and $12.9 billion in human illness in the United States, annually. Between $2.9 and $6.7 billion of these costs are attributed solely to foodborne causes (*51*).

With the growing public concern for food safety, the animal agriculture industry, especially the meat sector, is receiving extra scrutiny of their handling and processing techniques. The biggest food safety concern of today's red meat supply is the contamination of meat with *E. coli* O157:H7. It has become of increasing concern in today's food system due to multiple outbreaks and recalls especially in non-intact meat products such as: ground, blade tenderized,

and needle injected products. Between 2000 and 2004, three different outbreaks were linked to the consumption of non-intact products contaminated with *E. coli* O157:H7 (*32*). In 2005, the United States Department of Agriculture – Food Safety Inspection Service (USDA-FSIS) required plants producing mechanically tenderized and moisture enhanced beef products to reevaluate their Hazard Analysis and Critical Control Points (HACCP) plans to eliminate any bacterial contamination associated with such enhancement process.

Ground product is recommended to be cooked to an internal temperature of 70°C to insure destruction of pathogenic bacteria that may be present internally due to grinding. The current issue with blade tenderized beef is the potential to carry surface contamination internally into individual steaks that may be prepared rare to medium degrees of doneness for consumption. Thus, if these steaks have *E. coli* O157:H7 present internally it will not be destroyed at a rare to medium degree of doneness. The USDA–FSIS recognizes these products as non-intact beef and requires that antimicrobial interventions be in place to eliminate surface *E. coli* O157:H7 prior to mechanical tenderization. Most recently, an outbreak linked to a supplier of blade tenderized beef has raised concerns within the industry and the regulatory agency (53).

Escherichia coli O157:H7

Escherichia coli are Gram-negative, aerobic or facultative anaerobic, motile or nonmotile rods that belong to the family of Enterobacteriacea (*3, 22, 27*). Other microorganisms in the same family include: *Salmonella, Yersinia, Shigella, Citrobacter, Kelbsiella, Enterobacter*, and *Proteus* genera. Most strains of *E. coli* are non-pathogenic and are part of the normal microflora of the digestive tract in warm blooded animals including humans (*3, 22, 27*). Some studies show that *E. coli* serves a beneficial role in the body by synthesizing vitamins and outcompeting other consumed pathogenic bacteria (*24*).

There are six known classes of enteric *E. coli* (EEC): enteropahogenic (EPEC), enterotoxigenic (ETEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteroaggregative (EAEC), and diffusely adherent *E. coli* (DAEC; *27*). Among these classes, the EHEC group is

also referred to as the Shiga toxin producing *E. coli* (STEC) due to its ability to produce the potent cytotoxins, Shiga toxins I and/or II (*27*). *E. coli* serotypes are identified based on the combination of three different antigens: somatic lipopolysaccharide or cell wall antigens (O), the flagellar (H) antigens, and the capsular antigens (K; *22, 40*). Enterohemorrhagic (EHEC) is the most notorious EEC as it contains serotype O157:H7, one of the pathogens of greatest concern in today's food system. It is so named because it expresses the 157th O antigen identified with the 7th H antigen and is associated with gastrointestinal disease such as hemorrhagic colitis (HC; a clinical entity characterized by abdominal cramps and bloody diarrhea) and results in life threatening complications such hemolytic uremic syndrome (HUS; characterized by renal failure, thrombocytopenia, and microangiopathic haemolytic anameia) in humans (*11*).

E. coli O157:H7 was first recognized as a pathogen in 1982 (*39*) due to its association with two, nearly simultaneous, U.S. food related outbreaks of an unusual gastrointestinal illness (*11*). In the 30 years following, *E. coli* O157:H7 has emerged as a global public health concern. It causes the majority, and most severe, of gastrointestinal illnesses related to *E. coli* (*24, 35*) from infections that range from symptom free carriage to mild non-bloody diarrhea, HC, to fatal HUS. The severity of symptoms often depends on the status of the person infected with the pathogen, with young children or the immunocompromised suffering the worse. The average interval between exposure to infection is 3 d, with incubation periods as short as 1 d and as long as 8 d. The percentage of cases that progress to HUS range from 3-7% (*3, 5*) in sporadic cases and up to 20% or more in some outbreaks (*7, 55*).

E. coli O157:H7 was one of the first strains of EHEC believed to account for over 90% of all cases of HUS in industrialized countries (*42*). The full extent of how it causes HC and HUS is not fully understood. The infective dose is known to be relatively low (<10 cells). Mead (*31)* states that the organism is believed to adhere closely to the mucosal cells of the large bowel, disrupting the brush border and causing the onset of diarrhea. Shiga toxins have both local and systemic effects on the intestine. Hemolytic uremic syndrome (HUS) is thought to develop when Shiga toxins produced in the intestine enter the blood and bind to the cellular Gb3 rich endothelial

cells that is located in the vascular epithlium, colon, and kidneys (*31*). They then enter into the endosomes and become transported to the trans-Golgi network and the endoplasmic reticulum, where one of the toxin subunits enters the cytoplasmic matrix. This subunit inhibits protein production by enzymatic depurination of ribosomal ribonucleic acid (rRNA), which leads to cellular death (*19*). As a consequence of damage to cells, humans may experience platelet and fibrin deposition, leading to injury of passing erythrocytes (haemolysis) and occlusion of renal microvasculature (renal failure; *31*). Hemolytic uremic syndrome (HUS) is fatal in about 3-5% of cases with a similar percentage developing end stage renal disease (*42*); approximately 8% of the surviving patients experiencing a wide range of serious aftereffects including neurological disorders, blindness, paralysis, and renal compromise (*22*).

As of 2000, over 100 outbreaks of *E. coli* O157 have been documented with 52% being attributed to foods derived from cattle (*14*) especially meat or raw milk products. Healthy cattle have been implicated as a major reservoir of the pathogen and carry it without any clinical symptoms. The presence of this dangerous pathogen is a serious concern for the beef and meat industries. The USDA noted in one survey that 63 of 100 feedlots had at least one positive *E. coli* O157:H7 fecal sample (*50*). The high occurrence of it in shedding from ruminants, suggests these animals provide a special niche for the bacterium. However, the pathogen has been isolated in deer, sheep, goats, horses, dogs, birds, and flies as well.

Cost of *E. coli* 0157:H7 in the food industry

Based on the CDC estimation that O157 STEC caused 73,480 illnesses in 1997, resulting in 2,168 hospitalizations and 61 deaths (*32*). Of the estimated 73,480 annual cases, 78% of the individuals did not visit a physician, 19% visited a physician but were not hospitalized, and 3% required hospitalization (*16*). Of the 2,168 hospitalized patients, 16% developed HUS, <1% developed end stage renal disease, and 3% died (*16*). Frenzen, concluded the annual cost of illness due to this pathogen was $405 million (in 2003 dollars), including $370 million for premature deaths, $30 million for medical care, and $5 million for lost productivity (*16*).

Interventions

Meat typically becomes contaminated at the time of slaughter when fecal material comes in contact with the carcass, either during the dehiding or evisceration steps. A study reported that *E. coli* O157:H7 was found on 43% of beef carcasses prior to evisceration, 18% of the carcasses after evisceration, and 2% of the carcasses after processing (*13*). Meat can also be cross contaminated from processing tools/equipment, human contact, carcass to carcass contact, and from structural components of the facility. This contamination can compound during further processing steps if the pathogen is introduced into the interior of meat, where it is more likely to survive during the cooking processes.

Due to the ease of contamination, as part of their HACCP plan all processors and plants must implement steps to reduce pathogen likelihood in the end product. The beef industry has taken action to reduce the potential for contamination by incorporating scientifically proven antimicrobial interventions. These interventions can be applied individually or in conjunction with other treatments to reduce pathogen loads on carcass surfaces.

The hurdle technology approach to microbial carcass interventions combines routine activities such as sanitary hide removal and rapid chilling with a series of physical and chemical interventions to achieve a lower likelihood of carcass contamination (*23*).

Physical interventions include hot water spray, steam pasteurization, steam vacuuming, water wash cabinet, and knife trimming. The use of hot water (>74°C) on beef carcasses is widely practiced and accepted to reduce pathogens. Exposing carcasses to water above 70°C has been found to reduce bacterial counts by 1- to 3-log_{10} cycles against pathogenic bacteria including *Salmonella, Y. enterocoliticia, E. coli* 0157:H7, and *L. monocytogenes (8, 9, 20, 26, 44*). An extension of hot water rinsing is steam pasteurization. This process is the use of condensed steam to accomplish thermal destruction of bacteria (*23*). Another physical intervention known as

steam vacuuming is a variation on the use of steam followed by a hand held vacuum wand, to remove and/or inactivate surface contamination (*23*).

Chemical interventions include organic acids, polyphosphates, chlorine, acidified sodium chlorite, ozone, peroxyacetic acid, nisin, and lactoferrin. United States Department Agriculture – Food Safety Inspection Service (USDA-FSIS) has approved the use of acetic, lactic, and citric acids at concentrations of 1.5-2.5%. The most widely accepted chemical interventions are acetic and lactic acids, and work best if sprayed on the entire carcass while it is still hot. Lactic acid (1-3%) solutions have been shown to reduce bacterial numbers by 1- to 3- log_{10} (*8, 20, 26, 38, 45, 46*).

Tenderness

Beef tenderness is a major concern of producers because the most critical appraisal of meat quality occurs when the consumer eats the product. Consumer evaluation is based upon flavor, juiciness, and tenderness. Of these attributes, studies have shown tenderness to be the factor of most importance. Tenderness is the main source of consumer complaint and the primary cause of failure to repurchase (*4, 49*). This fact is shown by the positive association between the relative tenderness of a cut of meat and its premium price, thus there is an economic incentive for tender meat.

The tenderness of meat is highly variable. It varies among the same or different species, between anatomically different muscles, and is influenced by both pre slaughter and post slaughter factors. Much research has been dedicated to establishing and improving of tenderness including the effects of pre harvest factors such as, species, breed, age, sex, nutrition, environment, and exercise; and post harvest factors such as slaughter techniques, changing carcass suspension, aging, and further processing.

Blade tenderization

The National Cattlemen's Beef Association (NCBA) estimated that approximately 94% of beef processing plants utilize mechanical tenderization of lower value cuts and that 18% of retail

beef products are either mechanically tenderized or moisture enhanced (*34*). Beef tenderness is affected by two primary attributes: background tenderness (the amount and type of connective tissue in a given cut) and protein (muscle fiber) tenderness. Blade tenderization is a type of mechanical tenderization that uses very sharp blades (needles) to penetrate meat cutting through muscle fibers and connective tissues. Blades can vary in size, thickness, and number of blades per square cm, depending on the distributor. Blade tenderization can be performed on whole muscle cuts or individual steaks. It is commonly practiced to improve tenderness on whole muscle cuts such as chucks, ribs, strip loins, and tenderloins.

Blade tenderization usually takes place at the processing plant or purveyor level. The process varies among manufactures and the specifications required by the processor. It is used on raw product, generally after rigor (*14*) and can be done before or after packaging and aging with equal effectiveness (*10, 41*)

The product is commonly placed on a conveyor belt system where the belt speed can be controlled. By controlling the belt speed, processors can control how many times a cut is tenderized and the number of times the blades enter the meat. Studies show that one pass through a blade tenderizer at medium to fast conveyor belt speed is adequate to improve tenderness (3*6*). This process is also termed by the industry as “needling” or “Jaccarding” (Jaccard™ is a company that makes needle tenderization devices commonly used in homes and restaurants) (*5*).

Outbreaks

E. coli O157:H7 has become the most frequent STEC serotype in North America (*22*). Foodborne Diseases Active Surveillance Network (FoodNet) data showed that in the U.S. in 2008 the relative incidence of STEC *E. coli* was 1.2 cases per hundred thousand individuals (3*2*). Ground beef has been shown to be the primary vehicle of outbreaks. As of 2002, 41% of the over 350 outbreaks of *E. coli* O157:H7, covering 49 states were traced to ground beef (*24, 25, 37*). In 1993, a multistate outbreak sickening 501 individuals, resulting in 151 hospitalizations and 3

deaths were traced back to undercooked hamburgers from a fast-food restaurant chain (*1*). The frozen contaminated patties from a single plant were involved with the outbreak 6 weeks after the production date (*1, 15*). In 1994, under the Federal Meat Inspection Act (FMIA), the USDA-FSIS declared *E. coli* 0157:H7 to be an adulterant in raw ground beef (*32, 54*). Afterwards, the FSIS established new provisions for all meat and poultry plants, requiring the mandatory implementation of a HACCP system in order to identify risky and potentially hazardous practices that account for microbial contamination (*12*).

Non-Intact Meat Outbreaks

In 2005, FSIS published notice that establishments producing non-intact beef products were required to reassess their HACCP plans because recent outbreaks indicated that *E. coli* O157:H7 was a hazard likely to occur (*53, 55*) due to pathogen translocation from the external surface of the meat to the internal deep tissue muscles. According to the FSIS, a non-intact product is defined as “ground beef; beef that has been injected with solutions; beef that has been mechanically tenderized by needling, cubing, Frenching, or pounding devices; and beef that has been reconstructed into formed entrees” (*53*). These products inevitably lend themselves to a potential for pathogen internalization if on the surface of the product (*28*). The internalization can occur by direct translocation via contaminated blades or needles, recycled injection fluid, or by combining pieces into reconstructed forms (*30, 47, 48*).

It has been shown that only 3 to 4% of surface bacteria may be transferred from the surface to interior muscles (*48*). Most of the translocation happens within the topmost 1 cm, but cells have been shown to further contaminate the deep tissue muscles (*30*). However, cooking to the proper temperature (63°C) may ensure microbial safety (*17, 18*). The National Advisory Committee for the Microbiological Criteria of Foods found that the risk of *E. coli* O157:H7 infection from contaminated non-intact beef is low (1 illness per 14.2 million steaks) and similar to that from intact steaks (1 illness per 15.9 million steaks; *33*). Even with this reported data, multiple outbreaks have been linked to non-intact beef products.

Outbreak 1:

The outbreaks associated with these new provisions started with an non-intact incident in Michigan in 2000 (*53*) when a laboratory analysis with pulsed field gel electrophoresis (PFGE), a technique used to determine the relatedness of bacteria, identified two human *E. coli* O157:H7 strains with matching patterns. Follow up studies linked the outbreak to consumption of rare-medium degree of doneness prepared steaks. This outbreak did not result in a recall of product; however, the steak supplier implemented changes in the sanitizing procedures used on their tenderizer machine and incorporate *E. coli* testing program.

Outbreak 2:

An outbreak in 2003 was traced back to blade tenderized, marination injected steaks sold vacuum packaged and frozen by door-to-door vendors (*28*). During this outbreak a total of twelve people were infected and identified by state health departments from Minnesota, Michigan, Kansas, Iowa, and North Dakota (*28*). In the following investigation of the processing plant, it was shown that the steaks were passed multiple times through an injector/blade tenderizer apparatus (*28*). The equipment was cleaned and sanitized on a daily basis, but only completely disassembled once a week (*28*). This allowed the machine to harbor pathogens that were transferred to multiple steaks. Furthermore, the investigation showed that the steaks were cooked directly from the frozen state without prior thawing, which could have led to undercooked product (*28*). The company voluntarily recalled 335,204.8 kg of frozen beef product and implemented changes in their standard sanitation operation procedures (SSOP) by dismantling, washing, and sanitizing the equipment on a daily basis (*28*).

Outbreak 3:

The third outbreak that initiated FSIS's new provisions was linked to an epidemiological case control study in 2004 determining that consumption of tenderized, marinated beef steaks served at four different locations of a national restaurant chain confirmed four cases of human

infection with *E. coli* O157:H7 (*53*). As a result of the outbreak, the producing company in Bolingbrook, IL, recalled approximately 184,158.5 kg of frozen beef product (*53*).

Conclusion

Much research has been dedicated to making meat more tender for consumer acceptability. Non-intact beef from mechanical tenderization has become an overwhelming process that takes place in today's processing facilities and is extensively utilized in the commercial hotel, restaurant, and retail sectors. There is no federal regulation to regulate the labeling of these beef products, leaving consumers at the risk of cooking them as an intact beef cut. As observed from the reported outbreaks, there is a potential hazard of pathogen translocation into the deep tissue muscles from mechanical tenderization. Thus, if these cuts are not treated as non-intact product consumers are at the risk of unintentionally undercooking the product and putting themselves in danger of a potential health risk.

The use of organic acids and other antimicrobials have been shown to reduce microbial loads on beef carcasses, trim, and ground products. However, there is a need in the beef industry to further investigate the use of these antimicrobials in conjunction with mechanical tenderization. This type of research would have the potential to demonstrate if pathogen entry can be reduced or eliminated when interventions are applied.

CHAPTER III

METHODOLOGY

ABSTRACT

The purpose of this study was to determine the effectiveness of seven proven effective antimicrobial intervention strategies (AvGard – XP, Stabilized NA- Chlorite, Cytoguard Plus, Lactic Acid FCC 88%, AFTEC 3000, Citrilow, and HB2) to control the presence *E. coli* O157:H7 in mechanically tenderized beef subprimals. Boneless top sirloin beef subprimals cap off (IMPS #169) were inoculated on the lean side with 2 X 10^4 CFU/cm^2 of a four-strain rifamycin-resistant cocktail of *Escherichia coli* O157:H7 and passed once, lean side up, through a blade tenderizer. Inoculated subprimals that were water sprayed without tenderization served as the negative control and inoculated subprimals that were water sprayed with tenderization served as the positive control. Four core samples (20.25 cm^2) were obtained from each subprimal and cut into four consecutive 2.54 cm sections: sections A and B comprised the top 5.08 cm and sections C and D comprised the deepest 5.08 cm. The recovered cores were passed through an Infrared Grill (211.0°C at 5 hz) to further eliminate surface contamination. After aseptic separation into A, B, C, and D sections, each individual core section was blended at a volume of 2.5:1 with enrichment broth and incubated for 18 h at 37°C. After incubation 1 ml of the blended samples was extracted with *E. coli* specific immunomagnetic beads, allowing for selective recovery. The entire amount of recovered immunomagentic beads was plated onto Levine's eosin methylene blue agar containing 10μg/ml Rifamycin. Presence of *E coli* O157:H7 (positive or negative) was

recorded for each individual core section (A-D). In section A, AvGard-XP was more effective (P < 0.05) at reducing microbial presence then AFTEC 3000, HB2, and Stabilized NA-Chlorite. Section B revealed that AvGard-XP was more effective at reducing microbial presence then Lactic Acid, AFTEC 3000, HB2, and Stabilized Na- Chlorite. From segment C, AvGard-XP, Citrilow, Cytoguard Plus, Lactic Acid, and AFTEC 3000 were the most effecive (P < 0.05) for the reduction of microbial presence when compared to W+BT. In section D no differences were observed (P > 0.05) between AvGard-XP, Cytoguard Plus, Citrilow, Lactic Acid, AFTEC 3000, HB2, and Stabilized NA-Chlorite. The positive controls (W+BT) consistently showed the highest microbial presence across sections. Similarly, the negative controls (W-BT) consistently showed the lowest microbial presence across sections.

INTRODUCTION

In recent decades there has become an increased awareness of the dangers of foodborne pathogens. More than 200 known diseases are transmitted through viruses, bacteria, parasites, toxins, metals, and prions. Of these diseases, *E. coli* serotype O157:H7 has become an overwhelming concern. *E. coli* O157:H7 is shed from healthy cattle that have a specific niche for carrying the bacteria. These cattle show no sign of the bacteria and shed it mostly during the warmer summer months. The USDA found in one study that 63 of 100 feedlots sampled had at least one positive *E. coli* O157:H7 fecal sample.

E. coli O157:H7 is part of the enterohemorrhagic class of Enterobacteriacea, that are known for there ability to produce the potent cytotoxins, Shiga toxin I and/or II. These potent toxins have both local and systematic effects on the intestine. The infective dose is relatively low and has the ability to cause hemorrhagic colitis and lead to hemolytic uremic syndrome (renal failure).

E. coli O157:H7 is most prevalent in food sourced from animal products. One of the leading sources of this contamination is red meat. Beef is mainly contaminated during the harvest process when fecal material from the hooves and hide come into contact with the carcass. Contamination also can occur from cross contamination from processing tools/equipment, human contact, carcass-to-carcass contact, and from structural components of the facility. This contamination can compound during mechanical tenderization if the pathogen is present on the surface of the meat.

Due to consumers desire for tender meat many processors have utilized the technology of mechanical tenderization. This process uses a series of sharp blades to penetrate the meat and disrupt background tenderness and protein tenderness by cutting through muscle fibers and connective tissues. Due to the possibility of translocating surface contamination via the penetration of blades into the interior of the meat all processors must implement steps to reduce pathogen likelihood in the end product.

Many physical and chemical interventions are utilized to prevent contamination on carcass surfaces. However, these interventions have not been researched as to their effectiveness when used in combination with mechanical tenderization. The objective of this experiment is to validate the use of proven antimicrobial solutions on the reduction of microbial presence of *E. coli* O157:H7 on blade tenderized top sirloin butt beef subprimals.

MATERIALS AND METHODS

Bacterial Strains. A four-strain cocktail mixture of *E. coli* O157:H7 (ATCC 43890, ATCC 43894, ATCC 43895, ATCC 35150) was used to inoculate samples. Strains ATCC 43890, ATCC 43894, and ATCC 43895 are human isolates associated with recent outbreaks related to beef. The strains were made resistant to rifamycin (10 μg/ml; Sigma-Aldrich, St. Louis, MO) by passage on media containing this antibiotic. Stock strains were cultured separately by transferring 100 μl of culture grown the day prior to testing to separate test tubes containing 10 ml of Difco™ Tryptic

Soy Broth (Becton, Dickinson & Company; Sparks, MD) and incubated at 37°C for 24 h. The entire 10 ml of each of the freshly grown cultures was combined in a 50 ml centrifuge tube, centrifuged slightly and resuspended in 0.1% buffered peptone water to the appropriate density. The use of antibiotic resistant strains allowed for recovery of viable and heat-injured cells on all-purpose plating media containing these antibiotics or recovery from samples containing other background microflora.

Inoculation and mechanical tenderization of subprimals. Top sirloin butt beef subprimals (IMPS #169; ca. 4.54 to 6.80 kg each) were purchased fresh from a local wholesale distributor and stored at 4°C for no longer than 7 d of total postmortem storage. The caps were removed and fat was trimmed so that a contiguous intact core could be obtained. These experiments occurred at Oklahoma State University's Food and Agriculture Products Center (Stillwater, OK) in the Biosafety Level 2 pathogen processing facility.

For each tenderization treatment, four 5.08 cm diameter circles were marked using edible ink on the lean surface of the beef subprimals (Fig. 1). One hundred μl of 2 X 10^5 CFU/ml of the bacterial cocktail was applied within each 20.25 cm^2 circle and then thoroughly spread with a gloved finger within the circle. This gave an inoculation level of approximately 1 X 10^4 CFU/cm^2. The inoculated top sirloin butt beef subprimal was then allowed to sit at 4°C for 30 min to promote attachment of the cells to the meat prior to any further processing. Each of the subprimals were moved via a conveyor belt (inoculated side up) beneath two sets of blades in a series on the TC 7000M tenderizer (Ross Industries, Midland, VA; Fig. 2) equipped with a custom-built multi nozzle spray system (Dositron), with 3 nozzles spraying from above the conveyor belt and 2 nozzles spraying from below the conveyor belt.

Each of the blade sets consisted of seven rows that each contained 12 stainless steel blades (each 3 mm wide), which made 32 incisions per in^2. The belt advanced by increments of 3.6 cm with the blades penetrating the subprimal after each advancement. Thus, each subprimal was penetrated multiple times by both sets of blades during its 18-s dwell time. The custom-built

spray nozzles sprayed each antimicrobial and control treatment at a rate of 5.68 L per min with 2.46 L per cm^2 pressure.

There were two control treatments per trial: 1.) inoculated with water spray treatment and blade tenderization (W+BT) as the positive control and 2.) inoculated with water spray treatment without blade tenderization (W-BT) as the negative control. Each treatment passed once longitudinally through the tenderizer lean side (inoculate side) up with an antimicrobial spray. One top sirloin butt beef subprimal (with four 20.25 cm^2 circles per top sirloin butt) was run for each antimicrobial treatment. One concentration was run per antimicrobial as recommended by the manufacturer's technical personnel.

Antimicrobial solutions. Seven proven antimicrobials which were identified as being effective in previous research *(56)* were evaluated in this study for reduction of *E. coli* O157:H7 presence in subsurface sections. These products were applied as recommended by the manufacturer's technical personnel. The antimicrobials observed were: 1) disodium metasilicate (AvGard-XP; Danisco A/S, Copenhagen, Denmark), 2) Na chlorite/citric acid/Na hydroxide (Stabilized Na Chlorite; Alliance Analytical Laboratories Inc., Coopersville, MI), 3) lauric arginate and peroxyacetic acid (CytoGuard Plus), 4) hydroxypropanoic acid (Lactic Acid FCC 88%; Archer Daniels Midland Company, Decatur, Illinois), 5) buffered sulfuric acid (AFTEC 3000; Advanced Food Technologies, LLC, Shreveport, LA), 6) hydrochloric and citric acids (Citrilow; Safe Foods Corp, Little Rock, Arkansas), and 7) hydrobromic acid (HB2; Enviro Tech Chemical Services, Modesto, CA). The application strength (strength of actual product dilution is listed, not active ingredient concentrations; the active ingredient concentrations are protected for proprietary purposes by respective manufacturers), and pH for each particular antimicrobial is presented in Table 1.

Microbial sampling of core samples. After tenderization, subprimals were aseptically transferred onto sterilized cutting boards inoculated side up for cores to be taken. Four core samples were taken from each subprimal using a sterilized, stainless steel metal coring device 13 cm long and 5.08 cm in diameter (20.25 cm^2 area; Fig. 3). Each core was taken from within the

circle (20.25 cm^2) previously marked on the beef subprimal. Each core was removed from the coring device, the top 0.635 cm of the inoculated surface was removed and the core was placed in a sterilized, stainless steel container and kept on ice until further processed (within 1 hr after processing).

Core samples were then passed through an Infrared Grill Pasteurizer (Unitherm Food Systems, Inc., Bristow, OK) at 211.4°C at 5 hz (Fig. 4). They were removed from the grill aseptically and placed horizontally on a sterilized cutting board. Each core sample was cut into four consecutive 2.54 cm segments using an alcohol-sterilized knife starting from the inoculated surface: segments A and B comprised the top 5.08 cm of the core and segments C and D comprised the lower 5.08 cm of the core (Fig. 5). The knife was alcohol and flame sterilized between each cut and a freshly autoclaved knife was used between each individual core. Each segment was weighed using a top loading balance (XP- 1500, Denver Instrument, Arvada, CO) and diluted at a volume of 2.5:1 with modified tryptone soya broth (Oxoid Ltd., Basingstoke, UK) supplemented with novobiocin (10 mg/L; Sigma Chemical CO, St. Louis, MO) and Protein Hydrolysate Amicase (10%). This was then blended (Single Speed Blenders, 1L, Waring) for 30 s and transferred to a sterile filter homogeniser bag and incubated at 37°C for 18 h.

Immunoconcentration: Dynabeads anti- *E. coli* O157:H7. For immunomagnetic separation (IMS), Dynabeads anti-*E. coli* O157:H7 (Dynal, Oslo, Norway) were used following the manufacturer's instructions. Dispoable sample tube strips were labeled and placed in the BeadRetriever™ sample rack (Fig. 6A). Dynabeads anti- *E.coli* O157:H7 were vigorously vortexed for a minimum of 10 s until properly mixed and using aseptic techniques 10 μl were dispensed into sample tubes 1 and 2 within the sample strip. Then, 500 μl of wash buffer (PBS Tween: pH 7.4, with 0.05% Tween-20) was added to sample extraction tubes 1 and 2, 1 ml of wash buffer was added to sample wash tubes 3 and 4, and 100 μl of wash buffer was added to sample wash tube 5 within the strip. After mixing the pre-enriched sample thoroughly once more by homogenizing, 500 μl was added to extraction tubes 1 and 2. The test rack was placed inside the BeadRetriever instrument using the EPEC/VTEC program sequence (Fig. 6B).

Selective isolation and confirmation. Each sample was direct plated after the IMS procedure onto Levine's eosin methylene blue agar (Oxoid, Cambridge, UK) containing Rifamycin SV Sodium Salt (10 μg/ml; MP Biomedicals, LLC; Solon, OH). The plates were read after 24 h of incubation at 37°C for presence [positive (those with the characteristic *E. coli* metallic sheen) or negative] of *E. coli* O157:H7 (Fig. 7).

Statistical analysis. Data were analyzed by section of the core using a one-way analysis of variance (ANOVA) model with treatment as the fixed effect in version 9.1 of the SAS statistical package (SAS Institute, Inc., Cary, NC) and presence as the dependent variable. Multiple comparisons were performed by using Fisher's Protected least significant difference test (LSD). Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Translocation of *E. coli* O157:H7 into section A of core. The occurrence of *E. coli* O157:H7 recovered from uppermost section (A) of top sirloin butt subprimals treated with various antimicrobials revealed that AvGard-XP was more effective ($P < 0.05$) at reducing microbial presence (Fig. 8) then AFTEC 3000, HB2, and Stabilized NA-Chlorite. HB2. AFTEC 3000, and Stabilized NA-Chlorite were shown to be less effective ($P < 0.05$) then W-BT (Fig 8).

Translocation of *E. coli* O157:H7 into section B of core. The occurrence of *E. coli* O157:H7 recovered from section B of subprimals treated with various antimicrobials revealed that AvGard-XP was more effective ($P < 0.05$) at reducing microbial presence then Lactic Acid, AFTEC 3000, HB2, and Stabilized NA-Chlorite (Fig. 9). Cytoguard Plus was more effective ($P < 0.05$) at reducing microbial presence then Stabilized NA-Chlorite (Fig 9). Both AvGard-XP and Cytoguard Plus were more effective ($P < 0.05$) then W+BT (Fig 9). Lactic acid, AFTEC 3000, HB2, Stabilized NA-Chlorite were shown to be less effective ($P < 0.05$) then W-BT (Fig. 9).

Translocation of *E. coli* O157:H7 into section C of core. From segment C, which represents the section 7.62 cm from the inoculated surface, Av-Gard-XP, Citrilow, Cytoguard Plus, Lactic Acid, and AFTEC 3000 were the most effective (P < 0.05) for the reduction of microbial presence when compared to W+BT (Fig. 10). HB2 and Stabilized NA-Chlorite showed no significant difference (P < 0.05) at reducing pathogen presence when compared to W+BT (Fig. 10).

Translocation of *E. coli* O157:H7 into section D of core. It was possible to recover the pathogen from all four sections of the core sample, representing a total depth of translocation of 10.16 cm into the interior. No differences were observed (P > 0.05) between AvGard- XP, Cytoguard Plus, Citrilow, Lactic Acid, AFTEC 3000, HB2, and Stabilized NA- Chlorite (Fig. 11). W+BT had the lowest (P < 0.05) reduction of *E. coli* O157:H7 microbial presence (Fig. 11).

DISCUSSION

Foodborne illness remains a major concern for today's food industry evidenced by several recent recalls. The CDC estimates that approximately 76 million illnesses, 325,000 hospitalizations, and 5,00 deaths are caused by foodborne pathogens in the United States, annually (32). The contamination of beef with *E. coli* O157:H7 is becoming increasingly prevalent. Since *E. coli* O157:H7's first documented recognition in 1982, there have been an estimated 62,000 cases occurring annually with 1,800 hospitalizations, and 53 deaths. Shortly after 1982, the USDA-FSIS recognized ground beef to be an adulterant under the Federal Meat Inspection Act. Ground beef has been shown to be the primary vehicle of outbreaks. In addition to these problems there have also been several recalls related to non-intact beef products that has sparked an interest in understanding the risk of such products. In 2005, FSIS published notice that establishments producing non-intact beef products – ground beef; beef that has been injected with solutions; beef that has been mechanically tenderized by needling, cubing, Frenching, or pounding devices; and beef that has been reconstructed into formed entrees *(53)* -

were required to reassess there HACCP plans, because recent outbreaks indicated *E. coli* O157:H7 was a hazard likely to occur (53, 55).

Studies have shown the translocation of *E. coli* O157:H7 into the deeper tissue muscles of meat in non-intact products *(30).* In 2008, a study indicated that regardless of inoculation level (0.5, 1.5, 2.5, 3.5 log CFU/cm^2) possible translocation existed between segments 1-3, which represented the topmost 3 cm of the top sirloin butt beef subprimal *(30).* Also, there were no differences ($P > 0.05$) in recovery of pathogens between segments 2-6 (representing a total of 8 cm) regardless of inoculation levels *(30)*. However, it is important to note that for inoculation levels up to 1.5 log CFU/cm^2 there was no pathogen recovery in the lower segments 4-6 *(30).* These experimental designs were able to prove that *E. coli* O157:H7 is a potential threat in non-intact meat due to blade translocation.

The results of previous studies that have addressed the internalization of surface microflora into the deeper tissues of mechanically tenderized beef along with continued consumer concern has sparked the need for data looking at the use of interventions in combination with blade tenderization. If the level of surface pathogens could be eliminated by applying interventions to the surface, then presumably there would be fewer cells translocated into the interior upon subsequent blade tenderization.

The present study was conducted to evaluate the effectiveness of antimicrobial interventions, and therefore a high inoculation level was used; this needs to be kept in perspective when evaluating this data. Results from the present study showed that with initial innoculum levels of 1×10^4 CFU/cm^2 there was translocation between sections A through D, which represented 10.16 cm depth penetration. The highest microbial presence were detected in the topmost sections A, with decreasing microbial presence in section B and C and the lowest microbial presence in section D.

We characterized the effects of seven proven effective antimicrobial sprays (AvGard – XP, Citrilow, Cytoguard Plus, Lactic Acid, AFTEC 3000, HB2, and Stabilized NA Chlorite) as

interventions to reduce the translocation of *E. coli* O157:H7. The lowest microbial presence was observed in those samples treated with AvGard – XP followed by Citrilow and Cytogard Plus. The order of effectiveness for remaining antimicrobials was Lactic Acid, AFTEC 3000, Stabilized Na-Chlorite, and HB2. The positive controls (W+BT) consistently showed the highest microbial presence across sections. Similarly, the negative controls (W-BT) consistently showed the lowest microbial presence across sections. It is important to notice that translocation does not always occur at a constant or with decreasing levels of contamination from top to bottom of the core sample. We observed higher microbial presence on the top sections as expected, but in some cases, such as: W-BT pathogen presence in section C were higher than pathogen presence in section B and with Citrilow pathogen presence in section D were higher than pathogen presence in section C. The higher numbers in the descending sections could either be due to cross contamination during processing or intermittent translocation to lower sections. In general, all the interventions were effective in reducing translocation of *E. coli* O157:H7, with AVGard – XP, Citrilow, and Cytoguard Plus presenting the most consistent and greatest reduction effect.

The use of antimicrobial spray interventions prior to blade tenderization can help to reduce the presence of *E. coli* O157:H7 to lower levels than occur prior to spray treatment and therefore, can reduce the likelihood of translocation to internal sections of beef subprimal.. The novelty of antimicrobial intervention as demonstrated herein, may be applied immediately prior to blade tenderization and incorporated in the development of Critical Control Points for non-intact product. This information will also be useful in the improvement of safe cook guidelines for non-intact meat. Studies are ongoing to validate use of different antimicrobials, heated antimicrobials, and incorporating hurdle technology.

TABLE 1. *Antimicrobial products used for reduction of E. coli O157:H7 on tenderized top sirloin beef subprimals, active ingredients of these products, applied dilution strength and pH upon application.*

Trade Name	Active Ingredient(s)	Application Strength	pH
AvGard-XP	Disodium Metasilicate	6%	13.1
Stabilized Na Chlorite	Na Chlorite/Citric Acid/Na Hydroxide	<1%, <1%, <1%	6.5
CytoGuard Plus	Lauric Arginate & Peroxyacetic Acid	5% CytoGuard .15% Perasan MP2	3.0
Lactic Acid FCC 88%	Hydroxypropanoic Acid	5%	1.9
AFTEC 3000	Buffered Sulfuric Acid	5%	1.0
Citrilow	Hyrdrochloric & Citric Acids	18%	0.8
HB2	Hydrobromic Acid	2.8%	0.8

Figure 1. *Top sirloin butt, cap off, with pre-marked 20.25 cm^2 (5.08 cm diameter) circles to show approximate location of where the 4 core samples were taken from the tenderized subprimal.*

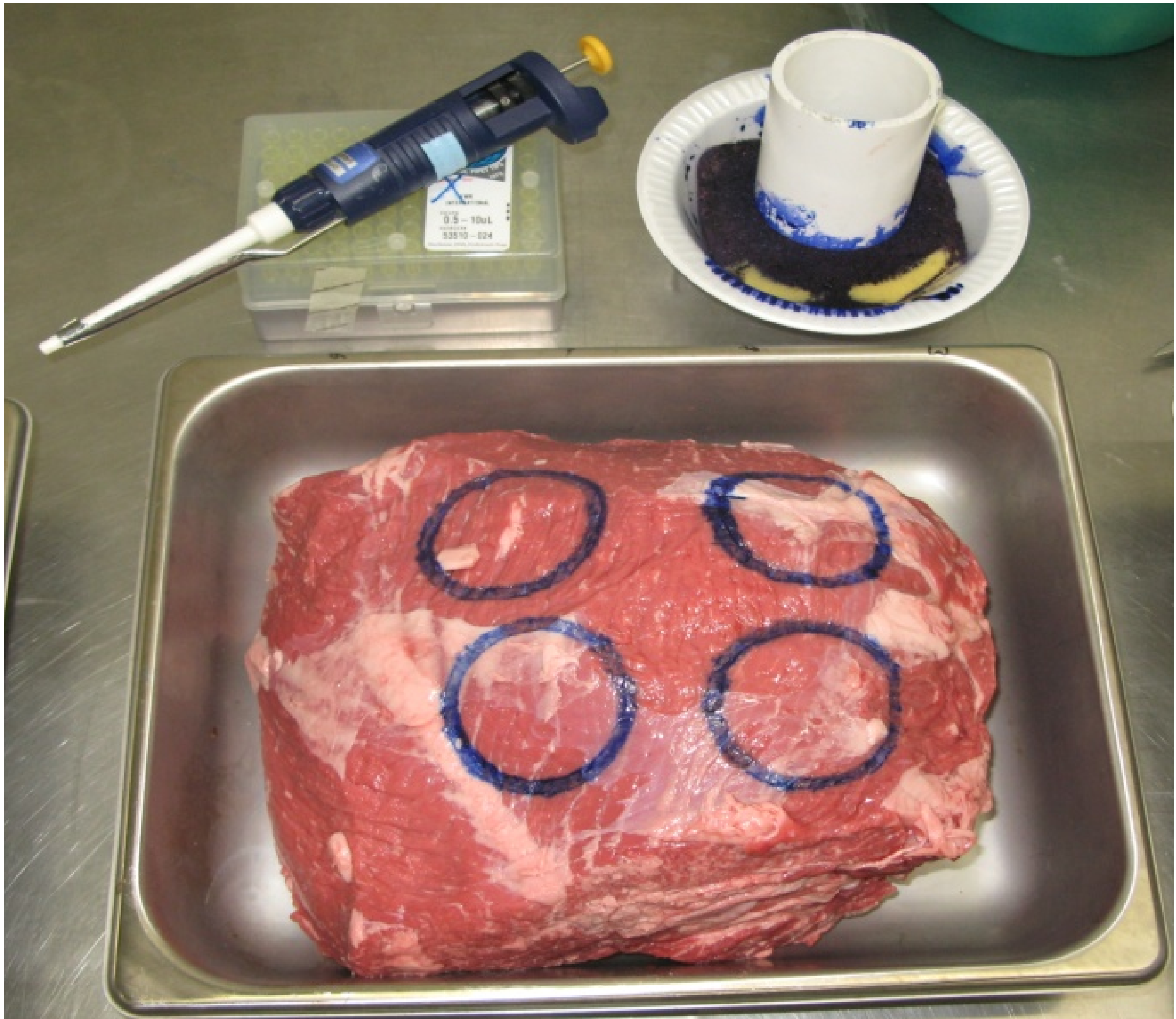


Figure 2. *Ross tenderizer TC700MC equipped with a custom-built Dositron multi nozzle spray system .*

Figure 3. *After exiting the Ross Integrated Tenderizer, beef cores were extracted using a 5.08 cm (20.25 cm^2) stainless steel circular drill bit from each subprimal.*

FIGURE 4. *To further eliminate surface contamination from recovered beef cores each sample passed through an Infrared Grill Pasteurizer at 412.5°F at 5 hz with a 5 min dwell time.*

Figure 5. *Photograph depicting approximate location of each of the four sections obtained to evaluate the effects of antimicrobial interventions and to determine the levels of bacteria transferred into muscle during mechanical tenderization; segments were 2.54 cm each.*

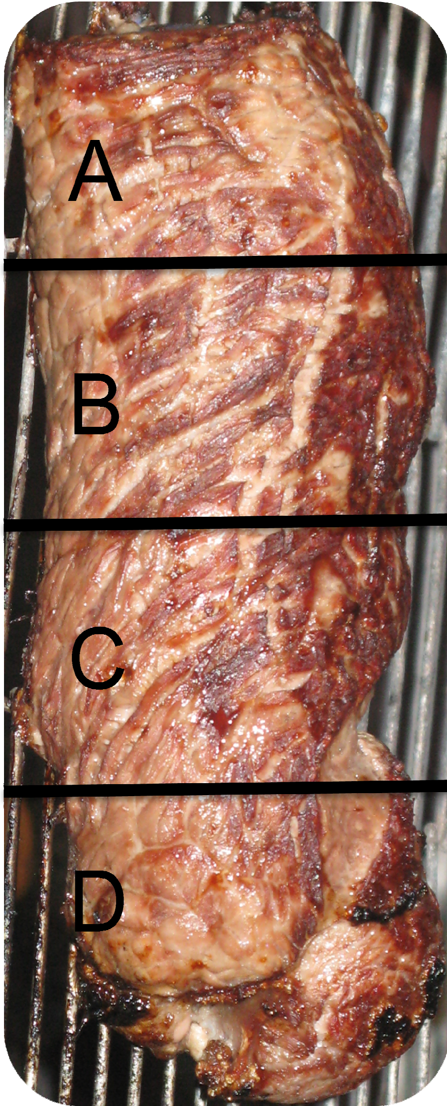


Figure 6 A. *Picture of BeadRetriever sample rack filled with sample strips depicting sample tubes 1-5 of each sample strip.*

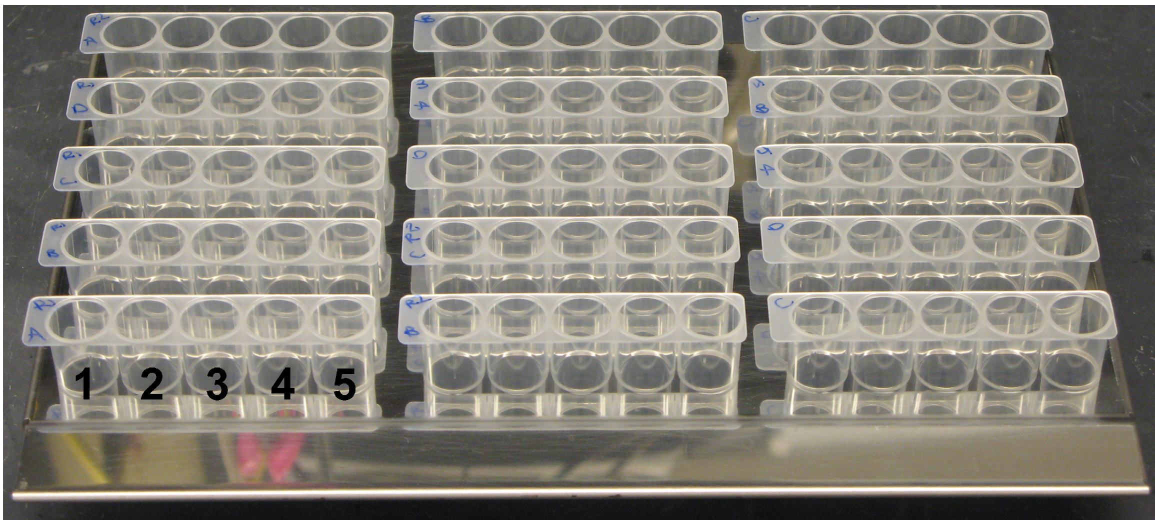


Figure 6 B. *The BeadRetriever instrument used for immunomagnetic bead separation.*

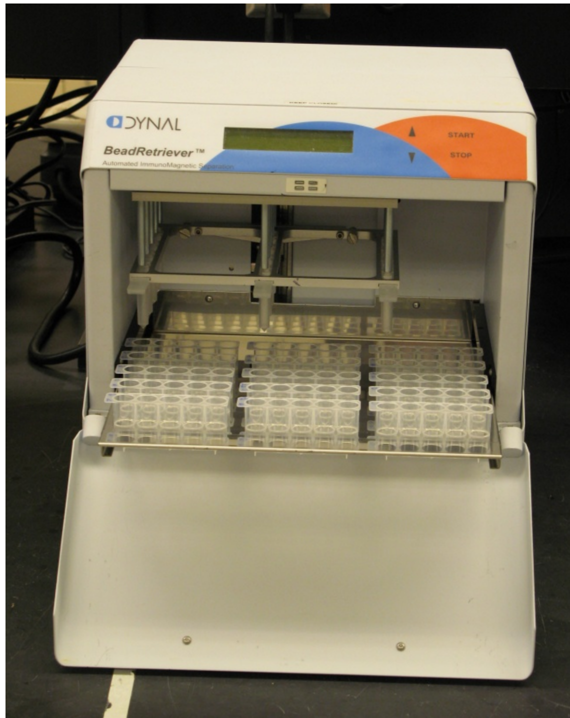


Figure 7. Selective recovery of immunomagnetic beads and differential detection (characteristic green sheen) depicting positive *E. coli* O157:H7 *from Levine's eosin methylene blue agar containing Rifamycin SV Sodium Salt*.

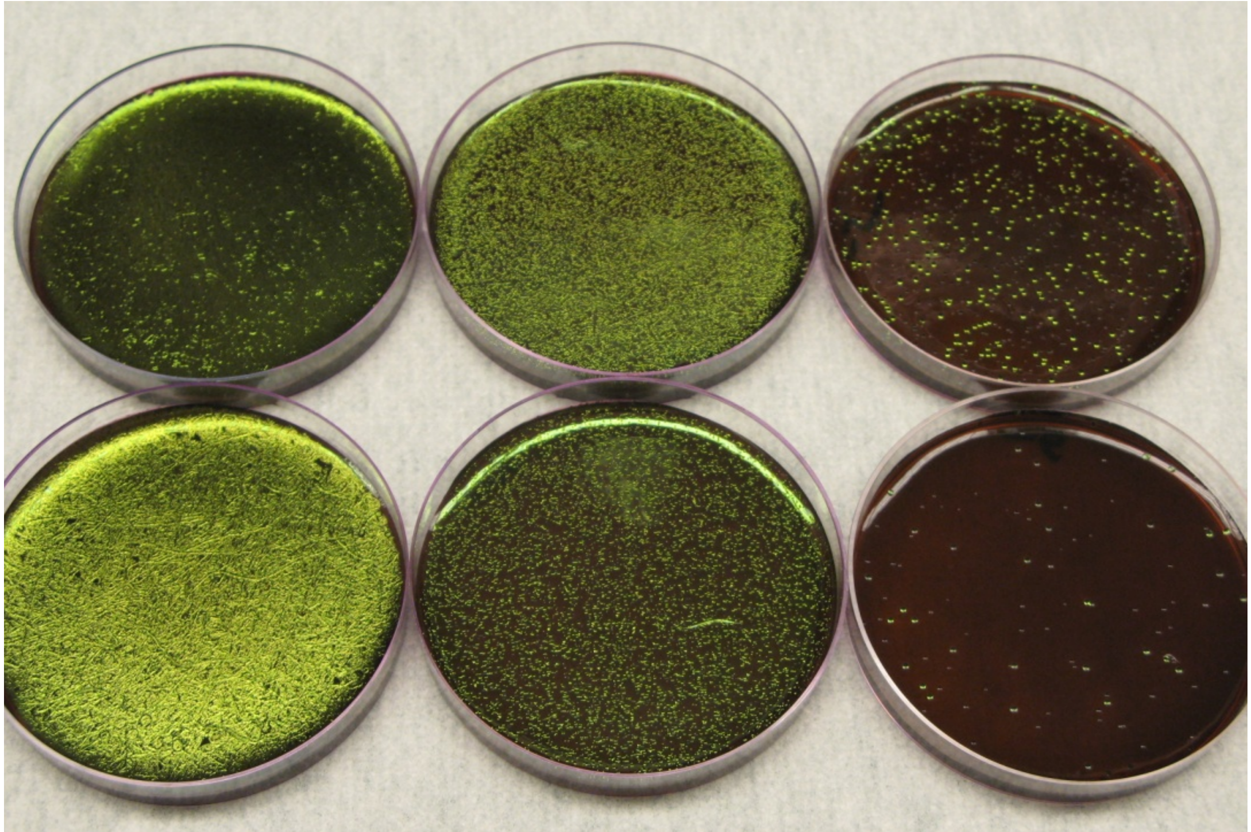


Figure 8. *Effects of antimicrobials on the reduction of E. coli O157:H7 in section A of subprimal cores.*

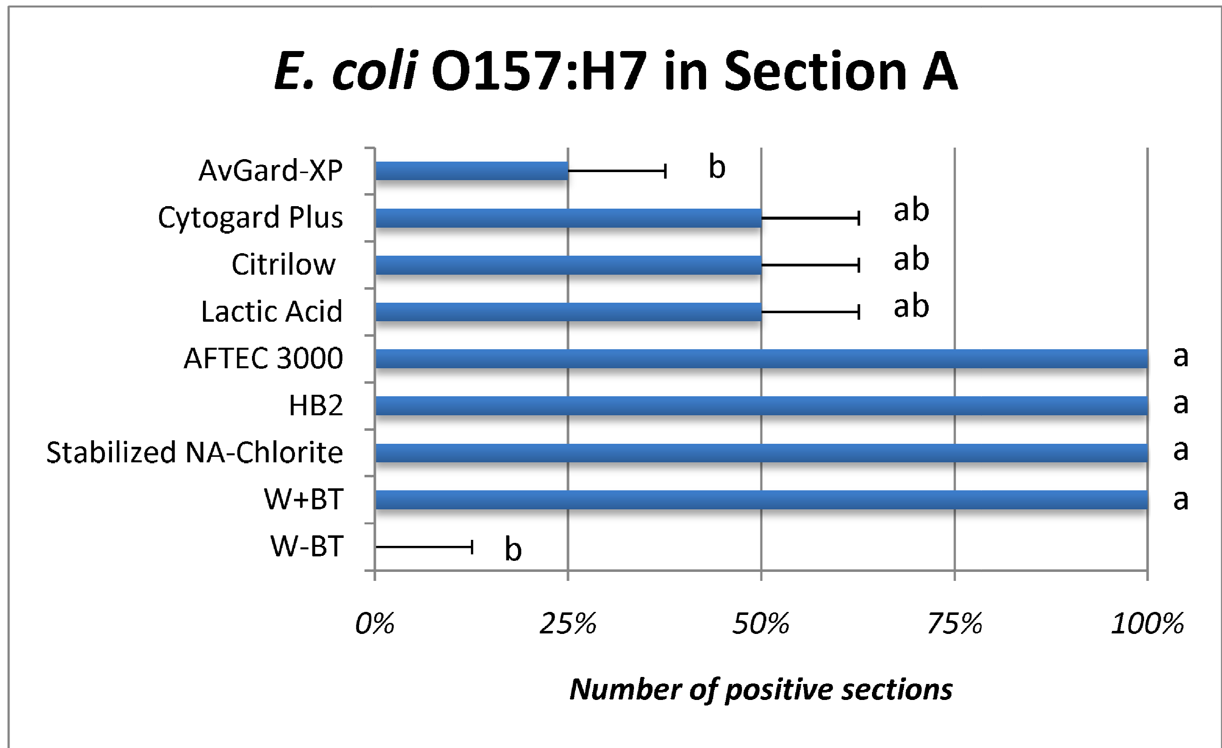


Figure 9. *Effectiveness of antimicrobials on the reducing of E. coli O157:H7 in section B of subprimal cores.*

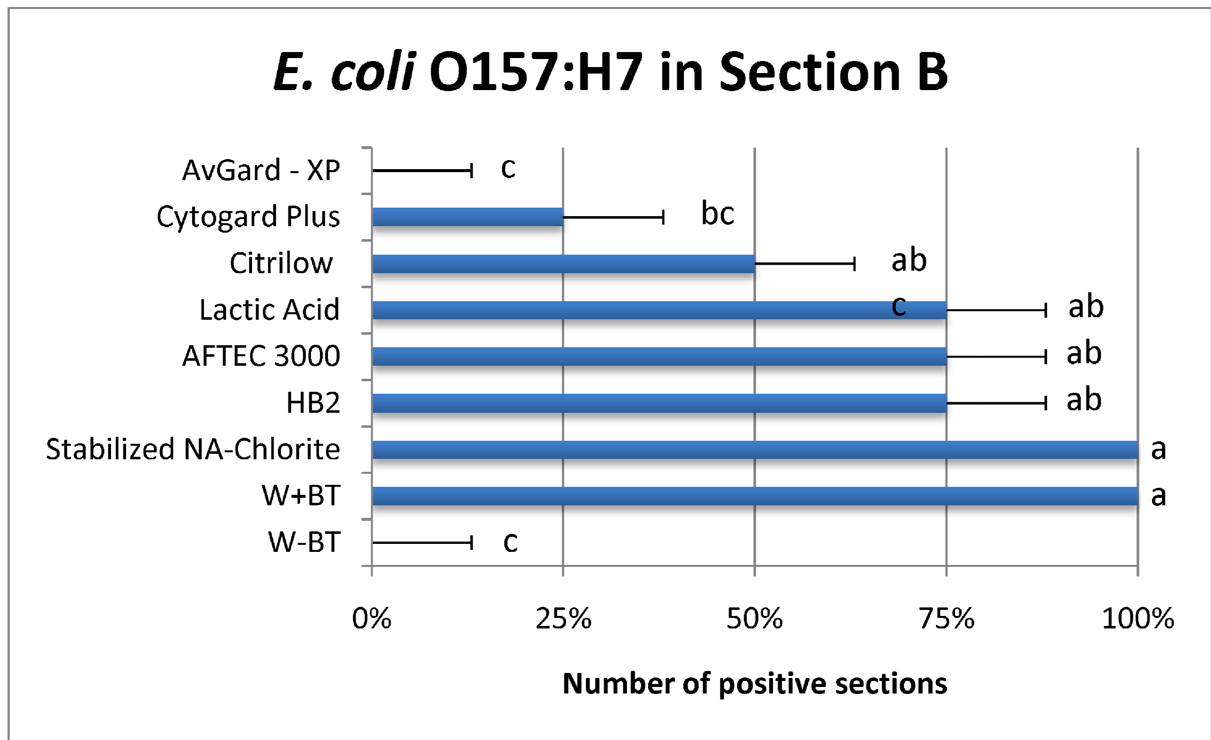


Figure 10. *Effectiveness of antimicrobials at reducing E. coli O157:H7 in section C of subprimal cores.*

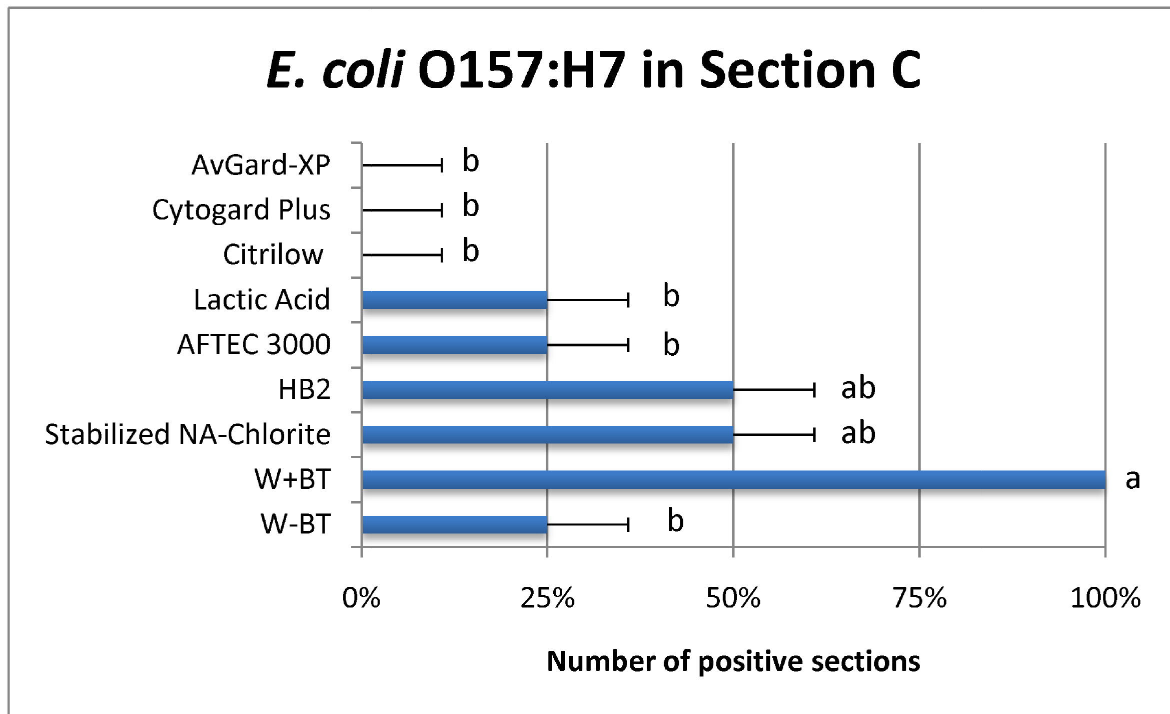
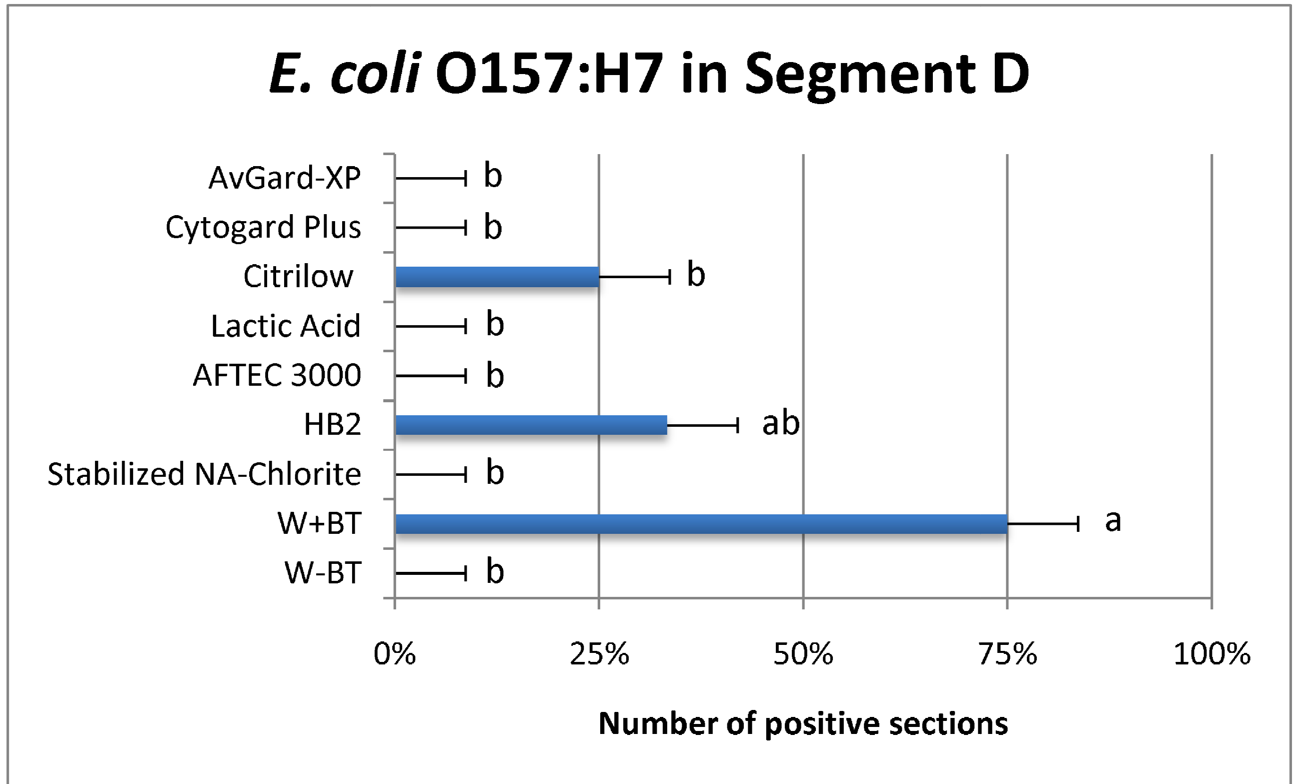


Figure 11. *Effectiveness of antimicrobials at reducing E. coli O157:H7 in section D of subprimal cores.*



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VITA

Jacqulynn Marie Eager

Candidate for the Degree of

Master of Science

Thesis: VALIDATION OF VARIOUS ANTIMICROBIAL SOLUTIONS ON THE REDUCTION OF MICROBIAL PRESENCE OF *E.COLI* O157:H7 ON BLADE TENDERIZED BEEF SUBPRIMALS

Major Field: Type Animal Science

Biographical:

Education:

Completed the requirements for the Master of Science in Animal Science at Oklahoma State University, Stillwater, Oklahoma in December, 2011.

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Name: Jacqulynn Marie Eager Date of Degree: December, 2011*

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: VALIDATION OF VARIOUS ANTIMICROBIAL SOLUTIONS ON THE REDUCTION OF MICROBIAL PRESENCE OF *E.COLI* O157:H7 ON BLADE TENDERIZED BEEF SUBPRIMALS

Pages in Study: 41 Candidate for the Degree of Master of Science

Major Field: Animal Science

Scope and Method of Study: Seven proven effective antimicrobial spray interventions were validated for their effectiveness of reducing *E. coli* O157:H7 on blade tenderized beef subprimals. The antimicrobial treatments consisted of AvGard- XP, Citrilow, Cytogard Plus, Lactic Acid, AFTEC 3000, Stabilized Na-Chlorite, and HB2. Each antimicrobial was applied to fresh boneless top sirloin butt subprimals inoculated with 1 X 10^4 CFU/cm^2 of a four-strain *E. coli* O157:H7 cocktail. Each subprimal was passed through a blade tenderizer after an intervention spray treatment was applied. Immunomagnetic bead separation along with selective, antibiotic resistant media was used for the isolation of pathogen presence.

Findings and Conclusions: The lowest microbial presence was observed in those samples treated with AvGard – XP followed by Citrilow and Cytogard Plus. The order of effectiveness for remaining antimicrobials was Lactic Acid, AFTEC 3000, Stabilized Na-Chlorite, and HB2. The positive controls (W+BT) consistently showed the highest microbial presence across sections. Similarly, the negative controls (W-BT) consistently showed the lowest microbial presence across sections.

ADVISER’S APPROVAL: ____________________