COMPARISON OF DIFFERENT ANTIMICROBIAL TREATMENTS IN THE DECONTAMINATION OF FRESH BEEF HEAD AND CHEEK MEAT

A Thesis

by

KARLA MARÍA SOLÍS SALAZAR

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

MASTER OF SCIENCE

Chair of Committee,	Gary R. Acuff
Co-Chair of Committee,	Alejandro Castillo
Committee Member,	Rhonda K. Miller
Head of Department,	Steve Searcy

August 2019

Major Subject: Food Science and Technology

ABSTRACT

Shiga toxin-producing *Escherichia coli* are enteric pathogens that can cause severe illnesses in humans including Hemolytic Uremic Syndrome (HUS) and Hemorrhagic Colitis (HC). These pathogens may be present in the gastrointestinal tract and hide of cattle and during the harvesting process might be transmitted to the meat. It is of significant importance to prevent the contamination of check and head meat, as these are often used in the production of ground beef. If the head or cheek meat has been contaminated with pathogens during head processing, that contamination can further spread during grinding.

The purpose of this study is to examine the application of commonly used carcass antimicrobial interventions [lactic acid (LA), peroxyacetic acid (PAA), acidified peroxyacetic acid (aPAA), and 1,3-Dibromo-5,5-dimethylhydantoin (DBDMH)] for the reduction of STEC on fresh head and cheek meat during beef head processing. On the first phase of the study four replications were performed. Beef heads (n=20) were inoculates with STEC surrogates and assigned to one of 10 different treatments for 5 or 15 min: 1. Control (no treatment), 2. Water, 3. 1% LA, 4. 2% LA, 5. 200 ppm PAA, 6. 400 ppm PAA, 7. 200 ppm aPAA, 8. 400 ppm aPAA, 9. 200 ppm DBDMH and 400 ppm DBDMH. For the second phase of the study two additional replicates of 4 different treatments were performed: control, water, 2% LA, and 400 ppm PAA. After each treatment, the heads were allowed to drip for 5 min, and were then sampled to obtain head and cheek meat subsamples. The subsamples were subjected to serial dilutions using BPW and plated into 3M™ Petrifilm™ E. coli/Coliform count plates for enumeration.

Results from this study showed that dipping treatments including 2% LA, and 400 ppm PAA are capable of significantly ($P < 0.05$) reduce surface bacteria on fresh head and check meat by more than 1.2 log CFU/g. Neither water nor DBDMH dipping treatment produced a significant reduction on head or cheek meat surface bacteria. Further investigations are recommended in order to validate an adequate system to be employed within beef processing facilities to provide adequate decontamination of heads while preventing cross-contamination.

DEDICATION

To my family and friends for their invaluable love and moral support despite the distance. Specially to Erick, for his encouragement, advice, help, support and love.

ACKNOWLEDGEMENTS

I want to express my gratitude to Dr. Gary Acuff, and Dr. Alejandro Castillo for providing me with the opportunity of pursuing this degree and for all their support and invaluable guidance during the progression of my graduate studies.

I wish to thank Dr. Rhonda K. Miller for serving on my advisory committee, and Dr. Randall Phebus and Dr. Harshavardhan Thippareddi, for their help and assistance in conducting this research project.

I also wish to express my gratitude to Mrs. Lisa M. Lucia for her friendship, technical assistance and her guidance, and to Nooshin Moradi, Daniel Vega and Katia Pozuelo for their technical assistance in the collection and processing of the samples in this research.

I want to express my gratitude to CRUSA Foundation, who sponsored my studies conducting to the Master's Degree at Texas A&M University.

Finally, thanks to my mother and father for their encouragement and to my husband Erick for his patience and love.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis dissertation committee consisting of Dr. Gary R. Acuff of the Department of Nutrition and Food Science, Dr. Alejandro Castillo and Dr. Rhonda Miller of the Department of Animal Science.

Funding Sources

Graduate study was supported by a fellowship from Texas A&M University and a fellowship from CRUSA Foundation.

This work was also made possible in part by STEC CAP, under Grant Number 25-6239-0235-304. This based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture (USDA-NIFA), under award number #2012-68003-30155. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA-NIFA.

NOMENCLATURE

AA	Acetic acid
aPAA	Acidified peroxyacetic acid
ATCC	American Type Culture Collection
BPW	Buffered peptone water
CDC	Centers for Disease Control and Prevention (Atlanta, GA)
DAEC	Diffusely adherent *E. coli*
DBDMH	1,3-Dibromo-5,5-dimethylhydantoin
EAEC	Enteroaggregative *E. coli*
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
FDA	United States Food and Drug Administration
FoodNet	Foodborne Disease Active Surveillance Network
FSIS	Food Safety and Inspection Service
GRAS	Generally recognized as safe
HACCP	Hazard Analysis Critical Control Points
HC	Hemorrhagic colitis
HOBr	Hypobromous acid
HOCl	Hypochlorous acid
HUS	Hemorrhagic-uremic syndrome

HW	Hot Water
LA	Lactic Acid
PAA	Peroxyacetic acid
STEC	Shiga toxin-producing *E. coli*
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
USDA-FSIS	United States Department of Agriculture - Food Safety and Inspection Service (Washington, DC)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Pathogenic *Escherichia coli* cause several outbreaks worldwide each year and therefore are considered to be major foodborne pathogens. Shiga toxin-producing *E. coli* (STEC) has been linked to several outbreaks (*35*, *37*, *38*, *40*) and in recent reports it has been estimated to cause 175,905 illnesses annually (63,153 STEC O157:H7, and 112,752 STEC non-O157:H7) (*116*). The most common STEC serogroups related to human disease are O157, O26, O45, O103, O111, O121, and O145, and these serotypes are recognized as food adulterants by the Food Safety and Inspection Service (FSIS) (*17*, *131*).

STEC transmission to humans occurs through consumption of contaminated food, water or direct contact with infected people or animals (*106*), and the fecal-to-oral route is the primary pathway for infection. This pathogen has a low infective dose that has been estimated to be 10 to 100 organisms (*81*, *136*). Once ingested, the pathogen enters the human digestive system via the mouth and must survive the stomach acidic environment to reach the colon where it attaches and releases Shiga toxin (*63*, *106*). The diarrheal disease produced by this pathogen may range from mild to bloody. Infected patients may develop severe complications, such as hemorrhagic colitis (HC), and hemolytic-uremic syndrome (HUS) characterized by acute kidney failure, microangiopathic hemolytic anemia, and thrombocytopenia (*13*, *70*).

Beef products may become contaminated with STEC during the slaughter and dressing of cattle. Ruminant species have been recognized as STEC natural reservoirs (*74*, *110*). It is commonly accepted that internal and intact muscles of an animal are sterile until

the hide is removed during harvesting. When the knife cuts through the hide and into muscle, as the tissue is exposed, the meat may become contaminated. (*3, 134*). During the hide removal step the head is usually hanging down. This positioning of the carcass may allow in microbial contamination to migrate from carcass surfaces to the head (*56, 111*).

Different decontamination methods have been designed to effectively reduce microbial contamination on carcass, primal and subprimal cut surfaces, and their effectiveness has been widely tested (*15, 30, 44, 79, 112*). However, use of these methods to obtain an optimal reduction of pathogens on head and cheek meat has not been widely studied or implemented. The intent of this study is to examine the application of commonly used carcass antimicrobial interventions [lactic acid (LA), peroxyacetic acid (PAA), acidified peroxyacetic acid (aPAA), and 1,3-Dibromo-5,5-dimethylhydantoin (DBDMH)] for the reduction of STEC on fresh head and cheek meat during beef head processing.

CHAPTER II

LITERATURE REVIEW

Impact of foodborne illness

Foodborne pathogens are a significant cause of illness and death. Recognized foodborne pathogens include protozoa, helminths, fungi, bacteria, viruses, and prions (*75*). It is estimated that in the U.S. approximately 48 million people suffer foodborne diseases annually, however, only 20% of these cases can be linked to a specific pathogen (*69*). The economic burden of foodborne pathogens in the U.S. has been reported to be approximately $15.5 billion per year (*69*).

Foodborne diseases result from the consumption of contaminated food products. These illnesses may be caused by pathogens, allergens and chemicals that contaminate the food at some point in the production chain (*123*). Foodborne illnesses have an impact on the world economy. It is estimated that 76 million illnesses associated with the consumption of food occur each year in the U.S., resulting in productivity losses and medical costs that range from $6.6 billion to $37.1 billion (*22*, *95*). The costs associated with foodborne diseases include, but are not limited to, workers productivity, medical costs, and, in the worst scenario, death expenses. The economic losses also include the recall of products and the image damage that may be produced by the association of an outbreak to a specific product or company. In order to prevent foodborne illnesses, government agencies and food industries spend a significant amount of resources on prevention (*69*).

The Foodborne Disease Active Surveillance Network (FoodNet) data gathering system is a collaborative program among the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS), Centers for Disease Control and Prevention (CDC), and the U.S. Food and Drug Administration (FDA). It conducts surveillance on nine foodborne pathogens in California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New Mexico, New York, Oregon and Tennessee. The pathogens monitored by FoodNet include *Campylobacter* spp., *Cryptosporidium* spp., *Cyclospora, Listeria monocytogenes, Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC) O157 and non-O157, *Shigella* spp., *Vibrio* spp., and *Yersinia.* (*36*, *66*).

Foodborne illnesses are often underreported. The most common causes for this are: people with acute diarrhea usually do not seek medical care, or in the cases that do pursue medical care sometimes it is not possible to identify the cause, or the cause is not adequately reported (*54*). FoodNet monitors changes in the incidence of the selected pathogen, attributes illnesses produced by each pathogen to food sources, and estimates the total numbers of foodborne illnesses in the country (*66*). Pathogenic *E. coli, Salmonella,* and *Campylobacter* spp. constitute the greatest burden of foodborne diseases for which etiology is known (*87*).

Salmonella is recognized as one of the main causes of foodborne disease in developed countries (*24*). Most salmonellosis cases do not result in a medical visit, however, *Salmonella* infections are potentially serious and may be fatal for people that are immunocompromised (*54*). FoodNet estimates that in the U.S. 1.3 million cases of salmonellosis occur annually due to the consumption of food contaminated by *Salmonella*

(*54*). The economic burden due to this pathogen in the U.S. is estimated at $2.8 billion to $3.6 billion annually (*4*, *66*, *69*).

Campylobacter together with *Salmonella* are recognized as the two most common causes of bacterial foodborne illnesses in the U.S. (*93*). The annual cost of foodborne illness estimates for *Campylobacter* enteritis range from $800 million to $5.6 billion (*23*, *69*). Campylobacteriosis disease can range from mild diarrhea that may resolve in less than a week without professional medical attention, to severe bloody diarrhea that requires medical attention. In some cases *Campylobacter* may lead to the development of Guillain-Barré syndrome a few weeks after infection (*22*, *75*).

Shiga toxin-producing *E. coli* is widely recognized as an important cause of foodborne diseases. The severity of the diseases may range from a mild diarrhea to severe complications such as hemorrhagic-uremic syndrome (HUS) and hemorrhagic colitis (HC). It was reported that the estimated cost of illness due to STEC O157:H7 is approximately $405.2 million per year (*53*). The reported economic cost associated with STEC O157 infections has been shown to be higher than the cost related to non-O157 cases (*69*).

The study and prevention of foodborne diseases is dynamic and in constant evolution. New pathogens could emerge, and the currently recognized pathogens may appear on different food vehicles or acquire new virulence or resistance characteristics.

Escherichia coli

The genus *Escherichia*, named after the German pediatrician Theodor Escherich, consist of facultative anaerobic Gram-negative, mesophilic, rod shaped bacteria that belong to the *Enterobacteriaceae* family (*49, 75*). *E. coli* species include nonpathogenic bacteria that are a part of the normal microbiota of the intestinal tract of humans and animals, and a small number of pathogenic bacteria that have acquired genes that enable them to cause intestinal or extra-intestinal disease (*58, 63*). *E. coli* can be serologically classified based on their somatic (O), flagellar (H), and capsular (K) antigens, The O antigen identifies the serogroup of a strain, and the H antigen identifies its serotype (*80, 96*). Several *E. coli* serogroups have been identified, however, not all of them have been shown to cause illness.

E. coli pathogens are one of the most important etiological agents of diarrheal disease. Diarrheagenic *E. coli* strains have acquired particular sets of virulence factors through horizontal gene transfer, and the specific combination of these factors determine pathotype characteristics, regarding preferential host colonization site, virulence mechanisms, and clinical symptoms (*58*). Pathogenic *E. coli* can be categorized into six pathotypes according to virulence factors and mechanisms by which they cause disease. The pathotypes described include: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enterohemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli* (STEC) (*80*).

Enteropathogenic *E. coli* (EPEC)

EPEC was first described as a causative agent of infant diarrhea, characterized by watery stools, often accompanied by vomiting and low grade fever (*68*). This *E. coli* pathotype does not causes diarrhea by toxin production, EPEC-induced diarrhea is a multi-factorial process that involves disturbances in ion transport, intestinal permeability disruption, intestinal inflammation, serotonin butyrate transporters modulation and loss of absorptive surface area resulting from microvillus effacement (*68*, *75*, *80*)

EPEC produces a characteristic attaching and effacing (A/E) lesion which is marked by effacement of microvilli on the epithelial surface at the site of bacterial attachment (*80*). A/E lesions are encoded by a pathogenicity island (PAI) of around 35kb known as locus of enterocyte effacement (LEE), which is organized into five operons (LEE1 to LEE5) (*58*). The A/E lesion begins with a nonintimate bacterial attachment, followed by the injection of type III proteins, and the secretion of various effector proteins including *tir*, *espF*, *espG*, *espH* and *Map*, that are translocated into the host cell. The bacteria binds through the *tir*-intimin interaction, leading to the activation of protein kinase C (PKC), phospholipase Cγ, myosin light-chain kinase and mitogen-activated protein (MAP) kinases, producing several downstream effects, which include permeability increase due to loosened tight junctions that effect cytoskeletal changes and microvilli effacement (*80*).

The infection of intestinal epithelial cells with EPEC decreases epithelial ion absorption. The type III secretion system of EPEC releases *E. coli* secreted proteins (*esp*) into the host cytosol, *espF* inhibits the function of the Na^+/H^+ exchanger isoform 3

(NHE3), whereas *espG*, affect the apical $Cl^-/HCO3^-$ exchanger downregulated in adenoma (DRA) via disruption of microtubules, leading to a decrease in the exchange activity (*68*). All these together lead to the diarrhea production.

Enterotoxigenic *E. coli* (ETEC)

ETEC is the recognized cause of travelers' diarrhea, characterized by watery diarrhea with little or no fever, abdominal pain, nausea and vomiting (*50, 97*). Humans are the main reservoir of ETEC strains associated with human diarrheic illness (*96*). The infective dose of ETEC has been estimated to be 10^6 to 10^{10} cells, but in immunocompromised people, elderly and children the infective dose may be lower (*50, 75, 97*). Pathogenesis is due to enterotoxin production, which gives rise to intestinal secretion (*80*). ETEC strains attach to and colonize the small intestine by the use of fimbrial colonization factor antigens, followed by toxin secretion once attached (*75*). ETEC enterotoxins include a 91kDa heat-labile toxin (LT) that is destroyed at 60°C in approximately 30 min and a 4kDa heat-stable toxin (STa or ST-1, and STb or ST-II) that can resist 100°C for 15 min (*50, 75*).

ETEC enterotoxins produce diarrhea by various mechanisms. The LT has a mechanism of action similar to that of the cholera toxin, increasing Cl^- secretion via activation of cAMP (cyclic adenosine monophosphate) (*50, 68*). The STa binds to a specific high-affinity non-ganglioside receptor, starting a transmembrane signal that triggers the production of intracellular cyclic guanosine monophosphate (cGMP). The increased levels of mucosal cGMP lead to loss of fluids and electrolytes, producing

diarrhea (*75*, *80*). While, the STb induces elevation of cytosolic Ca^{2+} concentrations, stimulating prostaglandin E_2 and serotonin release, which lead to increased ion and water secretion, resulting in diarrhea (*80*).

Enteroinvasive *E. coli* (EIEC)

EIEC cause an invasive inflammatory colitis, with watery diarrhea, indistinguishable from that due to other pathogenic *E. coli* infection and occasionally dysentery (*80*). The infective dose of EIEC has been estimated to be 10^6 cells (*50*). EIEC pathogenesis is primarily due its ability to invade and destroy colonic tissue, it possess 140MDa enteroinvasive plasmids (pINV) that are similar to those found in *Shigella flexneri* and are essential for their invasiveness (*75*). Epithelial cell penetration is followed by lysis of the endocytic vacuole, intracellular multiplication, directional movement through the cytoplasm and extension into adjacent epithelial cells (*80*).

Enteroaggregative *E. coli* (EAEC)

EAEC are recognized by a distinctive adherence to HEp-2 cells in an aggregative stacked brick-like pattern (*101*). EAEC strains cause a persistent diarrhea that may last more than 14 days, especially in children and immunocompromised individuals (*75*, *99*, *100*). The watery diarrhea may be accompanied by occasional presence of mucus and blood, abdominal pain, nausea, vomiting and low-grade fever (*58*, *83*).

EAEC carry a 60MDa plasmid that is needed for the production of fimbriae that are responsible for the aggregative expression, and for a specific outer membrane protein

involved in bacteria-eukaryotic cell adherence, bacteria-bacteria adherence and biofilm formation (*75*, *92*). Pathogenesis initiates with the adherence of the bacteria to the intestinal mucosa and the mucus layer in an aggregative pattern (*101*). The initial adherence is mediated by the aggregative adherence fimbriae (AAF), it promotes auto-agglutination of EAEC and mediate bacterial adherence to extracellular matrix proteins such as fibronectin, laminin, and collagen IV (*92*, *101*).

In order to neutralize negative charge polysaccharides of the bacteria, EAEC secrete a protein named dispersin that permit the positively charged AAF fimbriae to mediate distant interactions (*99*). EAEC form mucoid biofilms at the surface of the intestinal epithelium of the terminal ileum and of the colon and some strains may elaborate toxins (*64*, *99*). The oligomeric enterotoxin called *Shigella* enterotoxin 1 (ShET1) and the *E. coli* heat-stable enterotoxin EAST1 contribute to the watery, secretory diarrhea; and the plasmid encoded toxin (Pet), a cytotoxin that modifies the cytoskeleton of enterocytes, leads to cell elongation and exfoliation and cell detachment (*58*, *83*, *99*).

Diffusely adherent *E. coli* (DAEC)

DAEC are recognized by a characteristic, diffuse adherence pattern on HeLa and HEp-2 cells, in which the bacteria uniformly cover the entire cell surface (*80*, *115*). It may cause intestinal and urogenital infections (*62*, *118*).

DAEC strains induce a cytopathic effect that is characterized by the development of long cellular extensions, which wrap around the adherent bacteria (*80*). This adherent pattern is mediated through afimbrial (Afa) and fimbrial adhesins that interact with the

brush border-associated complement decay-accelerating factor (*43*). The secreted autotransporter toxin (Sat), a serine protease autotransporter, act as a virulence factor by promoting lesions in the tight junctions of polarized epithelial cells (*62*). EAEC also triggers a Ca^{2+} dependent signaling cascade, that induce the elongation and lesions in the brush border microvilli through the disorganization of key components of the cytoskeleton (*43*). The structural damage of brush border is accompanied by a decrease in the enzyme activity and expression of functional brush border-associated proteins (*118*).

Shiga toxin-producing *E. coli* (STEC)

STEC is capable of expressing one or more subtypes of Shiga toxin (*stx*). This cytotoxic toxin possess a structure and function similar to the Shiga toxin produced by *Shigella dysenteriae* (*17*, *63*). Numerous STEC strains are capable of producing one or both Shiga toxins (*stx*1 and s*tx*2), but not all of them are significant human pathogens (*82*). Patients with a STEC infection will present diarrhea from mild to bloody and may develop severe complications as HC, and HUS. HC symptoms include bloody diarrhea, abdominal cramps, fever, and vomiting, while HUS is characterized by thrombocytopenia, renal failure, and hemolytic anemia (*13*, *70*).

Different STEC serotypes have been implicated as the cause of foodborne outbreaks. The most common serogroups related to human disease are O157, O26, O45, O103, O111, O121, and O145 (*17*), and these serotypes are recognized as food adulterants by FSIS in raw intact and non-intact beef products that are intended for use in raw non-intact products (*131*).

STEC virulence factors

STEC virulence factors vary to some extent among strains, but all strains by definition produce Shiga toxin (*82*). Described virulence factors include Shiga toxin (*stx*), intimin (*eae*), hemolysin (*hly*A), extracellular serine protease gene (*esp*P), catalase peroxidase gene (katP), and etpD among others (*13*, *48*)

Most of the primary virulence determinants of STEC are chromosomally encoded, however, some virulence factors encoded in plasmids have been reported to play a role in the pathogenesis of STEC (*14*). The *stx* genes are encoded on bacteriophages integrated in the chromosome of the bacteria (*27*, *63*). Two types of Shiga toxin that share approximately 55% amino acid homology and different antigenic variants of them have been identified: *stx*1 (*stx*1a, *stx*1c, *stx*1d), and *stx*2 (*stx*2a, *stx*2b, *stx*2c, *stx*2d, *stx*2d-activatable, *stx*2e, *stx*2f and *stx*2g) (*58*, *80*, *121*).

The Shiga toxin consists of one enzymatically active A subunit non-covalently associated to five B subunits that are responsible for binding the toxin to the glycolipid globotriaosylceramide (Gb3) on the target cell surface (*76*, *80*, *103*). Various cell types are sensitive to this toxin, including enterocytes, renal, aortic, and brain endothelial cells, platelets and erythrocytes, among other cell types (*110*). The A subunit is responsible for the ribosomal RNA cleavage, leading to protein synthesis disruption, pro-inflammatory cytokine expression and cell death (*76*, *80*).

The toxin is produced in the colon, where it causes local damage resulting in bloody diarrhea and hemolytic colitis. It may travel to the kidney by the bloodstream, where it damages the endothelial cells and occludes the microvasculature, leading to

inflammation that may contribute to HUS (*80*). Different studies have indicated that strains carrying *stx*2 have a higher virulent potential than those with *stx*1 or both *stx*1 and *stx*2 (*12*, *55*)

STEC strains may carry the pathogenicity island of 43kb, LEE, that contains a cluster of genes whose products contribute to their pathogenesis (*80*). Genes associated with adherence to the host cell include the chromosomally located genes *iha*, long polar fimbriae (*lfp*) and genes located in the LEE (*14*, *98*). The locus of enterocyte effacement is functionally composed by three modules: one that encodes for a type III secretion system, one that encodes the secreted proteins *espA*, *espB* and *espD*, and one that encodes for intimin (*eae*) and the translocated intimin receptor (*tir*) (*27*).

Intimin is encoded by the *eae* gene, and it allows the STEC tight adherence to the host intestine during infection (*14*). Fourteen antigenic variants of the *e*ae gene that encode 14 different intimin types and subtypes can be identify using intimin type-specific PCR assays (α1, α2, β1, β1, γ1, γ2/θ, δ/κ, ε, ξ, η, ι, λ, μ, ν) (*11*). Different STEC serotypes express different intimin subtypes for example the subtype *eae*-γ1 is associated with highly pathogenic STEC serotypes O157:H7 and O145:H-, intimin ε is associated to serogroups O103 and O121 and the subtype β have been linked to serogroup O26 (*14*, *27*, *48*). Intimin along with the *tir* gene are responsible for the adherence and effacing (A/E) lesion, an arrangement of the intestinal epithelial cell architecture in a characteristic pedestal formation (*63*, *100*)

STEC O157 possess a large virulence plasmid of approximately 90kb named plasmid pO157. This is a dynamic structure, which consists of different mobile genetic

elements such as prophages, transposons, and parts of other plasmids assembled together by recombination events (*27*). This plasmid carries a type II secretion system, *toxB, hly* operon, katalase-peroxidase (*katP*) and a serin protease (*espP*) (*27*). The extracellular serine protease (*esp*P) is able to cleave pepsin and coagulation factor V, leading to hemorrhagic colitis exacerbation, it also cleaves multiple complement system components protecting bacteria from the immune system-mediated elimination (*18, 110*). Large plasmids similar to pO157 that carry the *hly* operon can be found in most non-O157 STEC, while the other markers are not usually found as part of these plasmids (*27*).

Pathways of infection- animal reservoirs

Cattle and other ruminant animals have been recognized as natural reservoirs of STEC. *E. coli* O157:H7 is considered a member of the normal microbiota in cattle and does not cause disease (*74*, *110*). Cattle are able to carry STEC asymptomatically due to the lack of the Gb3 receptor in the blood vessels of their gastrointestinal tract, allowing STEC to colonize without causing clinical symptoms typically observed in humans (*102*).

Meat products may be contaminated through the harvesting and processing of STEC colonized animals. In whole carcasses, the prevalence rates will range from 0.01% to 43.4% for STEC O157, and from 1.7% to 58.0% for non-O157 STECs (*70*).

Shedding by these animals can also contaminate the soil and water, leading to the contamination of other products (*19*). Produce can become contaminated with STEC before harvest when grown in fields fertilized with contaminated manure or irrigated with contaminated water. The presence of cattle feed yards near farms where produce is

cultivated, is a big risk factor, as the feces of the cattle may contaminate the water that is used for irrigation (*73*, *122*). STEC O157:H7 can remain viable in bovine feces for more than 7 weeks (*135*) and can survive in soil fertilized with contaminated manure or irrigated with contaminated water for more than 7 months (*73*). The long persistence of the pathogen in the environment increases the risk for transmission through wash-off to nearby farms. Because of this, proper management of cattle waste is critical in controlling STEC spreading into vegetable crops.

STEC transmission occurs through consumption of contaminated food, water or direct contact with people or animals (*106*). The fecal-to-oral route is the primary pathway for *E. coli* infections in humans. In several outbreaks, ingestion of contaminated food products has been recognized as one of the main causes of infection. Consumption of undercooked ground meat has been associated with up to 40% of outbreaks. Products such as apple juice, unpasteurized dairy products, vegetables and other meat products have also been implicated in outbreaks worldwide (*19*, *110*).

The low infectious dose of this foodborne pathogen is of particular interest (*28*, *80*). The infectious dose has been estimated to be as low as 10 to 100 cells (*81*, *136*). Once ingested, the pathogen enters the gastrointestinal system through the mouth and must survive the acidic environment of the stomach before moving through the intestines to the colon, where the bacteria attaches to the cell wall, producing cellular damage and releasing Shiga toxin (*63*, *106*).

Symptoms usually appear 3 to 4 days after exposure, most of the people infected with STEC will experience mild diarrhea, abdominal cramps, nausea, or no symptoms at

all (*69*). On average, the acute illness ends 6 to 8 days after onset (*24*), and a high percentage of infected people recover without medical care, but around 15% of cases experience more serious symptoms, such as bloody diarrhea or severe complications as HUS (*69*).

STEC outbreaks

An outbreak occurs when two or more people report illnesses that are traced to the same exposure source (*61*). From 1998 to 2016, there have been 142 outbreaks, 2,075 illnesses, 498 hospitalizations and 9 deaths from *Escherichia*-related incidents in beef registered in the National Outbreak Reporting System (NORS) (*35*).

The first outbreak linked to STEC was reported in 1982. *E. coli* O157:H7 was found in stool samples of patients with gastrointestinal illness who developed bloody diarrhea and abdominal cramps after eating hamburgers (*96*, *113*). In January 1993, the Jack in the Box STEC O157:H7 outbreak caused the deaths of 3 children under the age of 3 years old (*88*). The presence of *E. coli* O157:H7 in ground beef is considered adulterated and is not allowed into commerce (*86*).

According to the CDC more than 265,000 illness each year have been associated with STEC, causing more than 3600 hospitalizations annually (*39*). Fey et al., (*51*) found a 4.2% STEC prevalence in stool samples associated with sporadic diarrhea cases in Nebraska. The largest percentage of foodborne outbreaks caused by STEC is attributed to beef (58%), followed by leafy vegetables (17%), dairy, and fruits/nuts commodities (*59*).

In the U.S., 55.2% of O157 foodborne outbreaks and 40% of non-O157 STEC outbreaks between 1999 and 2008 were linked to beef (*9*).

E. coli O157:H7 causes 63,000 cases of foodborne illness in a typical year while non-O157 STECs causes around 112,000 cases. This represents less than 1% and 1.2% of the cases of foodborne illnesses acquired in the U.S. for which a specific pathogen cause can be found respectively, with almost half of the STEC O157 cases requiring hospitalization (*69*).

In 2014, a STEC O157:H7 outbreak linked to ground beef occurred and twelve people from 4 different states (Michigan, Ohio, Missouri, and Massachusetts) reported illness. Patient's ages ranged from 16 to 46 years old and 58% of the patients were hospitalized; however, there were no deaths associated with the outbreak (*38*). Traceback investigations indicated that contaminated ground beef produced by Wolverine Packing Company (Detroit, Michigan) was the likely source of this outbreak, leading to the recall of approximately 1.8 million pounds of ground beef products (*129*).

In 2018 a multistate outbreak of STEC O157:H7 infections linked to romaine lettuce produced in the Yuma region (Arizona) affected 210 people from 36 states. The illnesses occurred from March 13, 2018 to June 6, 2018 with 96 hospitalizations, 27 HUS cases and five deaths reported. The strain of *E. coli* O157:H7 associated whit this outbreak was identified in canal water samples taken from the Yuma growing region (*37*). The same year, on September 19, Cargill Meat Solutions (Fort Morgan, Colorado) recalled ground beef products that were produced and packaged on June 21 and shipped to retailers. This recalled ground beef was associated with 18 STEC O26 cases in 4 states (Colorado,

Florida, Massachusetts and Tennessee). Six hospitalizations and 1 death were reported in this outbreak (*40*).

In 2019 a multistate *E. coli* O103 outbreak involving 196 people from 10 states was related to ground beef. The illnesses occurred from March 01, 2019 to April 19, 2019 with 28 hospitalizations, 2 HUS cases and no deaths reported. Two companies, Grant Park Packing (Franklin Park, Illinois) and K2D Foods-Colorado Premium Foods (Carrollton, Georgia), recalled ground beef products that were sold to restaurants and institutions because they may be contaminated with *E. coli* O103 (*41*).

Meat byproducts (offal-variety meat): beef head and cheek meat

Animal byproducts, or offal, include all parts of a live animal that are not part of the dressed carcass. In the case of cattle it is approximately 44% of the live weight of the animal (*94*). These byproducts may be classified into edible and inedible for humans. Inedible offal consists of the hide and skin, blood, and fat. While edible offal includes variety meats such as liver, brain, heart, kidney, tongue and feet (*3*).

Variety meats are an excellent source of essential nutrients required in the human diet. In some developing countries, consumption is associated with low-income populations as they are an inexpensive way to obtain high-quality protein (*1*, *104*), while in other countries offal products are part of traditional dishes associated with cultural and religious practices (*3*, *71*). In some Asian countries, edible offal products such as liver and heart are eaten raw. For example, *sashimi*, a food that consists of very fresh raw meat or fish sliced into thin pieces, is consumed in Japan (*8*).

Meat industry byproducts have economic importance as they can be used in a variety of industries, including pharmaceutical, textile, cosmetic, and human or animal food (*3*, *108*). Offal attribute for 4.9% of the total revenue for beef slaughter (*42*). In the U.S., edible offal are primarily used as ingredients in products such as sausages and ground meat (*94*). Animal bones and skin can be used in gelatin production, intestines are used as sausage casings, cheek and head trimmings are commonly used in sausages and hamburger patty manufacturing, and kidneys and spleens can be found as an ingredient in pet food (*3*, *56*).

When beef cheek meat is used in the preparation of ground beef, the amount used is limited to 25%, and it should be declared on the label if used in higher proportions (*131*). A 24.3% non-O157 STEC prevalence was observed in commercial ground beef samples obtained from numerous manufacturers across the U.S. (*14*). When beef is ground, the surface area of the product is increased and microorganisms that may be present on the exterior of the meat will be spread through the rest of the meat, possibly increasing the chance of exposure to contamination. Ground beef contaminated with pathogens represents a high risk if hamburger patties are not cooked well done and the center of the patty is not exposed to lethal temperatures (160 ^{o}F or 71.1^{o}C) (*106*).

Harvest of beef head and cheek meat

The muscle tissue of healthy animals before slaughter is considered sterile, however, microbial contamination of carcasses can occur at beef harvesting facilities

during processing (*3*). Higher bacterial counts have been found on head and cheek meat when compared to other beef trimmings (*28*)

Bacteria on the hides of animals at slaughter, including pathogens such as STEC O157:H7 and *Salmonella*, have been identified as the main source of contamination during the processing of beef carcasses (*7*, *13*, *134*). Pathogens may be part of the cattle hide microbiota due to the close proximity of animals during transport and lairage (*20*).

When harvesting beef head tissues, critical hygiene points may be applied in order to avoid cross-contamination from the carcass, the equipment, workers and the environment (*91*, *111*). Identification of sites where antimicrobial interventions should be applied on the harvest floor is critical. This, along with strict hygiene practices and adequate zone division can help to reduce the incidence of pathogens in the processing environment.

At harvesting the animal is rendered unconscious, typically by mechanical stunning that uses air pressure. Buncic et al., (*20*) showed that penetrative stunning of food animals carries a risk of introducing pathogenic bacteria from the skin into edible parts of the animal. The possible dissemination of Bovine Spongiform Encephalitis (BSE) agents from the brain of infected animals to edible tissues also exists during this procedure (*111*).

After stunning, the animal is positioned with the hind legs in the air and the head toward the ground. The exsanguination process is carried out immediately after stunning and the animal is allowed to bleed out before going through a hide-on carcass wash that is usually used as the first microbial intervention on the harvest floor (*56*).

In typical beef harvesting processes applied in the U.S., the head, as the lowest part of the animal during the early stages of dressing, is at particular risk of contamination due to splash, draining of surface water, and worker handling (*56*, *111*). The head is usually removed from each carcass after dehiding, prior to evisceration, and the heads are carried by chain conveyors through the processing floor (*28*, *56*). Proper procedures including flushing nasal, throat, and mouth cavities, and washing the whole head and tongue, must be followed when processing whole heads (*65*). During removal of meat tissue from the head and cheek, the meat is usually dropped onto a conveyor belt for transport to drums where it can be washed, drained and packed (*56*).

Variety meats tend to be more perishable than carcass meat, due to a higher glycogen and lower fat content. Therefore, these products must be handled with sanitary procedures and chilled quickly after slaughter (*3, 104*). During the washing of head meat several interventions may be applied in order to reduce the possible presence of bacteria. Keen et al., detected STEC O157:H7 in the cattle oral cavity during harvesting (*84*). This may act as a source of contamination that is not affected by antimicrobial treatments applied to the head exterior surface throughout processing.

Meat inspection and federal regulations

Food safety regulations are designed to reduce the risk of human illnesses through prevention and control of the presence and amount of foodborne pathogens and other hazards in food products (*24*). The Federal Meat Inspection Act (FMIA) of 1906 and the Wholesome Meat Act of 1967 were designed and implemented to provide the public with

a safe, wholesome meat supply (*72*). FMIA established sanitary standards for slaughter and processing establishments and mandated ante- and post-mortem inspection of every carcass, including cattle, hogs, sheep and goats (*3*).

Required programs such as the implementation of Hazard Analysis Critical Control Point system (HACCP) and Sanitary Standard Operating Procedures (SSOPs), allow establishments to adjust their practices to fit the needs of their operations and meet microbiological performance standards (*130*, *133*). This control system has been used in food production to prevent problems by applying control points in a food production process where hazards could be controlled, reduced, or eliminated (*128*). HACCP was adopted voluntarily in 1996 by a group of beef harvesting facilities, but USDA made the implementation of this program mandatory between 1998 and 2000, starting with large plants with more than 500 employees on January 26, 1998, small establishments (between 10 and 500 employees) on January 25, 1999 and finalizing with very small plants (less than 10 employees or annual sales of less than $2.5 million) on January 25, 2000 (*130*).

STEC O157:H7 was declared an adulterant in 1994 by the USDA-FSIS, establishing a zero-tolerance policy for this pathogen. In 2011 another six STEC serogroups (O26, O45, O103, O111, O121 and O145) where declared as adulterants by FSIS (*131*). In food processing environments the presence of non-pathogenic *E. coli* may serve as an indicator of process failure, and meeting performance criteria for *E. coli* determines if a beef harvesting facility is operating within the limits of Title 9 of the U.S. Code of Federal Regulations (CFR), part 310.25. The CFR outlines criteria that

establishments must follow to verify their process through written procedures, sampling, and analysis for the reduction of *E. coli* (*125*).

Antimicrobial interventions

Interventions used in the U.S. must be approved by the FDA as generally recognized as safe (GRAS), and under the FMIA, FSIS is responsible for determining the suitability of FDA-approved substances in meat products. (*16*, *126*). The FSIS directive 7120.1 "Safe and suitable ingredients used in the production of meat, poultry, and egg products" provides a list of substances that may be used as an intervention in beef production, the amount of antimicrobial allowed, and the point in the process where it can be applied (*127*).

Carcass interventions

Throughout harvesting and subsequent processing, microbial contamination of meat products may occur. It may be introduced onto the edible product from the hide, gastrointestinal tract, workers, and the environment (*137*). Since beef is one of the major proteins consumed worldwide, in order to improve safety, the meat industry employs numerous methods to reduce pathogen loads in the meat products supply. Multiple carcass interventions have been implemented as a control measures for STEC in the meat industry.

Hot water

For a hot water (HW) treatment of carcass surfaces to be effective in reducing bacteria, temperature is the most important factor to be considered; however, other factors such as volume of the spray, type of nozzle, and distance from the nozzle to the carcass play an important role in influencing effectiveness of a HW treatment (*30*). It has been reported that 80 to 82°C water was required in order to deliver 72°C water to a carcass surface located 15 cm from the wash nozzle tip, and that any increase in water pressure above 20 psi would induce additional atomization of the water, resulting in a lower carcass surface temperature (*46*).

Castillo et al., (*32*) evaluated the use of HW for beef carcass contamination. They compared the effect of a warm water wash (manual wash at 25°C, 10 psi, 10s followed high pressure wash at 35°C, 250 to 400 psi, for 9 s) and a warm water wash followed by a HW spray (95°C, 24 psi, 5 s) on fecal contaminated carcass areas. In this investigation, inside and outside rounds, brisket, flank and clods were contaminated with inoculated bovine feces containing 10^6/g rifampicin-resistant *E. coli* O157:H7 or with uninoculated feces to evaluate indicator organisms (aerobic plate counts (APC), total coliforms and thermotolerant coliforms) (*32*). Antimicrobial treatments were evaluated according to two different time delays with respect to the inoculation (5 min and 20-30 min). The researchers reported that all treatments significantly reduced indicator organisms and pathogen levels from the initial inoculation level without being affected by the treatment delay (*32*). However, treatments which included HW were associated with significantly greater reductions than those obtained by water wash alone. In the case of indicator

organisms, a reduction of 1.3 to 2.3 log CFU/cm^2 was obtained by the water wash and an additional reduction of 0.5 to 2.3 log CFU/cm^2 was obtained after the use of the HW spray. Mean reductions of 3.8 log CFU/cm^2 and 2.1 log CFU/cm^2 of *E coli* O157:H7 were observed after applying wash and HW spray and water wash alone, respectively (*32*).

Dorsa et al., (*46*) evaluated the use of a water wash and the combination of water wash plus steam at three different temperatures (15.6°C, 54.4°C, and 82.2°C) on the treatment of beef and sheep carcasses inoculated with a fecal slurry (6 log CFU/cm^2). They reported that inoculated carcasses subjected to water wash treatments at 82.2°C experienced higher reductions in inoculated fecal bacterial populations, approximately 1 log unit more than carcasses treated at 54.4°C. When HW wash (82.2°C) was followed by the use of steam, the bacterial reductions where higher than when using HW alone, 4.0 log CFU/cm^2 and 3.3 log CFU/cm^2, respectively. It was observed that regardless of the level of inoculum (4 or 6 log CFU/cm^2) on inoculated carcasses, the bacterial population was not reduced more that 2.5 to 3.0 log CFU/cm^2, suggesting that extended hydration of a carcass before and during moist heat interventions protects a limited bacterial population (*46*).

Kalchayanand et al., (*77*) evaluated the effect of different spray treatments HW (85°C), 4% LA (25°C), acidified sodium chlorite (ASC, 1000 ppm, 25°C), and 200 ppm PAA (25°C) on fresh beef inoculated with non-O157 STEC serogroups O26, O45, O103, O111, O121, and O145 compared with *E. coli* O157:H7. The HW followed by the 4% LA were the most effective treatments at reducing the pathogens by 3.2 to 4.2 log CFU/cm^2 and 1.4 to 2.7 log CFU/cm^2 respectively. However, elimination of STEC present on meat

surfaces was not completely achieved. Some of the disadvantages of using HW in the beef industry are the high water volume used and the costs of maintain the water at high temperatures (*78*).

Organic acids - lactic acid

The antimicrobial activity of organic acids is influenced by pH, the dissociation of the acid and the specific effect of the acid molecule (*6*). The antimicrobial effect of LA is due to the pH reduction below the growth range and the metabolic inhibition by the undissociated molecule (*75*). LA is one of the most commonly used antimicrobials in beef harvesting facilities (*34*). Many studies have demonstrated the effectiveness of this organic acid throughout the harvest process from the time the hide is opened to post-chill interventions.

It has been shown that a combination of HW followed by LA spray treatment will reduce fecal contaminants. The temperature of the sprayed organic acid solution can affect the effectiveness of the treatment (*5, 30, 31, 33, 60*). Anderson and Marshall (*6*), demonstrated that an increase in the concentration or temperature in the application of a mixture of organic acids (acetic, lactic, citric and ascorbic acid), increased the reduction of microbial loads on beef tissues.

Bosilevac et al., (*15*) compared the use of 2% LA (42°C), HW washing (74°C, 5.5 s), and the combination of HW followed by 2% LA as pre-evisceration carcass interventions, resulting in reductions of 1.6, 2.7 and 2.2 log CFU/100cm^2 for APC and 1.0, 2.7 and 2.5 log CFU/100cm^2 for *Enterobacteriaceae* count, respectively. The prevalence

of *E. coli* O157:H7 was reduced 35% by LA wash, 81% by HW wash and 79% by the combined treatment. The researchers concluded that the combination of treatments did not achieve the expected reduction due to the low temperature of the LA sprayed on the carcasses after the HW wash (*15*). The temperature of the LA was significantly cooler than the carcass surface and thus may have accelerated the carcass surface cooling, reducing the effectiveness of the organic acid.

Immersion in 2% LA at 55° for 30s reduced *E. coli* O157:H7 populations on inoculated beef carcass tissues and lean tissue pieces by 3.3 log CFU/cm^2 and 1.3 CFU/cm^2, respectively (*112*). Castillo et al., (*34*) reported a significant bacterial reduction on outside rounds contaminated with *Salmonella* Typhimurium and *E. coli* O157:H7 after a pre-chill wash water treatment alone and wash water followed by 2% LA (55°C for 15s), with an additional reduction if a post-chill acid treatment was applied (4% LA, 55°C for 30s). In this study, the ground beef produced from the outside rounds that received pre-chill and post-chill acid spray treatments had significantly lower counts of both pathogens than the ground beef produced from those that only received a post-chill spray treatment (*34*).

The temperature of the acid solution has a profound effect on the magnitude of the microbial reduction in bacterial counts on carcass surfaces (*6, 34*). Synergistic interactions between organic acids and heat may increase the antimicrobial activity of a determined treatment. In the case of LA and acetic acid (AA) a synergistic effect has been shown in several reports (*5, 6, 30*). This interaction is dependent on the organic acid used, and did not always occur. For example, in the case of formic acid and heat a negative interaction

is observed, leading to lower inhibitions than expected at high temperatures and acid concentrations. This effect could be due to the high volatility of formic acid (*114*).

Peroxyacetic acid

Peroxyacetic acid (PAA) is an oxidizing agent that is commonly used in food processing. The use of a PAA spray at 200 ppm as an antimicrobial treatment was evaluated on beef flanks inoculated with non-O157 STEC serogroups (O26, O45, O103, O111, O121, and O145) and compared with *E. coli* O157:H7 (*77*). The reductions on STEC populations obtained ranged from 0.9 to 1.5 log CFU/cm^2, with no significant differences between the reduction of STEC O157:H7 and non-O157 STECs (*77*).

To evaluate the effect of PAA as a post-chilling intervention, beef carcass surfaces were inoculated with fecal matter or with fecal matter containing rifampicin-resistant STEC O157:H7 (*85*) and subjected to a simulated pre-chill carcass wash with and without 2% LA. After chilling, the carcass pieces were sprayed with 200 ppm PAA (0.85MPa, 43°C, for 15 s). This post-chill application of PAA had no effect on the counts of coliforms and STEC O157:H7 (*85*). When PAA was applied before chilling a 0.7-log CFU/cm^2 reduction on STEC O157:H7 was observed (*85*). These investigators reported that PAA was not an effective post-chilling carcass intervention.

Ransom et al., (*112*) obtained greater STEC O157:H7 reductions (1.4 log CFU/cm^2 and 1.0 log CFU/cm^2 on inoculated beef carcass tissues and lean tissue pieces, respectively) using PAA than reported by King et al., (*85*) (0.7 log CFU/cm^2). However, the application of the treatment between the two studies was different. King et al., (*85*)

used a PAA spray treatment (200 ppm, 15 s) while Ransom et al., (*112*) immersed the inoculated samples in 500 mL of 0.02% PAA, allowing for a longer and more uniform application of the treatment.

Ellebracht et al., (*47*) reported that PAA is effective in reducing populations of rifampicin-resistant *E. coli* O157:H7 on beef trim by approximately 1.0 log CFU/cm^2. When comparing the effectiveness of PAA and LA at reducing *E. coli* O157:H7 on fresh beef trimmings, the authors reported a reduction in *E. coli* O157:H7 of 0.7 log CFU/cm^2 due to PAA, whereas LA caused a reduction of 1.3 log CFU/cm^2.

Other antimicrobial interventions

Bromine compounds

The use of bromine compounds, such as 1,3-Dibromo-5,5-dimethylhydantoin (DBDMH), have been approved for decontamination of beef carcasses and variety meats (*127*). The hypobromus acid derived from this compound in aqueous solution is a strong oxidant, highly reactive with biomolecules (*29*).

Carr et al., (*29*) evaluated the reactivity of hypochlorous (HOCl) and hypobromous (HOBr) acids on *E. coli* purified phospholipid. When HOCl interact with *E. coli* phosphatidyleyhanolamine (PE) chloramines are predominantly produced, whereas when PE interact with HOBr, bromamines and bromo-hydrins are produced. HOBr contributes to disruption of cell membranes at reacting with double bonds of unsaturated fatty acids of the bacterial membrane (*78*).

Different studies using DBMDH as an antimicrobial treatment on beef products reported contradictory results. Sexton (*119*) compared the effectiveness of 650 ppm DBMDH, 3.5% LA, and water spray treatments (37°C) on the reduction of STEC (a cocktail containing the serotypes O26, O45, O103, O111, O121, O145, O157:H7) on inoculated cheek and head meat (7 log CFU/cm^2). LA significantly reduced STECs counts on head meat by 0.87 log CFU/cm^2; however, the reduction on cheek meat was not significant and neither the DBMDH nor the water treatment produced significant reductions on either head or cheek meat.

Kalchayanand et al., (*78*) exposed *cutaneous trunci* (CT) muscle sections and beef hearts inoculated with a cocktail mixture of pathogens (8 log CFU/cm^2), including *E. coli* O157:H7 (6 log CFU/cm^2), to different concentrations (75, 175 and 270 ppm) of DBDMH spray (25°C for 12 s CT and 28 s for hearts), in order to evaluate the use of this antimicrobial for washing carcasses and variety meats. DBDMH treatments on CT reduced *E. coli* O157:H7 in a range between a 1.6 and 1.8 log CFU/cm^2, while on hearts it caused a reduction ranging between 1.7 and 2.1 log CFU/cm^2 (*78*).

Colicins E1 (ColE1)

Colicins are antimicrobial proteins produced by certain *E. coli* strains and other members of *Enterobacteriaceae* that are able to kill or inhibit the growth of other *E. coli* strains (*26, 89*). Colicin V kills target cells by membrane potential disruption and it has been shown to be effective in killing *E. coli* O157:H7 in ground beef at 4°C (*2*).

The bacteriocin ColE1 was studied by Patton et al., (*107*) as a potential intervention strategy for controlling *E. coli* O157:H7 contamination on beef carcasses. The ability of sprayed ColE1 treatments to reduce STEC O157:H7 on inoculated beef round roast samples was evaluated at different concentrations (0, 100, 500, and 1000 µg/mL). Samples treated with 500 and 1000 µg/mL reduced *E. coli* O157:H7 by 5 log CFU/cm^2 or more. However, after 4 days, growth of the pathogen was observed, suggesting the ability of the pathogen to harbor resistance against ColE1 (*107*). Further studies are needed in order identify optimum conditions for the use of this compound as an antimicrobial against *E. coli* O157:H7.

Variety meat interventions

Few studies focus on variety meats interventions. Delmore et al., (*44*) evaluated treatments commonly used for decontaminating carcasses and tested their potential use on beef variety meats. They applied different treatments (2% AA at 50°C, 2% LA at 50°C, chlorine 0.005% at 50°C and HW at 80°C) using two application methods (spraying or immersion) on cheek meat, large intestine, lips, liver, oxtail, and tongue meat. LA spray and immersion, AA spray and immersion, and HW immersion, were effective in reducing APCs by 0.7 log CFU/g or more in five of the six of the variety meats studied.

Pokharel et al., (*109*) reported that a 4.5% LA spray treatment was effective in reducing indicator bacteria (APC, total coliforms and generic *E. coli*) on variety meats. Generic *E. coli* was reduced by 0.15, 0.41, and 0.5 log on head, heart and liver samples, respectively, while APC and coliform counts were reduced by 1.96 log and 0.41 log in

beef head samples, by 1.3 log and 1.06 log in heart samples and by 1.08 log and 1.16 log in liver samples, respectively. The efficacy of 4.55% LA and a 2.5 % blend of LA/AA (50/50) in reducing STEC (O157:H7, O26, O111, O45, O145, O103, O121) and *Salmonella* on offal meats was evaluated at three different temperatures, including: cool (21.11°C), warm (37.78°C), and hot (54.44°C) by Parks et al., (*105*). the investigators found that hot 4.55% LA caused the greatest reductions for both pathogens compared with the other treatments.

Kalchayanand et al., (*79*) evaluated the effectiveness of different antimicrobial interventions in the treatment of head and cheek meat inoculated with *E. coli* O157:H7 using a beef head wash cabinet. The antimicrobial treatments evaluated in the study included HW (74°C, 10 psi for 26 s), 2% LA (25°C, 25 psi for 26 s), and freshFx (FF) (25°C, 25 psi for 26 s), Electrolyzed oxidizing water (EO) applied in the acidic form (EO-I) (25°C, 13 s, 25 psi) and alkaline form (EO-II) (13 s, 25 psi, 25°C) and ozonated water (OZ) applied at 25°C for 26 s at 25 psi. The researchers reported reductions of 1.72, 1.52, and 1.06 log CFU/cm^2 for HW, LA and FF, respectively, while EO and OZ reduced *E. coli* O157:H7 less than 0.50 log CFU/cm^2.

Delmore et al., (*44*) evaluated different decontamination treatments on beef variety meats, including 2% AA (50°C, pH 2.8) immersion and spray, 0.005% sodium hypochlorite immersion (50°C, pH 6.5), HW immersion (80°C) and spray (78 to 80°C), 2% LA immersion (50°C, pH 2.8) and spray (40 to 50°C), steam pasteurization system (SPS, 76.6°C), steam cabinet (SC, 82.2°C for 15 and 30 s), steam vacuum (82°C), and 12% trisodium phosphate (50°C, pH 12.5) immersion and spray. The authors reported that

were no differences among treatments evaluated for the ability to reduce *E. coli* counts on cheek meat or tongue samples, with reductions ranging between -0.1 and 0.3 log CFU/g. The authors attributed these results to a low initial contamination level (<0.7 log CFU/g) on tongue meat and a possible protection of the microorganisms to exposure by the treatments due to the physical structure of cheek meat, which contains large amounts of connective tissue (*44*, *67*).

Beef cheek meat inoculated with O157:H7 STEC and non-O157:H7 STEC was immersed in seven antimicrobial solutions, including HW (80°C for 10 s), 1% Aftec 300, 2.5% Beefxide (BX), 2.5% LA, 5% LA, 200 ppm PAA, HOBr and 0.5% levulinic acid and 0.05% sodium dodecyl sulfate (LEV-SDS). These interventions were applied for 1, 2.5, or 5 min, in order to evaluate bacterial reductions due to the activity of the antimicrobials and compare obtained results to immersion in HW (*117*). HW immersion was the most effective intervention, reducing the STEC by 2.2 log CFU/cm^2 on adipose tissue and by 1.7 log CFU/cm^2 on the muscle surface. Inoculated cheek meat immersed for 1 min in Aftec 300, BX, 2.5% LA, 5% LA, and PAA reduced the pathogens ranging from 1.0 to 2.0 log CFU/cm^2 on the adipose surface and ranging from 0.7 to 1.4 log CFU/cm^2 on the muscle surface. In general, increasing the duration of immersion did not significantly increase the antimicrobial effect of each treatment (*117*). The bacterial reductions results obtained after 1 min immersion in HOBr and LEV-SDS did not significantly differ from the control treatment, suggesting that these antimicrobials in the conditions employed in the study are not effective as an intervention in cheek beef treatment (*117*).

CHAPTER III

MATERIALS AND METHODS

Experimental design

The first phase of the study consisted of treating hot beef heads in 3 replications of 4 different antimicrobial treatments at 2 different concentrations and dipping times (5- and 15-min) and included 2 non-treated inoculated controls per replication. Hot beef heads were obtained from the production line of a Tyson beef harvesting facility in Dakota Dunes, SD.

Each replication consisted of 20 beef heads surface inoculated with nonpathogenic STEC surrogates. Following a 30-min inoculum attachment period, each head was assigned to one of the following treatments for 5 or 15 min: control (no antimicrobial treatment), water wash, LA (1% or 2%), DBDMH (200 ppm or 400 ppm), aPAA (200 ppm or 400 ppm), or PAA (200 ppm or 400 ppm).

For the second phase of the study, the two antimicrobial treatments that provided better bacterial reductions in the first phase were selected and compared again in two additional replications. Each replication consisted of 8 (4 different treatments and 2 application times for each) beef heads surface inoculated with nonpathogenic STEC surrogates. Following a 30-min inoculum attachment period, two heads were assigned to one of the following treatments: control (no antimicrobial treatment), water wash, 2% LA, and 400 ppm PAA, for each treatment two different dipping times (5 and 15 min) were evaluated. For the analysis of the respective treatments on the second part, the data

analyzed included that obtained in both phases of the experiment for the respective antimicrobial treatments.

Inoculum preparation and meat inoculation

The inoculum was prepared from stock cultures obtained from the Kansas State University Food Safety and Security Laboratory (Manhattan, KS). It consisted of a mixed 5-strain cocktail solution (10^8 CFU/ml) of non-pathogenic *E. coli* Biotype I (Non-RifR) strains (STEC surrogates), containing strains of the American Type Culture Collection (ATCC), *E. coli* (Migula) Castellani and Chalmers ATCC® BAA-1427™, ATCC® BAA-1428™, ATCC® BAA-1429™, ATCC® BAA-1430™, and ATCC® BAA-1431™. Previous research (*25*, *52*, *124*) has validated that these microorganisms demonstrate similar thermal and acid resistance properties to STEC O157:H7. These marker microorganisms were combined into a cocktail to represent possible contamination with enteric pathogens of fecal origin such as STEC.

Each *E. coli* strain was grown in 40 ml of sterile brain-hearth infusion broth (BHI; Difco Laboratories, Detroit, MI) and incubated at 37°C for 24 h. Equal volumes of each strain were mixed into 250 ml sterile centrifuge bottles to prepare an inoculum mixture containing approximately 10^8 CFU/ml. Concentrated pellets of the microorganism were obtained by centrifugation at 6000 rpm for 15 min at 4°C using a Beckman J2-21M/E centrifuge (Beckman Coulter, Inc., Indianapolis, IN).

Each respective day of the study a concentrated cocktail pellet was resuspended in 200 ml of 0.1 % buffered peptone water (BPW) and diluted to a final volume of 2000 ml

using 0.1% BPW in order to achieve a concentration of approximately 10^5 CFU/g on the inoculated heads. For the beef head inoculation, 3 ml of the nonpathogenic STEC surrogate cocktail was sprayed within the internal oral cavity using a refillable disposable spray bottle and 50 ml of the inoculum was painted on the external head tissues using a foam paint brush (SeaChoice, Pompano Beach, FL). The inoculated heads were left undisturbed for 30 min at room temperature to allow for inoculum attachment before proceeding with the assigned antimicrobial treatments.

To conduct this investigation, normal harvesting procedures were followed. Hot heads were separated from the production line to a place in the plant where no other production was in process. This to ensure that sanitary conditions were maintained, products were controlled, and there was no potential for cross-contamination with non-experimental products.

Initial bacterial counts

After the 30-min attachment period, two heads were used as controls and were immediately deboned to obtain head and cheek meat. Subsamples of 100-120 g representing both head and cheek meat were collected to determine initial inoculation levels for all treatments. The subsamples were placed in sterile Whirl-Pak® bags containing 225 ml of Dey-Engley (D/E) neutralizing broth before transportation to the laboratory for analysis.

Antimicrobial treatments

Antimicrobial solutions tested on the first phase of the study included water wash, LA (1% and 2%) PAA (200 ppm and 400 ppm), aPAA (200 ppm and 400 ppm), and DBDMH (200 ppm and 400 ppm). For the second phase of the study the antimicrobial solutions tested included water wash, 2% LA and 400 ppm PAA. Treatment solutions were prepared in clean 55-gal drums according to manufacturer`s recommendations and chilled to 3°C (38°F) using ice. Solution concentration, temperature and pH was measured before and after each treatment. Corporate representatives of Zoetis Services LLC (Parsippany, NJ) and Passport™ Food Safety Solutions (West Des Moines, IA) prepared and verified the concentration of aPAA and DBDMH solutions, respectively, during the first phase of the study.

After a 30-min attachment period, heads were treated with their assigned antimicrobial. The inoculated head was dipped into the drum containing the assigned solution and was manually agitated for 5 or 15 min as determined by the treatment. Heads were removed from the drum and hung with steel hooks to drip for 5 min. Each head was visually observed following application of each decontaminating treatment to document any changes in product color or appearance. Heads were deboned after the 5-min drip time and head and cheek meat samples of 100-120 g were collected to determine bacterial levels after treatment. The subsamples were placed in sterile Whirl-Pak® bags containing 225 ml of D/E neutralizing broth in order to neutralize the effects of the antimicrobial treatments.

In the second phase of the study, a 50-ml sample of the assigned dipping solution was collected after each head treatment, in order to evaluate the presence of microorganisms in the dipping solution. Each 50-ml sample was mixed with 100 ml D/E neutralizing broth and kept chilled on ice until transportation to the laboratory for analysis.

Post treatment bacterial counts

Homogenate samples were processed using a stomacher. Serial dilutions of each subsample were made using 0.1% buffered peptone water (BPW), and these were plated on 3M™ Petrifilm™ *E. coli*/Coliform count plates for enumeration.

Statistical analysis

Data was analyzed for significant differences between carcass treatments. The bacterial counts were transformed into log CFU/g values prior to statistical analyses. All data were analyzed using JMP® Software (JMP Pro, v14.0.0, SAS Institute Inc., Cary, NC). The Fit Model function was used for analysis of variance (ANOVA), determining interactions from the full model. Least squares mean comparisons were performed using Student's t tests with an $\alpha = 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

First phase

Four replicates of the different 18 treatments where performed in the first phase to evaluate STEC reduction; however, only the last 2 replicates were used during the analysis and evaluation of the different treatments on the first phase. The first two replicates did not have reliable results due to the use of water containing an unknown concentration of DBDMH to prepare the antimicrobial solutions. As the concentration of DBDMH was not being monitored, it is not possible to assume a constant concentration of the compound in the water, and the results obtained in the replicates 1 and 2 could be biased. For these reasons, it was decided to eliminate replicates 1 and 2 from the study, and only include the results from replicates 3 and 4 from phase one.

Bacterial attachment

The desired STEC inoculation level on the heads after attachment was approximately 10^4 CFU/g, as illustrated in Figure 1. The concentration of the STEC cocktail was 6.7 ± 0.1 log CFU/ml, while the control mean counts were 3.9 ± 0.02 log CFU/g and 3.6± 0.4 log CFU/ g on head and cheek meat, respectively.

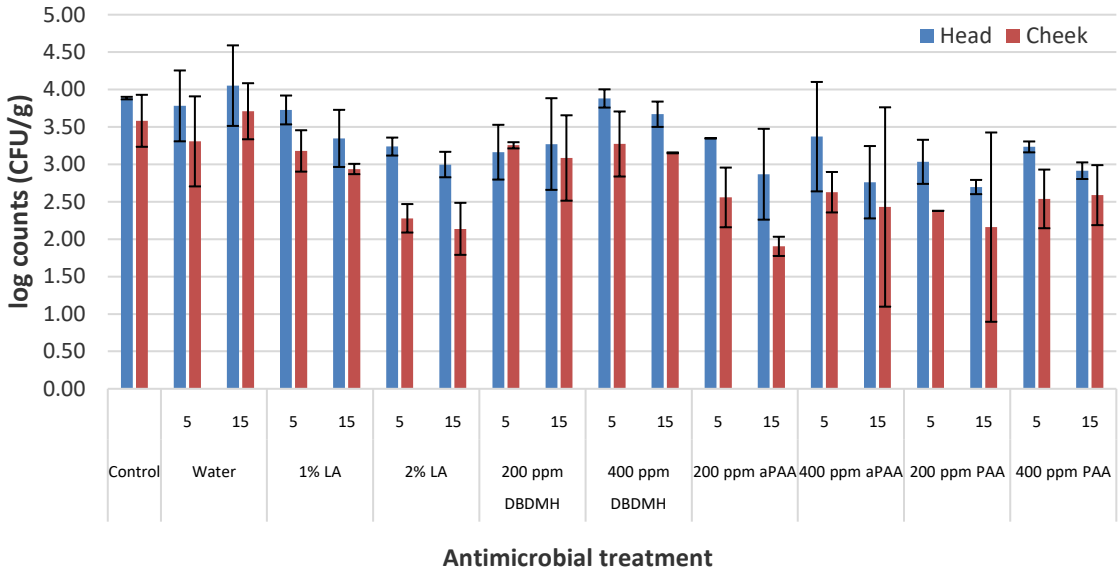

Figure 1. Mean log counts (CFU/g) of cheek and head meat treated with different antimicrobials.

Surrogate reductions

Reductions of the STEC surrogate microorganisms from the 5- and 15-min post-intervention sampling interval for LA, PAA, aPAA, DBDMH were similar for head and cheek meat beef products (Table 1), demonstrating slightly higher reductions on cheek meat. More favorable reductions where obtained with the 15-min dipping time using 200 ppm aPAA, 2% LA and 200 ppm PAA on cheek meat (1.7, 1.5 and 1.4 log CFU/g respectively), and 200 ppm PAA on head meat (Table 1). The bacterial log counts (CFU/g) after treatment are shown in figure 1.

The mean log reductions recorded after the respective 5- or 15-min DBDMH (200 ppm and 400 ppm) treatments ranged between 0.1 and 0.7 log CFU/g in both head and cheek meat are comparable to those obtained with water treatment alone (0.0 to 0.3 log

CFU/g). This results agrees with Sexton (*119*) who study the effect of a DBMDH 650 ppm (38°C) spray treatment on the decontamination of inoculated head and cheek meat, and found that the treatment was not able to significantly reduce STEC or *Salmonella* counts on either cheek meat or head meat.

This study results also agree with Schmidt et al. (*117*) who studied the effect of immersion in different antimicrobials on cheek meat. They tested a concentration of 300 ppm HOBr, which is the end product of DBMDH hydrolyzed in water. In Schmidt et al., beef cheeks were inoculated with a cocktail containing STECs and *Salmonella* at a concentration of 7.7 log CFU/cm², and treated by dipping into the treatment solution at intervals of 1, 2.5, and 5 min. It was determined that while HOBr produced a 0.1- to 0.4-log CFU/cm² reduction, this reduction was not significantly different ($P < 0.05$) from reductions obtained when the cheek meat was immersed in room temperature water (*117*).

Kalchayanand et al., (*78*) reported greater reductions (1.8 to 2.2 log CFU/cm^2) on inoculated heart and *cutaneous trunci* surfaces after applying a spray of DBDMH (270 ppm, 25°C, 35 psi) and HW (85°C, 20 psi) on inoculated fresh meat. The observed reduction may be due the temperature and method employed to apply the antimicrobial treatment. However, the results of the present study agrees with those of Kalchayanand et al. in the sense that the DBMDH treatment was not effective in reducing pathogen counts since the treatment was not significantly different than the water treatment (*78*).

TABLE 1. Least square means of the interaction treatment x time on STEC reductions (log CFU/g) on inoculated beef head and cheek meat.

Antimicrobial treatment	Reduction post treatment (mean log_{10} CFU/g)	
	Head	Cheek
5 min		
Water	0.1	0.3
1% LA	0.2	0.4
2% LA	0.6	1.3
200 ppm PAA	0.9	1.2
400 ppm PAA	0.7	1.0
200 ppm aPAA	0.5	1.0
400 ppm aPAA	0.5	1.0
200 ppm DBDMH	0.7	0.3
400 ppm DBDMH	0.0	0.3
15 min		
Water	-0.2	-0.1
1% LA	0.5	0.6
2% LA	0.9	1.5
200 ppm PAA	1.2	1.4
400 ppm PAA	1.0	1.0
200 ppm aPAA	1.0	1.7
400 ppm aPAA	1.1	1.2
200 ppm DBDMH	0.6	0.5
400 ppm DBDMH	0.2	0.4
SEM[2]	0.140	0.315
p-value	0.052	0.166

[1]SEM is the standard error of the least square means.

Appearance of beef heads after treatment

Color changes were observed on beef heads treated with LA, PAA and aPAA after 5 and 15 min, where a browning effect was observed on heads after the respective treatment (Figures 3 to 5) This discoloration was not observed on the heads immersed on

DBDMH 5- and 15-min treatment, where the observed color was similar to that seen with water treatment for the respective dipping times. (Figures 2 and 6).

Several researchers have studied the effect of organic acids on sensory properties, primarily meat color. It has been reported that potential discoloration of carcasses might occur when high acid concentrations and/or longer exposure time is used in decontamination treatments (*45, 90, 138*). Woolthuis and Smulders (*138*) evaluated LA in concentrations of 0.75% to 2.5% on calf carcasses and determined that concentrations up to 1.25% LA resulted in substantial reductions in total aerobic counts with minimal carcass discoloration. They reported that for LA concentrations up to 2%, discoloration was restricted to the subcutaneous fat, but at 2.50% there was denaturation of the lean and cut surfaces (*138*).

Bell et al., (*10*) reported that treating beef with 1.2% v/v AA for 1 min did not result in discoloration; however, a solution of 0.6% LA for 10 min resulted in significant discoloration when compared with untreated controls. In contrast, Goodard et al., (*57*) reported that meat color of acid-sprayed strip loins was not affected by a treatment of a mixture of 50:50 2% LA and 2% AA.

Figure 2. Control beef heads inoculated with STEC cocktail (A,B) and dipped on cold water (3°C) for 5 min (C) and 15 min (D).

Figure 3. Beef heads inoculated with STEC cocktail and dipped on cold (3°C) 1% LA for 5 min (A) and 15 min (B) and 2% LA for 5 min (C) and 15 min (D)

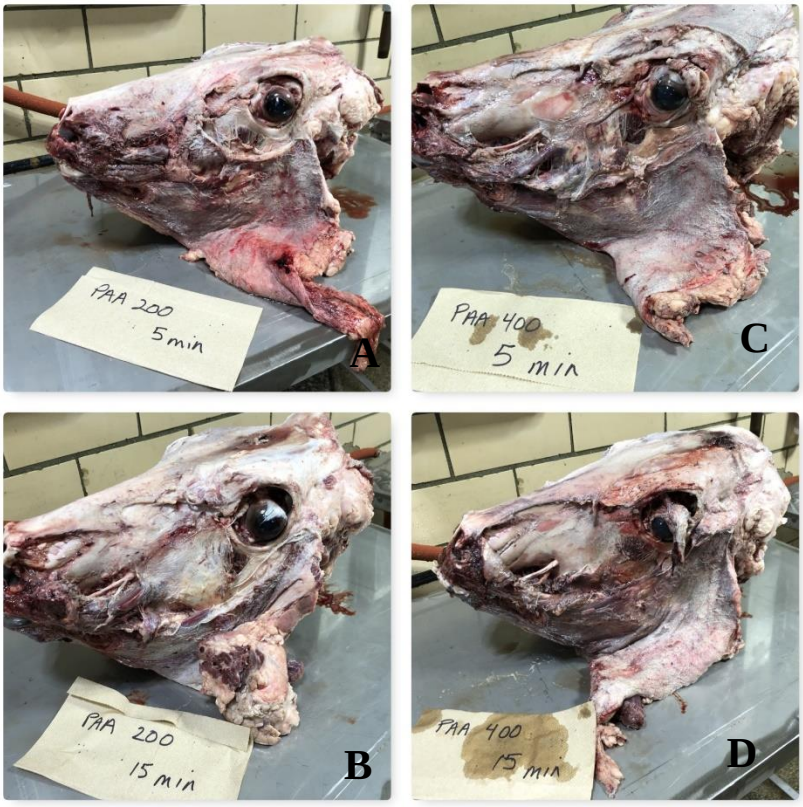

Figure 4. Beef heads inoculated with STEC cocktail and dipped on cold (3°C) 200 ppm PAA for 5 min (A) and 15 min (B) and 400 ppm PAA for 5 min (C) and 15 min (D)

Figure 5. Beef heads inoculated with STEC cocktail and dipped on cold (3°C) 200 ppm aPAA for 5 min (A) and 15 min (B) and 400 ppm aPAA for 5 min (C) and 15 min (D)

Figure 6. Beef heads inoculated with STEC cocktail and dipped on cold (3°C) 200 ppm DBDMH for 5 min (A) and 15 min (B) and 400 ppm DBDMH for 5 min (C) and 15 min (D)

Second phase

Bacterial attachment

The desired STEC inoculation level on the heads after attachment was approximately 10^4 CFU/g as illustrated in Figure 7. The concentration of the STEC cocktail was 7.1 ± 0.5 log CFU/ml, while the control counts means were 4.2 ± 0.3 log CFU/g and 3.8± 0.4 CFU/ g on head and cheek meat, respectively.

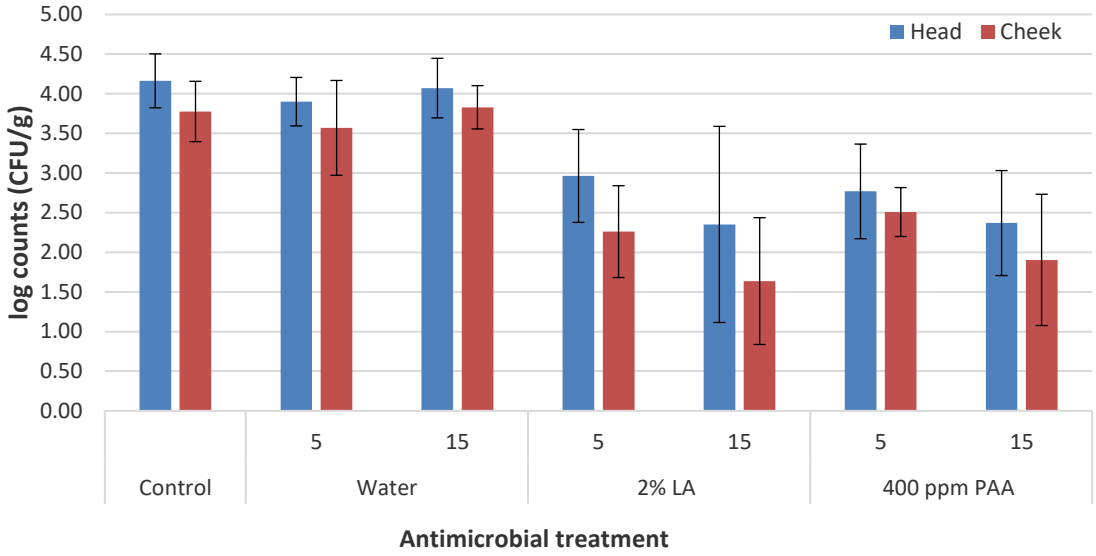

Figure 7. Mean log counts (CFU/g) of cheek and head meat treated with different antimicrobials.

Surrogate reductions

The two antimicrobials that were used in the second phase of the study were chosen according to their common use in industry (*21*, *86*, *112*, *120*, *132*). No difference was observed among the bacterial counts obtained after treating the inoculated head and cheek meat with the different antimicrobial treatments in the first phase of the study (Figure 1). Therefore, 2% LA and 400 ppm PAA, commonly used antimicrobials in beef harvesting facilities, were chosen to be compared in the second phase of the study.

2% LA and 400 ppm PAA provided significantly higher bacterial reductions ($p < 0.05$) than water dipping alone on cheek meat 5- and 15-min treatment and on 15-min head treatment (Table 2). The effect of time did not manifest itself as having a significant

role in the comparison (p >0.05), and the difference observed relied primarily on the antimicrobial treatment employed (p <0.05). This agrees with Schmidt et al., (*117*) who found that, in general, increasing the immersion duration in antimicrobial solutions did not significantly increase the effectiveness of the treatment.

TABLE 2. Least square means of the interaction treatment x time on STEC reductions (log CFU/g) on inoculated beef head and cheek meat.

Antimicrobial treatment	**Reduction post treatment (mean log_{10} CFU/g)**	
	Head[1]	Cheek[1]
5 min		
Water	0.3^{a}	0.2^{A}
2% LA	1.2^{ab}	1.5^{B}
400 ppm PAA	1.4^{ab}	1.3^{B}
15 min		
Water	0.1^{a}	-0.1^{A}
2% LA	1.8^{b}	2.1^{B}
400 ppm PAA	1.8^{b}	1.9^{B}
SEM[2]	0.825	0.498
p-value	0.054	0.001

[1]Numbers within a column with different letters differ (*P < 0.05*).
[2]SEM is the standard error of the least square means.

The effectiveness of water as a decontamination treatment is determined by the temperature, pressure and time at which the treatment is applied; therefore, increasing these factors correlate with an enhancement in the bacterial reduction (*32*). In the present study, the water dipping treatment was applied at a cold temperature 3°C (38°F), and no significant reductions were observed. The counts obtained after the respective 5- or 15-min water treatment were similar to the control (Figure 2), with mean reductions ranging between 0.1 and 0.3 log CFU/g.

In previous research, Ellebracht et al., (*47*) evaluated PAA as a decontamination method applied to beef trimmings. PAA reduced rifampicin-resistant *E. coli* O157:H7 on beef trim by approximately 1.0 log CFU/cm2. In this study the use of 400 ppm PAA significantly ($P < 0.05$) reduced STEC counts on head and cheek meat, with no significant difference reported between the two types of meat.

Immersion in either 2% LA or 400 ppm PAA effectively reduced STEC surrogate counts on head and cheek meat. Mean reductions between 1.2 and 2.1 log CFU/g were obtained with these treatments in the present study. Similar results were reported by Schmidt et al., (*117*) who found that immersion in LA (2.5%, and 5% for 5 min) resulted in significant reductions ($P < 0.05$) of STEC O157:H7 and non-O157:H7 on cheek meat, ranging from 1.3 to 2.1 log CFU/cm^2 . In the same study significant reductions ($P < 0.05$) ranging from 1.0 to 1.3 log CFU/cm^2 were obtained after a 220 ppm PAA, 5-min treatment.

LA is one of the most widely studied organic acids currently used in the beef industry. The effects of the use of LA differ among published studies, but generally suggest the achievement of a 1.0- to 2.0-log reduction, and its effect is enhanced by an increase in the application temperature (*33*, *34*, *44*, *112*). In this study the use of 2% LA significantly ($P < 0.05$) reduced STEC counts on both head and cheek meat, with a slightly higher reduction of the total bacterial populations observed on the cheek meat.

The observed results in the present study correlated with those found in the literature. Sexton (*119*) found LA (3.5%, 21°C) to be effective in reducing STEC counts on head meat by 0.87 log CFU/cm^2 ($P < 0.05$). Kalchayanand et al., (*79*) reported a 1.5-

log CFU/cm² reduction on bovine head inoculated with *E. coli* O157:H7 using 2% LA sprayed at 25 psi at 25 °C. Ramson et al., (*112*) obtained a reduction of 3.1 log CFU/cm^2 when using a 2% LA (55°C) dipping treatment on beef carcass tissue inoculated with *E. coli* O157:H7, while the reductions obtained using water and PPA dipping treatments were 0.6 and 1.4 log CFU/cm^2, respectively.

In general, higher counts were observed on head meat compared with cheek meat after the respective antimicrobial interventions (Figure 2). One theory for this difference would be that the antimicrobial treatments can access crevices of cheek meat, due to the greater exposed surface of the cheek tissue compared with head meat, and this may facilitate the contact between the antimicrobial treatment and the meat tissue. In contrast to the results seen in the present study, Schmidt et al., (*117*) did not observe a significant difference between the reductions obtained with different antimicrobial treatments, including 2.5% LA, 5% LA, 2.5% Beefxide, 1% Aftec, 220 ppm PAA, 0.5% LEV-SDS and tap water, on adipose and muscle cheek surfaces when comparing them.

Solutions after treatment

Samples (50 ml) of the different antimicrobial treatments were collected after each dipping treatment in order to evaluate the presence of surviving bacteria in the head dipping solutions. The results are presented in Table 3. The D/E neutralizing broth was unable to buffer the 50-ml samples of LA, and this was observed by a change in color of the broth from purple to yellow. In this case, if there were surviving bacteria present in the solution, the acid likely continued to affect survival for a longer period prior to analysis.

To evaluate this possibility, an additional sample consisting of 5 ml of 2% LA after the head treatment was added to 100 ml D/E broth, and this dilution revealed a bacterial count of 1.9 log CFU/ml. The presence of residual bacteria in the head dipping solution is of significance, as it could serve as a point of cross-contamination between heads during treatment. It is possible that a counter-flow treatment system similar to that used in poultry carcass chill tanks might alleviate potential cross-contamination issues. Further studies are recommended to assess the use of a recirculated antimicrobial treatment as well as the disposition and flow of the treatment.

TABLE 3. Bacterial counts on the corresponding washing solutions after the determined treatment.

Antimicrobial treatment		***Count on washing solution post treatment (mean log10 CFU/ml)***
5 min	**Water**	3.3
15 min	**Water**	3.4
5 min	**2% LA**	<0.0[a]
15 min	**2% LA**	<0.0[a]
5 min	**400 ppm PAA**	<0.0[a]
15 min	**400 ppm PAA**	<0.0[a]

[a] Plate count was below the detection limit of the method used (3 CFU/ml).

CHAPTER V

CONCLUSIONS

Results from this study showed that dipping treatments including lactic acid, and peroxyacetic acid are capable of significantly reducing surface bacteria on fresh head and check meat. Heavy bacterial contamination on the heads may inoculate the antimicrobial solution, which might allow cross-contamination of head and cheek meat during treatment; however, it is unlikely that naturally occurring contamination levels would resemble those used for evaluation in this study. Further investigations are recommended in order to validate an adequate system to be employed within beef processing facilities to provide adequate decontamination of heads while preventing cross-contamination.

REFERENCES

1. Abd-El-Malek, A. M., and T. El-Khateib. 2018. Microbiological evaluation of some edible bovine by-products. *Int. J. Curr. Microbiol. Appl. Sci.* 7:3449–3458.
2. Abercrombie, J. G., M. J. B. Paynter, and S. S. Hayasaka. 2006. Ability of colicin V to control Escherichia coli in ground beef. *J. Food Saf.* 26:103–114.
3. Aberle, E. D., J. C. Forrest, D. E. Gerrard, and E. W. Mills. 2012. Principles of Meat Science, 5th ed. Kendall Hunt Publishing Company, Dubuque. Iowa.
4. Adhikari, B., F. Angulo, and M. Meltzer. 2004. Economic burden of Salmonella infections in the United States. Centers for Disease Control and Prevention. American Agricultural Economics Association Annual Meeting, Denver, Colorado.
5. Anderson, M. E., and R. T. Marshall. 1989. Interaction of concentration and temperature of acetic acid solution on reduction of various species of microorganisms on beef surfaces. *J. Food Prot.* 52:312–315.
6. Anderson, M. E., and R. T. Marshall. 1990. Reducing microbial populations on beef tissues: concentration and temperature of an acid mixture. *J. Food Saf.* 10:181–190.
7. Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2007. Effects of a minimal hide wash cabinet on the levels and prevalence of Escherichia coli O157:H7 and Salmonella on the hides of beef cattle at slaughter. *J. Food Prot.* 70:1076–1079.
8. Asakura, H., E. Saito, Y. Momose, T. Ekawa, M. Sawada, A. Yamamoto, A. Hasegawa, J. Iwahori, T. Tsutsui, K. Osaka, T. Matsushita, M. Kakinuma, K.

Motoyama, Y. Hayama, H. Kitamoto, S. Igimi, and F. Kasuga. 2012. Prevalence and growth kinetics of Shiga toxin-producing Escherichia coli (STEC) in bovine offal products in Japan. *Epidemiol. Infect.* 140:655–664.

9. Batz, M. B., S. Hoffmann, and J. G. Morris. 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. *J. Food Prot.* 75:1278–1291.

10. Bell, M. F., R. T. Marshal, and M. E. Anderson. 1986. Microbiological and sensory tests of beef treated with acetic and formic acids. *J. Food Prot.* 49:207–210.

11. Blanco, J. E., M. Blanco, M. P. Alonso, A. Mora, G. Dahbi, M. A. Coira, and J. Blanco. 2004. Serotypes , virulence genes , and intimin types of Shiga toxin (Verotoxin)-Producing Escherichia coli isolates from cattle in Spain and identification of a new intimin variant gene (eae-ξ). *J. Clin. Microbiol.* 42:311–319.

12. Boerlin, P., S. A. McEwen, F. Boerlin-Petzold, J. B. Wilson, R. P. Johnson, and C. L. Gyles. 1999. Associations between virulence factors of Shiga toxin-producing Escherichia coli and disease in humans. *J. Clin. Microbiol.* 37:497–503.

13. Bosilevac, J. M., R. Wang, B. E. Luedtke, S. Hinkley, T. L. Wheeler, and M. Koohmaraie. 2017. Characterization of enterohemorrhagic Escherichia coli on veal hides and carcasses. *J. Food Prot.* 80:136–145.

14. Bosilevac, J. M., and M. Koohmaraie. 2011. Prevalence and characterization of non-O157 Shiga toxin-producing Escherichia coli isolates from commercial ground beef in the United States. *Appl. Environ. Microbiol.* 77:2103–2112.

15. Bosilevac, J. M., X. Nou, G. A. Barkocy-Gallagher, T. M. Arthur, and M.

Koohmaraie. 2006. Treatments using hot water instead of lactic acid reduce levels of aerobic bacteria and Enterobacteriaceae and reduce the prevalence of Escherichia coli O157:H7 on preevisceration beef carcasses. *J. Food Prot.* 69:1808–1813.

16. Brashears, M. M., and B. D. Chaves. 2017. The diversity of beef safety: A global reason to strengthen our current systems. *Meat Sci.* 132:59–71.

17. Brooks, J. T., E. G. Sowers, J. G. Wells, K. D. Greene, P. M. Griffin, R. M. Hoekstra, and N. A. Strockbine. 2005. Non-O157 Shiga toxin-producing Escherichia coli infections in the United States, 1983-2002. *J. Infect. Dis.* 192:1422–1424.

18. Brunder, W., H. Schmidt, M. Frosch, and H. Karch. 1999. The large plasmids of Shiga toxin-producing Escherichia coli (STEC) are highly variable genetic elements. *Microbiology* 145:1005–1014.

19. Bryan, A., I. Youngster, and A. J. McAdam. 2015. Shiga toxin-producing Escherichia coli. *Clin. Lab. Med.* 35:247–272.

20. Buncic, S., J. McKinstry, C. A. Reid, and M. H. Anil. 2002. Spread of microbial contamination associated with penetrative captive bolt stunning of food animals. *Food Control* 13:425–430.

21. Buncic, S., G. J. Nychas, M. R. F. Lee, K. Koutsoumanis, M. Hébraud, M. Desvaux, N. Chorianopoulos, D. Bolton, B. Blagojevic, and D. Antic. 2014. Microbial pathogen control in the beef chain: Recent research advances. *Meat Sci.* Elsevier Ltd 97:288–297.

22. Buzby, J. C., and T. Roberts. 1996. ERS updates U.S. foodborne disease costs for

seven pathogens. *Food Rev.* 19:20–25.

23. Buzby, J. C., and T. Roberts. 1997. Estimated annual costs of Campylobacter-associated Guillain-Barré syndrome. Economic Research Service, U.S. Department of Agriculture. Agricultural Economic Report No. 756. Washington, DC.

24. Buzby, J. C., T. Roberts, C. J. Lin, and J. M. Macdonald. 1996. Bacterial foodborne disease: Medical costs and productivity losses. Economic Research Service, USDA. Agricultural Economic Report No. 741. Washington, DC.

25. Cabrera-Diaz, E., T. M. Moseley, L. M. Lucia, J. S. Dickson, A. Castillo, and G. R. Acuff. 2016. Fluorescent protein–marked Escherichia coli biotype I strains as surrogates for enteric pathogens in validation of beef carcass interventions. *J. Food Prot.* 72:295–303.

26. Callaway, T. R., T. S. Edrington, G. H. Loneragan, M. A. Carr, and D. J. Nisbet. 2013. Current and near-market intervention strategies for reducing Shiga toxin-producing Escherichia coli (STEC) shedding in cattle. *Agric. Food Anal. Bacteriol.* 3:103–120.

27. Caprioli, A., S. Morabito, H. Brugere, and E. Oswald. 2005. Enterohaemorrhagic Escherichia coli: Emerging issues on virulence and modes of transmission. *Vet. Res.* 36:289–311.

28. Carney, E., S. B. O'Brien, J. J. Sheridan, D. A. McDowell, I. S. Blair, and G. Duffy. 2006. Prevalence and level of Escherichia coli O157 on beef trimmings, carcasses and boned head meat at a beef slaughter plant. *Food Microbiol.* 23:52–59.

29. Carr, A. C., J. J. M. Van den Berg, and C. C. Winterbourn. 1998. Differential

reactivities of hypochlorous and hypobromous acids with purified Escherichia coli phospholipid: Formation of haloamines and halohydrins. *Biochim. Biophys. Acta, Lipids Lipid Metab.* 1392:254–264.

30. 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.

31. 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.

32. 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.

33. Castillo, A., L. M. Lucia, I. Mercado, and G. R. Acuff. 2001. In-plant evaluation of a lactic acid treatment for reduction of bacteria on chilled beef carcasses. *J. Food Prot.* 64:738–740.

34. 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.

35. Centers for Disease Control and Prevention. Beef related outbreaks from 1998 to 2016. National Outbreak Reporting System (NORS).

36. Centers for Disease Control and Prevention. Foodborne Diseases Active Surveillance Network.

37. Centers for Disease Control and Prevention. 2018. Multistate outbreak of E. coli O157:H7 infections linked to romaine lettuce.

38. Centers for Disease Control and Prevention. 2014. Multistate outbreak of Shiga toxin-producing Escherichia coli O157:H7 infections linked to ground beef.

39. Centers for Disease Control and Prevention. 2012. National enteric disease surveillance: STEC surveillance overview.

40. Centers for Disease Control and Prevention. 2018. Outbreak of E. coli infections linked to ground beef.

41. Centers for Disease Control and Prevention. 2019. Outbreak of E. coli O103 infections linked to ground beef.

42. Corkran, S. C. 2016. Comparison of processing parameters in small and very small beef processing plants and their impact on Escherichia coli prevalence. Thesis dissertation for the degree of Master of Science. Purdue University, West Lafayette, IN.

43. Croxen, M. A., and B. B. Finlay. 2010. Molecular mechanisms of Escherichia coli pathogenicity. *Nat. Rev. Microbiol.* 8:26–38.

44. Delmore, R. J., J. N. Sofos, G. R. Schmidt, K. E. Belk, W. R. Lloyd, and G. C. Smith. 2000. Interventions to reduce microbiological contamination of beef variety meats. *J. Food Prot.* 63:44–50.

45. Dickson, J. S., M. D. Hardin, and G. R. Acuff. 1997. Organic acid rinses. Proc. Recip. Meat Conf. American Meat Science, Chicago, IL. 49:129-131.

46. Dorsa, W. J., C. N. Cutter, G. R. Siragusa, and M. Koohmaraie. 1995. Microbial

decontamination of beef and sheep carcasses by steam, hot water spray washes, and a steam-vacuum sanitizer. *J. Food Prot.* 59:127–135.

47. Ellebracht, J. W., D. A. King, A. Castillo, L. M. Lucia, G. R. Acuff, K. B. Harris, and J. W. Savell. 2005. Evaluation of peroxyacetic acid as a potential pre-grinding treatment for control of Escherichia coli O157:H7 and Salmonella Typhimurium on beef trimmings. *Meat Sci.* 70:197–203.

48. Etcheverría, A. I., N. L. Padola, M. E. Sanz, R. Polifroni, A. Krüger, J. Passucci, E. M. Rodríguez, A. L. Taraborelli, M. Ballerio, and A. E. Parma. 2010. Occurrence of Shiga toxin-producing E. coli (STEC) on carcasses and retail beef cuts in the marketing chain of beef in Argentina. *Meat Sci.* 86:418–421.

49. Ewing, W. H. 1986. Edwards and Ewing's identification of Enterobacteriaceae, 4th ed. Elsevier, New York.

50. Feng, P., S. Weagant, and K. Jinneman. 2002. Bacteriological Analytical Manual: Diarrheagenic Escherichia coli. United States Department of Agriculture.

51. Fey, P. D., R. S. Wickert, M. E. Rupp, T. J. Safranek, and S. H. Hinrichs. 2000. Prevalence of non-O157:H7 Shiga toxin-producing Escherichia coli in diarrheal stool samples from Nebraska. *Emerg. Infect. Dis.* 6:530–533.

52. Frenzel, M. A. 2017. Evaluation of antimicrobial interventions applied during further processing of raw beef products to reduce pathogen contamination. Thesis dissertation for the degree of Doctor of Philosophy. Texas A&M University, College Station. TX.

53. Frenzen, P. D. 2007. An online cost calculator for estimating the economic cost of

illness due to Shiga toxin-producing E. coli (STEC) O157 infections, EIB-28., United States Department of Agriculture, Economic Research Service.

54. Frenzen, P. D., T. L. Riggs, J. C. Buzby, T. Breuer, T. Roberts, D. Voetsch, and S. Reddy. 1999. Salmonella cost estimate updated using FoodNet data. *Food Rev.* 22:10–15.

55. Friedrich, A., M. Bielaszewska, W. W. Zhang, M. Pulz, T. Kuczius, A. Ammon, and H. Karch. 2002. Escherichia coli harboring Shiga toxin 2 gene variants: Frequency and association with clinical symptoms. *J. Infect. Dis.* 185:74–84.

56. Gill, C. O., J. C. McGinnis, and T. Jones. 1999. Assessment of the microbiological conditions of tails, tongues, and head meats at two beef-packing plants. *J. Food Prot.* 62:674–677.

57. Goddard, B. L., W. B. Mikel, D. E. Conner, and W. R. Jones. 2016. Use of organic acids to improve the chemical, physical, and microbial attributes of beef strip loins stored at -1°C for 112 days. *J. Food Prot.* 59:849–853.

58. Gomes, T., W. Elias, I. Scaletsky, B. Guth, J. Rodrigues, R. Piazza, L. Ferreira, and M. Martinez. 2016. Diarrheagenic Escherichia coli. *Brazilian J. Microbiol.* 47:3–30.

59. Gould, L. H., K. A. Walsh, A. R. Vieira, K. Herman, I. T. Williams, A. J. Hall, and D. Cole. 2013. Surveillance for foodborne disease outbreaks-United States, 1998-2008. *Morb. Mortal. Wkly. Rep.* 62:1–34.

60. Greer, G. G., and B. D. Dilts. 1992. Factors affecting the susceptibility of meatborne pathogens and spoilage bacteria to organic acids. *Food Res. Int.* 25:355–

364.

61. Greig, J. D., and A. Ravel. 2009. Analysis of foodborne outbreak data reported internationally for source attribution. *Int. J. Food Microbiol.* 130:77–87.

62. Guignot, J., C. Chaplais, and A. L. Servin. 2007. The secreted autotransporter toxin, Sat, functions as a virulence factor in Afa/Dr diffusely adhering Escherichia coli by promoting lesions in tight junction of polarized epithelial cells. *Cell. Microbiol.* 9:204–221.

63. Gyles, C. L. 2007. Shiga toxin-producing Escherichia coli: an overview. *J. Anim. Sci.* 85:45–62.

64. Harrington, S. M., E. G. Dudley, and J. P. Nataro. 2005. Pathogenesis of enteroaggregative Escherichia coli infection. *FEMS Microbiol. Lett.* 254:12–18.

65. Harris, K. B., and J. W. Savell. 2016. Best Practices for Beef Slaughter, Beef Industry Food Safety Council. College Station, Texas.

66. Henao, O. L., T. F. Jones, D. J. Vugia, and P. M. Griffin. 2015. Foodborne diseases active surveillance network-2 decades of achievements, 1996-2015. *Emerg. Infect. Dis.* 21:1529–1536.

67. Hoagland, R., and G. Snider. 1926. Nutritive value of the protein in veal and calf sweetbreads; in beef cheek meat, lips, tongues, brains, spleen and tripe; and in hog brains and tongues. *J. Agric. Res.* 32:679–688.

68. Hodges, K., and R. Gill. 2010. Infectious diarrhea: Cellular and molecular mechanisms. *Gut Microbes* 1:4–21.

69. Hoffmann, S., B. Maculloch, and M. Batz. 2015. Economic burden of major

foodborne illnesses acquired in the United States, EIB-140.U.S. Department of Agriculture, Economic Research Service.

70. Hussein, H. S. 2007. Prevalence and pathogenicity of Shiga toxin-producing Escherichia coli in beef cattle and their products. *J. Anim. Sci.* 85:63–72.

71. Im, M. C., K. W. Seo, D. H. Bae, and Y. J. Lee. 2016. Bacterial quality and prevalence of foodborne pathogens in edible offal from slaughterhouses in Korea. *J. Food Prot.* 79:163–168.

72. Institute of Medicine. 1990. Cattle Inspection.Institute of Medicine. The National Academies Press, Washington, DC.

73. Islam, M., M. P. Doyle, S. C. Phatak, P. Millner, and X. Jiang. 2004. Persistance of enterohemorrhagic Escherichia coli O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure compost or irrigation water. *J. Food Prot.* 67:1365–1370.

74. Jacob, M. E., T. R. Callaway, and T. G. Nagaraja. 2009. Dietary interactions and interventions affecting Escherichia coli O157 colonization and shedding in cattle. *Foodborne Pathog. Dis.* 6:785–792.

75. Jay, J. M., M. J. Loessner, and D. A. Golden. 2005. Modern Food Microbiology, 7th ed. Springer Science and Business Media, Inc., New York.

76. Johnson, K. E., C. M. Thorpe, and C. L. Sears. 2006. The emerging clinical importance of non-O157 Shiga toxin-producing Escherichia coli. *Clin. Infect. Dis.* 43:1587–1595.

77. Kalchayanand, N., T. M. Arthur, J. M. Bosilevac, J. W. Schmidt, R. Wang, S. D.

Shackelford, and T. L. Wheeler. 2012. Evaluation of commonly used antimicrobial interventions for fresh beef inoculated with Shiga toxin-producing Escherichia coli serotypes O26, O45, O103, O111, O121, O145, and O157:H7. *J. Food Prot.* 75:1207–1212.

78. Kalchayanand, N., T. M. Arthur, J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2009. Effectiveness of 1,3-dibromo-5,5 dimethylhydanoiin on reduction of Escherichia coli O157:H7 and Salmonella inoculated fresh meat. *J. Food Prot.* 72:151–156.

79. Kalchayanand, N., T. M. Athur, J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, T. L. Wheeler, and M. Koohmaraie. 2008. Evaluation of various antimicrobial interventions for the reduction of Escherichia coli O157:H7 on bovine heads during processing. *J. Food Prot.* 71:621–624.

80. Kaper, J. B., J. P. Nataro, and H. L. T. Mobley. 2004. Pathogenic Escherichia coli. *Nat. Rev. Microbiol.* 2:123–140.

81. Karch, H., M. Bielaszewska, M. Bitzan, and H. Schmidt. 1999. Epidemiology and diagnosis of Shiga toxin-producing Escherichia coli infections. *Diagn. Microbiol. Infect. Dis.* 34:229–243.

82. Kaspar, C., M. E. Doyle, and J. Archer. 2009. White paper on non-O157 Shiga-toxin producing E. coli from meat and non-meat sources. *FRI. Food Saf. Rev. Saf. Rev.* 1–26.

83. Kaur, P., A. Chakraborti, and A. Asea. 2010. Enteroaggregative Escherichia coli : An emerging enteric food borne pathogen. *Interdiscip. Perspect. Infect. Dis.*

2010:1–10.

84. Keen, J. E., W. W. Laegreid, C. G. Chitko-McKown, L. M. Durso, and J. L. Bono. 2010. Distribution of Shiga-toxigenic Escherichia coli O157 in the gastrointestinal tract of naturally O157-shedding cattle at necropsy. *Appl. Environ. Microbiol.* 76:5278–5281.

85. King, D., L. Lucia, G. Acuff, K. B. Harris, and J. W. Savell. 2005. Evaluation of peroxyacetic acid as a post-chilling intervention for control of Escherichia coli O157:H7 and Salmonella Typhimurium on beef carcass surfaces. *Meat Sci.* 69:401–407.

86. Koohmaraie, M., T. M. Arthur, J. M. Bosilevac, M. Guerini, S. D. Shackelford, and T. L. Wheeler. 2005. Post-harvest interventions to reduce/eliminate pathogens in beef. *Meat Sci.* 71:79–91.

87. Koopmans, M., H. Kruse, H. Sprong, F. Scheutz, M. Langelaar, L. Verhoef, E. Duizer, D. G. Newell, J. Threfall, J. van der Giessen, M. Opsteegh, and A. Aidara-Kane. 2010. Food-borne diseases-The challenges of 20 years ago still persist while new ones continue to emerge. *Int. J. Food Microbiol.* 139:S3–S15.

88. Littlefield, R. S. 2005. Jack in the Box: Lessons learned by accepting responsibility, p. 35–48. *In* T.L. Sellnow, and R.S. Littlefield (eds.), Lessons learned about protecting America's food supply. Institute for Regional Studies, North Dakota State University.

89. Liu, S. M., D. M. Miller, and R. F. Roberts. 2011. Cloning of genes encoding colicin E2 in Lactococcus lactis subspecies lactis and evaluation of the colicin-producing

transformants as inhibitors of Escherichia coli O157:H7 during milk fermentation. *J. Dairy Sci.* 94:1146–1154.

90. Loretz, M., R. Stephan, and C. Zweifel. 2011. Antibacterial activity of decontamination treatments for cattle hides and beef carcasses. *Food Control*. Elsevier Ltd 22:347–359.

91. Mackey, B. M., and C. M. Derrick. 1979. Contamination of the deep tissues of carcasses by bacteria present on the slaughter instruments or in the gut. *J. Appl. Bacteriol.* 46:355–366.

92. Mainil, J. 2013. Veterinary immunology and immunopathology Escherichia coli virulence factors. *Vet. Immunol. Immunopathol.* 152:2–12.

93. Marder, E. P., P. M. Griffin, P. R. Cieslak, J. Dunn, H. S. Hurd, R. Jervis, S. Lathrop, A. Muse, P. Ryan, K. Smith, M. Tobin-D'Angelo, D. J. Vugia, K. Holt, B. Wolpert, R. V. Tauxe, and A. Geissler. 2018. Preliminary incidence and trends of infection with pathogens transmitted commonly through food-Foodborne diseases active surveillance network, 10 U.S. sites, 2006-2017. *Morb. Mortal. Wkly. Rep.* 64:324–328.

94. Marti, D. L., R. J. Johnson, and K. H. Mathews. 2011. Where's the (not) meat? Byproducts from beef and pork production. *J. Curr. Issues Glob.* 5:397–423.

95. 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.* 5:840–2.

96. Meng, J., J. Lejeune, T. Zhao, and M. P. Doyle. 2013. Enterohemorragic

Escherichia coli, p. 287–309. *In* M.P. Doyle, and R. Buchanan (eds.), Food Microbiology: Fundamentals and Frontiers, 4th ed. ASM Press, Washington, DC.

97. Mirhoseini, A., J. Amani, and S. Nazarian. 2018. Review on pathogenicity mechanism of enterotoxigenic Escherichia coli and vaccines against it. *Microb. Pathog.* 117:162–169.

98. Monaghan, Á., B. Byrne, S. Fanning, T. Sweeney, D. McDowell, and D. J. Bolton. 2012. Serotypes and virulotypes of non-O157 Shiga toxin-producing Escherichia coli (STEC) on bovine hides and carcasses. *Food Microbiol.* 32:223–229.

99. Nataro, J. P. 2005. Enteroaggregative Escherichia coli pathogenesis. *Curr. Opin. Gastroenterol.* 21:4–8.

100. Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic Escherichia coli. *Clin. Microbiol. Rev.* 11:142–201.

101. Nataro, J. P., T. Steiner, and R. L. Guerrant. 1998. Enteroaggregative Escherichia coli. *Emerg. Infect. Dis.* 4:251–261.

102. Nguyen, Y., and V. Sperandio. 2012. Enterohemorrhagic E. coli (EHEC) pathogenesis. *Front. Cell. Infect. Microbiol.* 2:1–7.

103. Obrig, T. G. 2010. Escherichia coli Shiga toxin mechanisms of action in renal disease. *Toxins (Basel).* 2:2769–2794.

104. Ockerman, H. W., and C. L. Hansen. 1988. Animal by-product processing. VCH and Ellis Horwood Ltd, New York.

105. Parks, A. R., M. F. Miller, K. Hanlon, S. Pokharel, K. Spivey, and M. M. Brashears. 2016. Validation of the efficacy of 4.55% FCC 88 lactic acid and 2.5% lactic

acid/acetic acid (50/50) blend on offal meats and carcass surfaces under simulated industry conditions. *Meat Sci.* 112:166–167.

106. Paton, J. C., and A. W. Paton. 1998. Pathogenesis and diagnosis of Shiga toxin-producing Escherichia coli infections. *Clin. Microbiol. Rev.* 11:450–79.

107. Patton, B. S., S. M. Lonergan, S. A. Cutler, C. H. Stahl, and J. S. Dickson. 2008. Application of Colicin E1 as a prefabrication intervention strategy. *J. Food Prot.* 71:2519–2522.

108. Pearson, A. M., and T. R. Dutson. 1992. Inedible meat by-products. Advances in Meat Research. Elsevier Science Publishers LTD, New York.

109. Pokharel, S., M. F. Miller, A. R. Parks, and M. M. Brashears. 2016. In-plant validation study to determine the efficacy of lactic acid as an antimicrobial intervention on beef heads and variety meats. *Meat Sci.* 112:166–167.

110. Rahal, E. A., N. Kazzi, F. J. Nassar, and G. M. Matar. 2012. Escherichia coli O157:H7-Clinical aspects and novel treatment approaches. *Front. Cell. Infect. Microbiol.* 2:1–7.

111. Ramantanis, S. B. 2006. Alternative cattle slaughtering technologies and/or measures reducing the dissemination of central nervous system tissue during head handling, harvesting of cheek meat and tongue and carcass splitting-a review. *Vet. Arh.* 76:19–36.

112. Ransom, J. R., K. E. Belk, J. N. Sofos, J. A. Stopforth, J. A. Scanga, and G. C. Smith. 2003. Comparison of intervention technologies for reducing Escherichia coli O157:H7 on beef cuts and trimmings. *Food Prot. Trends* 23:24–34.

113. Riley, L. W., R. S. Remis, D. S. Helgerson, H. B. MacGee, J. G. Wells, D. R. Betty, R. J. Herbert, E. S. Olcott, L. M. Johnson, N. T. Hagrett, P. A. Blake, and M. L. Chohen. 1983. Hemorragic colitis associated with a rare Escherichia coli serotype. *N. Engl. J. Med.* 308:681–685.

114. Rodríguez, I., J. A. Vázquez, P. Fuciños, J. Méndez, and L. Pastrana. 2013. Optimization of antimicrobial combined effect of organic acids and temperature on foodborne Salmonella and Escherichia coli in cattle feed by response surface methodology. *Foodborne Pathog. Dis.* 10:1030–1036.

115. Scaletsky, I. C. A., S. H. Fabbricotti, R. L. B. Carvalho, C. R. Nunes, M. B. Morais, and U. Fagundes-neto. 2002. Diffusely adherent Escherichia coli as a cause of acute diarrhea in young children in northeast Brazil : A case-control study. *J. Clin. Microbiol.* 40:645–648.

116. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States-Major pathogens. *Emerg. Infect. Dis.* 17:7–15.

117. Schmidt, J. W., J. M. Bosilevac, N. Kalchayanand, R. Wang, T. L. Wheeler, and M. Koohmaraie. 2014. Immersion in antimicrobial solutions reduces Salmonella enterica and Shiga toxin-producing Escherichia coli on beef cheek meat. *J. Food Prot.* 77:538–548.

118. Servin, A. L. 2005. Pathogenesis of Afa/Dr diffusely adhering Escherichia coli. *Clin. Microbiol. Rev.* 18:264–292.

119. Sexton, S. 2015. A validation of 1,3-dibromo-5,5-dimethylhyndation and lactic

acid on Salmonella and Shiga toxin-producing Escherichia coli on beef head and cheek meat. Thesis dissertation for the degree of Master of Science. Texas Tech University. Lubbock, TX.

120. Signorini, M., M. Costa, D. Teitelbaum, V. Restovich, H. Brasesco, D. García, V. Superno, S. Petroli, M. Bruzzone, V. Arduini, M. Vanzini, A. Sucari, G. Suberbie, T. Maricel, R. Rodríguez, and G. A. Leotta. 2018. Evaluation of decontamination efficacy of commonly used antimicrobial interventions for beef carcasses against Shiga toxin-producing Escherichia coli. *Meat Sci.* Elsevier 142:44–51.

121. Smith, J. L., P. M. Fratamico, and N. W. Gunther. 2014. Shiga toxin-producing Escherichia coli, p. 145–197. *In* S. Sariaslani, and G.M. Gadd (eds.), Advances in Applied Microbiology, 1st ed. Elsevier, San Diego, CA.

122. Solomon, E. B., S. Yaron, and K. R. Matthews. 2002. Transmision of Escherichia coli O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Appl. Environ. Microbiol.* 68:397–400.

123. Tauxe, R. V., M. P. Doyle, T. Kuchenmüller, J. Schlundt, and C. E. Stein. 2010. Evolving public health approaches to the global challenge of foodborne infections. *Int. J. Food Microbiol.* 139:S16–S28.

124. Ulbrich, C. J., L. M. Lucia, A. N. Arnold, T. M. Tayor, J. W. Savell, and K. B. Gehring. 2015. Reduction of surrogates for Escherichia coli O157:H7 and Salmonella during the production of nonintact beef products by chemical antimicrobial interventions. *J. Food Prot.* 78:881–887.

125. United States Department of Agriculture-Food Safety and Inspection Service. 2012.

Contamination with microorganisms; process control verification criteria and testing; pathogen reduction standards. 9 CFR Part 310.25. *Fed. Regist.* 135–137.

126. United States Department of Agriculture-Food Safety and Inspection Service. 2013. Food ingredients and sources of radiation listed and approved for use in the production of meat and poultry products. 9 CFR Part 424. *Fed. Regist.* 78:14636–14640.

127. United States Department of Agriculture-Food Safety and Inspection Service. 2018. Food Safety and Inspection Service Directive 7120.1. Safe and Suitable Ingredients used in the production of meat, poultry and egg products.

128. United States Department of Agriculture-Food Safety and Inspection Service. 2015. FSIS compliance guideline HACCP systems validation.

129. United States Department of Agriculture-Food Safety and Inspection Service. 2014. Michigan firm recalls ground beef products due to possible E. coli O157:H7.

130. United States Department of Agriculture-Food Safety and Inspection Service. 1996. Pathogen reduction; Hazard analysis and critical control point (HACCP) systems; Final rule. *Fed. Regist.* 61:38805–38839.

131. United States Department of Agriculture-Food Safety and Inspection Service. 2012. Shiga toxin-producing Escherichia coli in certain raw beef products. 9 CFR, parts 416, 417, and 430. *Fed. Regist.* 76:31975–31981.

132. Van Ba, H., H. W. Seo, S. Pil-Nam, Y. S. Kim, B. Y. Park, S. S. Moon, S. J. Kang, Y. M. Choi, and J. H. Kim. 2018. The effects of pre-and post-slaughter spray application with organic acids on microbial population reductions on beef

carcasses. *Meat Sci.* Elsevier 137:16–23.

133. Viator, C. L., S. C. Cates, S. A. Karns, and M. K. Muth. 2017. Food safety practices in the U.S. meat slaughter and processing industry: Changes from 2005 to 2015. *J. Food Prot.* 80:1384–1392.

134. Wambui, J., P. Lamuka, E. Karuri, J. Matofari, and P. M. K. Njage. 2018. Microbial contamination level profiles attributed to contamination of beef carcasses, personnel, and equipment: Case of small and medium enterprise slaughterhouses. *J. Food Prot.* 81:684–691.

135. Wang, G., T. Zhao, and M. P. Doyle. 1996. Fate of enterohemorrhagic Escherichia coli O157:H7 in bovine feces. *Appl. Environ. Microbiol.* 62:7–11.

136. Willshaw, G. A., J. Thirlwell, A. P. Jones, S. Parry, R. L. Salmon, and M. Hickey. 1994. Vero cytotoxin-producing Escherichia coli O157 in beefburgers linked to an outbreak of diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome in Britain. *Lett. Appl. Microbiol.* 19:304–307.

137. Winkler, D., and K. B. Harris. 2009. Reference Document: Antmicrobial interventons for beef. Texas A&M University. College Station, Texas.

138. Woolthuis, C. H. J., and F. J. M. Smulder. 1985. Microbial decontamination of calf carcasses by lactic acid sprays. *J. Food Prot.* 48:832–837.