MODELING THE EFFICACY AND EFFECTIVENESS OF ESCHERICHIA COLI O157:H7 PRE-HARVEST INTERVENTIONS

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MODELING THE EFFICACY AND EFFECTIVENESS OF

*ESCHERICHIA COLI O157:H7* PRE-HARVEST INTERVENTIONS

by

Amanda R. Vogstad

A THESIS

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science

Major: Veterinary Science

Under the Supervision of Professor David R. Smith

Lincoln, Nebraska

May, 2012
Shiga toxin-producing *Escherichia coli* O157:H7 (STEC O157) is one of the leading causes of hemolytic uremic syndrome in humans. Reducing the prevalence of STEC O157 in live cattle may reduce ground beef prevalence and subsequent human illness. Type III secreted protein vaccines (TTSP) reduce fecal shedding of STEC O157 in cattle. However, pre-harvest vaccines have yet to be adopted by the beef industry. The objectives of this thesis were to 1) conduct a meta-analysis to test factors effecting efficacy of a 3-dose regimen TTSP vaccine product, and 2) use stochastic simulation to model the usefulness of preharvest control measures. In the meta-analysis, data was used from four randomized controlled vaccine trials conducted from 2002-2008 at the University of Nebraska–Lincoln (n=184 pens, 1,462 cattle). Results indicated, study, challenge load, and days from administration of the last dose of the vaccine did not modify the measure of vaccine efficacy for a 3-dose regimen TTSP vaccine product. Model adjusted efficacy was 48% (95%CI, 0.37-0.57). In the modeling study, we simulated the pen-level fecal shedding prevalence distribution of cattle fed in the summertime and vaccinated with a TTSP vaccine and compared it to the winter fecal shedding prevalence distribution. Model inputs were previously observed frequency distributions for number of animals within a pen, and pen-level fecal shedding prevalence for summer and winter. Uncertainty about vaccine efficacy was modeled as a log-normal
distribution ($\mu=58$, SE=0.1393). The simulation was performed 5,000 times. Vaccination with a TTSP vaccine reduced summertime pen prevalence distributions of STEC O157 to levels comparable to wintertime pen prevalence, with the major effect being reduced variability in fecal shedding prevalence. Our simulation model should be a useful tool for food safety decision makers evaluating the usefulness of pre-harvest interventions.
DEDICATION

I dedicate this thesis to Grandpa Edmund, Grandma Joy, Dad, Mom, Danielle, Amber, Shawni Jo, and Robert – for all their love, support, and encouragement.
AUTHOR’S ACKNOWLEDGEMENTS

I would like to acknowledge my committee members Dr. Smith, Dr. Erickson, Dr. Moxley, and Dr. Randle. I am thankful for their time and patience.

I owe my deepest gratitude to my advisor Dr. Smith for his inspiration, sincerity, and unfailing support. Without his guidance, I would not have completed this master’s thesis.

A special thanks to Dr. Erickson for his valuable advice over the years. Also, thanks to Dr. Erickson and Dr. Klopfenstein for giving me the opportunity to participate in Ruminant Nutrition events, and seminars. These activities were always very rewarding to me.

I would also like to thank Dr. Moxley for providing feedback and constructive criticism, especially after I presented my first seminar. This was very helpful and appreciated. Thank you to Dr. Randle for his insightful discussions on the use of data in the beef industry.

I would like to acknowledge everyone in the Veterinary Sciences Department who assisted me over the years, especially Lee Johnson. Thank you to Dr. Hostetler for the many conversations.

To all the professors and teachers I had during my masters programs for their knowledge.

I am grateful for the memorable times spent with friends. A special thanks to the members of the Ruminant Nutrition Department and to my colleague Giselle Kruse.

To my friends and family in Canada who kept in contact, even when I was so far away. I thank my mom, dad, and sisters for their many words of encouragement. Lastly, I would like to thank my boyfriend for his patience and loving support.
GRANT INFORMATION

This work was supported in part by USDA CSREES AFRI Competitive Grants Program (Proposal # 2009-04248).
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CHAPTER I: LITERATURE REVIEW
Emerging Foodborne Pathogen

Shiga toxin-producing Escherichia coli O157:H7 (STEC O157) was first identified as a foodborne pathogen in 1982 (Riley et al., 1983). In the years following, STEC O157 became a leading cause of hemorrhagic colitis in humans (Mead et al., 1999). In 1993, one of the largest historical STEC O157 multistate outbreaks occurred known as the Jack and the Box scare. Consumption of undercooked STEC O157 contaminated hamburgers were attributed to causing 732 illnesses, 195 hospitalizations, and 3 deaths (Bell et al., 1994; Rangel et al., 2005). During the outbreak 255,000 frozen hamburgers were recalled (Rangel et al., 2005). The event became widely publicized, and ended up spurring research and regulations in the area of STEC O157 food safety. One of the outcomes of this research effort was discovery of the linkage between cattle and STEC O157 (Wells et al., 1991; Chapman et al., 1997). Cattle became recognized as asymptomatic carriers (Cray et al., 1995) and the most important reservoir for human exposure. Transmission of STEC O157 to humans was determined to occur either directly or indirectly through contact with contaminated cattle feces (Sargeant et al., 2003b).

Human STEC O157 infection became nationally notifiable in 1994 (Rangel et al., 2005), resulting in the establishment of FoodNet by the CDC. FoodNet is a system that aids in monitoring and early detection of foodborne outbreaks (Kassenborg et al., 2004). In 1996, under the Federal Meat Inspection Act, FSIS declared STEC O157 an adulterant to meat (Food Safety and Inspection Service, 1996). Along with the declaration that STEC O157 was an adulterant to meat was the implementation of the Hazard Analysis Critical Control Points (HACCP) program (Food Safety and Inspection Service, 1996). This program, which is still in place today, requires
slaughter and processing plants to systematically and safely produce meat & poultry products for the public. HACCP relies on the identification of hazards, and points in the process where these hazards can be controlled, called critical control points (United States Food and Drug Administration, 2011). At these critical control points, interventions are put into place to prevent or reduce hazards from occurring during the slaughter process (United States Food and Drug Administration, 2011). Examples of interventions to reduce STEC O157 on carcasses at slaughter include steam cabinets, lactic acid washes, and trimming of carcasses. The Centers for Disease Control attributes part of the decline in STEC O157 human infection rates to HACCP and these post-harvest technologies (Centers for Disease Control and Prevention, 2011a). For 2010, rates of STEC O157 human illness were at the lowest of 0.9 cases per 100,000 people (Centers for Disease Control and Prevention, 2011b). Healthy People 2020, a program that sets national goals to promote healthy living of the U.S. population, has targeted a reduction in STEC O157 infections to 0.6 cases per 100,000 by 2020 (Department of Health and Human Services, 2012). It is believed that post-harvest interventions may be overwhelmed and incapable of reducing STEC O157 human infection rates further. In order to achieve the Healthy People 2020 goal or to maintain reduced rates of human infection, pre-harvest interventions may need to be implemented.

Pre-harvest interventions reduce STEC O157 in live cattle prior to harvest. Some have proposed that the successful implementation of pre-harvest interventions in the industry will require both efficacy and effectiveness to be demonstrated (Smith et al., 2012). Efficacy refers to the ability of the intervention to decrease the likelihood of recovering STEC O157 from live
cattle. Effectiveness refers to the actual usefulness of the intervention in the beef production system (Smith et al., 2012). A number of pre-harvest intervention technologies have been researched in vivo and have demonstrated efficacy (Potter et al., 2004; Younts-Dahl et al., 2005; Anderson et al., 2005; Sargeant et al., 2007). For example, a bovine vaccine product that utilizes three doses of type three secreted proteins has been shown to reduce fecal populations of STEC O157 in live cattle anywhere from 43% to 73% (Smith et al., 2012).

Despite the demonstration of efficacy of pre-harvest control measures, adoption of these technologies has yet to occur. In May 2010, FSIS introduced a pre-harvest guidance document for slaughter facilities. This document outlined research on known pre-harvest technologies and management practices. It also recommended that packing plants only receive live cattle from beef producers implementing one or more of these pre-harvest interventions (Food Safety and Inspection Service, 2010). Others in the industry responded to this document with concern. This concern was due primarily to the lack of research indicating the practicality and ease of implementing many of these pre-harvest technologies in the beef industry (American Meat Institute, 2010). Since there is a gap in research indicating the usefulness of pre-harvest interventions, the main goal of this thesis was to evaluate both the efficacy and effectiveness of a pre-harvest intervention in a beef production setting. Preceding the discussion on this pre-harvest technology is a review of the current literature on STEC O157 in beef cattle. This review will include topics such as: STEC O157 human infection, the beef cattle reservoir, epidemiology of STEC O157 in beef cattle, sources and transmission of STEC O157 to cattle, and pre-harvest technologies.
STEC O157 Human Illness

*Shiga Toxin-Producing Escherichia coli O157:H7*

STEC O157 is a type of *Escherichia coli*. Commonly *E. coli* inhabit the gastrointestinal tract of mammals. Some *E. coli* strains are commensal causing no harm to the host, while other strains, such as STEC O157 may be pathogenic causing life-threatening disease. STEC O157 possesses both O and the flagellar H antigens as well as Shiga-like toxins leading to the name Shiga toxin-producing *Escherichia coli* O157:H7 (STEC O157).

**STEC O157 Human Infection**

STEC O157 is a zoonotic pathogen that may cause illness, hospitalization, and even death in humans that become infected (Mead *et al.*, 1999). In 2010, the CDC reported that approximately 63,153 illnesses, 2,138 hospitalizations, and 20 deaths occur each year due to STEC O157 foodborne illnesses (Scallan *et al.*, 2011). Signs of clinical infection in cases include diarrhea, hemorrhagic colitis, or hemolytic uremic syndrome (HUS) (Besser *et al.*, 1999). Often the young, the elderly, and the immuno-compromised are more susceptible to severe STEC O157 illness, and approximately 3-5% of individuals that develop HUS will die from complications (Besser *et al.*, 1999). For children it is even greater, 5-10% (Besser *et al.*, 1999). Complications arising from HUS infection include hemolytic anemia, thrombocytopenia, and renal disease (Griffin *et al.*, 1991). Antibiotics have not proven useful for treating severe STEC O157 infection (Griffin *et al.*, 1991). Therefore treatment consists mainly of intense fluid therapy (Besser *et al.*, 1999).
Cost of Human Illness

STEC O157 is an important food safety concern not only because it causes severe illness and death but also because the cost of human infection is substantial. The USDA Economic Research Service estimates total costs incurred for STEC O157 foodborne illnesses in 2010 were $488,771,183 for both hospitalized and non-hospitalized individuals, with the average cost per case estimated at $6,652 (Economic Research Service, 2011). Expenditures for individuals that required treatment for HUS, including end-stage renal disease, averaged just over 6 million per patient (Economic Research Service, 2011). These estimates accounted for medical costs, lost productivity, disutility, and premature death but did not include losses related to legal settlements, and costs for childcare and nursing homes (Economic Research Service, 2011).

Impact of STEC O157 on the Beef Industry

STEC O157 food borne outbreaks have also contributed to economic losses in the beef sector. From 1992-2002 it was estimated that these losses were as high as $2.7 billion (Kay, 2003). Accounting for these expenditures were the decline in beef demand, decreased beef prices, increased packer operating costs, recall losses, and expenditures for research and technology (NCBA, 2004).

A North American consumer survey indicated with elevated food safety concerns twenty percent of consumers reduce beef consumption. Included in this assessment were consumer’s perceptions for the risk of STEC O157 infection (Schroeder et al., 2007). Beef, pork, and poultry recalls reported by the Food Safety Inspection Service (FSIS), from 1992-1998, also were shown to negatively impact consumer demand (Marsh et al., 2004). While in 1993 the STEC O157 Jack
in the Box outbreak caused an estimated 2.9% drop in beef demand (Schroeder et al., 2000). STEC O157 recall events have also been linked to a decline in boneless beef prices during the short term (McKenzie et al., 2001). Others reported reductions in boneless beef prices accounted for a loss of $172 million over ten years (NCBA, 2004). Foodborne outbreaks often appear to have a visual impact on live cattle prices, although research has not supported this (McKenzie et al., 2001; Lusk et al., 2002). While historically, outbreaks leading to STEC O157 human illness have had little no reaction in boxed beef prices (McKenzie et al., 2001; Lusk et al., 2002).

Packer expenditures due to increased operating costs, research and development, and recall losses associated with STEC O157 outbreaks are substantial. More than 26 million pounds of beef have been recalled since 2006 due to contamination by Shiga toxin-producing E. coli (United States Government Accountability Office, 2012). From 1994 to 2004, the top ten packers spent an estimated $400 million for beef research on STEC O157 (NCBA, 2004). A Class I recall event, which included a STEC O157 associated event, reduced packer share prices as much as 1.5-3% (McKenzie et al., 2001). Increased packer operating costs due to the implementation of the 1996 Pathogen Reduction/Hazard Analysis and Critical Control Point Regulations (PR-HACCP) added additional expenditures upwards of $250 million. The PR-HACCP Regulations were also estimated to have impacted beef and poultry production costs by one third a cent per pound (Ollinger et al., 2004). However, 1.9 billion dollars in medical costs were estimated to be prevented by the PR/HACCP rule (Crutchfield et al., 1997).
Sources and Transmission of STEC O157 to Humans

STEC O157 is spread by the fecal-oral route and exposure of humans to the pathogen may be direct or indirect. Transmission to people occurs mainly through contact with contaminated food, water, the environment, animals or other humans carrying the pathogen (Feng, 1995).

Foodborne outbreaks account for a significant source of STEC O157 illness in humans. From 1998-2002, CDC surveillance data implicated 52% of STEC O157 outbreaks to food sources (Rangel et al., 2005). Food sources linked to human illness include contaminated fruits (Rangel et al., 2005), vegetables (Berger et al., 2010), cider (Besser et al., 1993), meat (Riley et al., 1983; Ryan et al., 1986; Ostroff et al., 1990; Keene et al., 1997b), and milk products (Feng, 1995; Keene et al., 1997a). Of these foods, undercooked ground beef has been the predominate source of STEC O157 outbreaks (Codex, 2002). For instance, of the 350 U.S. outbreaks reported from 1982-2002, 41% of these were traced back to contaminated ground beef. Over the past decade however, there has been a decline in human illnesses attributed to ground beef and a rise in human illnesses due to contaminated fruits and vegetables (Codex, 2002; Williams et al., 2010). Primarily the rise in STEC O157 fresh produce outbreaks (Besser et al., 1999) have been attributed to contaminated leafy greens and sprouted seeds (Berger et al., 2010). In 2006, a large multi-state outbreak encompassing twenty-six U.S. states was linked to contaminated pre-packaged spinach (Grant et al., 2008). This incident resulted in 205 illnesses and 3 deaths. Of the infected individuals twenty-nine percent ended up developing HUS, which was a much higher rate of HUS development than previous outbreaks (Grant et al., 2008). STEC O157 is also
capable of surviving in foods with a low pH (Glass et al., 1992). Unpasteurized juice and ciders have supported STEC O157 survival and have also been implicated in foodborne outbreaks (Besser et al., 1993; Leyer et al., 1995).

STEC O157 inhabits the gastrointestinal tract (GIT) of mammals, including domestic and wild animals (Hancock et al., 1998). Unlike humans, animals may carry STEC O157 without development of severe disease. Shedding of STEC O157 in the feces of animals results in the dissemination of STEC O157 via the fecal-oral route (Rice et al., 1995; Kudva et al., 1996; Hancock et al., 1998). Some of the species STEC O157 has been isolated from include: Dogs (Hancock et al., 1998), horses (Hancock et al., 1998), cattle (Chapman et al., 1997), pigs (Chapman et al., 1997), sheep (Chapman et al., 1997), deer (Sargeant et al., 1999; Renter et al., 2001), bison (Reinstein et al., 2007), rabbits (Scaife et al., 2006), raccoons (Shere et al., 1998), wild birds (Wallace et al., 1997; Hancock et al., 1998), rats (Cizek et al., 1999), flies (Hancock et al., 1998) and opossums (Renter et al., 2003). People mainly come into contact with animals carrying STEC O157 on the farm, at agricultural exhibits, and at zoos (Rangel et al., 2005). Transmission of STEC O157 between animal species could also account for the widespread dissemination of STEC O157. For instance, European starlings in confinement were capable of carrying and transmitting STEC O157 to other starling birds and 12-week-old-calves (Kauffman et al., 2011).

Another important route of exposure is direct person-to-person contact. An infected individual carries STEC O157 in their feces and transmits the organism through poor hygiene. Direct human exposure is often reported in day cares, schools, nursing homes, and medical
centers, where susceptible people are in close proximity to each other (Spika et al., 1986; Carter et al., 1987; Griffin et al., 1991; Belongia et al., 1993).

Outbreak investigations have determined contaminated water sources may also lead to STEC O157 human illnesses. In particular, drinking and recreational water supplies contaminated with STEC O157 (Rangel et al., 2005). One of the earliest known water-associated outbreaks took place in a small rural town with an un-chlorinated drinking water supply (Swerdlow et al., 1992). Recreational water sources implicated in outbreaks include swimming pools, lakes, and ponds (Rangel et al., 2005). Data has also indicated that STEC O157 is capable of surviving in water at cool temperatures for extended periods of time, possibly aiding in the pathogens transmission to humans (Wang et al., 1998a).

Dissemination of STEC O157 to humans may occur through contact with environmental sources. Possible environmental sources of STEC O157 include soil, manure, water, feed, and slurry (Sargeant et al., 2003b). In vitro STEC O157 revealed the ability to survive 70 days at 5°C and 49 days at 37°C in manure samples (Kudva et al., 1996). Common routes of STEC O157 entry into the environment are excretion of STEC O157 in bovine feces, fertilization of farm ground or gardens with STEC O157 contaminated bovine feces, and contamination of irrigation or run-off water with bovine feces containing STEC O157 (Sargeant et al., 2003b). Studies have also shown humans are at a greater risk of becoming infected with STEC O157 when contacting environments where cattle were previously housed (O'Brien et al., 2001; Locking et al., 2001). In 2000, twenty boy scouts were infected with STEC O157 while camping. Infected individuals evidently contacted environmental sources of STEC O157 with their hands. Inadequate hand
washing then led to the ingestion of the pathogen and subsequent infection. The original source of STEC O157 at the camping site was believed to be contaminated sheep feces (Howie et al., 2003).

**Beef Cattle Reservoir**

Beef cattle are an important reservoir for STEC O157 human exposure (Chapman et al., 1997). STEC O157 colonizes the gastrointestinal tract of beef cattle (Cray et al., 1995; Grauke et al., 2002; Naylor et al., 2003), and is subsequently shed in the feces at variable magnitudes (Zhao et al., 1995). Both calves and mature beef animals experience transient shedding, though neonatal calves reportedly shed for longer durations and at higher magnitudes (Zhao et al., 1995; Cray et al., 1995; Besser et al., 1997). Unlike humans, cattle do not develop hemolytic uremic syndrome (Pruimboom-Brees et al., 2000). Much of the earlier research demonstrated that adult cattle carrying STEC O157 also did not exhibit clinical signs of STEC O157 infection (Cray et al., 1995). Neonatal calves however, were reported to be capable of developing non-bloody diarrhea (Cray et al., 1995; Dean-Nystrom et al., 1997). It was later discovered that tissue from adult cattle were susceptible to lesion formation as a result of STEC O157 colonization (Baehler et al., 2000). Researchers now confirm STEC O157 is not a commensal organism of cattle (Naylor et al., 2003; Nart et al., 2008; Moxley et al., 2010). In this section STEC O157 virulence factors, mechanism of attachment, colonization, and expression of Shiga toxins in beef cattle will be discussed briefly. Also, described are methods for isolating STEC O157 from cattle feces.
**Virulence Factors**

Diarrheagenic *E. coli* have been classified into six groupings based on different virulence mechanisms (Nataro *et al.*, 1998). STEC O157 belongs to the Enterohemorrhagic *E. coli* (EHEC) (Nataro *et al.*, 1998). Other groupings of diarrheagenic *E. coli* include: Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Diffusively Adhesive *E. coli* (DAEC) and Enterotoxigenic *E. coli* (ETEC) (Viazis *et al.*, 2011). Similar to EPEC, STEC O157 possesses the ability to create attaching and effacing lesions on host enterocytes (Nataro *et al.*, 1998). Other virulence factors that may attribute to the pathogenicity of STEC O157 consist of a pO157 plasmid, and toxins (Law, 2000a).

Attaching and effacing lesions on host epithelial cells represent colonization by STEC O157 (Nataro *et al.*, 1998). These lesions are characteristic of the bacterium’s intimate adherence which leads to the destruction of microvilli and rearrangement of host actin filaments (Nataro *et al.*, 1998). Lesion formation is carried out by effector molecules belonging to a Type III secreted system (TTSP) and coded for on the locus for enterocyte effacement (LEE) (Nataro *et al.*, 1998). STEC O157 also possesses a pO157 plasmid that encodes genes. Once expressed, these genes generate other virulence factors, such as cytotoxic hemolysins and catalase-peroxidase (Viazis *et al.*, 2011). Another important feature of STEC O157 is the possession of Shiga toxins. These toxins account for the vascular damage that may result in patients with severe kidney failure. STEC O157 possesses two types of toxins, stx1 and stx2, both resembling the Shiga toxin produced by *Shigella dysenteriae* (O’Brien *et al.*, 1987). Studies of human patients with disease suggest that stx2 may be more virulent than stx1 (Kleanthous *et al.*, 1990; Law, 2000b).
Conversely, infection in calves appears to be associated with STEC O157 producing stx1 compared to stx2 (Moxley et al., 2010).

**Colonization Region in Live Cattle**

Colonization of cattle occurs mainly in the large intestine (Cray et al., 1995; Grauke et al., 2002; Naylor et al., 2003), though is probably not limited to this region (Keen et al., 2010). Originally it was suggested the rumen was the primary site for STEC O157 proliferation (Rasmussen et al., 1993; Armstrong et al., 1996). Another report found survival of STEC O157 occurred predominately in the forestomach of calves (Brown et al., 1997). However, other researchers indicated challenged calves possessed higher concentrations of STEC O157 in the lower gut (Cray et al., 1995). Experimentally challenged sheep and cattle were also more likely to be colonized with STEC O157 in the colon (Grauke et al., 2002). In particular the persistence of STEC O157 was determined to localize specifically to the terminal rectal region (TRM), at the lymphoid follicle-dense mucosa (Naylor et al., 2003). Others confirmed the terminal rectal region was a common site of colonization in experimentally challenged cattle (Lim et al., 2007). Cattle shedding high levels of STEC O157 in the feces were also found to be significantly associated with STEC O157 persistence at the TRM region (Low et al., 2005). This discovery was made using swabs of the TRM region of cattle. More recent work has presented the argument that STEC O157 may be found throughout the GIT and does not preferentially colonize any region (Keen et al., 2010). STEC O157 was recovered from tonsils, reticulum, rumen, omasum, abomasum, duodenum, jejunum, cecum, spiral colon, rectum, and liver of naturally infected cattle. Of all these sampling sites no single location accounted for a greater
proportion of STEC O157 positive study animals. However, researchers only observed the occurrence of STEC O157 along the GIT tract, colonization status was not assessed (Keen et al., 2010).

**STEC O157 Mechanism of Attachment and Colonization of Cattle**

STEC O157 intestinal attachment is mediated by contact with the host cell using a type III secretion system (TTSS) (Nataro et al., 1998). Also thought to assist in intestinal attachment, are the quorum sensing mechanism (Moxley et al., 2010) and the H7 flagellin (Bretschneider et al., 2007; Mahajan et al., 2009), although little is known about their role in this process. A major component of the type III secretion system is a needlelike structure used to secrete proteins from the bacterial cell into the host cytoplasm. Of the proteins secreted through the TTSS, EspA, EspB, EspD, and Tir are most important for mediating lesion formation (Nataro et al., 1998). EspA in particular, begins the attachment process by forming a translocation tube on the surface of STEC O157. The proteins EspB, EspD, and Tir are then secreted into the host cell through this translocation tube (Ebel et al., 1998; Frankel et al., 1998; Knutton et al., 1998). Following the secretion of these LEE-encoded genes, intimin the outer membrane protein binds to the translocated intimin receptor (Tir) located in the host cell plasma membrane (DeVinney et al., 1999). Studies have indicated intimin is a requirement for A/E lesion formation on intestinal epithelial cells in calves and humans (Donnenberg et al., 1993; Dean-Nystrom et al., 1998). Succeeding Tir-intimin binding, are the cytoskeleton rearrangements of the host, and the destruction of brush border micorvilli (Vallance et al., 2000). Host cell cytoskeleton
reorganization results in the creation of pedestals known as attaching and effacing lesions (Campellone, 2010; Hamada, 2010).

It has not always been clear if adult cattle were susceptible to A/E lesion formation when naturally colonized with STEC O157. However, using colon and rectum mucosal epithelial explants from animals 18 months of age, A/E lesions were demonstrated on tissues after experimental STEC O157 inoculation (Baehler et al., 2000). Attaching and effacing lesions were also later discovered in both natural and experimental cattle colonized preferentially at the terminal rectum (Naylor et al., 2005). This study provided evidence that adult cattle are in fact susceptible to A/E lesions, resulting from STEC O157 colonization.

Shiga Toxin Expression in Cattle

Succeeding intestinal attachment of STEC O157 to the host is the expression of the extracellular cytotoxins, stx1 and stx2. Either one or both of the Shiga toxins (stx1, stx2) may be produced by a STEC O157 cell (Law, 2000b). Once secreted by STEC O157, these toxins are capable of causing death to susceptible host cells through the: 1) binding of the host outer membrane; 2) absorption via receptor-mediated endocytosis; and 3) inhibition of protein synthesis within the cell; (Sandvig et al., 1996; Law, 2000b). Attachment of Shiga toxins to host cells, occurs through the globotriaosylceramide (Gb3) receptors (O'Brien et al., 1987) and is common among humans and cattle (Pruimboom-Brees et al., 2000). In humans, these receptors have been found in endothelial cells of the arteries, kidneys, brain, and gastro-intestinal mucosa (Mainil et al., 2005). In cattle, these receptors are found specifically in the epithelial crypt regions of the intestine but are not present on vasculature (Hoey et al., 2002). Hemorrhagic
colitis (HC) and HUS in humans is a result of Shiga toxins binding Gb3 in vascular cells (Kaplan et al., 1990). The lack of Gb3 in vascular cells is hypothesized to explain why cattle do not develop HC or HUS (Hoey et al., 2002). Also, Shiga toxins endocytosed into bovine intestinal cells, do not make it to the golgi apparatus where stx activation generally occurs (Hoey et al., 2003). Instead, the toxin appears to be in lysosomes were it remains incapable of inhibiting protein synthesis and causing cell death.

**Detection of STEC O157**

STEC O157 can be identified from cattle feces by enrichment, and direct plating onto selective media, followed by confirmation with the multiplex polymerase chain reaction (mPCR) (Moxley, 2003). The initial isolation of STEC O157 requires enrichment of the fecal sample by addition of media containing antibiotics and nutrients (Moxley, 2003). Nutrients in the media promote STEC O157 growth and replication while the antibiotics such as vancomycin, cefixime, and cefsulodin inhibit growth of other background organisms. Without enrichment, STEC O157 isolation techniques are less sensitive (Sanderson et al., 1995). Following enrichment, immunomagnetic separation is sometimes used (Chapman et al., 1994). This procedure utilizes anti-O157 antibodies bound to magnetic beads. The final IMS separation is then plated on selective agar media, generally sorbitol MacConkey agar (Farmer, III et al., 1985) containing tellurite (Zadik et al., 1993) and cefixime (Chapman et al., 1991). Since STEC O157 does not ferment sorbitol within 24 hrs, STEC O157 colonies can be isolated using the CT-SMAC plates. Non-sorbitol fermenting colonies are then picked from the CT-SMAC plates and tested further to identify possible STEC O157 colonies which ferment lactose but do not produce β-glucuronidase.
Presumptive STEC O157 isolates then undergo further testing using latex agglutination (Chapman et al., 1989). Using the lipopolysaccharide O and flagellar H antigens, isolates agglutinating anti-O157 and anti-H7 latex beads are identified. Occasionally there is a tendency for the H7 flagellar antigen to go unrecognized, due to lack of expression in the serum sample (Moxley, 2003). In order to avoid these false negatives, isolates that are non-sorbitol fermenting, β-glucuronidase negative, lactose fermenting, and O157 positive up until this point are subjected to mPCR (Smith et al., 2005). Isolates hybridizing primers of the virulence genes rfbO157:H7 and fliC, along with two additional virulence genes; eae, stx1, or stx2, are considered to be STEC O157 (Potter et al., 2004).

**Epidemiology of STEC O157 in Beef Cattle**

Understanding the occurrence and distribution of STEC O157 in beef cattle populations is important for the development of pre-harvest control measures. In particular, describing fecal and hide prevalence patterns, and factors that affect prevalence patterns in individual animals and pens of animals, will aid in a greater understanding of the epidemiology of STEC O157 carriage in cattle. This section will describe the frequency of STEC O157 in susceptible beef populations by animal-unit, place, and time. A review of how pen condition, super-shedders, and stress are thought to influence the carriage of STEC O157 in the bovine reservoir will also be discussed.
Geographical Distribution

STEC O157 is ubiquitous to fed cattle populations (Hancock et al., 1997b; Smith et al., 2001; Sargeant et al., 2003a), with detection in cattle occurring throughout the globe. Various studies have surveyed STEC O157 in cattle populations by geographical region (Hancock et al., 1997a; Heuvelink et al., 1998; Smith et al., 2001; Sargeant et al., 2003a). Clustering of STEC O157 in cattle by region was not evident in fecal samples collected in one hundred feedlots across the U.S. (Hancock et al., 1997b). A regional difference in the probability to detect STEC O157 in the feces of cattle was also not detected in a large cross sectional survey of four Midwestern states (Sargeant et al., 2003a). Altogether 52% of pens and 96% of feedlots possessed one STEC O157 fecal culture positive sample in this study. Despite the fact that these large scale survey studies have indicated no regional disparity in STEC O157 fecal prevalence, regional differences based on STEC O157 hide prevalence were suggested in one report. Cattle arriving for harvest at two packing facilities located in the north and south were sampled for 5 months. In the end, regional disparities were attributed to hide prevalence levels that were significantly different between the two plants locations (Rivera-Betancourt et al., 2004).

Prevalence

The distribution of STEC O157 in live cattle encompasses all beef production systems; cow-calf, dairy, stocker, and feedlot. No differences exist in STEC O157 fecal prevalence between cattle grown in organic or conventional production systems (Kuhnert et al., 2005; Cho et al., 2006; Reinstein et al., 2009). Similarly there were no differences in the concentration or probability of detecting STEC O157 in feces of cattle fed in lot confinement versus cattle fed out
on pasture (15% vs. 10% prevalence; 1.3 vs. 0.6 log concentration) (Fegan et al., 2004). Both adult and young cattle may become positive for STEC O157 (Laegreid et al., 1999; Van Donkersgoed et al., 1999). Initial infection likely occurs very early in the life of an animal (Laegreid et al., 1999). Studies estimating herd fecal prevalence for feedlot cattle have reported 1.3%, 13%, and 23% (Sargeant et al., 2000; Smith et al., 2001; LeJeune et al., 2004). Fecal shedding and hide prevalence of feedlot cattle at slaughter was reportedly 28% in a survey of four Midwestern packing plants (Elder et al., 2000). Observed STEC O157 herd fecal prevalence levels for weaned range calves were previously described as 2.5%, 6.9%, 2.5%, and 5% (Laegreid et al., 1999; Renter et al., 2003; Dunn et al., 2004; Arthur et al., 2009).

**Pen Prevalence**

For a particular population, prevalence is described as the proportion of animals with disease or infection at a specific point in time. Prevalence is the best measure for describing the occurrence of STEC O157 in cattle populations, largely since STEC O157 fecal shedding is dependent on time and pen related factors (Khaitsa et al., 2003). STEC O157 pen prevalence has been described as highly variable, with as great as 80% of cattle in a pen shedding STEC O157 in the feces at one point in time (Smith et al., 2001). Pen fecal shedding variability is commonly displayed between feedlots but when contrasting different feedlots the proportion of cattle shedding STEC O157 in the feces is comparable (Smith et al., 2001). Pen-level fecal shedding of the organism is also directly a function of its duration and incidence (Khaitsa et al., 2003). Production or management strategies that target the incidence and duration of STEC O157 in live...
cattle are important for pathogen mitigation. These data also suggest longitudinal studies may be useful for studying STEC O157 carriage in the feces of live cattle (Khaitsa et al., 2003).

**Hide Prevalence**

A major source of carcass contamination at slaughter is prevalence of STEC O157 on the hides of cattle (Elder et al., 2000; Arthur et al., 2004). Hide prevalence represents the proportion of animals’ culture positive for STEC O157 on the hides. Different sampling strategies have indicated STEC O157 is distributed at multiple locations on the hide of animals (Keen et al., 2002; Arthur et al., 2004; Kalchayanand et al., 2009). In some reports the back was the most likely region to detect the pathogen on the hide (Keen et al., 2002), others reported the hock and perineum region (Stephens et al., 2007c) and finally, the belly was a significant source of contamination in a more recent report (Kalchayanand et al., 2009). Despite the differences in optimal hide recovery sites, researchers generally agree multiple sampling locations give a more complete picture of the pathogen load on the hide (Keen et al., 2002; Stephens et al., 2007c).

The prevalence of STEC O157 on the hides of cattle is variable, similar to STEC O157 fecal shedding prevalence. Some of this variation is explained by season, with a greater likelihood for hide contamination in the summer months (Barkocy-Gallagher et al., 2003; Arthur et al., 2009; Stanford et al., 2011). In the feedlot environment the probability to detect STEC O157 on the hides of cattle may fluctuate by pen and between pens of cattle (Arthur et al., 2009). There are also differences in the proportion of animals carrying STEC O157 on hides within lots at both large and small packing facilities (Brichta-Harhay et al., 2008; Bosilevac et al., 2009). Two separate studies found hide prevalence of cattle at harvest to be 6.3% (50/784) and 76%
(218/288) (Arthur et al., 2004; Dewell et al., 2008). There is little data on hide prevalence of cattle grazing pasture, however in a longitudinal study 54% of weaned calves coming off pasture carried STEC O157 on their hides (Arthur et al., 2009).

Carriage of STEC O157 on the hides of cattle may be influenced by the feedlot environment, other animals, transport, and holding at lairage (Arthur et al., 2007; Fox et al., 2008). Through the use of molecular genotyping, 29%, 69%, and 2%, of the hide contamination seen post-harvest was attributed to the feedlot, lairage, and hauling environments, respectively (Arthur et al., 2007). Survival of the pathogen is also believed to be short-lived. A recent study showed STEC O157 may only survive on the hide of an animal for up to 9 days (Arthur et al., 2011).

Because hide contamination is a correlate of carcass contamination (Arthur et al., 2004), significant efforts have been made over the years to reduce the presence of STEC O157 during the slaughter process. These efforts included the implementation of HACCP and post-harvest interventions in beef processing plants. Studies have indicated STEC O157 hide and carcass prevalence at slaughter has been reduced and may be attributed to these interventions (Arthur et al., 2002; Barkocy-Gallagher et al., 2003). At present, the focus has turned to decreasing the probability of recovering STEC O157 from the hides of cattle in the feedlot environment prior to slaughter. Controlling STEC O157 hide contamination pre-harvest should result in less environmental contamination, and less carriage of STEC O157 into packing plants by live cattle.
**Season**

Shedding of STEC O157 in the feces of cattle is influenced by season. A greater proportion of both beef and dairy cattle are found carrying STEC O157 in the summer months versus the winter months, (Hancock et al., 1994; Chapman et al., 1997; Hancock et al., 1997a; Heuvelink et al., 1998; Garber et al., 1999; Van Donkersgoed et al., 1999; Dunn et al., 2004; Smith et al., 2005). The seasonal relationship of STEC O157 carriage in live cattle was demonstrated in a cross sectional study where both fecal and hide samples were collected from pre-evisceration carcasses. Fecal shedding prevalence of STEC O157 was 43 times greater in cattle sampled in the summer versus cattle sampled in the winter (Summer prevalence =12.9%; Winter prevalence=0.3%) (Barkocy-Gallagher et al., 2003). Hide prevalence was not significantly different by season, although the proportion of cattle with STEC O157 on the hides was 73.5% and 29.4%, for summer and winter, respectively. Others have reported in the winter, there is a high probability for no animals in a pen to shed STEC O157 in the feces, with pen prevalence ranging from 0%-56% (Williams et al., 2010). While in the summer there is a high probability for at least one animal in every single pen to shed STEC O157 in the feces, with pen prevalence ranging from 1%-80% (Williams et al., 2010). Not all studies have indicated STEC O157 fecal shedding in cattle is seasonal (Sargeant et al., 2000; Synge et al., 2003; Ogden et al., 2004). Cattle arriving at a Scottish abattoir in the winter were 1.5 times more likely to shed STEC O157 in the feces compared to cattle arriving at the abattoir in the summer. The higher proportion of animals positive for STEC O157 in the winter may have been partially explained by the practice of commingling cattle indoors during the winter in Scotland (Ogden et al., 2004).
A number of factors have been proposed to explain the seasonality of STEC O157 fecal shedding; daylight hours, ambient temperature, precipitation and insect populations (Edrington et al., 2006a). These factors are thought to lead to physiological changes in the animal or environmental conditions conducive to STEC O157 survival.

Possibly in the summer, environmental conditions are conducive to STEC O157 transmission and in the winter, environmental conditions are conducive to STEC O157 survival. For instance, research has indicated STEC O157 is capable of surviving cool temperatures, in water, manure, and soil samples (Wang et al., 1996; Kudva et al., 1998; Rice et al., 2000; Berry et al., 2007). Others have indicated with increasing temperatures, the likelihood for recovering STEC O157 in the feedlot environment was greatly improved (Van Donkersgoed et al., 2001; Smith et al., 2005; Berry et al., 2010a). In one of these studies, air temperature was tested for an association with the seasonal recovery of STEC O157 from ropes (Smith et al., 2005). In both the summer and winter, the prior 7-day mean air temperature was found to explain the probability of detecting STEC O157 in ropes positive feedlot pens (Smith et al., 2005). The odds of recovering STEC O157 from ropes were 1.5 times greater for every 10°C weekly increase in summer air temperature and for every 10°C weekly increase in winter air temperature the odds of recovering STEC O157 from ropes were 3.7 times greater. Ropes were hung in pens for cattle to chew in this study. Therefore ropes testing positive for STEC O157 at the pen-level represented the oral challenge of the pathogen in the pen environment (Smith et al., 2005).

Others have tried to explain seasonal variability in relation to STEC O157 fecal shedding by evaluating factors that may lead to physiological changes in the animal. Physiological
changes favoring STEC O157 persistence in the gut could be induced by factors such as stress, temperature, and daylight hours (Edrington et al., 2006a). Longer hours of daylight have been hypothesized to result in higher levels of STEC O157 fecal shedding (Edrington et al., 2006a). Researchers reported, cattle allocated to a control natural light group, receiving less hours of daylight than an artificial light group had reduced STEC O157 fecal shedding 53 days post-treatment. When the artificial lighting was removed, the proportion of STEC O157 culture positive animals was similar between the two treatments. To explain this difference, researchers performed a trial comparing two levels of melatonin supplemented orally, to no melatonin supplemented orally to cattle (Edrington et al., 2008). Melatonin levels secreted within an animal are known to increase with fewer hours of daylight, and decrease with greater hours of daylight (Edrington et al., 2008). Increased levels of melatonin may influence the shedding of STEC O157 in the feces of cattle by affecting the normal microflora of the GI tract. In this trial, cattle supplemented with 0.5mg/kg per day of melatonin, were similar to control cattle in STEC O157 fecal shedding prevalence. However, in cattle supplemented with 5 mg/kg per day melatonin there was a reduced percentage of fecal samples positive for STEC O157 over the control non-melatonin supplemented cattle. Similarly, researchers evaluated Vitamin D intake on the ability to seasonally recover STEC O157 from the feces of cattle. In the end no correlation between Vitamin D levels and STEC O157 fecal shedding were determined (Edrington et al., 2012).

The seasonal variation in STEC O157 fecal shedding by cattle appears to be a common pattern occurring in many regions of the world (Chapman et al., 1997; Hancock et al., 1997a; Hancock et al., 1997b; Heuvelink et al., 1998; Bonardi et al., 1998). Outbreaks of human
infection also coincide with the peaks in STEC O157 fecal shedding prevalence of live cattle (Rangel et al., 2005; Williams et al., 2010). Factors that lead to the variation in recovering STEC O157 from the feces of cattle may be a result of physiological changes in the animal or possibly favorable ecological conditions of the feedlot pen.

**Pen Condition**

Commercial feedlot pens exhibit a wide array of pen conditions throughout the seasons. The condition of the pen as described by the amount of mud, manure, or standing water present may affect the level of STEC O157 in both a commercial feedlot environment (Smith et al., 2001) and on the farm (Garber et al., 1999). Flushing alleyways to clear away manure on dairy farms increased the risk of finding STEC O157 by 8 times compared to clearing away manure by other removal methods (Garber et al., 1999). Fecal shedding of STEC O157 by commercial feedlot cattle was more commonly associated with muddy pen conditions as compared to drier more ideal surface conditions (Smith et al., 2001; Sargeant et al., 2004). This again was illustrated in a feedlot trial utilizing ropes devices to detect STEC O157 positive pens. Cattle fed in the winter were more likely to test ropes positive if the pen conditions were dry (OR= 3.09) or wet (OR=3.88) compared to ideal (Smith et al., 2005). Wet bedding in pens was also associated with a greater odds of recovering STEC O157 from pen floor fecal pats of cattle housed in doors in farms in England and Wales (wet bedding OR= 3.43, very wet OR=4.24) (Ellis-Iversen et al., 2007).
*Super-shedders*

A super-shedder is an animal that is colonized by, and excretes high levels of STEC O157 in the feces (Chase-Topping *et al.*, 2008). Super-shedders have been defined a couple of different ways, either as: 1) animals colonized at the terminal rectal region 2) animals shedding STEC O157 in the feces at levels > $10^3$ CFU/g or 3) animals shedding at levels in excess of other animals (Berry *et al.*, 2010a). Individual cattle that are super-shedders have a greater potential to disseminate the pathogen in the feedlot environment (Bach *et al.*, 2005a; Stephens *et al.*, 2008). For instance, pens of cattle with high level shedders were more likely to have higher mean fecal prevalence levels and higher mean rectal-anal junction colonization (Cobbold *et al.*, 2007). In the same study, the odds of cattle not shedding STEC O157 throughout the sampling period, when housed without a super-shedder were 5 times the odds of those housed with a super-shedder (Cobbold *et al.*, 2007). A quantitative model illustrating transmission dynamics at a Scottish farm described how eighty percent of STEC O157 fecal transmission arises from 20% of high-level shedders (Matthews *et al.*, 2006a; Matthews *et al.*, 2006b).

STEC O157 rectal anal junction colonization has been correlated with super-shedding status in cattle (Low *et al.*, 2005; Cobbold *et al.*, 2007). A greater likelihood of detecting lots of cattle fecal culture positive for STEC O157 were associated with colonization of STEC O157 at the terminal rectal region (Low *et al.*, 2005). Excessive excretion of STEC O157 in the feces has also been correlated with increased hide prevalence (Arthur *et al.*, 2007; Arthur *et al.*, 2009). One study showed that if pen prevalence levels were greater than 20%, likely possessing a super-shedder, then hide pen prevalence levels exceeded 80% (Arthur *et al.*, 2009). High level shedders
present on cattle liners destined for slaughter, may also increase the risk for carcass contamination (Fox et al., 2008).

At this point it is somewhat unclear as to why certain animals shed STEC O157 in the feces at higher concentrations. Factors leading to a greater incidence and duration of STEC O157 infection in animals are thought to play a role (Chase-Topping et al., 2008). Control of STEC O157 in the future may involve the identification and removal of super-shedders from pens of cattle prior to slaughter (Naylor et al., 2003).

**Other Stress Factors**

Weaning, heat, confinement, animal handling, cattle transport, and lairage are some of the factors that could induce stress in live animals (Fike et al., 2006). Stress can lead to suppression of immune function, causing animals to become more susceptible to pathogens (Brown-Brandl et al., 2009). The effect of stress on STEC O157 carriage by cattle is unclear.

A number of trials have indicated weaning results in increased carriage of STEC O157 in calves (Herriott et al., 1998; Garber et al., 1999; Bach et al., 2004; Chase-Topping et al., 2007). In a case-control study to identify risk factors explaining STEC O157 shedding in dairy calves, weaning was significant. After weaning, dairy calves were 3 times more likely to shed STEC O157 in the feces compared to before weaning (Garber et al., 1999). Others reported abrupt compared to gradual weaning lead to increased persistence of STEC O157 in calves, 1.67% and 0.82%, median prevalence for abrupt and gradual weaning, respectively (Herriott et al., 1998). While in another study, pre-conditioning calves was found to reduce the likelihood for detecting
STEC O157 in the feces of calves following shipment for weaning (Bach et al., 2004). On Scottish farms, one of the risk factors for a super-shedder was identified as cattle stress due to weaning or movement (Chase-Topping et al., 2007). However, weaning was not observed to be a risk factor for recovery of STEC O157 in another Scottish study (Synge et al., 2003).

Heat and handling stress factors were not correlated with STEC O157 fecal shedding in a study evaluating heifers exposed to summer weather and chute confinement (Brown-Brandl et al., 2009). Others explored temperament of cattle, to discover if calm, intermediate, or excitable animals, where more predisposed to shedding STEC O157 (Schuehle et al., 2009). In the end, a higher proportion of calm animals shed STEC O157 as compared to more intermediate and excitable animals (Schuehle et al., 2009).

Events surrounding cattle shipment may increase fecal and hide prevalence of STEC O157. This is primarily thought to be due to exposure of susceptible animals to infected pen mates and contaminated trucks during transport (Arthur et al., 2007; Cuesta Alonso et al., 2007). Various studies have confirmed STEC O157 hide prevalence and concentration increases due to shipment of cattle for slaughter (Bach et al., 2004; Arthur et al., 2007; Dewell et al., 2008; Miller et al., 2008; Smith et al., 2009b). Conversely, others have found the probability to detect STEC O157 on the hides of cattle does not increase following transportation (Barham et al., 2002; Minihan et al., 2003; Reicks et al., 2007; Fegan et al., 2009; Stanford et al., 2011).

Aspects of shipment that have been shown to affect the recovery of STEC O157 from the hides or carcasses of cattle at post-harvest are the: distance cattle are hauled (Dewell et al., 2008), the presence of a high-shedder on the truck (Fox et al., 2008), and the use of a
commercial hauler. Cattle were three times more likely to have a contaminated hide if they were transported greater than 161 km to a packing plant versus less than 161 km (Dewell et al., 2008). Animals designated as high-shedders on truckloads destined for slaughter had a 16-fold increased odds for detection of STEC O157 on beef carcasses in a Midwestern packing plant (Fox et al., 2008). Hide cross-contamination was also significantly explained by the use of a commercial hauler during transport as compared to a non-commercial hauler (farmer) (OR=5.7, 95%CI 0.99-33) (Mather et al., 2008). Cross contamination of the hide was defined as the detection of a STEC O157 phage/VT type previously not identified from the farm of origin. Exposure to dust generated during a mock cattle handling and loading exercise caused an increase in the bacterial counts of STEC O157 (0.80 to 2.30 log MPN/cm²) on the hides of cattle although there was no effect on STEC O157 prevalence (Miller et al., 2008). Conversely, transportation factors such as temperature relative humidity index, length (time) of hauling, and number of animals in a truck were not significantly associated with hide contamination of heifers followed to slaughter in a western plains feedlot trial (Stanford et al., 2011).

Lairage is a holding area for cattle prior to harvest at packing facilities. The lairage environment has more recently been implicated as a significant source of STEC O157 for hide contamination (Arthur et al., 2007; Arthur et al., 2008; Mather et al., 2008). Using pulse field gel electrophoresis (PFGE) patterns, it was determined that postharvest STEC O157 genotypes off the hides of cattle were more often attributed to lairage than the feedlot environment (Arthur et al., 2008). Some research however, has shown that carriage of STEC O157 on the hides and in
the feces of cattle at lairage was actually lower than when cattle left the feedlot for harvest (Barham et al., 2002; Fegan et al., 2009).

**Sources & Transmission of STEC O157 to Cattle**

**Feedlot Environment**

STEC O157 is capable of surviving in the environment and being transmitted via water (LeJeune et al., 2001), soil (Ogden et al., 2001; Miller et al., 2008), manure (Kudva et al., 1998), slurry (Kudva et al., 1998), hides (Elder et al., 2000; Arthur et al., 2004), and feed (Lynn et al., 1998). Fecal shedding, and hide contamination result from exposure of cattle to environmental sources of STEC O157 (Smith et al., 2005; Arthur et al., 2009). Although, STEC O157 may also be recovered from the feedlot environment from where cattle are being housed and test fecal culture negative (Davis et al., 2005). Once cattle are exposed to STEC O157, oral-ingestion may occur, which leads to shedding of the pathogen in the feces. Fecal shedding increases the dose load of STEC O157 in the environment for further transmission. Contaminated cattle hides are also important for the spread of the pathogen to pen mates during commingling events. During the slaughter process the hides have been identified as the primary source for carcass contamination. Therefore identifying and understanding sources for STEC O157 survival and persistence may be important for reducing the maintenance of the pathogen in the beef production environment. This section will discuss research surrounding non-bovine reservoirs for STEC O157.
**Water**

STEC O157 may be found in water sources in the beef production environment, particularly water troughs (Faith *et al.*, 1996; Hancock *et al.*, 1998; Van Donkersgoed *et al.*, 2001; LeJeune *et al.*, 2001). Cross-sectional prevalence values of STEC O157 in water troughs from U.S. and Canadian cattle operations have been reported at 3.1%, 1.3%, and 12% (Hancock *et al.*, 1998; LeJeune *et al.*, 2001; Van Donkersgoed *et al.*, 2001). Data from an in vitro lab experiment confirmed STEC O157 were capable of surviving for long periods of time in water samples at relatively cold temperatures (Wang *et al.*, 1998b). After 6 months, STEC O157 isolated from sediments in an experimental microcosm remained infectious to calves (LeJeune *et al.*, 2001). Water troughs were therefore hypothesized to serve as a source and play a role in the dissemination of STEC O157. Others identified, similar STEC O157 isolates among water samples and fecal samples from cattle operations using molecular sub typing. Similar isolates suggests there may be a common source of STEC O157 on farms (Faith *et al.*, 1996; Hancock *et al.*, 1998; Shere *et al.*, 1998; Van Donkersgoed *et al.*, 2001).

In a cross-sectional study of 5 Midwestern feedlots, the probability of recovering STEC O157 from pens of cattle was not associated with culture positive water or feed samples from a pen. There was also no relationship with fecal recovery of STEC O157 and temperature, pH, or cleanliness of the water trough (Smith *et al.*, 2001). Similarly others found, drinking water management practices (emptying and cleaning water troughs) were not related with the risk for STEC O157 carriage in live cattle (Ellis-Iversen *et al.*, 2009). These studies, suggest water troughs may not be as an important of a source of STEC O157 as originally believed.
Manure

Contaminated cattle feces are likely the main vehicle for STEC O157 exposure to other cattle. For instance, dissemination of STEC O157 from experimentally inoculated calves to uninfected pen mates has been attributed to fecal-contaminated hides and pen floors (Cobbold et al., 2002). Cattle manure is capable of supporting STEC O157 survival under a variety of conditions (Kudva et al., 1998; Fukushima et al., 1999). Over a 21 month period, STEC O157 was reported to survive in an ovine manure pile exposed to the natural environment (Kudva et al., 1998). In vivo, persistence of STEC O157 inoculated into manure samples (10⁵ CFU/g) occurred for 70 days at 5°C. At 37°C experimentally inoculated STEC O157 was capable of surviving in manure samples for 49 days (Wang et al., 1996).

Slurry

Persistence of STEC O157 in cattle slurry (manure, urine, and water mixture) appears to be more limited (Hancock et al., 1994; Kudva et al., 1998). In vitro, the pathogen survived <9 days in slurry samples (Maule, 1997) and in vitro, aeration was determined to inhibit pathogen persistence in slurry samples (Kudva et al., 1998). STEC O157 was not detected in fecal/slurry samples taken from a survey of 47 dairy and beef herds in Washington State (Hancock et al., 1994). However, urine-contaminated animal bedding was capable of supporting STEC O157 survival in another report (Davis et al., 2005).
Soil and Dust

STEC O157 is known to survive in soil samples both in vivo and in vitro (Maule, 1999; Ogden et al., 2001; Islam et al., 2004; Miller et al., 2008). A study simulating cattle transport assessed dust generation and exposure as a factor contributing to STEC O157 and Salmonella populations on cattle hides. There was a significant increase in the concentration of these pathogens on cattle hides after exposure to dust but no effect on prevalence was seen. Altogether, 16% of pen soil samples and 30% of load out area soil samples were positive for STEC O157. Of the dust samples collected 6.6% were positive for STEC O157. The researchers concluded STEC O157 was persistent in the soil throughout the study and was likely a contributing factor to the increased concentration of STEC O157 on the hides of cattle after dust exposure (Miller et al., 2008).

Feed

STEC O157 has been isolated from feed commodities and feedbunks (Lynn et al., 1998; Shere et al., 1998; Van Donkersgoed et al., 2001; Smith et al., 2001; Dodd et al., 2003; Sanderson et al., 2006; Doane et al., 2007) and is capable of replicating in feeds (Lynn et al., 1998). One trial evaluated, 504 feedbunk samples collected from various feedlots for STEC O157 and found 75 positive (Dodd et al., 2003). A longitudinal study following twelve pens of cattle determined 1.25% of feed samples were positive for STEC O157 prior to animal access. After exposure of cattle to the feed, prevalence of STEC O157 in the feedbunk samples were increased to 3.25% (Sanderson et al., 2006). Cattle feces, saliva, and vermin, likely accounted for this slight increase in STEC O157 prevalence (Dodd et al., 2003).
The distribution of STEC O157 geographically was proposed to be from contaminated feeds (Rice et al., 1999). However, the probability of recovering STEC O157 from pens of cattle was not associated with cattle feed in a large survey of 3100 head in Midwestern feedlots (Smith et al., 2001). Additionally, the low probability of finding STEC O157 in feed samples demonstrates the low likelihood that feed is a primary means of cattle exposure (Berry et al., 2010a).

**Flies**

Flies are common to the feedlot environment, and have been hypothesized to serve as vectors for STEC O157 transmission. A number of research trials have isolated flies carrying STEC O157 from cattle environments (Hancock et al., 1998; Shere et al., 1998; Alam et al., 2004). This is not surprising because flies can be found on the hides of cattle throughout most of the summer months, which also coincides with peaks in STEC O157 fecal prevalence in cattle (Rasmussen et al., 2001). To evaluate flies as a source for spread of STEC O157, a trial was conducted where fruit flies, highly contaminated internally and externally with STEC O157, were given access to exposed apple tissues (Janisiewicz et al., 1999). After flies had 48 hrs of exposure to the tissue, a high rate of STEC O157 contamination was found in the apple tissues. This study confirmed that experimentally flies were capable of carriage of STEC O157 and transmission of the organism. Others reported challenged house flies excreted STEC O157 in the feces up to 3 days post-infection (Kobayashi et al., 1999). In vivo however, there is little evidence to suggest that flies are important in the transmission of STEC O157 to cattle (Berry et
Rather flies are simply co-infected in environments where STEC O157 exposure is high.

**Wild or Domestic Animals**

Another route of STEC O157 transmission is animal to animal. Cattle may be important for spreading the pathogen to other animals or possibly other animals play a role in the spread of STEC O157 to cattle. In both domestic and wild animals STEC O157 has been isolated (Kudva et al., 1996; Chapman et al., 1997; Wallace et al., 1997; Hancock et al., 1998; Shere et al., 1998; Sargeant et al., 1999; Cizek et al., 1999; Renter et al., 2001; Feder et al., 2003; Renter et al., 2003; Nielsen et al., 2004; Keen et al., 2006; Doane et al., 2007).

**Pre-Harvest Interventions**

Currently the primary method of controlling STEC O157 carcass contamination is within slaughter facilities post-harvest (Loneragan et al., 2005). However, some have indicated high pathogen loads on the hides of cattle during slaughter may overwhelm post-harvest interventions leading to increased STEC O157 carcass contamination (Elder et al., 2000; Arthur et al., 2004; Loneragan et al., 2005). Survival of STEC O157 in the environment also remains unaffected by post-harvest interventions. Interventions that mitigate pathogen carriage in live cattle prior to harvest have the potential to target both environmental and cattle gut populations of STEC O157. Management strategies aimed at decreasing sources of STEC O157 in the feedlot environment include cleaning water troughs (LeJeune et al., 2004) and feedlot pen floors (Smith, unpublished data). Unfortunately, these strategies have been somewhat unsuccessful at reducing STEC O157 carriage in beef cattle. Pre-harvest technologies targeting STEC O157 in the gut of animals
through competitive microorganisms, chemicals, feed additives, and immune modulation are also being considered. Some of these pre-harvest technologies have proven successful, such as vaccination and DFM treatment (Younts-Dahl *et al.*, 2004; Potter *et al.*, 2004). In the following section, efficacy of various pre-harvest interventions tested in live cattle will be discussed. A few of these interventions are available for use but many still require further development or FDA approval before being implemented.

**Other Management Practices**

Strategies for controlling STEC O157 carriage in live cattle fall under pre-harvest management practices and pre-harvest technologies (Loneragan *et al.*, 2005). In general, good management practices are important in beef production for maintaining animal health and welfare (Callaway, 2011). Management strategies may also be beneficial for reducing STEC O157 fecal shedding in live cattle because they are easily implementable, practical, and economical in comparison to new pre-harvest technologies (Loneragan *et al.*, 2005). Some of the management strategies that have been tested in vivo include; clean bedding, condition of the pen floor, cleaning of water troughs, and reduced animal density. Unfortunately, the majority of commercial field studies indicate management practices show little benefit in reducing carriage of STEC O157 by cattle (Dargatz *et al.*, 1997; LeJeune *et al.*, 2007; Cobbaut *et al.*, 2009; Berry *et al.*, 2010b).

In twenty commercial dairy farms, the probability of recovering STEC O157 from the feces of cattle housed with sawdust compared to sand bedding were evaluated. Herds bedded in pens with sawdust were 2.2 times more likely to shed STEC O157 in the feces compared to cattle
bedded in sand (LeJeune et al., 2005). Using a quantitative model, hygiene defined as replacing bedding frequently and cleaning of water troughs, was tested for an association with reduced fecal shedding in cattle. A greater than 89% reduction in STEC O157 herd fecal prevalence was simulated from the model. However, this was only when hygiene was used in conjunction with one other pre-harvest intervention (Vosough et al., 2007). Conversely, in vivo cleaning of water troughs in feedlot pens has not been a successful management strategy for reducing STEC O157 carriage in cattle (Smith et al., 2002).

The condition of the feedlot pen surface has been associated with the probability of detecting STEC O157 culture positive animals in a pen (Smith et al., 2001; Smith et al., 2005). Average pen prevalence for cattle housed in muddy, dusty, and normal pen surfaces conditions were reported at 22.4%, 17.9%, 6.5%, respectively (Smith et al., 2001). Under less ideal pen surface conditions (muddy or dusty), it is believed that the survival of STEC O157 may increase the inoculum dosage that pen mates may ingest orally, resulting in increased carriage of STEC O157 by cattle (Smith et al., 2005). Others have evaluated methods for reducing STEC O157 in cattle populations by scraping surface material in pens (Smith, unpublished data). Pen scraping was not found to be effective, nor was utilizing pens with pond ash as the surface material compared to standard soil surface material (Berry et al., 2010b). Contaminated cattle liners have also been implicated in exposing cattle to STEC O157 (Cuesta Alonso 2007). Cleaning of cattle liners may be a management practice that reduces the potential risk for STEC O157 transmission (Cuesta Alonso et al., 2007).
In a randomized controlled trial evaluating pre-harvest control measures, a closed herd reduced STEC O157 spread (Ellis-Iversen et al., 2008). Other researchers have found not introducing new animals into herds does not prevent the spread of STEC O157 (Cobbaut et al., 2009). A stochastic simulation model suggested reducing animal density in pens would likely decrease the recovery of STEC O157 from live cattle (Stacey et al., 2007), although a cross-sectional study in 100 feedlots found no association with animal density and fecal shedding (Dargatz et al., 1997). Another management strategy that has been recommended is the testing and removal of super-shedders either during the finishing stage in the feedlot or at time of shipment for slaughter (Cobbold et al., 2007; Arthur et al., 2010b). However, presently studies have not been conducted to determine if removing high-shedders from pens will be an effective method for controlling STEC O157 in beef populations.

**DFMs, Prebiotics and Competitive Exclusion**

Feeding direct fed microbials (DFM) to live cattle has been proposed as an approach to reduce STEC O157 in the feces of feedlot cattle. Direct fed microbials are made-up of living and naturally occurring bacteria, or fungi (Yoon et al., 1995). They act to stimulate or inhibit growth of microorganisms in the rumen or gastrointestinal tract of cattle (Krehbiel et al., 2003). As a result, feed efficiency and animal health may be greatly improved from feeding these products to cattle (Krehbiel et al., 2003). A variety of research studies have evaluated DFM as a pre-harvest intervention against STEC O157 carriage in live cattle in commercial feedlots (Elam et al., 2003; Brashears et al., 2003; Younts-Dahl et al., 2004; Younts-Dahl et al., 2005; Peterson et al., 2007a; Stephens et al., 2007a; Stephens et al., 2007b; Tabe et al., 2008; Stephens et al., 2010;
Arthur et al., 2010a). One of the most commonly studied DFM to reduce STEC O157 fecal shedding and hide contamination in cattle is *Lactobacillus acidophilus* (LA) strain NP51, also called strain NPC747 (Elam et al., 2003; Brashears et al., 2003; Younts-Dahl et al., 2004; Younts-Dahl et al., 2005; Peterson et al., 2007a; Stephens et al., 2007a; Stephens et al., 2007b; Tabe et al., 2008). Other bacterial and yeast products administered to target STEC O157 in live cattle include *Lactobacillus cristatus*, *Propionibacterium freudenreichii*, *Bacillus subtilis* and *Saccharomyces cerevisiae* (Brashears et al., 2003; Stephens et al., 2010; Arthur et al., 2010a).

In several feedlot trials addition of LA NP51 (10⁹ CFU/animal/day) to finisher rations has demonstrated a reduced odds for detecting STEC O157 in the feces of cattle by 49%-77% compared to control cattle (Brashears et al., 2003; Younts-Dahl et al., 2004; Younts-Dahl et al., 2005; Stephens et al., 2007a; Stephens et al., 2007b; Tabe et al., 2008). Another trial showed two independent groups of cattle receiving LA NP51 throughout a 2-year period were 35% less likely to shed STEC O157 in the feces versus cattle receiving no LA NP51 (Peterson et al., 2007a). Some have also reported a linear decrease in the proportion of cattle shedding STEC O157 in the feces when cattle were fed low, medium, and high concentrations of LA NP51 (Younts-Dahl et al., 2004; Younts-Dahl et al., 2005; Stephens et al., 2007b). Conversely, others found steers supplemented with LA NP51 in increasing dosages did not exhibit a dose effect. In this study, low (10⁷) and high (10⁹) LA NP51 treatments significantly reduced fecal shedding of STEC O157 over controls. However the medium (10⁸) LA NP51 treatment was not effective (Stephens et al., 2007b). Similarly, *L. cristatus and B. subtilis* were not capable of reducing
STEC O157 carriage pre-harvest when fed to cattle in finishing trials (Brashears et al., 2003; Arthur et al., 2010a).

Studies examining combinations of bacterial strains in DFM products have demonstrated inconsistent results at lowering STEC O157 carriage in live cattle (Elam et al., 2003; Younts-Dahl et al., 2004; Younts-Dahl et al., 2005; Stephens et al., 2007a). Cattle administered LA NP45 and 51 strains in combination were no more likely to have reduced fecal shedding of STEC O157 compared with non-DFM treated cattle (Elam et al., 2003; Younts-Dahl et al., 2004; Younts-Dahl et al., 2005), while strains LA NP35 and 51 significantly reduced the overall fecal concentration and proportion of cattle with STEC O157 in the feces compared with non-DFM fed cattle (Stephens et al., 2007a). Cattle fed a L. acidophilus and S. cerevisiae diet were tested against traditionally fed cattle with no DFM, however detectable levels of STEC O157 in fecal and hide swab samples were not different between the two groups (Stephens et al., 2010).

Other DFM of interest for reducing STEC O157 in the feces of cattle are non-pathogenic E. coli strains that produce colicins. Colicins are toxic compounds produced by commensal E. coli and other members of the Enterobacteriaceae family to destroy competing bacteria (Cascales et al., 2007). Many of them function by binding to and killing other bacteria through one of the following methods; inhibiting protein synthesis, hydrolyzing RNA, cleaving DNA or enabling production of other macromolecules of the target cell (Cascales et al., 2007). In vitro, a number of colicin strains have been identified that could act inhibitory against STEC O157 in the gastrointestinal tract of live cattle (Zhao et al., 1998; Schamberger et al., 2002). Experimental challenge studies have also suggested that colicins fed to cattle may be useful for reducing fecal
shedding of STEC O157 (Tkalcic et al., 2003; Schamberger et al., 2004). STEC O157 has the potential to become resistant to single strain colicins (Schamberger et al., 2005). However, the potential for resistance with multiple strain colicins was greatly reduced when tested in vitro (Schamberger et al., 2005). Currently, there have been no experimental field studies assessing efficacy of colicins at reducing carriage of STEC O157 in live cattle.

The correlation of STEC O157 hide prevalence with carcass contamination has led researchers to evaluate DFM for reducing STEC O157 on the hides of cattle. In two different studies, the presence of STEC O157 on cattle hides was decreased by 88% and 74% in LA NP51 treated cattle as compared to control non-DFM treated cattle (Brashears et al., 2003; Stephens et al., 2007b). Recovery of STEC O157 from the hides of cattle was not affected by DFM treatment in other reports (Younts-Dahl et al., 2005; Stephens et al., 2010; Arthur et al., 2010a).

Overall DFM supplementation to reduce STEC O157 prevalence in live cattle does not appear to affect carcass characteristics or feedlot performance of study animals (Elam et al., 2003; Brashears et al., 2003; Younts-Dahl et al., 2004; Peterson et al., 2007a; Stephens et al., 2010). A DFM product, consisting of LA NP51 has been approved for commercial application in U.S. feedlots and appears to be widely used today (Callaway et al., 2004).

**Diet**

The extent to which specific diets impact STEC O157 colonization and fecal shedding in live cattle is unknown. Part of the complexity of the relationship may be due to variation between individual animals, feed components, and the environment (Jacob et al., 2009). For
example, season may influence the recovery of STEC O157 from the feces of feedlot cattle. Also, many beef production systems utilize different grain types and processing methods, based on price and availability. Because of this, a variety of different diets are fed to cattle, and often the diets may consist of commodities that vary in nutritional value. With the variability in rations fed, it becomes difficult for researchers to directly identify specific diets or dietary components that are associated with STEC O157 fecal shedding in cattle. Dietary components and management feeding practices suggested to influence STEC O157 fecal shedding include; forages, fasting, grain type, extent of grain processing, and by-product use. For a review of the literature on the effect of diet against STEC O157 carriage in live cattle refer to the appendix.

STEC O157 primarily inhabits and colonizes the gastrointestinal tract of cattle (Naylor et al., 2003; Naylor et al., 2005). Consequently, carriage of STEC O157 by live cattle may be affected by 1) dietary components that physiologically alter the hindgut environment or; 2) nutrients available in the gut that are used as energy sources for STEC O157 survival (Fox et al., 2007; Klopfenstein et al., 2008). Environmental conditions in the hindgut of cattle could be a result of dietary factors such as, the level of: pH, VFAs, ammonia, oleic acid, or L-lactate (Berg et al., 2004; Bach et al., 2005b; Fox et al., 2007; Klopfenstein et al., 2008; Wells et al., 2009; Jacob et al., 2009). The presence and quantity of these dietary factors in the gut of cattle have been suggested to act anti-bactericidal against STEC O157. An alternate hypothesis may be that dietary nutrients, such as starch and fiber serve as energy sources for survival of STEC O157 in the hindgut region (Klopfenstein et al., 2008; Wells et al., 2009). Or rather, competing gut microbial populations may preferentially utilize these dietary nutrients for survival. With further
understanding of microbial populations and conditions of the gastrointestinal tract of cattle, methods to reduce STEC O157 pre-harvest through diet may become possible.

**Orange Peels**

Cattle rations often include byproducts that can add value and nutritional quality to the ration (Callaway, 2011). In some areas of the US, orange peels and citrus pulp are fed to cattle as a by-product feed. Researchers have had some interest in citrus products fed to cattle because of their antimicrobial properties. In vitro, STEC O157 inoculated into ruminal fluid samples was reduced when orange peel and pulp were added at levels above 1% (w/v) (Callaway et al., 2008a). Feeding citrus products to sheep (5%-10% DM Basis) and pigs (10% As Fed Basis) in preliminary challenge studies have also indicated STEC O157 may be reduced in the GI tract (Collier et al., 2010; Callaway et al., 2011). At this point no commercial studies have focused on evaluating citrus products as a pre-harvest intervention against STEC O157 in beef cattle.

**Phenolic Compounds**

Phenolic acids are antimicrobials produced by plants. Plants release phenolic acids to inhibit bacterial pathogens (Levin, 1976). Examples of these compounds include tannins and lignin (Callaway, 2011). In vitro, phenolic acids have significantly reduced the survival of STEC O157 populations (Wells et al., 2005; Min et al., 2007; Wang et al., 2009). When STEC O157 inoculated manure samples were incubated with trans-cinnamic acid, para-coumaric acid, and ferulic acid and compared to control samples, reductions in STEC O157 populations were shown (Wells et al., 2005). Results from an experimental challenge study also indicated tannin
supplementation significantly reduced fecal shedding of generic *E. coli* in cattle compared to controls (Min *et al.*, 2007).

Tasco-14 is a brown seaweed product (*Ascophyllum nodosum*) supplemented to cattle to reduce STEC O157 populations. Phlorotannins in the seaweed are thought to be responsible for the anti-oxidant effect of Tasco-14 on STEC O157 (Allen *et al.*, 2001). Administration of Tasco-14 is also reported to improve carcass characteristics, and shelf life of beef (Braden *et al.*, 2007). A natural exposure study found Tasco-14 included in the finishing ration at 2% just prior to slaughter compared to no Tasco-14 in the finishing ration, was significantly protective against STEC O157 fecal (RR=0.31, *p*<0.05) and hide prevalence (RR=0.21, *p*<0.05) of cattle (Braden *et al.*, 2004). Similarly, STEC O157 fecal and hide levels were decreased when Tasco-EX was fed to cattle (Behrends *et al.*, 2000). A comparison in experimentally inoculated calves of Tasco at: 1) 10 g/kg for 14 days; 2) 20g/kg for 7 days; 3) 20g/kg for 14 days and 4) 0 g/kg (control), found the most promising treatment was the 20 g/kg level of Tasco™ fed for 7 days. This treatment reduced both the duration and concentration of STEC O157 in the feces of cattle (Bach *et al.*, 2008).

**Bacteriophages**

Bacteriophages or phages are highly specific bacterial viruses naturally found in the environment. Often phages inhabit reservoirs populated by bacteria, in mammals they are commonly recovered from the gastrointestinal tract (Klieve *et al.*, 1988). Bacteriophages are also non-toxic to mammals and show promise as antimicrobial agents against bacterial pathogens (Alisky *et al.*, 1998). In beef production particularly, these viruses have received attention as an
intervention to reduce STEC O157 carriage in the gut and on the hides of cattle (Callaway, 2011). Currently there is a phage product (Finalyse™, Elanco Food Solutions) available on the market for reducing STEC O157 on the hides of cattle immediately prior to slaughter (Callaway, 2011). Phage products are still however in developmental stages for preharvest control of STEC O157 on the farm. These products have been evaluated in preliminary reports, mainly conducted in a laboratory setting or in a challenge study design.

Phage therapy thus far has aimed at reducing STEC O157 populations in the gastrointestinal tract of experimentally inoculated cattle (Sheng et al., 2006; Rozema et al., 2009; Stanford et al., 2010; Rivas et al., 2010), sheep (Bach et al., 2003; Raya et al., 2006; Callaway et al., 2008b; Bach et al., 2009), and mice (Tanji et al., 2005; Sheng et al., 2006). Researchers have documented a successful decrease in the concentration (Sheng et al., 2006; Callaway et al., 2008b), and duration of STEC O157 in the feces (Stanford et al., 2010) of cattle provided with a phage treatment. However, others have found no difference between phage treatment and no-phage treatment in the recovery of STEC O157 from the feces of cattle (Rozema et al., 2009; Rivas et al., 2010). Similarly, sheep studies have indicated variable results following phage treatment. Sheep supplemented with phage possessed either a significantly lower concentration of STEC O157 in the feces (Bach et al., 2009) or were no different from control sheep in the concentration of STEC O157 in the feces (Bach et al., 2003; Raya et al., 2006). The successful reduction of STEC O157 in one of these study comparisons was reportedly 22% in rams receiving phage as compared to rams receiving no-phage (Bach et al., 2009).
Inconsistent results of the phage treatment in these challenge studies may be partly due to the method of delivery of the virus product. A goal of some researchers has been to determine an effective means of delivering the phage to the gastrointestinal tract of the animal for targeting a meaningful decline in STEC O157 populations (Stanford et al., 2010). Application methods tested include; oral administration: either via a bolus, top dress in the feed, or in the drinking water (Stanford et al., 2010; Rivas et al., 2010); rectal administration: by applying phage to the rectal anal junction region of the animal (Rozema et al., 2009); or some combination of these oral and rectal applications (Rozema et al., 2009). An assessment of oral supplementation (bolus vs top-dressed), found administration of phage via a bolus decreased fecal shedding of STEC O157 in phage treated cattle over control non-phage treated cattle (Stanford et al., 2010). Also effective for reducing STEC O157 in the fecal matter of cattle, was the addition of phage to the drinking water in conjunction with the addition of phage to the rectal anal junction region of the animal (Sheng et al., 2006).

The mechanism of bacterial infection by phages may be useful for determining the long term relevance of phage therapy for pre-harvest food safety against STEC O157. Phage infection is initiated through the attachment of the virus to specific cellular receptors of the bacteria. Once attached the phage inserts its genetic material into the bacterial cell where multiplication of phage viruses can occur. Lysing of the bacterial cell then follows for lytic phages, causing the release of new virus particles. Some phages however, are not lytic, and there is evidence that these non-lytic phages are capable of integrating their genome into the bacterial genome. This is of interest to researchers since the integration process may lead to the introduction of new
virulence genes in the STEC O157 genome. New virulence genes within the STEC O157 genome could pose a risk to humans (Callaway, 2011).

Another potential drawback of phage treatment against STEC O157 is resistance. It is believed however, that a cocktail of phages, specific to all strains of STEC O157, may mitigate the risk for development of bacterial resistance. In vitro work particularly, has been important for showing phage cocktails are capable of destroying STEC O157 cell cultures and may also suppress phage resistance (Kudva et al., 1999; Tanji et al., 2004). Additionally in vivo studies utilizing a combination of phages appear to be effective in controlling STEC O157 (Sheng et al., 2006; Callaway et al., 2008b; Bach et al., 2009).

Understanding the relationship between specific STEC O157 phages and STEC O157 in the environment may be important for developing phage therapy as a pre-harvest intervention. Observational reports have indicated STEC O157 specific phage are transient similar to STEC O157 in the feedlot environment (Niu et al., 2009). Others have hypothesized that the presence of phages may explain some of the transient shedding of STEC O157 by cattle (Oot et al., 2007). For instances, the likelihood of an animal shedding STEC O157 in the feces was determined to decrease if phage were present at high prevalence levels in the feedlot pen environment (Niu et al., 2009). Environmental factors may play a role in the survival of STEC O157 phage in the feedlot environment, as well. The recovery of phage in two commercial feedlots was significantly more likely among manure slurry samples as compared to fecal pat samples, rectal samples, and water samples (p<0.001) (Niu et al., 2009).
Despite the concerns for STEC O157 resistance and the passage of virulence genes to STEC O157 through phage, phage therapy to reduce this pathogen in cattle is promising. Further understanding of the relationship between phage and STEC O157 persistence in the environment may also prove useful.

**Antibiotics**

Research to date is limited on use of antibiotics against STEC O157 fecal shedding in live cattle. Antibiotics, such as neomycin sulfate and ceftiofur are another means for controlling STEC O157 populations in cattle, although controversial due to resistance issues in humans. Neomycin sulfate is a member of the aminoglycoside family, and resembles the antibiotics streptomycin, kanamycin, and gentamycin, commonly administered as a treatment to humans (LeJeune et al., 2007). Currently neomycin sulphate is approved for feeding to cattle, and may be administered in the water or feed. However, cattle being fed neomycin sulphate must be withdrawn from the antibiotic 24 hrs prior to shipment for harvest (LeJeune et al., 2007). In vivo studies have indicated STEC O157 shedding in the feces of cattle reduces over the short term feeding of neomycin sulphate (Elder et al., 2002; Ransom et al., 2003; Woerner et al., 2006). Alternately, experimentally challenged calves fed milk replacer with an antibiotic (neomycin and oxytetracycline) did not demonstrate reductions in STEC O157 in the feces compared to calves given milk replacer with no antibiotic (Alali et al., 2004).

**Ionophores**

Ionophores are antimicrobial products that are used in cattle feedlot production systems most commonly to improve production and efficiency (Vogel, 1995). These products directly
inhibit gram-positive bacteria in the rumen of the animal causing reduced variation in feed intake and reduced variation in rumen pH, leading to improved weight gain and feed efficiency. Some of these products are also approved for prevention of coccidiosis (Watkins et al., 1986). In the U.S. commercially available ionophores include monensin, lasalocid, and laimedlomycin propionate. Although ionophores are recognized as safe and widely used in feedlot operations today, there is some concern that these compounds may competitively select for gram negative bacteria such as STEC O157 and serve as a risk factor for greater STEC O157 carriage in live cattle (Edrington et al., 2003c; McAllister et al., 2006).

Researchers have documented that ionophore feeding may have an effect on the prevalence of STEC O157 in live cattle (Herriott et al., 1998; Van Baale et al., 2004; Lefebvre et al., 2005; McAllister et al., 2006). For instance, an evaluation of 36 dairies determined herds more likely to be culture positive for STEC O157 were also significantly more likely to be supplemented with monensin and lasalocid (RR=1.45) (Herriott et al., 1998). Similarly, monensin treated cattle were more likely to shed STEC O157 in the feces compared to cattle receiving no monensin. However this relationship was only significant on one sampling date throughout the test period (Lefebvre et al., 2005). In contrast, a reduced proportion of ruminal fluid and fecal samples testing positive for STEC O157 were associated with ionophore feeding from forage fed cattle (Van Baale et al., 2004; McAllister et al., 2006). This same effect with ionophore feeding was not seen in ruminal fluid and fecal samples collected from grain fed cattle (Van Baale et al., 2004; McAllister et al., 2006).

While some reports have indicated ionophore feeding may affect STEC O157 populations, the majority of reports assessing ionophore feeding in cattle and sheep have
demonstrated no association with STEC O157 fecal shedding (Garber et al., 1995; Dargatz et al., 1997; Bach et al., 2002; Edrington et al., 2003b; Edrington et al., 2003c; Sargeant et al., 2004; Dewell et al., 2005; McAllister et al., 2006; Edrington et al., 2006c; Jacob et al., 2008; Swyers et al., 2011). Observational studies both on dairy farms and at commercial feedlots found no relationship between ionophores, supplemented in the feed at the time of sampling, and shedding of STEC O157 in the feces of cattle (Garber et al., 1995; Dargatz et al., 1997; Sargeant et al., 2004; Dewell et al., 2005). Others reported cattle randomized to ionophore and control treatments showed no difference in risk for STEC O157 in the feces under conditions of natural exposure (Edrington et al., 2006c; Jacob et al., 2008; Swyers et al., 2011). Similarly, grazing calves fed a mineral supplement with lasalocid or a mineral supplement with no lasalocid for 60 days did not differ in the incidence of STEC O157 by treatment (Edrington et al., 2006c). Even when STEC O157 was grown in pure culture and mixed ruminal fluid there was no affect on STEC O157 populations after addition of commercial ionophores (Bach et al., 2002; Edrington et al., 2003c) at ruminal concentrations approved for feeding in cattle (Bach et al., 2002). To date the balance of evidence suggests ionophores present no increased risk for STEC O157 fecal shedding by cattle.

**Beta-Agonists**

β-Agonists are molecules capable of binding to muscle and fat cell β-adrenergic receptors, subsequently leading to increased protein accretion and decreased fat synthesis (Ricks et al., 1986). When supplemented to market ready cattle β-agonists increase carcass weight, dressing percentage, and feed efficiency (Vogel et al., 2009). β-agonist feeding was initially
hypothesized to have stimulatory effects on the presence of STEC O157 in the feces of cattle (Edrington et al., 2006b). This was hypothesized because STEC O157 utilizes a system of bacterial cell-to-cell signaling known as quorum sensing. During this signaling process STEC O157 virulence genes are up regulated. Quorum sensing is mediated by catecholamine hormones, and β–agonists structurally resemble these hormones. Since β-agonists are fed just prior to slaughter, this could result in increased risk of STEC O157 spilling over to the post-harvest sector.

Currently there are two β-agonists approved for feeding to beef cattle; ractopamine and zilpaterol. Ractopamine (Optaflexx™, Elanco Animal Health), was licensed in 2003, and can be fed 28-42 days before slaughter with no necessary withdrawal. Zilpaterol (Zilmax™, Intervet/Schering-Plough Animal Health, Millsboro, DE) a more recently approved β-agonist may be fed to cattle 20-42 days prior to harvest but has a 3-day withdrawal requirement. Findings from reports evaluating ractopamine feeding in cattle have either found no association with STEC O157 fecal shedding (Edrington et al., 2009; Paddock et al., 2011) or reduced fecal shedding of STEC O157 (Edrington et al., 2006b). Over 28 days, cattle housed in small pens and fed ractopamine were 28% less likely to shed STEC O157 in the feces than control non-ractopamine supplemented cattle housed in small pens (p=0.0006) (Edrington et al., 2006b). While a large pen commercial trial found 2 out 3 replicates of cattle receiving either 0 mg/hd/day ractopamine or 200 mg/hd/day ractopamine where not different in respect to the probability of detecting STEC O157 in the feces (Edrington et al., 2006b). The third replicate however reported significantly reduced probability of recovering STEC O157 in the feces of the ractopamine 200 mg/hd/day treated cattle. Overall β-agonist supplementation was reported as effective at
reducing STEC O157 populations in the feces of live cattle in this study (Edrington et al., 2006b). Another randomized control trial indicated no association between STEC O157 fecal shedding and ractopamine feeding in cattle, with 4.4% and 4% fecal prevalence in the ractopamine treatment and non-ractopamine treatment, respectively (p=0.89) (Paddock et al., 2011).

Studies evaluating zilpaterol feeding on the effect of STEC O157 carriage in live cattle have been two-fold. Some field studies showed no association between STEC O157 fecal shedding (Edrington et al., 2009) and zilpaterol feeding in cattle while in another study zilpaterol feeding increased STEC O157 fecal shedding (Edrington et al., 2009). In the latter study, zilpaterol supplementation increased the probability of recovering STEC O157 fecal positive samples in cattle by 70% over the non-zilpaterol supplemented group (Edrington et al., 2009). Altogether these research findings suggest β-agonist (zilpaterol and ractopamine) feeding in cattle prior to harvest have minimal effects on the fecal prevalence of STEC O157.

**Sodium Chlorate**

Another strategy for reducing STEC O157 populations in cattle is the supplementation of sodium chlorate in feed or water supplies. At low concentrations sodium chlorate is not harmful to cattle (Anderson et al., 2002; Callaway et al., 2002; Anderson et al., 2005), but has bactericidal properties against STEC O157 and other pathogenic bacteria that contain the enzyme nitrate reductase (Anderson et al., 2000). Nitrate reductase is important for anaerobic respiration in these bacteria (Stewart, 1988; Anderson et al., 2000). The enzyme normally helps the bacteria reduce nitrate to nitrite but is also capable of reducing chlorate to chlorite (Stewart, 1988;
Anderson et al., 2000). Chlorite has been shown to be toxic to STEC O157, but harmless to potentially beneficial bacterial populations (Anderson et al., 2000; Anderson et al., 2002). When tested in challenge studies, cattle, sheep, and pigs, orally supplemented with sodium chlorate demonstrated significantly reduced concentrations of STEC O157 throughout their gastrointestinal tract (Anderson et al., 2001; Callaway et al., 2001; Callaway et al., 2002; Callaway et al., 2003; Edrington et al., 2003a).

Use of sodium chlorate as a pre-harvest intervention has been suggested for short-term supplementation immediately prior to slaughter (Anderson et al., 2000; Edrington et al., 2003a). A natural exposure trial indicated sodium chlorate treatments supplied in the feed 1- week prior to harvest resulted in cattle less likely to carry STEC O157 in the feces compared to cattle receiving no treatment in their feed (Anderson et al., 2005). In this study sodium chlorate was also evaluated in drinking water 12 hrs prior to shipment and was not found to affect STEC O157 levels in rumen, fecal, or hide samples. Conversely, cattle with access to sodium chlorate treated water 24 hours prior to shipment for slaughter possessed decreased levels of experimentally infected STEC O157 in their feces and rumen compared to animals not receiving sodium chlorate treated water (Callaway et al., 2002).

Sodium chlorate supplementation to cattle may be an effective means for reducing the overall presence of STEC O157 in the gastrointestinal tract of cattle without detrimental effects on commensal bacteria (Anderson et al., 2000). Short-term feeding of this product to food-animals immediately prior to harvest has been recommended (Anderson et al., 2000). At this time sodium chlorate is not approved for use in cattle (Callaway, 2011).
**Water Treatment**

To reduce the survival and transmission of STEC O157 from water troughs chlorination, electrolyzed oxidation, and sodium chlorate treatment have been suggested. In a commercial feedlot trial chlorination was not effective at reducing STEC O157 from water troughs (LeJeune *et al*., 2004), even though 17% of water samples from the chlorinated water troughs and 26% of water samples from the non-chlorinated water troughs were positive for STEC O157. Chlorination was determined to be ineffective because feed particles and sediments accumulated in the water trough, causing inactivation of the biocidal activity of chlorine (LeJeune *et al*., 2004). Evidently, electrolyzing oxidizing water was effective in vitro against $10^4$ CFU/mL of STEC O157 (Stevenson *et al*., 2004). Of the in vivo studies evaluating sodium chlorate in the water, one study found reduced STEC O157 fecal shedding in treated cattle compared to controls (Callaway *et al*., 2002). Studies demonstrating efficacy are necessary before water treatments may be considered potentially effective pre-harvest interventions.

**Vaccines**

Pre-harvest vaccines show promise in reducing STEC O157 in beef cattle populations (Snedeker 2011). Vaccination against STEC O157 carriage by cattle creates an unfavorable gut environment for pathogen survival, and in some studies has shown reductions in the presence of STEC O157 in the feces, on the hides, and at the TRM region (Smith *et al*., 2012). Mitigation of STEC O157 populations in the feces, on the hides, and at the TRM region of cattle could be important for reducing subsequent carcass contamination (Elder *et al*., 2000; Arthur *et al*., 2004). Currently there are two types of STEC O157 pre-harvest vaccines that have
become available for producers, the type three secreted proteins vaccine product (TTSP) and the siderophore and receptor porin (SRP) protein vaccine product. Both the TTSP and SRP vaccines work differently to inhibit STEC O157 populations in the gut of cattle. Immunization of cattle with TTSP proteins (EspA, EspB, Tir, Intimin) blocks the intimate adherence of STEC O157 to intestinal epithelial cells (Potter et al., 2004), while immunization of cattle with SRP proteins inhibits STEC O157 iron uptake by depriving the cell of required nutrients (Thornton et al., 2009). In live cattle both products have demonstrated efficacy at reducing the proportion of cattle testing culture positive for STEC O157 in the feces (Snedeker et al., 2011). Further, both products have been shown to have no effect on cattle performance (Peterson et al., 2007b; Peterson et al., 2007c; Thomson et al., 2009; Wileman et al., 2011).

Experimental Challenge Studies

A number of experimental challenge studies have evaluated STEC O157 pre-harvest vaccines in cattle, pigs, and lambs (Dean-Nystrom et al., 2002; Potter et al., 2004; Dziva et al., 2007; McNeilly et al., 2008; Thornton et al., 2009; McNeilly et al., 2010; Yekta et al., 2011; Allen et al., 2011). These include vaccine products that contain TTSP (Dean-Nystrom et al., 2002; Potter et al., 2004; Allen et al., 2011), flagellin (McNeilly et al., 2008), and SRP proteins (Thornton et al., 2009). Piglets inoculated with Shiga toxin negative STEC O157 and ingesting colostrum containing anti-intimin antibodies were protected from STEC O157 colonization and lesion formation (Dean-Nystrom et al., 2002). Others reported vaccination of cattle with supernatant proteins containing TTSP reduced the concentration, duration, and number of animals shedding STEC O157 in the feces (Potter et al., 2004). An evaluation of the same
vaccine product yielded similar results, a decrease in the concentration and number of animals shedding STEC O157, following challenge with $10^9$ CFU of STEC O157 (Allen et al., 2011). No differences were reported in the duration of fecal shedding between the calves vaccinated and non-vaccinated (Allen et al., 2011). Vaccination of challenged calves with a purified EspA protein was not effective at decreasing the concentration or duration of STEC O157 in the feces (Dziva et al., 2007). Cattle immunized with H7 flagella intramuscularly and mucosally at the terminal rectum region, have shown reduced colonization by STEC O157 (McNeilly et al., 2008). While a vaccine consisting of TTSP and H7 flagellin proteins was found to have lower efficacy in comparison to a TTSP alone (McNeilly et al., 2010). An assessment of the SRP vaccine product in challenged calves suggested a reduction in the concentration and prevalence of STEC O157 in the feces. However, these reductions were only approaching statistical significance (Thornton et al., 2009).

### Field Efficacy Studies

Trials evaluating the efficacy of STEC O157 pre-harvest vaccines have focused mainly on the commercially available TTSP and SRP vaccine products.

Data has suggested three doses of type three secreted proteins reduce fecal populations of STEC O157 in live cattle anywhere from 43% to 73% (Smith et al., 2012). Two-doses of a TTSP vaccine has also demonstrated efficacy against STEC O157 carriage in cattle (Peterson et al., 2007c; Moxley et al., 2009; Smith et al., 2009a; Smith et al., 2009b). Although, 2-doses of the vaccine were not effective at reducing fecal populations of STEC O157 in a large commercial trial conducted early in the development of the TTSP vaccine product (Van Donkersgoed et al.,
When comparing 2- and 3-dose regimen treatments there has been evidence of a dose effect, with greater efficacy indicated by the higher dosage of TTSP (Peterson et al., 2007c; Moxley et al., 2009). For instance, the probability of recovering STEC O157 from the feces of vaccinated cattle compared to control cattle was reduced 65%, and 33%, in the 3-dose regimen, and 2-dose regimen treatments, respectively (Moxley 2009). STEC O157 colonized at the terminal rectal region of cattle was also decreased by TTSP vaccination, 92% and 98% in two separate reports (Peterson et al., 2007c; Smith et al., 2009b). In addition, the probability of recovering STEC O157 from the hides of cattle was less likely through vaccination with 3-doses of a TTSP vaccine (Smith et al., 2009a).

A TTSP vaccine product may provide herd-level protection (Peterson et al., 2007c). Cattle unvaccinated that were penned with vaccinated cattle were 55% less likely to shed STEC O157 in the feces compared to cattle in pens that were unvaccinated (Peterson et al., 2007c). Environmental contamination, measured using ropes hung in pens for cattle to chew, may also be decreased with TTSP vaccination. For instance, the odds of recovering STEC O157 from ropes of vaccinated pens of cattle was 41% less than the odds of recovering STEC O157 from ropes of non-vaccinated pens of cattle (Smith et al., 2008). Vaccinating cattle over a wide region in the feedlot may also be important for reducing environmental levels of STEC O157 (Smith et al., 2009a). Regional vaccination was determined to be more effective for reducing hide contamination than commingling non-vaccinated and vaccinated cattle within a pen (Smith et al., 2009a). These studies demonstrate herd-level effects play a role in the efficacy of the vaccine,
which may useful when considering the practical applications of a pre-harvest vaccine in a feedlot environment (Smith et al., 2012).

There have been few studies assessing the practical application of the SRP vaccine in a feedlot setting (Fox et al., 2009; Thomson et al., 2009). In one of these studies, cattle were prescreened for STEC O157 in the feces prior to allocation to control, 2 mL, and 3 mL of an SRP vaccine. Cattle vaccinated with 3mL of SRP proteins were 47% less likely to shed STEC O157 in the feces compared to control cattle (Fox et al., 2009). Conversely, 2 mL of the SRP vaccine did not significantly reduce fecal shedding in vaccinated cattle compared to non-vaccinated cattle (Fox et al., 2009). While in another report three doses of the SRP vaccine decreased the recovery of STEC O157 from the feces of cattle by 85% at day 98 (Thomson et al., 2009). A 1.7 log reduction of the count of STEC O157 in the feces was also found in vaccinated cattle at day 98. No difference in the carriage of STEC O157 was detected between cattle receiving two-doses of the SRP vaccine and no vaccine in another report (Thomson et al., 2009). However on the last sampling date STEC O157 in the feces, on the hides, or at the rectal anal mucosal region was significantly less in vaccinated cattle (Thomson et al., 2009).

Researchers have also evaluated STEC O157 fecal shedding in SRP vaccinated beef calves born to cows vaccinated prepartum (Wileman et al., 2011). Longitudinally cows and their calves were followed from branding to slaughter to find differences in STEC O157 fecal shedding after administering vaccination treatments. No significant treatment differences were detected from branding to slaughter between any of the study treatments: 1) cows receiving the vaccine prepartum; 2) calves receiving the vaccine at branding; 3) cows receiving the vaccine
prepartum and calves receiving the vaccine at branding; and 4) cows not receiving the vaccine prepartum and calves not receiving the vaccine at branding (Wileman et al., 2011).

**Efficacy and Effectiveness of Pre-Harvest Interventions**

For pre-harvest vaccines to reduce STEC O157 throughout the beef production system some measure of efficacy and effectiveness must be demonstrated (Smith et al., 2012). A review of the literature indicates commercially available vaccines, are efficacious at reducing STEC O157 in beef cattle populations, but the measure of efficacy may vary over studies (Snedeker 2011). Factors leading to heterogeneity in the measure of vaccine efficacy could be a result of comparing studies with different sampling designs, dose-regimens, and outcomes. In addition, studies demonstrating the usefulness of pre-harvest vaccines are limited. Vaccine products are available for beef producers to use, but have yet to be widely adopted by the beef industry. For adoption to occur, producers must find pre-harvest vaccines valuable, implementable, and practical. Based on this information the goal of this thesis was to evaluate further the efficacy and effectiveness of a TTSP vaccine at reducing STEC O157 fecal shedding in live cattle. In particular, our objectives were to 1) determine if 3-doses of a TTSP vaccine administered to cattle demonstrated heterogeneity in the measure of vaccine efficacy; 2) create a model that evaluated the effectiveness of pre-harvest vaccination on fecal pen prevalence distributions.
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CHAPTER II:

META-ANALYSIS OF THE EFFICACY OF A THREE-DOSE REGIMEN OF A TYPE III SECRETED PROTEIN VACCINE FOR REDUCING STEC O157 IN FECES OF FEEDLOT CATTLE
CHAPTER II

META-ANALYSIS OF A THREE-DOSE REGIMEN OF A TYPE III SECRETED PROTEIN VACCINE FOR EFFICACY AT REDUCING STEC O157 IN FECES OF FEEDLOT CATTLE

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Abstract

Pre-harvest control of *Escherichia coli* O157:H7 (STEC O157) may prevent human illness by reducing the presence of STEC O157 throughout the beef production chain. Immunization of cattle with a type III secreted protein (TTSP) vaccine inhibits colonization of cattle with STEC O157 and reduces the probability for fecal shedding and hide contamination. Our objectives were to perform a meta-analysis to estimate efficacy of a three-dose regimen of TTSP vaccine at reducing the presence of STEC O157 in the feces of feedlot cattle and to test factors that might modify vaccine efficacy. Pen-level data (n=184 pens, 1,462 cattle) from four randomized controlled vaccine trials conducted from 2002-2008 at the University of Nebraska–Lincoln Agricultural Research & Development Center (Mead, NE) were analyzed. Factors explaining the probability for a positive fecal sample were tested in a generalized estimating equations (GEE) logistic regression model. An autoregressive correlation structure was defined to account for clustering of repeated test-periods within block. Clustering or potential confounding by study was accounted for by treating study as a fixed effect. Relative risk was estimated from a corresponding log-binomial GEE model. STEC O157 was detected from 661 of
5,451 post-vaccination fecal samples. The probability to detect STEC O157 post-vaccination was 8.4% and 15.8% in vaccinated and unvaccinated cattle, respectively. Interactions between vaccination and 1) study; 2) prevalence of control pens within each time-place cluster and 3) days from vaccination were not significant or fit poorly with observed data. Adjusting for study, cattle in pens receiving three doses of vaccine were less likely to shed STEC O157 (OR=0.46 p<0.0001). Model adjusted vaccine efficacy was 48% (95% CI, 0.37 - 0.57). We concluded that a three-dose regimen TTSP vaccine was efficacious at reducing the probability to detect STEC O157 in the feces of cattle and vaccine efficacy was not modified by study, prevalence of control pens, or days from vaccination.

Introduction

Shiga-toxin producing *Escherichia coli* O157:H7 (STEC O157) is a human pathogen that may lead to diarrhea, hemorrhagic uremic syndrome, or death (Griffin et al., 1988). Cattle populations serve as reservoir for STEC O157 exposure of humans via direct contact or indirect contact with contaminated food, water or other environmental sources (Sargeant et al., 2003). The probability to detect STEC O157 in the feces of cattle is variable by season, with higher pathogen levels found in the summer months (Hancock et al., 1997; Chapman et al., 1997; Heuvelink et al., 1998; Van Donkersgoed et al., 1999; Smith et al., 2005). Cattle hides contaminated with STEC O157 have been correlated with STEC O157 carcass contamination in packing facilities (Elder et al., 2000; Arthur et al., 2004). A recent study has shown that the seasonal peak in STEC O157 fecal shedding in beef cattle precedes the peak of both the prevalence of STEC O157 contaminated beef, and incidence of STEC O157 human illness
(Williams et al., 2010). These data suggest that reducing STEC O157 fecal shedding in beef cattle might result in less frequent human illness.

Interventions against STEC O157 carriage in live cattle may aid in reducing STEC O157 throughout the beef production chain. Several studies have demonstrated vaccinating cattle using type III secreted proteins (TTSP) decreases the probability to detect STEC O157 from cattle feces (Potter et al., 2004; Peterson et al., 2007a; Peterson et al., 2007b; Moxley et al., 2009; Smith et al., 2009a; Rich et al., 2010). Risk modelers have also predicted that a STEC O157 vaccine administered to live cattle might have the greatest potential impact at reducing STEC O157 carcass contamination (Jordan et al., 1999). A systematic review on commercially available vaccines concluded that vaccination of cattle has efficacy as a pre-harvest intervention (Snedeker et al., 2011). In this review, the odds of detecting STEC O157 in the feces of cattle vaccinated with TTSP were reduced by 62% compared to control cattle. Another important finding from this study was that the measure of efficacy for the TTSP vaccine product demonstrated statistically significant heterogeneity. Factors accounting for the heterogeneity of vaccine efficacy between studies were left unaccounted for in this report (Snedeker et al., 2011). Vaccine efficacy is the percent reduction in the disease rate among vaccinated subjects that is attributable to vaccination. Factors that lead to heterogeneity (variability in the effect of the vaccine) could be important if vaccination is adopted widely as a pre-harvest intervention. Heterogeneity could be a result of evaluating efficacy over different studies, challenge loads, or days from administration of the last dose of the vaccine. Since we possessed the complete datasets for these TTSP vaccine trials, our objectives were 1) to determine the efficacy of a three
dose regimen of a TTSP vaccine product at reducing the probability to detect STEC O157 in the feces of feedlot cattle; and 2) to test factors that may modify the overall efficacy of the STEC O157 TTSP vaccine in vaccinated pens of cattle.

**Materials and Methods**

*Data Selection and Coding*

Eight randomized controlled TTSP vaccine studies were screened for possible use in this study (Figure 2.1). Trials were performed at the University of Nebraska- Lincoln within the years 2003-2008. Studies selected to test for heterogeneity in the measure of efficacy of a TTSP vaccine product had a dose-regimen according to the vaccine label, and a similar study design, and study outcome. Regional vaccination and herd immunity studies were excluded (Peterson *et al.*, 2007b; Smith *et al.*, 2009a), since we were only interested in studies comparing vaccinated and non-vaccinated cattle. Studies where the outcome was a measure of STEC O157 fecal shedding were selected. The vaccine dose-regimen was 3-doses. Vaccinated cattle received a commercial TTSP vaccine product provided by Bioniche Life Sciences, Belleville, Ontario, Canada as previously described (Potter *et al.*, 2004; Peterson *et al.*, 2007a; Moxley *et al.*, 2009; Rich *et al.*, 2010). Animals in the control treatment were given either a placebo vaccination or no placebo. Fecal samples were collected longitudinally from cattle following administration of the vaccination treatment. For the analysis, we were left with four studies with four comparisons. Variables that were tested were study, treatment, and test-period (Table 2.1). Factors calculated from the raw data were the challenge load, and days from vaccination (Table 2.1).
A generalized estimating equations (GEE) logistic regression model (Proc Genmod, SAS Institute, Cary, N.C.) was used to test the null hypothesis that there was no difference in the probability for a positive STEC O157 fecal sample between vaccinated and unvaccinated cattle. The GEE model specified a logit link function for a binary response, which was the number of culture positive animals in a pen divided by the total number of animals in that pen. To account for clustering of repeated test-periods within block an autoregressive correlation structure was defined. Potential confounding and clustering by study was accounted for by treating study as a fixed effect. Fixed effects tested to explain the probability for a positive fecal sample were vaccination, days from vaccination, challenge load, and study. Two-way interactions tested to assess heterogeneity in the effect of the vaccine were vaccination and the factors: days from vaccination, study, and challenge load. The final multivariable model was determined using a manual forward selection process based on; 1) significance and 2) model fit. Significance level was set at $\alpha \leq 0.05$ and p-values were obtained from the Type III Score statistic. Model fit was assessed using the quasi-likelihood independence criterion (QIC), with the better fitting model possessing a lower value (Pan, 2001). Data points unduly influencing statistical significance remained in the model.

A corresponding log-binomial model was specified to obtain model adjusted-probabilities and relative risk. Explanatory variables represented in the final logistic regression model were specified in the log-binomial model. Model-adjusted probabilities were calculated for each level of categorical variables explaining the probability to detect STEC O157 in the feces of cattle.
using least squares means. Model-adjusted relative risk estimates and 95% confidence intervals were obtained from the contrast estimate results. Vaccine efficacy represents the proportion of cases prevented by vaccination. Efficacy of the vaccine treatment was then derived as one minus the relative risk.

**Results**

Features of the four trials included in the meta-analysis are presented in Table 2.2 (Potter *et al.*, 2004; Peterson *et al.*, 2007a; Moxley *et al.*, 2009; Rich *et al.*, 2010). 184 pens were represented by the analysis. Overall STEC O157 was detected from 661/5451 (12%) of post-vaccination fecal samples. The probability to detect STEC O157 in the feces of cattle over all studies and post-vaccination time periods were 8.4% (231/2734) and 15.8% (430/2717) in the vaccination and control treatments, respectively.

Study was significant as a univariate in the logistic regression model (p<0.01). Of the 2-variable models tested only vaccination (p<0.0001) with study, significantly explained the probability of detecting a STEC O157 positive fecal sample. All interactions were evaluated while keeping study and vaccination treatment as fixed effects. Interactions between study and vaccination (p=0.32), and challenge load and vaccination (p=0.21) were not significant. An interaction between days from vaccination and vaccination treatment was significant (p=0.05), although the interaction reduced model fit. Further evaluation of the interaction showed that significance was dependent on a single fecal collection day in the final test-period of the Peterson *et al.*, 2007 study. On the day cattle were transported to harvest a major rain event had
occurred, possibly resulting in pass through shedding of the organism. At harvest, colonization at the rectal-anal junction was 98% less for vaccinated compared to non-vaccinated cattle (Peterson et al., 2007a). The interaction was not significant when this single date was removed. Therefore due to poor model fit the interaction between days from vaccination by vaccination treatment was not included in the final model.

In the final model, vaccination treatment accounted for the probability of cattle to shed STEC O157 in the feces, after adjusting for study. The final logistic and log-binomial regression models are summarized in Tables 2.3 and 2.4, respectively. Vaccinated cattle had a 54% lowered odds of shedding STEC O157 in the feces than non-vaccinated cattle (OR=0.46 CI= 0.37-0.58; p<0.0001). The model-adjusted probability of recovering STEC O157 from the feces of cattle immunized and non-immunized were 0.068 and 0.131, respectively. Model adjusted vaccine efficacy was 48% (95% CI, 0.37 - 0.57; p<0.0001).

**Discussion**

A three dose regimen of TTSP vaccine significantly reduced fecal shedding of STEC O157 in cattle by 48% compared to non-vaccinated cattle under conditions of natural exposure. Also, days from vaccination, challenge load, and study did not modify the efficacy of the vaccine.

We chose to perform our meta-analysis using four formerly published studies that evaluated efficacy of a 3-dose regimen TTSP vaccine. In these individual studies vaccine efficacy was 59% (OR=0.36, p=0.04), 15% (OR=0.81, p=0.57), 65% (OR=0.34, p=0.002), and
43% (OR=0.50, P<0.01) (Potter et al., 2004; Peterson et al., 2007a; Moxley et al., 2009; Rich et al., 2010). Our combined measure of efficacy over these studies was comparable. This finding was consistent with the results of a recent systematic review and meta-analysis of eight TTSP treatment comparisons (OR=0.38, 95CI=0.29, 0.61) (Snedeker et al., 2011). The authors of that paper reported heterogeneity or variation in the efficacy of the TTSP vaccine. Heterogeneity was not attributed to the dose-regimen, or type of control treatment used (Snedeker et al., 2011). Other factors responsible for this variation were suggested; challenge load and number of animals in a pen. However these variables were not tested, since the researchers did not have full access to the original datasets. Using the original datasets, we were able to assess additional factors that could possibly account for any variation in vaccine efficacy. Factors specifically tested in our model to explain the probability of a positive fecal sample, were: days from vaccination, study, and challenge load. From our analysis, the level of efficacy of the TTSP vaccine product was not modified by any of these variables.

Immunization of cattle with type three secreted proteins blocks attachment of STEC O157 to gut epithelial cells (Potter et al., 2004). As a result, STEC O157 colonization, fecal shedding, and resulting environmental levels of STEC O157 may be reduced (Potter et al., 2004; Peterson et al., 2007a; Smith et al., 2008). Culture of ropes is used to reflect the environmental exposure of cattle to STEC O157 in feedlots (Smith et al., 2004). Vaccinated pens of cattle have been shown to be less likely to test STEC O157 ropes positive compared to non-vaccinated pens of cattle (Smith et al., 2008). STEC O157 localization at the TRM region is an important indication of colonization (Naylor et al., 2003; Naylor et al., 2005). Colonization measured by
culturing cells from the rectal-anal junction was reduced 92% and 98% in 2-dose and 3-dose regimen TTSP vaccine studies, respectively (Peterson et al., 2007a; Smith et al., 2009b).

Identification of factors that modify vaccine efficacy is important for considering the practical application of a pre-harvest vaccine. This study assessed efficacy of 3-doses of the TTSP vaccine where the outcome was STEC O157 fecal prevalence. However, two doses of a TTSP vaccine product have also successfully demonstrated a reduction in STEC O157 carriage in cattle populations (Peterson et al., 2007b; Moxley et al., 2009; Smith et al., 2009a).

In this study, days from administering the last dosage of the vaccine was not associated with efficacy of the TTSP vaccine product. It is of practical importance that a TTSP vaccine offer effective immunity in cattle from administration of the last dosage up until shipment for slaughter. Previous studies have shown no difference in the efficacy of the vaccine at reducing the probability of cattle to shed STEC O157 in the feces over the entire trial period (Potter et al., 2004; Peterson et al., 2007a; Moxley et al., 2009).

Challenge load did not modify the probability to detect STEC O157 from the feces of vaccinated cattle. Environmental factors mediate the transmission of STEC O157 in a feedlot production setting. For instance, there is a greater probability to detect STEC O157 in the feces of cattle housed on feedlot pen surfaces characterized as muddy as compared to feedlot pen surfaces characterized as normal (Smith et al., 2001). When cattle are housed in a pen with a high shedder they are also at a greater risk of shedding STEC O157 in the feces compared to when not housed with a high shedder (Cobbold et al., 2007). In this study, we hypothesized that the beneficial effect of the TTSP vaccine might be overwhelmed by an increase in STEC O157
in the feedlot environment or conversely, that the beneficial effect of the vaccine would be more observable during periods of high challenge.

Study did not explain the heterogeneity in the efficacy of a TTSP vaccine administered to cattle. Different batches of the vaccine product may be more or less immunogenic leading to variation in the estimate of vaccine efficacy. To test this hypothesis we used the variable study to represent inherent study differences over the years. Study significantly described the probability to detect STEC O157 in the feces of cattle. Of the studies assessed, some studies were more likely to have pens of cattle shedding STEC O157 in the feces compared to others.

**Conclusions**

We concluded there was a single estimate for the efficacy of a 3-dose regimen TTSP vaccine for reducing STEC O157 in the feces of cattle. Factors such as the challenge load, study, or days from administration of the last dose of vaccine did not appear to modify the efficacy of the vaccine product.
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Smith DR, Moxley RA, Klopfenstein TJ, and Erickson GE. A randomized longitudinal trial to test the effect of regional vaccination within a cattle feedyard on Escherichia coli O157:H7 rectal colonization, fecal shedding, and hide contamination. Foodborne Pathog Dis 2009a;6:885-892
Smith DR, Moxley RA, Peterson RE, Klopfenstein T, Erickson GE, and Clowser SL. A two-dose regimen of a vaccine against *Escherichia coli* O157:H7 type III secreted proteins reduced environmental transmission of the agent in a large-scale commercial beef feedlot clinical trial. *Foodborne Pathog Dis* 2008;5:589-598


Williams MS, Withee JL, Ebel ED, Bauer NE, Scholosser WD, Disney WT, Smith DR, and Moxley RA. Determining relationships between the seasonal occurrence of *Escherichia coli* O157:H7 in live cattle, ground beef, and humans. *Foodborne Pathog Dis* 2010;7:1-8
Figure 2.1: Flow chart displaying trials included/excluded in the meta-analysis of a three-dose regimen of a type III secreted protein vaccine for efficacy at reducing STEC O157 in feces of feedlot cattle
Table 2.1: Factors included in the meta-analysis of a three-dose regimen of a type III secreted protein vaccine for efficacy at reducing STEC O157 in feces of feedlot cattle

<table>
<thead>
<tr>
<th>Factor</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>Corresponds to feedlots, regions within a feedlot, and feedlot arrival dates</td>
</tr>
<tr>
<td>Challenge load</td>
<td>STEC O157 positive animals in a pen over the number of animals in a pen averaged over each test-period within a block</td>
</tr>
<tr>
<td>Days from vaccination</td>
<td>Days from the date of administration of the third dose of the vaccine/placebo treatment subtracted by the test-period sampling date</td>
</tr>
<tr>
<td>Test-period</td>
<td>Corresponds to a rectal fecal sampling event in the post-treatment phase</td>
</tr>
<tr>
<td>Treatment</td>
<td>i) 3 doses of a TTSP Vaccine  ii) Adjuvant</td>
</tr>
</tbody>
</table>
Table 2.2: Descriptive statistics for four natural exposure randomized controlled trials used in the meta-analysis of a three-dose regimen of a type III secreted protein vaccine for efficacy at reducing STEC O157 in feces of feedlot cattle

<table>
<thead>
<tr>
<th>Study</th>
<th>Blocks</th>
<th>Pens/Treatment</th>
<th>Animals/Pen</th>
<th>Sample Size</th>
<th>Test-periods</th>
<th>Days Post-vaccination</th>
<th>Positive Samples</th>
<th>Odds Ratio</th>
<th>P-value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moxley et al 2009</td>
<td>4</td>
<td>20</td>
<td>8</td>
<td>320</td>
<td>4</td>
<td>21, 35, 49, 70</td>
<td>79/1273</td>
<td>0.34</td>
<td>0.002</td>
<td>Vaccine reduced shedding of STEC O157</td>
</tr>
<tr>
<td>Peterson et al 2007</td>
<td>1</td>
<td>18</td>
<td>8</td>
<td>288</td>
<td>4</td>
<td>14, 28, 42, 56</td>
<td>79/1127</td>
<td>0.81</td>
<td>0.57</td>
<td>No difference between vaccine and control</td>
</tr>
<tr>
<td>Potter et al 2003</td>
<td>3</td>
<td>24</td>
<td>8</td>
<td>384</td>
<td>3</td>
<td>21, 42, 62/64</td>
<td>134/1152</td>
<td>0.36</td>
<td>0.04</td>
<td>Vaccine reduced shedding of STEC O157</td>
</tr>
<tr>
<td>Rich et al 2010</td>
<td>3</td>
<td>30</td>
<td>8</td>
<td>480</td>
<td>4</td>
<td>21/22, 42/43, 63/64, 84/85</td>
<td>369/1899</td>
<td>0.5</td>
<td>&lt;0.0001</td>
<td>Vaccine reduced shedding of STEC O157</td>
</tr>
</tbody>
</table>
Table 2.3: Multivariable multilevel logistic regression model for the probability of recovering STEC O157 from the feces of feedlot cattle (n=5451) sampled from 4 natural exposure studies from within 92 pens of vaccinated cattle and 92 pens of non-vaccinated cattle, while accounting for clustering of repeated test-periods within block and study as a fixed effect.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>Parameter Estimate</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>-1.8555</td>
<td></td>
<td></td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>Vaccine</td>
<td>-0.7704</td>
<td>0.463</td>
<td>0.369-0.580</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>Referent</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Moxley</td>
<td>-1.3090</td>
<td>0.27</td>
<td>0.14-0.52</td>
<td>0.0043</td>
</tr>
<tr>
<td></td>
<td>Peterson</td>
<td>-1.1822</td>
<td>0.31</td>
<td>0.18-0.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Potter</td>
<td>-0.6117</td>
<td>0.54</td>
<td>0.29-1.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rich</td>
<td>0</td>
<td>Referent</td>
<td>Referent</td>
<td></td>
</tr>
</tbody>
</table>
**Table 2.4:** Multivariable multilevel log-binomial model for the probability of recovering STEC O157 from the feces of feedlot cattle (n=5451) sampled from 4 natural exposure studies from within 92 pens of vaccinated cattle and 92 pens of non-vaccinated cattle, while accounting for clustering of repeated test-periods within block and study as a fixed effect.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>Parameter Estimate</th>
<th>Relative Risk</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>-2.0250</td>
<td></td>
<td></td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>Vaccine</td>
<td>-0.6573</td>
<td>0.518</td>
<td>0.427-0.629</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0</td>
<td>Referent</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Moxley</td>
<td>-1.1332</td>
<td>0.32</td>
<td>0.18-0.58</td>
<td>0.0042</td>
</tr>
<tr>
<td></td>
<td>Peterson</td>
<td>-1.0237</td>
<td>0.36</td>
<td>0.22-0.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Potter</td>
<td>-0.4992</td>
<td>0.61</td>
<td>0.36-1.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rich</td>
<td>0</td>
<td>Referent</td>
<td>Referent</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER III

STOCHASTIC SIMULATION MODEL COMPARING DISTRIBUTIONS OF STEC O157 FECAL SEDDING PREVALENCE BETWEEN CATTLE VACCINATED WITH TYPE III SECRETED VACCINES AND NON-VACCINATED CATTLE FED IN DIFFERENT SEASONS
CHAPTER III

STOCHASTIC SIMULATION MODEL COMPARING VACCINATION OF CATTLE WITH TYPE III SECRETED PROTEINS TO SEASONAL DISTRIBUTIONS OF STEC O157 FECAL SHEDDING PREVALENCE

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Abstract

Pens of cattle with high *Escherichia coli* O157:H7 (STEC O157) prevalence at harvest may present a greater risk to food safety than pens of lower prevalence. Vaccination of live cattle against STEC O157 has been proposed as an approach to reduce STEC O157 prevalence in live cattle. Our objective was to create a stochastic simulation model to evaluate the effectiveness of pre-harvest interventions. We used the model to compare STEC O157 prevalence distributions for summer- and winter-fed cattle to summer-fed cattle immunized with a Type III secreted protein (TTSP) vaccine. Model inputs were an estimate of vaccine efficacy, observed frequency distributions for number of animals within a pen, and pen-level fecal shedding prevalence for summer and winter. Uncertainty about vaccine efficacy was simulated using a log normal distribution (\(\mu=58\), SE=0.14). The outcome was STEC O157 fecal pen prevalence of summer-fed cattle unvaccinated and vaccinated, and winter fed cattle unvaccinated. The simulation was
performed 5,000 times. Summer fecal prevalence ranged from 0% to 80% (average = 30%), ninety percent of the values fell between 2%-76% prevalence. Thirty-six percent of summer-fed pens had STEC O157 prevalence greater than 40%. Winter fecal prevalence ranged from 0% to 60% (average = 10%), ninety percent of the values fell between 1%-52% prevalence. Seven percent of winter-fed pens had STEC O157 prevalence greater than 40%. Fecal prevalence for summer-fed pens vaccinated with a 58% efficacious vaccine product ranged from 0% to 52% (average = 13%), ninety percent of the values fell between 0%-32% prevalence. Less than one percent of vaccinated pens had STEC O157 prevalence greater than 40%. In this simulation, vaccination mitigated the risk STEC O157 fecal shedding to levels comparable to winter, with the major effects being reduced average shedding prevalence, reduced variability in prevalence distribution, and a reduction in the occurrence of the highest prevalence pens. Food safety decision-makers may find this modeling approach useful for evaluating the value of pre-harvest interventions.

**Introduction**

Shiga toxin-producing *Escherichia coli* O157:H7 (STEC O157) is an enteric pathogen common to fed cattle populations (Smith *et al.*, 2001). Humans become infected with STEC O157 through direct or indirect exposure to contaminated cattle feces (Sargeant *et al.*, 2003). Carriage of STEC O157 by cattle is variable within season and may be dependent upon the condition of the pen floor (Smith *et al.*, 2001; Smith *et al.*, 2005). Variation in fecal shedding also occurs by season, with a greater proportion of cattle shedding STEC O157 in the summer months compared to the winter months (Hancock *et al.*, 1994; Chapman *et al.*, 1997; Hancock *et
al., 1997; Heuvelink et al., 1998; Van Donkersgoed et al., 1999). The proportion of cattle shedding STEC O157 has been suggested to influence ground beef prevalence, and rates of subsequent human illness (Williams et al., 2010). Therefore it has been hypothesized that the occurrence of beef-related illnesses would be decreased with reductions in the proportion of cattle carrying STEC O157.

Hides of cattle have been implicated as a major source for STEC O157 contamination of beef carcasses at slaughter (Elder et al., 2000; Arthur et al., 2004). Hide contamination occurs at the feedlot, during transport, and at lairage (Arthur et al., 2007). Some suggest the implementation of pre-harvest mitigation strategies for reducing carcass contamination at slaughter (Arthur et al., 2009) and for controlling transmission of STEC O157 in the feedlot environment (Berry et al., 2010). Potential pre-harvest technologies include direct-fed microbials, vaccination, and sodium chlorate treatment. The efficacy of pre-harvest interventions at reducing fecal shedding in cattle has been reviewed (Sargeant et al., 2007; Snedeker et al., 2011). Vaccination in particular has demonstrated efficacy and may be useful for mitigating STEC O157 in cattle prior to harvest (Snedeker et al., 2011).

Simulation modeling has previously been used to evaluate pre-harvest interventions in live cattle (Jordan et al., 1999; Withee et al., 2009; Dodd et al., 2011). For example, combinations of pre-harvest technologies were most important for mitigating carcass contamination when the seasonal carriage of STEC O157 in cattle was high (Dodd et al., 2011). Others found pre-harvest vaccination demonstrated the greatest potential for reducing contaminated beef carcasses at slaughter (Jordan et al., 1999). The public health value of a pre-harvest vaccine at reducing STEC O157 human illnesses was also evaluated relative to marginal
cost of the vaccine product (Withee et al., 2009). Our goal was to compare the seasonal
distributions of pen-level fecal shedding prevalence to the fecal shedding prevalence distribution
of cattle fed in the summer and vaccinated with a pre-harvest vaccine.

**Materials and Methods**

*Model Overview*

We developed a stochastic simulation model using @Risk 5.7 (Palisade Corporation, Ithaca, NY) to evaluate the effectiveness of type III secreted proteins (TTSP) vaccine at reducing STEC O157 pen-level fecal shedding prevalence. Specifically we simulated fecal pen prevalence distributions for: 1) cattle fed in the summer; 2) cattle fed in the winter; and 3) cattle fed in the summer and vaccinated with a TTSP vaccine.

*Model Parameters*

Simulation inputs representing variability in the model were winter-fed fecal pen prevalence (Figure 3.1i), summer-fed fecal pen prevalence (Figure 3.1ii), and number of animals in a pen (Figure 3.1iii). These variables were represented as relative frequency distributions from seventy-four pens of cattle sampled from five Nebraska feedlots. (Smith et al., 2001; Williams et al., 2010). The percentage of cattle shedding STEC O157 in each pen was determined by culturing feces collected from the rectum of each animal in each pen while the animal was restrained in a chute for routine management procedures. Culturing methods for fecal samples are described by Smith et al 2001.

The five feedlots voluntarily participated in the study and were typical of commercial feedlots in Nebraska. The capacities of these feedlots ranged from 3,000 to 12,000 animals: approximately 40,000 total capacity. Pens were open-dirt lots. The feedlots involved in this study
fed rations consisting primarily of dry rolled corn, high-moisture corn, wet corn gluten feed, wet distillers’ grains, alfalfa hay, corn silage, and supplement. Feces from all cattle in the pen were tested at the time of routine administration of growth implants. The month pens were sampled was used to classify pens into summer (May-October) and winter (November-April) seasons.

A total of 44 and 30 pens were fecal sampled in the summer and winter, respectively. STEC O157 was detected in 1501 of 4,952 individual fecal samples collected in the summer. STEC O157 was detected in 179 of 2,941 individual fecal samples collected in the winter. The range in pen-level fecal shedding prevalence was 0%-80% in the summer (average=30%), and 0% -60% in the winter (average=10%). The distribution of pen sizes ranged from 36 head to 231 head (average=107).

Uncertainty around vaccine efficacy was represented by a log normal distribution. The parameter estimate used to model the uncertainty around vaccine efficacy was taken from a systematic review on pre-harvest vaccines (Snedeker et al., 2011). The model adjusted odds ratio estimate for vaccination was 0.38 (95% CI 0.29-0.51). We converted the odds ratio to a relative risk (Zhang, 1998) using a prevalence level of 15% (Snedeker et al., 2011). The natural log of the relative risk provided the mean parameter estimate used to describe the log normal distribution (μ=-0.87, SE=0.14).

**Model Outputs**

Model output scenarios were the simulated summer-fed fecal pen prevalence, winter-fed fecal pen prevalence, and the vaccinated summer-fed fecal pen prevalence. Both the summer-fed and winter-fed fecal pen prevalence output distributions were sampled from the original relative
frequency distributions. To estimate the pen-level distribution of cattle shedding STEC O157 in the feces following vaccination, the following calculations were performed:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control Positives</strong></td>
<td>Summer-Fed Fecal Pen Prevalence × Number of Animals in a Pen</td>
</tr>
<tr>
<td><strong>Vaccinated Positives</strong></td>
<td>Control Positives × Relative Risk</td>
</tr>
<tr>
<td><strong>Vaccinated Summer-Fed Fecal Pen Prevalence</strong></td>
<td>Vaccinated Positives × Number of Animals in a Pen</td>
</tr>
</tbody>
</table>

**Model Simulation**

The model was run for five thousand simulations. Results for each of the output scenarios, winter-fed fecal pen prevalence, summer-fed fecal pen prevalence, and vaccinated summer-fed fecal pen prevalence, were plotted as relative frequency histograms and cumulative probability distributions. Descriptive statistics were taken from the @Risk outputs. Fifth to ninetieth percentiles were calculated for each output distribution.

**Model Validation**

Model validation was performed by comparing the simulated distributions for summer-fed and winter-fed pen prevalence with the original summer-fed and winter-fed pen prevalence distributions. For example, descriptive statistics were compared between input distributions in Figure 3.1i and simulated output distributions in Figure 3.3i.

**Results**
Over 500,000 head of cattle were represented by each scenario of the model. Cattle fed in the summertime demonstrated the highest risk for STEC O157 fecal shedding compared to both winter and vaccinated summertime prevalence distributions. The mean pen prevalence was 30%, 10%, and 13%, in the summer, winter, and vaccinates, respectively. Non-vaccinated summer-fed fecal prevalence ranged from 0%-80%, ninety percent of the values fell between 2%-76% prevalence. Non-vaccinated winter-fed fecal prevalence ranged from 0%-60%, ninety percent of the values fell between 1%-52% prevalence. Vaccinated summer-fed fecal prevalence ranged from 0%-52%, ninety percent of the values fell between 0%-32% prevalence. Results from simulating the model with a 58% efficacious TTSP vaccine were shown as relative frequency probability distributions for the three output distributions studied (Figure 3.3).

Cumulative probability distributions from the three output distributions are presented in Figure 3.4. The percent of pens with greater than 40% fecal prevalence was the greatest among summer-fed cattle at 36% (Figure 3.4i). In the wintertime the percent of pens with greater than 40% fecal prevalence were 7% (Figure 3.4ii). Of the pens of cattle receiving a TTSP vaccine <1% of pens had greater than 40% fecal prevalence (Figure 3.4iii).

Discussion

We used a simulation model to assess the possible effects of pre-harvest interventions for controlling STEC O157 carriage in live cattle. As an example, we chose to examine the effect of vaccination of cattle with type III secreted proteins on the seasonal distribution of STEC O157 pen-level fecal shedding prevalence. In particular, the benefits of vaccination were determined by comparing the summer and wintertime STEC O157 fecal shedding prevalence distributions of
One of the outcomes of this model was a reduction in the percent of high prevalence pens for cattle fed in the summer and immunized with a Type III secreted protein vaccine. For example, with a 58% efficacious vaccine product, the percentage of pens with greater than 40% STEC O157 pen prevalence was <1%, 7%, and 36% in the vaccinated, winter-fed, and summer-fed distributions. These results indicate using an intervention in cattle prior to slaughter may prevent incidents where pathogen contamination may overwhelm post-harvest interventions. However these results do not take into consideration the possibility for the efficacy of a pre-harvest intervention to become compromised from cross-contamination at harvest (Smith et al., 2012). For instance, hide contamination of cattle during transport to harvest (Arthur et al., 2007), or cross-contamination of carcasses during harvest (Jordan et al., 1999) may cancel out the benefits of pre-harvest interventions.

In this simulation model, vaccination mitigated the risk STEC O157 fecal shedding to levels comparable to winter, with the major affect being a reduced range of shedding prevalence. A decreased range in seasonal fecal shedding prevalence suggests a more uniform distribution and possibly a form of process control for subsequent carcass contamination. Higher rates of human incidence in the summer have been attributed to the seasonal increase in ground beef prevalence, which are in turn due to the seasonal recovery of STEC O157 from the feces of live cattle (Williams et al., 2010). Therefore reducing the seasonal variability in pen-level fecal shedding prevalence through vaccination, as was suggested by this model, should lower the likelihood of STEC O157 infection in humans.
Development of a relevant model requires valid input assumptions. In our model we used observed distributions, by season, for cattle shedding STEC O157 in the feces. The seasonal increase in the probability of recovering STEC O157 from the feces of cattle in the summer months is widely recognized (Hancock et al., 1994; Chapman et al., 1997; Hancock et al., 1997; Heuvelink et al., 1998; Van Donkersgoed et al., 1999; Williams et al., 2010). We used previously observed fecal shedding patterns of cattle, so that we were able to accurately reflect the variability in fecal shedding within and between seasons. These observed seasonal prevalence distributions do not represent all fed cattle populations throughout the U.S., however the model serves to predict the prevalence distribution the cattle might have had, had they been vaccinated and then compared to a similar population fed in a different season.

In this simulation, the most likely value used for vaccine efficacy was 58%, with a distribution representing our uncertainty about that value. This value was a summary measure of eight treatment comparisons from natural exposure trials evaluating the recovery of STEC O157 from the feces of non-vaccinated and TTSP vaccinated cattle (Snedeker et al., 2011). A recent meta-analysis indicated efficacy of a 3 dose-regimen TTSP vaccine product did not appear to be modified by study, challenge load, or days from administration of the last dose of the vaccine (Vogstad et al., 2011). For this reason we chose to model vaccine efficacy with uncertainty around a single estimate.

Pen-level prevalence distributions for summer and winter-fed cattle were generated from data we possessed. Because of this we were able to validate our results. For instance, we compared the predicted range of outcomes for the non-vaccinated summer and winter-fed cattle to the original summer- and winter-fed prevalence distributions. This included comparing
descriptive statistics of the original prevalence data to the simulated prevalence data. Model validation confirmed that both the unvaccinated summer-fed and winter-fed prevalence distributions were consistent with the original data.

This model may be used to evaluate other pre-harvest interventions. As more data becomes available on the feces to hide, and hide to carcass transfer ratio, this model could be updated to predict the effect of pre-harvest interventions on hide and carcass contamination outcomes as well.

**Conclusion**

In summary, vaccinating cattle fed in the summertime with a 3-dose regimen TTSP vaccine reduced the average pen prevalence and decreased the variability in STEC O157 fecal pen shedding to levels comparable to the wintertime. Vaccination was also effective at reducing STEC O157 high-risk pens. This model may be useful for evaluating the effectiveness of pre-harvest interventions.
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Figure 3.1: Observed relative frequency distributions of i) pen-level fecal shedding prevalence for summer-fed cattle, ii) pen-level fecal shedding prevalence for winter-fed cattle, and iii) number of animals in a pen for a longitudinal study conducted from 1999-2002 in five feedlots, 44 pens in the summer and 30 pens in the winter.
Figure 3.2: Log normal distribution ($\mu=-0.54$, $SE=0.13$) representing efficacy of a TTSP vaccine product used to model the pen-level fecal shedding prevalence distribution of summer-fed cattle vaccinated.
Figure 3.3: Relative frequency distributions of simulated pen-level fecal shedding prevalence for
i) summer-fed cattle, ii) winter-fed cattle, and iii) vaccinated summer-fed cattle using a 58%
efficacious TTSP vaccine.
**Figure 3.4:** Cumulative probability distributions of simulated pen-level fecal shedding prevalence for i) summer-fed cattle, ii) winter-fed cattle, and iii) vaccinated summer-fed cattle using a 58% efficacious TTSP vaccine.
## Appendix

### Published Literature on Dietary Components that Affect the Carriage of Shiga Toxin-Producing Escherichia coli O157 (STEC O157) in Cattle

<table>
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<th>Author</th>
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<tr>
<td>Diez-Gonzalez et al., 1998</td>
<td>Generic E. coli Population</td>
<td>Observed generic E. coli population</td>
<td>Fecal samples</td>
<td>No grain vs. 60% rolled corn vs. 80% rolled corn</td>
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<td>Gilbert et al., 2005</td>
<td>Generic E. coli Population</td>
<td>Observed generic E. coli population</td>
<td>Fecal samples</td>
<td>Roughage vs. roughage + molasses vs. grain diet</td>
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<tr>
<td>Grauke et al., 2003</td>
<td>Generic coliforms</td>
<td>Observed generic coliforms</td>
<td>Rumen, duodenum, and fecal samples</td>
<td>90% Grain +10% triticale silage vs. 50% alfalfa + 50% timothy hay</td>
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<td>Krause et al., 2003</td>
<td>Generic E. coli Population</td>
<td>Observed generic E. coli population</td>
<td>Rumen, jejunum, ileum, caecum, and fecal samples</td>
<td>100% Rhodes grass vs. 70% rolled sorghum + 30% rhodes grass</td>
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<tr>
<td>Stanton et al., 2000</td>
<td>Generic E. coli Population</td>
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<td>Grauke et al., 2003</td>
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<td>No difference in concentration or duration of shedding</td>
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<td>Hovde et al., 1999</td>
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<td>Kudva et al., 1997</td>
<td>STEC O157 Experimental Challenge Study in</td>
<td>Fecal samples</td>
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<td>Diet/Challenge Study</td>
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<td>Tkalcic et al., 2000</td>
<td>STEC O157</td>
<td>Experimental Challenge Study</td>
<td>Fecal and rumen fluid samples</td>
<td>1.9 kg Bermuda grass + 3.8 kg concentrate mix vs. 3.8 kg bermuda grass +1.9 kg concentrate mix</td>
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<td>Van Baale et al., 2004</td>
<td>STEC O157</td>
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<td>85% Forage + 15% grain vs. 15% forage + 85% grain</td>
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<td>Diez-Gonzalez et al., 1998</td>
<td>Generic acid resistant E. coli</td>
<td>Observed generic E. coli population</td>
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<td>100% Timothy hay vs. 45% rolled corn vs. 90% rolled corn</td>
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<td>Grauke et al., 2003</td>
<td>Generic acid resistant coliforms</td>
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<td>Hovde et al., 1999</td>
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<td>Krause et al., 2003</td>
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<td>Van Kessel et al., 2002</td>
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<td>Grauke et al., 2003</td>
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<td>Experimental Challenge Study</td>
<td>Rumen, duodenum, and fecal samples</td>
<td>90% Grain +10% triticale silage vs. 50% alfalfa + 50% timothy hay</td>
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<td>Hovde et al., 1999</td>
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<td>Tkalcic et al., 2000</td>
<td>Acid resistant STEC O157</td>
<td>Experimental Challenge Study</td>
<td>Ruminal fluid samples</td>
<td>1.9 kg Bermuda grass + 3.8 kg concentrate mix vs. 3.8 kg bermuda grass +1.9 kg concentrate mix</td>
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**Production Systems**

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<td>Fegan et al., 2004</td>
<td>STEC O157</td>
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<td>Feedlot vs. pasture confinement production system</td>
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<td>Kuhnert et al., 2005</td>
<td>STEC O157</td>
<td>Observed STEC O157 population</td>
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<td>Reinstein et al., 2009</td>
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<td>Renter et al., 2004</td>
<td>STEC O157</td>
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<td>Fecal samples</td>
<td>Confinement vs. pasture production system</td>
<td>No difference in the probability to detect</td>
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**Abrupt Dietary Change**

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<td>Observed generic E. coli population</td>
<td>Fecal samples</td>
<td>Switching from high concentrate to high roughage diet</td>
<td>Lower concentration when switched to roughage diet</td>
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<td>Gregory et al., 2000</td>
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<td>Switching from pasture to hay diet</td>
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<td>Jordan et al., 1998</td>
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<td>Switching from high concentrate to 50% corn silage + 50% alfalfa hay vs. high concentrate</td>
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<td>Scott et al., 2000</td>
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<td>Observed E. coli population</td>
<td>Fecal samples</td>
<td>45% WCGF vs. 51% HMC vs. 85% DRC (Exp 1). All finishing diets then switched to alfalfa hay (Exp 2)</td>
<td>No difference in concentration (Exp 1). No difference in concentration (Exp 2). Reduced concentration in Exp 2 hay diets when compared to Exp 1 finishing diets</td>
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<td>Stanton et al., 2000</td>
<td>Generic E. coli</td>
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<td>Fecal samples</td>
<td>Switching from high concentrate to high roughage diet following fasting</td>
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<td>Buchko et al., 2000</td>
<td>STEC O157</td>
<td>Observed STEC O157 population</td>
<td>Fecal samples</td>
<td>80% barley diet, fasted 48hrs, then switched to 100% alfalfa silage, fasted 48hrs, re-fed alfalfa diet</td>
<td>No difference in number of culture positive animals when switched from barley to forage diet</td>
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<td>Keen et al., 1999</td>
<td>STEC O157</td>
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<td>Diez-Gonzalez et al., 1998</td>
<td>Generic acid resistant E. coli</td>
<td>Observed generic acid resistant E. coli population</td>
<td>Fecal samples</td>
<td>Switching from high concentrate to high roughage diet</td>
<td>Lower concentration when switched to roughage diet</td>
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<tr>
<td>Scott et al., 2000</td>
<td>Generic acid resistant E. coli</td>
<td>Observed generic acid resistant E. coli population</td>
<td>Fecal samples</td>
<td>45% WCGF vs. 51% HMC vs. 85% DRC (Exp 1). All finishing diets then switched to alfalfa hay (Exp 2).</td>
<td>Higher concentration in WCGF diet (Exp 1). No difference in concentration between diets (Exp 2). Reduced concentration in Exp 2 hay diets when compared to Exp 1 finishing diets</td>
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<td>Brownlie et al., 1967</td>
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<td>6.8 kg lucerne hay. Followed by withholding feed for 2 days</td>
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<td>Jordan et al., 1998</td>
<td>Generic E. coli</td>
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<td>50% corn silage + 50% alfalfa hay vs. high concentrate, then fasted 48hrs</td>
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<td>Brown et al., 1997</td>
<td>STEC O157</td>
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<td>Calves fasted 1-2 times (48hrs) vs. non-fasted control</td>
<td>Increased concentration in 7 fasting events, decreased concentration in 7 fasting events. No change in concentration 4 fasting events</td>
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<td>Buchko et al., 2000</td>
<td>STEC O157</td>
<td>Observed STEC O157 population</td>
<td>Fecal samples</td>
<td>80% barley diet, fasted 48hrs, then switched to 100% alfalfa silage, fasted 48hrs, re-fed alfalfa diet</td>
<td>Increased number of culture positive animals upon re-feeding forage diet after fast</td>
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<td>Cray et al., 1998</td>
<td>STEC O157</td>
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<td>Fecal samples</td>
<td>Fasted (following inoculation) vs. non-fasted animals (Exp 1). Fasted (48hrs prior to inoculation) vs. non-fasted animals (Exp 2)</td>
<td>No difference in concentration (Exp 1). Increased concentration and susceptibility for infection in fasted animals (Exp 2)</td>
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<td>Kudva et al., 1995</td>
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<td>Experimental Challenge Study in Sheep</td>
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<td>Fed alfalfa pellets then fasted (24hrs), then re-fed kochia weeds and fasted</td>
<td>Increased probability to detect after a fast followed by clearance in both dosed</td>
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### Grain Type

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<td>Berg et al., 2004</td>
<td>Generic E. coli</td>
<td>Observed generic E. coli population</td>
<td>Pen floor fecal samples</td>
<td>91% Barley + 7% silage vs. 86% corn + 7% silage + 5% protein supp</td>
<td>Higher mean concentration in corn diet</td>
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<tr>
<td>Bach et al., 2005</td>
<td>STEC O157</td>
<td>Experimental Challenge Study</td>
<td>Fecal and mouth swab samples</td>
<td>80% barley vs. 80% corn vs. 71% barley + 6% canola oil + 3% canola meal vs. 72.25% corn + 6% canola oil + 1.75% canola meal</td>
<td>No difference in concentration or number of culture positive animals (enumeration period – fecal samples). No difference in probability to detect (mouth swabs)</td>
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<td>Berg et al., 2004</td>
<td>STEC O157</td>
<td>Observed STEC O157 population</td>
<td>Pen floor fecal and hide swab samples</td>
<td>91% Barley + 7% silage vs. 86% corn + 7% silage + 5% protein supp</td>
<td>Greater mean probability to detect and concentration in barley diet (fecal samples). No difference in probability to detect (hide samples)</td>
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<td>Buchko et al., 2000</td>
<td>STEC O157</td>
<td>Experimental Challenge Study</td>
<td>Fecal samples</td>
<td>85% Barley + 15% AS' vs. 70% barley + 15% cottonseed + 15% AS vs.</td>
<td>Greater number of culture positive animals in barley diets</td>
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<td>Barley vs. no barley WCTS vs. no WCTS</td>
<td>Greater number of culture positive pens in barley diet. No difference in the number of culture positive pens for WCTS and no WCTS</td>
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<td>Dewell et al., 2005</td>
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<td>Barley vs. no barley</td>
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<td>Fox et al., 2007</td>
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<td>Observed STEC O157 population by prescreening cattle</td>
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<td>Observed STEC O157 in case and control herds</td>
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<td>No difference in odds for detection</td>
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**Grain Processing**

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<td>Fecal samples</td>
<td>Whole vs. steam-flaked vs. rolled vs. urea-ensiled in sorghum and barley diets</td>
<td>Higher concentration in SF sorghum and rolled-barley diets compared with whole sorghum or barley diets</td>
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<td>Depenbusch et al., 2008</td>
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<td>RAMS and fecal samples</td>
<td>DRC vs. SFC diets</td>
<td>Non-significant - greater probability to detect in SFC diet (RAMS samples). No difference in probability to detect (fecal samples)</td>
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<td>Edrington et al., 2010</td>
<td>STEC O157</td>
<td>Observed STEC O157 population</td>
<td>Fecal samples</td>
<td>0% vs. 20% WDG in DRC and SFC diets</td>
<td>No difference in probability to detect</td>
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<td>Jacob et al., 2008a</td>
<td>STEC O157</td>
<td>Observed STEC O157 population</td>
<td>Pen floor fecal samples</td>
<td>SFC + 15% CS + 0% DDG(^3) vs. SFC + 15% CS +25% DDG vs. SFC + 5% CS + 25% DDG</td>
<td>Greater probability to detect in 25% DDG diets</td>
</tr>
<tr>
<td>Jacob et al., 2008b</td>
<td>STEC O157</td>
<td>Observed STEC O157 population</td>
<td>Fecal samples</td>
<td>0% vs. 25% WDGS in SFC diets</td>
<td>Greater probability to detect in WDGS diet on one of two sampling dates</td>
</tr>
<tr>
<td>Jacob et al., 2008c</td>
<td>STEC O157</td>
<td>Experimental Challenge Study</td>
<td>Rumen, caecum, colon, fecal and RAMS samples</td>
<td>0% vs. 25% DDG in SFC diets</td>
<td>Greater probability to detect in DDG diet (fecal samples; day 35-42 and gut samples)</td>
</tr>
<tr>
<td>Jacob et al., 2009</td>
<td>STEC O157</td>
<td>Observed STEC O157 population</td>
<td>Pen floor fecal samples</td>
<td>0% vs. 25% DDG in SFC and SFC:DRC (2:1 ratio) diets</td>
<td>No difference in probability to detect</td>
</tr>
<tr>
<td>Jacob et al., 2010</td>
<td>STEC O157</td>
<td>Observed STEC O157 population</td>
<td>Pen floor fecal samples</td>
<td>0% vs. 20% vs. 40% DDG and WDG(^k) (Phase 1). Half of DDG</td>
<td>Greater probability to detect and number of high shedders in 40% DDG -WDG diets</td>
</tr>
</tbody>
</table>
pens transitioned to no-DDG (Phase 2).

(Phase 1). No difference in probability to detect (Phase 2).

Peterson et al., 2007a

| STEC O157 | Observed STEC O157 population | Fecal and TRM samples | 0% vs. 10% vs. 20% vs. 30% vs. 40% vs. 50% WDGS in HMC:DRC diets | Greater odds for detecting 40%-50% WDGS. Reduced odds for detecting 10%-30% WDGS (TRM samples). No difference in probability to detect (fecal samples). |

Rich et al., 2010

| STEC O157 | Observed STEC O157 population | Fecal samples | 0% vs. 40% WDGS in 3:2 ratio of HMC and DRC diets | Greater probability to detect in WDGS diets |

Wells et al., 2009

| STEC O157 | Observed STEC O157 population | Fecal and hide samples | 0% vs. 13.9% WDGS growing phase 0% vs. 40% WDGS finishing phase | Greater probability to detect in WDGS diets (fecal samples). Greater probability to detect in WDGS diet (finishing phase – hide samples) |

*aEscherichia coli, bRectoanal mucosal swabs, cWet corn gluten feed, dHigh moisture corn, eDry-rolled corn, fAlfalfa silage, gWhole cotton seed, hSteam-flaked corn, iReconstituted HMC, jDried distillers grains, kWet distillers grains, lTerminal rectal mucosal samples, mWet distillers grains solubles,
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