

**SIDEROPHORE RECEPTOR AND PORIN PROTEIN-BASED VACCINE
TECHNOLOGY: AN INTERVENTION STRATEGY FOR PRE-HARVEST CONTROL
OF *ESCHERICHIA COLI* O157 IN CATTLE**

by

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Abstract

Escherichia coli O157:H7 is a human food-borne pathogen and cattle feces are a major source of contamination. Immunization against *E. coli* O157 may be a practical pre-harvest intervention strategy. A siderophore receptor/porin proteins (SRP) based vaccine has been developed to decrease the prevalence of *E. coli* O157 in cattle. Two studies were conducted to determine the efficacy of the SRP vaccine.

In the first study, thirty calves were randomly assigned to one of two groups: control or SRP vaccine. Two weeks after the second vaccination, calves were orally inoculated with nalidixic acid-resistant (*Nal^R*) *E. coli* O157. Fecal samples were collected for five weeks. Calves were necropsied on day 35 to collect gut contents and tissue swabs to determine *Nal^R E. coli* O157:H7. The number of calves that were culture positive for *E. coli* O157 were lower ($P=0.07$) in vaccinated group compared to the control.

In the second study, cattle in two feedlots were randomized to SRP vaccine or control. Cattle were vaccinated on days 0 and 21. Rectal fecal samples were collected on day 0, and pen floor samples were collected on days 21, 35, and 70. Rectal fecal samples, RAMS, and hide swab samples were collected on d 85. Cattle were weighed on days 0, 21, and 85. Vaccination significantly reduced ($P = 0.04$) fecal *E. coli* O157 prevalence. There was also a decrease ($P < 0.05$) in *E. coli* O157 prevalence on hides and in fecal samples on day 85 in vaccinated cattle compared to the control.

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Dedication

I would like to dedicate my thesis to my parents, Danny and Vicki Thornton. They have always encouraged me to truly believe in myself and to strive for only the best.

I want to thank-you, Mom and Dad, for all of your encouraging words of wisdom and loving support. You put your lives on hold to make sure that I was raised in the best environment and received the best education. I will forever be grateful for the sacrifices that have you made for me. I owe all of my success to you. Thank-you.

Preface

The last two chapters in this thesis entitled: “Siderophore Receptor/Porin Proteins-based Vaccination Reduces Prevalence and Shedding of *Escherichia coli* O157:H7 in Experimentally Inoculated Cattle”, and “Effect of siderophore receptor/porin proteins-based vaccination on prevalence of *Escherichia coli* O157:H7 in feedlot cattle” were submitted for publication in the *Journal of Food Protection* and the *American Journal of Veterinary Research*, respectively. The text and figures within these chapters are formatted according to the guidelines that were specified by the journal.

CHAPTER 1 - LITERATURE REVIEW

Escherichia coli O157

Enterohemorrhagic *E. coli* O157:H7 is pathogenic microorganism that can cause severe hemorrhagic colitis, thrombocytopenic purpura, and hemolytic uremic syndrome (HUS) in humans. Illness usually occurs in children under the age of five, the elderly, and those who are immunosuppressed (Park et al., 1999). Based on an epidemiological study conducted in 1999, the CDC estimates that there are nearly 73,000 illnesses due to *E. coli* O157:H7 occurring annually in the United States. In 2006, specific outbreaks of *E. coli* O157:H7 were reported in more than 26 states affecting over 300 people and hospitalizing more than half of the population that was infected. Of those hospitalized, 39 people developed severe kidney failure and three people died (CDC, 2006).

Escherichia coli O157 is a Gram negative, rod-shaped, facultative anaerobe that is normally harbored in the intestines of healthy ruminants making manure an important source of environmental contamination (Rasmussen et al., 2001; Bach et al., 2002; CDC, 2006). According to Elder et al. in 2000, the prevalence of *E. coli* O157 is 11 to 28% in cattle feces, 11% on hides, and 3 to 28% on carcasses. Modes of transmission of this pathogen to humans are consumption of contaminated fruits and vegetables, consumption of improperly cooked contaminated ground beef, direct contact with animals (petting zoos), and exposure to contaminated water (Callaway et al., 2004).

Colonization and Virulence of *E. coli* O157

In order to effectively reduce the number of *E. coli* O157 shed by cattle and further reduce the risk of human illness, the bacteria's means of invading its host must be evaluated and understood. The ability of this organism to colonize in the intestines of its host requires several bacterial proteins which are important for proper attachment to the intestinal epithelial cells (Moxley, 2004). Intimin, which is encoded by the *eae* gene, is a bacterial outer membrane protein that mediates the intimate attachment of *E. coli* O157 to the intestinal epithelial cells. This attachment leads to the formation of A/E lesions which are encoded by the locus of enterocyte effacement (LEE) pathogenicity island (Gyles, 2007). The translocated intimin receptor (Tir), encoded by the *tir* gene, is a bacterial protein that is delivered to the host cell where it functions as a receptor for intimin. *E. coli* secreted proteins, or Esps, are proteins secreted by the type III secretion system which aid in bringing Tir into the host cell. Secretion proteins include: EspA which forms a filamentous structure that bridges the bacterial surface to the host cell's surface, EspB which is delivered into the host cell membrane where it becomes an integral membrane protein, and EspD which along with EspB, forms a pore structure in the membrane of the host cell (Kaper et al., 1998). Once the pathogen has invaded its host, it rearranges the cytoskeleton of each cell forming a pedestal where the bacteria then attach and reside (Vallance and Finlay, 2000).

Shiga toxins (Stx1 and Stx2) are two virulence factors that are very toxigenic to humans. Stx1 and Stx2 are both verotoxins that closely resemble those of *Shigella dysenteriae*. Shiga toxin 1 is a toxin that is commonly found in sheep and causes little to no disease in humans (Friedrich et al., 2004). In contrast, Shiga toxin 2 tends to cause severe endothelial damage and is responsible for acute renal failure (HUS) and bloody diarrhea in humans (Kaper et al., 1998).

The O-antigen, or lipopolysaccharide (LPS), and the H-antigen, which represents the flagella, are antigens that also play key roles in virulence (Gyles, 2007). LPS is a large complex molecule that is made up of lipids and carbohydrates (Prescott et al., 2005). It is very heat stable and consists of three unique parts: 1) the lipid A region, which is buried in the outer membrane of the bacterial cell and is responsible for toxicity of the LPS, 2) the inner and outer core region, which consists of several sugars that are needed for viability of the cells, and 3) the O-side chain, which is an antiphagocytic polysaccharide that extends outward from the core and is a major surface antigen for the bacteria (Prescott et al., 2005).

The H-antigen, or flagellar antigen, encoded by the *fliC* gene, is responsible for the movement of the microorganism within its host and is a major contributor to inflammation in humans (Gyles, 2007). *E. coli* O157:H7 has seven lophotrichous flagella (“H7”) that according to Berin et al. in 2002 play an important role in signaling proinflammatory cytokines (IL-8) in the human colonic epithelial cells. Researchers have found that the deletion of the *fliC* gene allows shiga-toxin producing *E. coli* to become less virulent (Gyles et al., 2007). Without flagella, bacteria cannot travel as effectively, thus, reducing the spread of the organism within the host (Prescott et al., 2005).

Environmental Conditions affecting prevalence of *E. coli* O157

Temperature and environmental conditions can often contribute to microorganism’s ability to thrive and therefore increase the exposure of the host animals. According to Hancock et al. in 1997, studies have shown that peak prevalence of *E. coli* O157 in cattle occurs in the late summer and early fall months in North America. This also correlates with documented increases in human cases of hemorrhagic colitis occurring from July to August relative to other times of the year (Armstrong, et al. 1996). *E. coli* O157 has been found to survive in manure for several

days at 37 degrees Celsius and for several months at 4 degrees Celsius due to its heat stable lipopolysaccharide (Wang et al, 1996; Kudva et al., 1998). Edrington et al. (2006) determined that there was a strong correlation between *E. coli* O157:H7 prevalence and day length. They found that after exposing four pens of cattle to an additional four hours of artificial daylight that experimentally infected cattle shed higher levels of *E. coli* O157:H7 than control cattle after 60 days of exposure (Edrington et al., 2006).

Smith et al. in 2001 found that there was a higher prevalence in cattle shedding *E. coli* O157:H7 in pens with muddy conditions when compared to cattle shedding in pens with normal conditions. This suggests that the condition of the pen floor may increase prevalence of fecal shedding of *E. coli* due to the increase exposure rate of cattle through drinking of contaminated ground water, increased contamination of the feed and intended water source, or increased stress on the cattle with the muddy conditions. Another study has shown that this organism is not only isolated from the feces of cattle (0.8%), but can also be isolated from feedbunks (1.7%), water troughs (12%), and incoming water supplies (4.5%) (Van Donkersgoed et al., 2005). These contaminated fomites may not only increase prevalence in cattle, but may also increase the chance of the organism to contaminate the environment.

Other environmental factors that may influence prevalence of *E. coli* O157 are house flies (*Musca domestica* L.). In 2006, eight calves were individually exposed to flies that were orally inoculated with a mixture of four strains of nalidixic acid-resistant (Nal^R) *E. coli* O157:H7. One day after exposure, all eight calves tested positive for the challenged organism. The fecal concentration of the Nal^R *E. coli* O157:H7 in exposed calves reached levels as high as 1.1×10^6 CFU/g. Calves remained positive for up to 11 days and 62% remained positive until the end of the study (Ahmad et al., 2007).

Intervention and Prevention

Farmers and scientists have been collaborating for the past twenty years to try to develop an appropriate pre/post-harvest intervention strategy to reduce *E. coli* O157 in cattle. Pre-harvest intervention would essentially reduce contamination of the microorganism in the environment, while post-harvest intervention would decrease contamination of beef products as the cattle are harvested. Pre-harvest strategies that are currently being researched to further reduce food borne illness include: probacterial/prebacterial intervention, the use of traditional antibiotics, bacteriophage therapy, dietary changes, and immunization.

Probiotics

According to Schrezenmeir and de Vrese (2001) probiotics are “products containing microorganisms in sufficient numbers which alter the microflora within the host and cause beneficial health effects.” These microorganisms cause competitive exclusion within the gut and essentially out-compete harmful bacteria for nutrients or receptor sites (Buchanan and Doyle 1997). Studies have shown that feeding a mixture of non-pathogenic strains of *E. coli* and *Proteus mirabilis* to cattle reduces colonization of *E. coli* O157 in the rumen and in the feces (Zhao et al., 1998). *Escherichia coli* strains capable of producing colicin E7 were also found to reduce (1.8 log₁₀CFU/g) serotype O157:H7 when administered to calves prior to oral challenge with nalidixic acid-resistant *E. coli* O157:H7. Colicins are antimicrobial proteins that are produced by some *E. coli* strains under high stress conditions and give that particular strain of bacteria a competitive advantage over pathogenic strains in the gut (Schamberger et al., 2004).

Additional studies conducted by Brashears et al. (2003) showed that *Lactobacillus*-based direct-fed microbials were effective in decreasing *E. coli* O157 in the feces and on hides of feedlot cattle. *Lactobacillus acidophilus*, particularly strain NP51, significantly reduced the

number of cattle shedding O157 resulting in the probability that “NP51 treated steers were 35% less likely to shed *E. coli* O157:H7 than control steers during a two year (2002-2003) period (Peterson, et al., 2007).”

Prebiotics

Oral prebiotics are another method used to out-compete pathogenic microorganisms for nutrients and space within the gut. Prebiotics are oligosaccharide polymers that are indigestible to the host and are highly digestible by commensal bacteria (LeJeune and Wetzel, 2007). Increasing the amount of nutrients available for commensal bacteria often creates an unfavorable environment for pathogens in means of space and nutrients. The addition of four fermentable carbohydrates (lactulose, inulin, wheat starch, and sugar beet pulp) were found to support growth of specific lactobacilli species (*Lactobacillus amylovorus*-like phylotype) in weaning piglets (Konstantinov et al. 2003). In 2007, an in vitro and in vivo trial was conducted to examine the effects of Cassiae Seeds (PCS) on improving the intestinal microflora of baby pigs (Deng et al. 2007). They found that there was a significant increase in *Lactobacillus* counts when 0.8% PCS was added to rejuvenation fluid and cecum content. Piglets (6.5 kg) were then fed diets supplemented with or without 0.4% or 0.8% PCS and upon necropsy, piglets that were fed 0.8% PCS had significantly more caecal bacterial microflora than those fed a normal ration or supplemented with 0.4% PCS. The use of prebiotics was, also, found to be beneficial in improving intestinal health of humans, yet little research has been done to test these products on cattle (de Vaux et al., 2002).

Antimicrobial Compounds

The administering of traditional antibiotics in cattle has been shown to effectively reduce *E. coli* O157:H7 in cattle. Neomycin sulfate has been tested in feedlot cattle and was found to

significantly decrease shedding of *E. coli* O157 in the feces and contamination on hides (98.2% and 95.0% reduction, respectively) (Loneragan and Brashears, 2005). Neomycin is an antimicrobial drug that is administered in the feed or in water and is used to treat colibacillosis in cattle (G.H. Loneragan and Brashears, 2005). The use of antibiotics as growth promotants in cattle has become a highly controversial topic making this form of intervention a less desirable strategy. “Bacteria have many complex mechanisms to resist antibiotics, and the widespread use of antibiotics in both human and animal medicine has led to the widespread dissemination of antibiotic resistance genes” (Callaway, et al., 2004). Although Neomycin is closely related to antibiotics that are used to treat human illness (aminoglycoside family) and may form a risk associated with antimicrobial residues, it is an ideal antibiotic for use in the cattle industry because it has a 24-hour withdrawal period (J.T. LeJeune and A.N. Wetzel, 2006, T.R. Callaway et al., 2003, Loneragan and Brashears, 2005). This allows for the proper reduction of *E. coli* O157:H7 to take place just prior to slaughter, thus reducing the risk of carcass contamination and further reducing the risk of food borne illness.

Supplementing sodium chlorate in the cattle’s drinking water is another method used to reduce *E. coli* O157:H7. This was tested in 2002 on four ruminally fistulated and four nonfistulated, nonlactating Holstein cows that were experimentally infected with three strains of *E. coli* O157:H7 (T.R. Callaway et al., 2003). Results indicated that a 24- hour treatment with sodium chlorate reduced *E. coli* O157:H7 by two logs (10^4 to 10^2) in the feces and by three logs (10^6 to 10^3) in the rumen. Sodium chlorate was described as a beneficial strategy because as chlorate is reduced to chlorite, by nitrate reductase, anaerobic bacteria die due to the chlorites bactericidal effects (T.R. Callaway et al. 2003; J.T. LeJeune and A. N. Wetzel, 2007). Though this chemical may be a beneficial strategy for reducing *E. coli* O157:H7 in the gut, it may

negatively impact beneficial microbial populations in the rumen, leading to a reduction in cattle's performance (J. T. LeJeune and A.N. Wetzel, 2007).

Bacteriophages

Another potential preharvest intervention strategy used to reduce *E. coli* O157:H7 in cattle are the use of bacteriophages. Bacteriophages are live viruses that are highly specific and can be used to kill bacterial pathogens within a mixed microbial population (T. R. Callaway, et al., 2003; J. T. LeJeune and Wetzel, 2007; Prescott et al., 2005). Bacteriophages have the ability to incorporate their DNA within the bacterial host's DNA, allowing the number of phages to increase exponentially, essentially exhausting all of the host's nutrients (Prescott et al., 2005; Callaway, et al., 2003).

Several research trials have been conducted on a variety of species to further examine the effects of bacteriophage therapy on reducing food borne pathogens (Tanji et al., 2005; Kudva et al., 1999; O'Flynn et al., 2004; Sheng et al., 2006). In 1999, researchers from the University of Idaho isolated and tested 53 *Escherichia coli* O157 antigen-specific bacteriophages from bovine and ovine fecal samples (Kudva, et al. 1999). Five of these phages were found to be *E. coli* O157 specific and three of these lytic phages (KH1, KH4, and KH5) lysed *E. coli* O157 after five days incubation at 4 degrees Celsius.

Sheng et al. in 2006 continued this research by orally administering a mixture of the previously tested bacteriophages (KH1 and SH1) to experimentally infected mice and found that phage treatment eliminated *E. coli* O157:H7 2 to 6 days after oral challenge. Phages were applied to the rectoanal junction mucosa of five Holstein steers and results indicated that there was a significant decrease in the concentration of *E. coli* O157:H7 when phage-treated steers were compared to the controls. This treatment did not, however, eliminate the bacteria in the gut

of most steers and did not reduce intestinal *E. coli* O157:H7 when the phage therapy was administered to sheep (Sheng et al., 2006). The use of bacteriophage therapy in food animals unfortunately is not frequently reported and further use of this intervention in the “real-world” setting may not be beneficial due to highly aerated conditions that are needed for this therapy to be effective (Kudva et al., 1999; Bach et al., 2002; Callaway et al., 2004).

Dietary Influence

Dietary changes may also influence the amount of *E. coli* O157:H7 found in the gastrointestinal tract of cattle (Duncan et al., 2000; Bach et al., 2002; Callaway et al., 2003; LeJeune and Wetzel, 2003). High concentrate diets that are fed to cattle during the finishing period often results in an increase of volatile fatty acids, which decreases the ruminal pH which creates an ideal environment for acid-resistant *E. coli* O157 (Bach et al., 2002). Published literature describing the effects of diet and diet change on the shedding of *E. coli* O157:H7 in ruminants have been controversial and inconclusive (Bach et al., 2002; Callaway et al., 2003; LeJeune and Wetzel, 2003). Some scientists have found that feeding forage-based diets allows animals to shed *E. coli* O157 longer than animals fed a grain-based diet (Hovde, et al. 1999), yet other scientists found that cattle that were abruptly switched from a finishing ration to 100% hay allowed fecal *E. coli* populations in the gut to decline 1,000-fold (Diez-Gonzalez et al., 1998; Hovde et al., 1999; Callaway et al., 2003; Van Baale et al., 2004).

Ingredients such as corn, barley, and canola oil have also been tested as dietary factors that may influence prevalence of *E. coli* O157 (Buchko et al., 2000; Berg et al., 2003; Bach et al., 2005). Buchko et al. in 2000 reported that there were significantly more animals culture positive for *E. coli* O157 when cattle were fed a finishing ration of 85% barley compared to cattle fed a finishing ration of 85% cracked corn. Berg et al. reported a similar hypothesis and

found that the average fecal concentration of *E. coli* O157 in cattle fed a barley-based diet compared to those fed a corn-based diet was 3.3 log CFU/g and 3.0 log CFU/g respectively. The addition of canola oil to the finishing ration was reported to have no effect on shedding of *E. coli* O157:H7 in feedlot cattle (Bach et al., 2005).

Fasting is a method commonly used to reduce contamination of hides prior to cattle being harvested (Stevens et al., 2002). The effect of dietary stress on fecal shedding of *Escherichia coli* O157:H7 in calves orally inoculated with 10^{10} CFU/animal was tested and revealed that withholding food for 48 hours after oral challenge did not significantly increase fecal shedding of *E. coli* O157 (Cray et al., 1998). Food withheld 48h prior to inoculation with 10^7 CFU/g of *E. coli* O157:H7 did result in a significant increase in the concentration of *E. coli* O157:H7 (1.4×10^4 CFU/g vs. 3.5×10^5 CFU/g; control vs. fasted, respectively) when fasted calves were compared to the controls (Cray et al., 1998). Kudva et al. (1995) witnessed the same phenomenon in sheep. They found that withholding feed prior to inoculation with *E. coli* O157 not only allowed the animals to shed bacteria at increased levels, but also increased the animal's susceptibility to other infections (Kudva et al., 1995). Fasting can cause the rumen pH to rise and the concentration of volatile fatty acids to drop, allowing for proliferation of *E. coli* O157:H7 due to favorable environmental conditions (Kudva et al., 1995). This suggests that interventions aimed at reducing food borne illness should be administered when cattle are experiencing dietary stress so that the spread of these pathogenic microorganisms is limited (Cray et al., 1998).

Vaccination

Exploiting the animal's immune system in order to decrease pathogenic microorganisms in the gastrointestinal tract is yet another intervention strategy that is currently being tested (Callaway et al., 2004; LeJeune and Wetzel, 2007, Bach et al., 2002). The use of vaccination to

prevent colonization of an organism that is a commensal in cattle can often be challenging. Nonetheless, investigators have developed and administered experimental vaccines containing proteins and cellular components that play key roles in bacterial adherence to the epithelial lining of the intestinal mucosa (LeJeune and Wetzel, 2007). Dean-Nystrom in 2002 used passive immunization to determine whether vaccination of dams with the *E. coli* O157 adhesin protein, intimin_{O157}, would reduce *E. coli* O157:H7 colonization and intestinal damage in neonatal piglets. Results indicated that vaccinated dams had colostral anti-intimin_{O157} titers greater than 100,000 and piglets that were nursing from vaccinated dams did not show signs of colonization of *E. coli* O157 10 days after inoculation (Dean-Nystrom et al., 2002).

Two other vaccines were developed and tested in hopes of reducing bacterial colonization of *E. coli* O157:H7. The first vaccine was developed by Konnadu et al. in 1999 and consisted of a detoxified lipopolysaccharide which was conjugated with a nontoxic B subunit of shiga-toxin 1. The second vaccine which was developed by Dziva et al. in 2007 was comprised of EspA, a Type III secretion protein (Konnadu et al., 1999; Dziva et al., 2007). Both vaccines were successful at promoting an immune response against the antigens of interest, yet the vaccine containing EspA was not effective in protecting calves against intestinal colonization of *E. coli* O157:H7 upon experimental infection.

Potter et al. in 2003 conducted an efficacy trial testing an additional experimental vaccine targeting the type III secretion system of *E. coli* O157. Researchers found that orally inoculated calves that were vaccinated with type III secretion proteins elicited an immune response and stopped shedding *E. coli* O157:H7 earlier than calves that were vaccinated with a placebo. In 2005, this vaccine was administered at a lower protein concentration to cattle at eight feedlots in Alberta, Canada and based on the results, scientists found that the pen prevalence was highly

variable (ranging from 0% to 80% at arrival, to 0% to 87% at revaccination, and 0% to 90% prior to slaughter) amongst both treatment groups (Type III vaccinates vs placebo controls) suggesting that the vaccine was not as efficacious in a natural feedlot setting (Van Donkersgoed et al., 2005).

E. coli O157:H7 SRP® vaccine

A recently developed vaccine that selectively targets the pathway at which bacteria acquires its nutrients was designed to reduce *E. coli* O157 in cattle by targeting siderophore receptors and porin proteins of gram negative bacteria (Emery et al., 2000). Iron is an essential nutrient for the growth and colonization of gram negative bacteria. Under low iron conditions, pathogenic bacteria produce a high affinity iron transport system to transport this required nutrient into the bacterial cell (Neilands, 1995). This siderophore receptor and porin protein-based (SRP) vaccine uses purified siderophore receptor and porin proteins to promote an immune response in cattle.

Siderophores are low molecular weight organic molecules that complex with ferric iron (Fe^{3+}) in tissues of the host and supply it to the bacterial cell. Siderophores bind with the siderophore receptors located on the outer membrane of the bacterial cell and allow for the passage of iron into the cell. Once the iron has reached the periplasmic space, it is reduced to the ferrous form (Fe^{2+}) to be used for the metabolic function (Prescott, 2005). Porin proteins, such as OmpA, OmpC, OmpD, and OmpF, are outer membrane proteins of certain gram negative bacteria that are expressed with or without the presence of iron (Emery et al., 2000). Porins often share a strong structural resemblance and may have slight sequence homology (Cowan et al., 1992).

The immunized animal produces anti-SRP antibodies which bind to the siderophore receptors and porins present on the outer membrane of the bacterial cell and block iron from being

transported into the cell. The blocking of this nutrient essentially starves the bacteria and further colonization of this organism is greatly reduced.

SRP technology has been utilized in several species for the control of gram negative bacterial pathogens in the gut. Field trials performed to aid in the development of an *E. coli* SRP vaccine to combat *E. coli* infections in turkeys showed that turkeys vaccinated with the *E. coli* SRP vaccine had an increase in antibody titers throughout a ten week period without revaccination (Emery et al., 2000). *E. coli* SRP vaccinated turkeys not only had decreased mortality rate (38.5%, $P < 0.01$) and decreased carcass condemnation (31%, $P < 0.01$), but, also, had increase in weight gain at harvest (9.3%, $P < 0.01$) when compared to the non-vaccinated controls (Emery et al. 2000).

REFERENCES

1. Ahmad, A., T. G. Nagaraja, and L. Zurek. 2007. Transmission of *Escherichia coli* O157:H7 to cattle by house flies. *Prev. Vet. Med.* 80:74-81.
2. Armstrong, G. L., J. Hollingsworth, and J. G. Moms. 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.* 18:29-51.
3. CDC Department of Health and Human Services website. *Escherichia coli* O157:H7 Available at <http://www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacolig.htm> Accessed on October 5, 2006.
4. Bach, S. J., T. A. McAllister, D. M. Veira, V. P. J. Gannon, and R. A. Holley. 2002. Transmission and control of *Escherichia coli* O157-a review. *Can. J. Anim. Sci.* 82:475-490.
5. Bach, S. J., L. J. Selinger, K. Stanford, et al. 2005. Effect of supplementing corn- or barley-based feedlot diets with canola oil on faecal shedding of *Escherichia coli* O157:H7 by steers. *J. Appl. Microbiol.* 98:464-475.
6. Berg, J., T. McAllister, S. Bach, et al. 2004. *Escherichia coli* O157:H7 Excretion by Commercial Feedlot Cattle Fed Either Barley- or Corn-Based Finishing Diets. *J. Food Prot.* 67:666-671.

7. Brashears, M. M., M. L. Galyean, G. H. Loneragan, et al. 2003. Prevalence of *Escherichia coli* O157:H7 and Performance by Beef Feedlot Cattle Given *Lactobacillus* Direct-Fed Microbials. *J. Food Prot.* 66:748-754.
8. Buchanan, R. L. and M. P. Doyle. 1997. Foodborne disease significance of *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli*. *Food Technol.* 51:69-76.
9. Buchko, S. J., R. A. Holley, W. O. Olson, et al. 2000. The Effect of Different Grain Diets on Fecal Shedding of *Escherichia coli* O157:H7 by Steers. *J. Food Prot.* 63:1467-1474.
10. Callaway, T. R., R. O. Elder, J. E. Keen, et al. 2003. Forage Feeding to Reduce Preharvest *Escherichia coli* Populations in Cattle, a Review. *J. Dairy Sci.* 86:852-860.
11. Callaway, T.R., R. C. Anderson, T. S. Edrington, K. J. Genovese, K. M. Bischoff, T. L. Poole, Y. S. Jung, R. B. Harvey, and D. J. Nisbet. 2004. What are we doing about *Escherichia coli* O157 in cattle? *J. Anim. Sci.* 82:E93-E99.
12. Cowan, S. W., T. Schirmer, G. Rummel, M. Steiert, R. Ghosh, R. A. Pauptit, J. N. Jansonius, and J.P. Rosenbusch. 1992. Crystal structures explain functional properties of two *E. coli* porins. *Nature* 358:727-733.

13. Cray, W. C. JR., T. A. Casey, B. T. Bosworth, et al. 1998. Effect of Dietary Stress on Fecal Shedding of *Escherichia coli* O157:H7 in Calves. *Appl. Environ. Microbiol.* 64:1975-1979.
14. Dean-Nystrom, E. A., L. J. Gansheroff, M. Mills, H. W. Moon, and A. D. O'Brien. 2002. Vaccination of pregnant dams with intimin O157 protects suckling piglets from *Escherichia coli* O157:H7 infection. *Infect. Immun.* 70:2414-2418.
15. Deng, Z. Y., J. W. Zhang, J. Li, et al. 2007. Effect of polysaccharides of cassiae seeds on the intestinal microflora of piglets. *Asia Pac. J. Clin. Nutr.* 16:143-147.
16. de Vaux, A., M. Morrison, and R. W. Hutkins. 2002. Displacement of *Escherichia coli* O157:H7 from rumen medium containing prebiotic sugars. *Environ. Microbiol.* 68:519-524.
17. Diez-Gonzalez, F., T. R. Callaway, M. G. Kizoulis, and J. B. Russell. 1998. Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. *Science* 281:1666-1668.
18. Duncan, S. H., I. R. Booth, H. J. Flint, et al. 2000. The potential for the control of *Escherichia coli* O157 in farm animals. *J. Appl. Microbiol. Symp. Suppl.* 88:157S-165S.

19. Dziva, F., I. Vlisidou, V. F. Crepin, et al. 2007. Vaccination of calves with EspA, a key colonization factor of *Escherichia coli* O157:H7, induces antigen-specific humoral responses but does not confer protection against intestinal colonization. *Vet. Microbiol.* 123:254-261.
20. Edrington, T. S., T. R. Callaway, S. E. Ives, et al. 2006. Seasonal Shedding of *Escherichia coli* O157:H7 in Ruminants: A New Hypothesis. *Foodborne Path. and Disease.* 3:413-421.
21. Emery, D. A., D. E. Straub, R. Huisinga, and B. A. Carlson. November 2000. Active Immunization using a siderophore receptor protein. U.S. Patent 6,027,736.
22. Friedrich, A. W., K. V. Nierhoff, M. Bielaszewska, A. Mellmann, and H. Karch. 2004. Phlogeny, clinical associations, and diagnostic utility of the pilin subunit gene (*sfpA*) of sorbitol-fermenting, enterohemorrhagic *Escherichia coli* O157:H7. *J. Clin. Microbiol.* 42:4697-4701.
23. Gyles, C. L. 2007. Shiga toxin-producing *Escherichia coli*: An overview. *J. Anim. Sci.* 85(E Suppl.):E45-E62.
24. Hovde, C., P. R. Austin, K. A. Cloud, et al. 1999. Effect of Cattle Diet on *Escherichia coli* O157:H7 Acid Resistance. *Appl. Environ. Microbiol.* 65:3233-3235.

25. Kaper, James B. and Alison D. O'Brien. 1998. *Escherichia coli* O157:H7 and Other Shiga Toxin-Producing *E. coli* Strains. Washington, D. C.: ASM Press. 465p.
26. Konnadu, E., A. Donohue-Rolfe, S. B. Calderwood, Vince Pozsgay, Joseph Shiloach, John B. Robbins and Shousun C. Szu. 1999. Syntheses and immunologic properties of *Escherichia coli* O157 O-specific polysaccharide and Shiga toxin 1 B subunit conjugates in mice. *Infect. Immun.* 67:6191-6193.
27. Konstantinov, S. R., A. Awati, H. Smidt, et al. 2004. Specific Response of a Novel and Abundant *Lactobacillus amylovorus*-Like Phylotype to Dietary Prebiotics in the Guts of Weaning Piglets. *Appl. Environ. Microbiol.* 70: 3821-3830.
28. Kudva, I. T., P. G. Hatfield, and C. J. Hovde. 1995. Effect of Diet on the Shedding of *Escherichia coli* O157:H7 in a Sheep Model. *Appl Environ Microbiol.* 61:1363-1370.
29. Kudva, I. T., K. Blanch, and C. J. Hovde. 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. *Appl. Environ. Microbiol.* 64:3166-3164.
30. Kudva, I. T., S. Jelacic, P. I. Tarr, et al. 1999. Biocontrol of *Escherichia coli* O157 with O157-Specific Bacteriophages. *Appl. Environ. Microbiol.* 65: 3767-3773.
31. Lejeune, J.T., and A.N. Wetzel. 2007. Preharvest control of *Escherichia coli* O157 in cattle. *J. Anim. Sci.* 85:E73-E80.

32. Lonergan, G H., and M. M. Brasheers, 2005. Pre-harvest interventions to reduce carriage of *E. coli* O157 by harvest-ready feedlot cattle. *Meat Sci.* 71:72-78.
33. Moxley, R. A. 2004. *Escherichia coli* O157:H7: an update on intestinal colonization and virulence mechanism. *Anim. Health Res. Rev.* 5: 15-33.
34. Neilands, J. B. 1995. Siderophores: Structure and function of microbial iron transport compounds. *J Biol Chem.* 270:26723-27726.
35. O'Flynn, G., R. P. Ross, G. F. Fitzgerald, et al. 2004. Evaluation of a Cocktail of Three Bacteriophages for Biocontrol for *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 70: 3417-3424.
36. Park, S., R. W. Worobo, and R. A. Durst.1999. *Escherichia coli* O157:H7 as an emerging food borne pathogen: A literature review. *Crit. Rev. Food Sci. Nutr.* 39:481-502.
37. Peterson, R. E., T. J. Klopfenstein, G. E. Erickson, et al. Effect of *Lactobacillus acidophilus* Strain NP51 on *Escherichia coli* O157:H7 Fecal Shedding and Finishing Performance in Beef Feedlot Cattle. 2007. *J. Food Prot.* 70:287-291.
38. Potter, A. A., S. Klashinsky, Y. Li, E. Frey, H. Townsend, D. Rogan, G. Erickson, S. Hinkley, T. Klopfenstein, R. A. Moxley, D. R. Smith, and B. B. Finlay. 2004. Decreased

shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. *Vaccine* 22:362-369.

39. Prescott, L. M., J. P. Harley, and D. A. Klein (6th ed.). 2005. Microbiology. Boston: McGraw Hill Higher Education, pp. 56-66,101-102, 371-385.

40. Rassmussen, M. A., and T. A. Casey. 2001. Environmental and food safety aspects of *Escherichia coli* O157:H7 infections in cattle. *Crit. Rev. Microbiol.* 27:57-73.

41. Schamerger, G. P., R. L. Phillips, J. L. Jacobs, and F. Diez-Gonzalez. 2004. Reduction of *Escherichia coli* O157:H7 Populations in Cattle by Addition of Colicin E7-Producing *E. coli* to Feed. *Appl. Environ. Microbiol.* 70:6053-6060.

42. Schrezenmeir, J., and M. de Vrese. 2001. Probiotics, prebiotics, and synbiotics- Approaching a definition. *Am. J. Clin. Nutr.* 73:361-364.

43. Sheng, H., H. J. Knecht, I. T. Kudva, and C. J. Hovde. 2006. Application of Bacteriophages To Control Intestinal *Escherichia coli* O157:H7 Levels in Ruminants. *Appl. Environ. Microbiol.* 72:5259-5366.

44. Smith, D., M. Blackford, S. Younts, R. Moxley, J. Gray, L. Hungerford, T. Milton, and T. Klopfenstein. 2001. Ecological Relationships between the Prevalence of Cattle

Shedding *Escherichia coli* O157:H7 and Characteristics of the Cattle or Conditions of the Feedlot Pen. J. Food Prot. 64:1899-1903.

45. Stevens, M. P., P. M. van Diemen, F. Dziva, et al. 2002. Options for the control of enterohaemorrhagic *Escherichia coli* in ruminants. Microbiology. 148:3767-3778.
46. Vallance, B. A. and B.B. Finlay. (2000). Exploitation of host cells by enteropathogenic *Escherichia coli*. PNAS. 97: 8799-8806.
47. Van Baale, M. J., J. M. Sargeant, D. P. Gnad, B. M. DeBey, K. F. Lechtenberg, and T. G. Nagaraja. 2004. Effect of forage or grain diets with or without monensin on ruminal persistence and fecal *Escherichia coli* O157:H7 in Cattle. Appl. Environ. Microbiol. 70:5336-5342.
48. Van Donkersgoed, J., D. Hancock, D. Rogan, and A. A. Potter. 2005. *Escherichia coli* O157:H7 vaccine field trial in 9 Feedlots in Alberta and Saskatchewan. Canadian Vet. J. 46:724-728.
49. Wang, L., K. R. Mankin, and G. L. Marchin. 2004. Survival of fecal bacteria in dairy cow manure. Trans Am Soc Agric Eng 47:1239-1246.

50. Zhao, T., M. P. Doyle, B. G. Harmon, et al. 1998. Reduction of Carriage of Enterohemorrhagic *Escherichia coli* O157:H7 in Cattle by Inoculation with Probiotic Bacteria. *J. Clin. Microbiol.* 36:641-647.

SUMMARY

Beef products have been commonly linked to several reported cases of human illness due to *Escherichia coli* O157:H7 infections (LeJeune and Wetzel, 2007). Pre-harvest, along with post-harvest control of this food-borne pathogen is important in providing a safe product for the consumer. The pre-harvest strategies must balance the implementation cost along with efficacy of the intervention. The use of vaccination to prevent pathogenic colonization and fecal excretion in cattle may be the most efficient pre-harvest intervention strategy for reducing *E. coli* O157:H7 in cattle. This is due to the familiarity by cattle producers, ease of acceptance, and ready incorporation into existing vaccination practices (Lonergan and Brashears, 2005).

A siderophore receptor/porin protein-based (SRP) vaccine was developed as an attempt to reduce human illness associated with *E. coli* O157:H7. Experimental trials used to evaluate the efficacy of this novel vaccine were performed in hopes of reducing prevalence and colonization of *E. coli* O157:H7 in cattle. Therefore, the objective of this thesis is to evaluate the ability of the *E. coli* SRP vaccine to reduce *E. coli* O157:H7 in cattle utilizing a challenge model and a feedlot setting.

**CHAPTER 2 - Siderophore Receptor/Porin Proteins-based
Vaccination Reduces Prevalence and Shedding of *Escherichia coli*
O157:H7 in Experimentally Inoculated Cattle**

ABSTRACT

The efficacy of a vaccine containing outer membrane siderophore receptor and porin (SRP) proteins in reducing prevalence of *E. coli* O157:H7 was evaluated in cattle inoculated with *E. coli* O157. Thirty, calves were randomly assigned to one of two groups and administered subcutaneously, on days 1 and 21, either a placebo (control) or a vaccine. Blood was collected weekly to monitor the serum anti-SRP antibody titers. Two weeks after the second vaccination, calves were orally inoculated with a mixture of five strains of nalidixic acid-resistant (*Nal^R*) *E. coli* O157:H7. Fecal samples and rectoanal mucosal swabs were collected daily for the first 5 days, and three times a week for the following four weeks to determine the presence and enumerate fecal concentration of *Nal^R* *E. coli* O157:H7. Calves were necropsied on day 35 to collect gut contents and tissue swabs to determine presence and concentration of *Nal^R* *E. coli* O157:H7. Vaccinated cattle had significantly higher anti-SRP antibody titers than the control with a significant treatment by week interaction ($P < 0.01$). Vaccination of cattle with the SRP tended to ($P = 0.10$) decrease fecal concentration of *E. coli* O157:H7. The number of calves that were culture positive for *E. coli* O157, were lower ($P = 0.07$) in vaccinated group compared to the control. The novel *E. coli* O157:H7 SRP vaccine appears to be effective in reducing prevalence and fecal concentration of *E. coli* O157 in cattle orally inoculated with *Nal^R* *E. coli* O157:H7 and may be useful as a possible pre-harvest intervention strategy.

INTRODUCTION

Escherichia coli O157:H7 is a foodborne pathogen that causes hemorrhagic colitis of varying severity in humans, particularly in children and elderly it could lead to a serious condition called hemolytic uremic syndrome (Park et al., 1999; Karmali, 2005). Cattle gastrointestinal tracts, particularly the hind gut, are the main reservoir and the organism is shed in the feces (Rasmussen et al., 2001; Bach et al., 2002). The primary site of *E. coli* O157 colonization in cattle has been shown to be the mucosal epithelium, proximal to the rectoanal junction, at the terminal rectum (Naylor et al., 2003; Low et al., 2005). Swabbing this region with a foam-tipped applicator, called rectoanal mucosal swab (RAMS), has been shown to be a more sensitive sampling method for detecting *E. coli* O157 in cattle (Rice et al., 2003; Greenquist et al., 2004; Davis et al., 2006).

Prevalence of *E. coli* O157 in cattle is variable among herds and individual animals, and feedlot cattle prevalence ranges from 10 to 28% and may be as high as 80% in the summer months (Elder et al., 2000; Gansheroff and O'Brien, 2000; Sargeant et al., 2003). Most incidence of *E. coli* O157:H7 illnesses have been associated with cattle feces contaminating food directly (ground beef) or indirectly (juices, produce, other foods, and water) (Besser et al., 1999).

Pre-harvest prevalence in cattle is associated with subsequent likelihood of post-harvest contamination of carcasses (Elder et al., 2000). Therefore, efforts to reduce carriage in harvest ready cattle will likely reduce the proportion of carcasses that are contaminated with *E. coli* O157:H7. Furthermore, interventions used to reduce shedding of this pathogen will also lower contamination of the pathogen to the environment.

Iron is an essential nutrient for all Gram negative bacteria including *E. coli* O157:H7 (Bolin and Jensen, 1987). A recently developed method that reduces the ability of gram-negative

bacteria to acquire iron (Emery, et al., 2000) may be a practical intervention to reduce infections with gram negative organisms. Under low iron conditions, pathogenic bacteria, including *E. coli*, have evolved high affinity iron transport systems, such as ferric iron chelators or siderophores, iron regulated outer membrane proteins (IROMPs), and siderophore receptor proteins (SRP), which are receptors for the siderophores on the outer membrane of the bacterial cell (Bolin and Jensen, 1987). Siderophores are low molecular weight organic molecules that complex with ferric iron (Fe^{3+}) in tissues of the host and supply it to the bacterial cell. Siderophores bind with the siderophore receptors located on the outer membrane of the bacterial cell and allow for the passage of iron into the cell. Once the iron has reached the periplasmic space, it is reduced to the ferrous form (Fe^{2+}) to be used for the metabolic function (Prescott, 2005). Porin proteins, such as OmpA, OmpC, OmpD, and OmpF, are outer membrane proteins of certain gram negative bacteria that are expressed with or without the presence of iron (Emery et al). Porins often share a strong structural resemblance and may have slight sequence homology (Cowan et al.).

Immunization that effectively targets the siderophore receptor and porin systems of *E. coli* O157:H7 should restrict iron transport into the bacterial cell. The purpose of this study, therefore, was to evaluate the efficacy of an experimental vaccine containing *E. coli* O157:H7 derived siderophore receptor and porin proteins in reducing prevalence and shedding of this pathogen in cattle that were orally inoculated with *E. coli* O157:H7.

MATERIALS AND METHODS

Cattle. Thirty, three or four-month old, mixed breed beef calves ($182 \pm$ kg) were used for this study. The calves on arrival were vaccinated with a *Mannhiemia haemolytica* bacterin with leukotoxin and 7-way clostridial bacterin (One Shot Ultra 7; Pfizer Animal Health, Kalamazoo, MI.; lot#1478032) and a modified live viral BVD type I and II, IBR, PI-3 and BRSV vaccine (Bovi-Shield Gold 5; Pfizer Animal Health, lot#1479203). Tilmicosin was injected subcutaneously (Micotil 300; Elanco Animal Health, Greenfield, IN.: lot#41820A) and an endectocide (Dectomax; Pfizer Animal Health, lot#KST01311) was also injected subcutaneously to each calf. Calves were allowed to acclimatize as a herd for approximately two months. The calves were fed group fed a commercial pelleted diet (Premium Starter 14, Wildcat Feeds, Topeka, KS) at approximately 3.1 kg per head per day and were allowed *ad libitum* access to brome grass hay and water throughout the study.

Approximately one month later, calves were randomly assigned to one of two treatment groups: 1) control that received a placebo (adjuvant only) or 2) treatment that received an experimental SRP *E. coli* O157:H7 vaccine (Epitopix LLC., Willmar, MN). There were fifteen calves in each group and the placebo or vaccine was administered subcutaneously on days 1 and 21. One week after the second injection (day 28), the calves were moved to a BSL-2 facility at Kansas State University and housed individually in pens with walls separating each pen. Each pen contained woodchip bedding, plastic buckets for drinking water, and individual feed bunks. Calves were continued on their starter ration diet (3.1 kg per head per day) and drinking water was provided *ad libitum*. On day 36, calves in both groups were orally inoculated, via gastric tube, with a mixture of five strains of *E. coli* O157:H7. The animal

housing and management procedures used in this study were approved by the institutional Animal Care and Use Committee.

Preparation of E. coli O157:H7 inoculum. Five strains of *E. coli* O157:H7 (FRIK920, FRIK1123, FRIK2000, 01-2-10561, and 01-2-08970), previously isolated from dairy or feedlot cattle feces (Kim et al., 1999; Sargeant et al., 2003), were made resistant to nalidixic acid (50 µg/ml; *Nal*^R). The stored strains were streaked onto sorbitol MacConkey agar (BD, Franklin Lakes, NJ) containing cefixime (50 ng/ml), potassium tellurite (2.5 µg/ml) (CT-SMAC), and supplemented with nalidixic acid (50 µg/ml) (CT-SMAC-N). After 24 h incubation, single colony of each of the five strains was subcultured into 10 ml of tryptic soy broth (TSB; BD). The broth was incubated overnight (14 to 18 h) and 1 ml of inoculum from each tube was transferred to 250 ml-pyrex bottles (Fisher, Palatine, IL) containing 100 ml of TSB, incubated for approximately 7 h and then pooled in a sterile 4 liter-pyrex bottle. Just prior to oral inoculation, 5 ml of the pooled culture was mixed with 195 ml of 1% sterilized skim milk (EMB, Gibbstain, NJ). A plate count of the pooled mixture was done by spread plating onto MacConkey agar (BD) and incubated overnight to determine the final concentration of cells in the inoculum. The final concentration of the inoculated skim milk was 1.5×10^9 CFU/ml and each calf received a dose of 7.5×10^9 CFU.

Samples and sampling schedule. Fecal samples were collected from each calf on the morning prior to oral inoculation with *Nal*^R *E. coli* O157:H7, which was 14 days after the second vaccination. After oral inoculation, fecal samples and RAMS were collected from each calf daily for the first five days, and subsequently three times a week (Monday, Wednesday and

Friday) for the following 4 weeks to test for the presence and determine the concentration of *Nal^R E. coli* O157:H7. Fecal samples were collected rectally and placed in Whirl-Pak bags (Nasco, Ft. Atikson, WI). Rectoanal mucosal swab sample was collected, using a sterile, foam-tipped applicator (VWR International, Buffalo Grove, IL), which was inserted approximately 2 to 5 cm into the anus of each calf to gently scrape the epithelium of the rectoanal junction with minimal amounts of fecal contamination of the swab (Rice et al., 2004; Greenquist et al., 2005). The swabs were immediately placed in culture tubes containing 3 ml of Gram-Negative (GN) broth (BD) with cefiximie (0.05 mg/liter), cefsulodin (10 mg/liter), and vancomycin (8 mg/liter; GNccv; Greenquist et al., 2005). Both fecal samples and swabs were held on ice and transported to the laboratory and processed within an hour after collection.

Blood samples were collected from each calf on day 1, just prior to the first vaccination (week 0), and thereafter at weeks 1, 2, 3 and 5, to monitor anti-SRP antibody titers in control and vaccinated cattle. The blood was transferred to the lab on ice, centrifuged for 10 min at 1,000 rpm, and serum was collected from each sample and placed in sterile cryogenic vials. The samples were shipped on ice to Epitopix LLC., for anti-SRP antibody analysis.

Necropsy examination. At the end of the study (day 35 following oral challenge), calves were euthanized and necropsied in the Kansas State University Veterinary Diagnostic Laboratory. Approximately 5 g of gut contents (rumen, omasum, abomasum, cecum, colon, and rectum) and tissue swab samples with foam-tipped applicators (gall bladder and rectoanal mucosa) were collected from each animal. The tissue swabs were immediately placed in 3 ml GNccv tubes. The gut contents in Whirl-Pak bags and tissue swabs in tubes were transported to the laboratory and analyzed to determine presence and concentration of *Nal^R E. coli* O157:H7.

Detection of E. coli O157 in pre-challenge fecal samples. Approximately 2 g of feces was placed in a tube containing 18 ml of GNccv broth. Fecal suspensions in the broth were vortexed for 1 min and incubated for 6 h at 37°C. The enriched fecal suspensions were then subjected to immunomagnetic separation (IMS; Dynal, Inc., New Hyde Park, NY) and spread-plated onto CT-SMAC. Plates were incubated overnight (16 to 18 h) and for each sample up to six sorbitol negative colonies were streaked onto blood agar plates (Remel, Lenexa, KS) and incubated for 12 to 18 h at 37°C. Colonies on blood agar plates were tested for indole production, O157 antigen using latex agglutination (Oxoid Limited, Basingstoke, Hampshire, England), and species confirmation was performed using API strips (Rapid 20E; Biomerieux, Inc., Hazelwood, MO).

Detection and quantification of NalR E. coli O157:H7 in post-challenge fecal, RAMS, and necropsy samples. Fecal or gut content (necropsy) samples were kneaded for 20 to 30 sec and approximately 2 g of each sample was placed in pre-weighed tubes containing 18 ml of GNccv broth. The tubes were reweighed to determine the amount of samples. The fecal suspensions or swab samples were vortexed for 1 min and 0.1 ml was pipetted into the first well of a 2.4 ml well capacity, 96 well Microtiter block (Corning Inc., Corning, NY) containing 0.9 ml of buffered peptone water (Sigma-Aldrich, St. Louis, MO) dilution blank per well. Serial 10-fold dilutions were made and 100 µl of the appropriate dilutions were spread plated onto CT-SMAC-N agar and incubated for 24 h at 37°C. After incubation, sorbitol-negative colonies with morphology characteristics of *E. coli* O157: H7 were counted and recorded. A maximum of

three colonies per sample were collected, streaked onto blood agar, and incubated for 24 h at 37°C and tested for indole production and for the O157 antigen using latex agglutination.

The detection limit of the direct plating method for *Nal^R E. coli* O157:H7 was 10² CFU per g of sample. Therefore, to detect low numbers of organisms (< 10²/g), an enrichment step was completed for each fecal, RAMS, gut content or tissue swab sample collected at necropsy (2.0 g in 18ml or swabs in 3 ml GNccv broth) by incubating the tubes for 6 h at 37°C. After incubation, 1.0 ml of enriched broth was transferred to another 9.0 ml GNccv broth and incubated overnight 18-24 h at 37°C. After incubation, 100 µl was spread plated onto CT-SMAC-N agar and incubated for 24 h at 37°C. A maximum of three sorbitol-negative colonies per sample were transferred to blood agar plate, incubated for 24 h at 37°C and tested for indole production and for the O157 antigen by latex agglutination.

Determination of anti-SRP antibody concentration in serum. Ninety-six well plates (Immulon-2 ELISA plates, Dynatech Laboratories, Chantilly, VA) were coated with *E. coli* O157:H7 SRP antigen diluted in a coating buffer (1.59 g/liter Na₂CO₃, 2.93 g/liter NaHCO₃, pH 9.6). The total antigen volume to coat each well was 100 µl. The antigen was allowed to bind to the plate for 2 h on a rotator at 37°C. Unbound antigen was removed from the plate and replaced with a blocking buffer (8.0 g/liter NaCl, 0.2 g/liter KCl, 1.44 g/liter Na₂HPO₄, 0.24 g/liter KH₂PO₄, 20 ml/liter fish gelatin). The plate was placed at 4°C overnight on a rotator. Serum from a calf that was hyper-immunized with the *E. coli* O157:H7 SRP vaccine was used as a positive control. The positive control sera and test sera samples were added to the plate in duplicate. One-hundred µl of each serum was added to the well at 1:50 dilution in phosphate buffered saline. The sera samples were incubated on the plate for 1 h on a rotator at 37°C. The

serums were then removed and the plate was washed three times with wash buffer (8.0 g/liter NaCl, 0.2 g/liter KCl, 1.44 g/liter Na₂HPO₄, 0.24 g/liter KH₂PO₄, 5 ml/liter Fish gelatin, 0.5 ml/liter Tween-20). A 1:15,000 dilution of alkaline phosphatase coated anti-bovine IgG H & L conjugate (Sigma-Aldrich, St. Louis, MO) was added to each well and the conjugate was incubated for 1 h with rotation at 37°C. The conjugate was then removed and the plate was washed three times with wash buffer. One-hundred µl of 4-Nitrophenyl phosphate (PNPP) substrate, prepared according to manufacturer's instructions (Sigma-Aldrich), was added to each well and incubated on a rotator at 37°C for 30 min. The absorbance of each well was determined with a spectrophotometer at a 405 nm wavelength. The blank wells were subtracted from all sample wells so that the actual sample absorbance could be determined. Results for samples were then determined by taking the absorbance of the unknown sample and dividing it by the absorbance of the standard positive serum to get a sample to positive (S:P) ratio.

Statistical analysis. Analysis of variance was performed using commercially available statistical analysis software (SAS System for Windows, Version 9.1, SAS Institute, Cary, NC) to analyze fecal or gut contents *Nal*^R *E. coli* O157:H7 concentrations. Bacterial counts were log₁₀ transformed before analysis. Data were analyzed as a repeated measure design. The statistical models included fixed effects of vaccination, sampling day, and the two-way interaction between vaccination and sampling day. The random effect consisted of experimental animal within treatment. Paired t-tests were used to determine statistical significance between treatment groups, sampling days or the interaction of treatment and sampling day. Binomial data (presence or absence of *E. coli* O157:H7 by enrichment) were analyzed using logistic regression. Initially, the interaction between treatment and day was assessed and if this interaction was not

statistically significant, it was not included in final models. Final logistic regression models included treatment and sampling day as main effects and repeated measures on animal was accounted for because of the lack of independence of these samples. Logit models in PROC GENMOD of SAS, were used to analyze the necropsy data with the numerator being the number of positive samples taken from the eight sampling sites and the denominator being the total samples collected for each animal (n=8).

RESULTS

Analysis of fecal samples collected just prior to oral inoculation revealed that two calves in the control group were culture positive for *E. coli* O157. No RAMS sample was collected before the inoculation. Statistical analysis of the fecal concentration data, after oral inoculation, of the two culture positive cattle were not different ($P = 0.87$) from the remaining cattle in the control group. Also, statistical analysis of the fecal concentration data in the control and vaccinated cattle showed only a small difference in with ($P = 0.10$) or without ($P = 0.12$) inclusion of the two culture positive cattle. Therefore, we decided to include the two culture positive cattle in all subsequent data analyses.

Antibody response in the control or vaccinated group. The mean and standard errors of anti-SRP antibody titers (serum to standard positive ratio) for each treatment group before vaccination (week 0), after first vaccination (weeks 1, 2, and 3), and after the second vaccination (week 5) are shown in figure 1. Before the first injection, both groups had low but similar antibody titers. There was a significant increase ($P = 0.01$) in the anti-SRP antibody titer in the

vaccinated group in all samples collected after the first vaccination. There was no change in anti-SRP antibody titer in the control group during the study (Figure 2-1). Therefore, there was a treatment by day interaction ($P < 0.01$).

Figure 2-1 Anti-siderophore-porin (SRP) antibody response

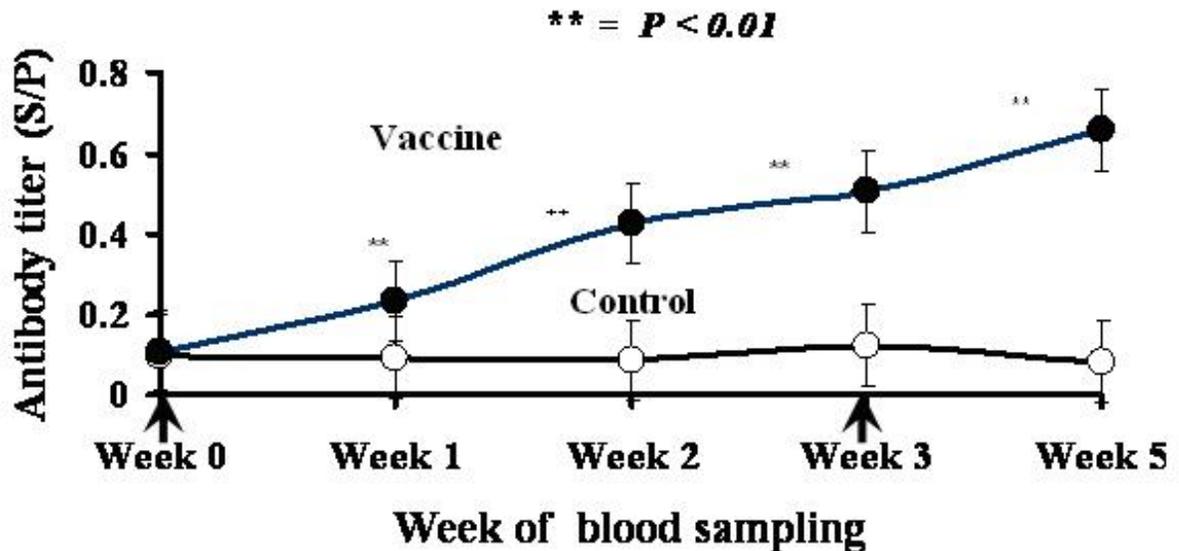


Figure 2-1 Anti-siderophore-porin (SRP) antibody response in control (open circles) or vaccinated (closed circles) cattle before first vaccination (week 0), after first vaccination (weeks 1, 2, and 3), and after second vaccination (week 5). Cattle were vaccinated on weeks 0 and 3 (Arrows). S/P represents the ratio of the absorbance of the unknown sample divided by the absorbance of the standard positive serum.

E. coli O157 in fecal or RAMS samples in the control or vaccinated group.

Average initial concentrations of *Nal^R E. coli O157:H7* in fecal samples of both groups were in the range of 10^3 to 10^5 CFU/g during the first 4 days following oral challenge (day 1) with 10^{10} per animal (Figure 2.2). Fecal concentrations (\log_{10} CFU/g) of *Escherichia coli O157* in control (open circles) or vaccinated (closed circles) cattle following oral challenge (day 0) with nalidixic acid-resistant *Escherichia coli O157*.

Figure 2-2 Fecal concentrations (\log_{10} CFU/g) of *Escherichia coli* O157

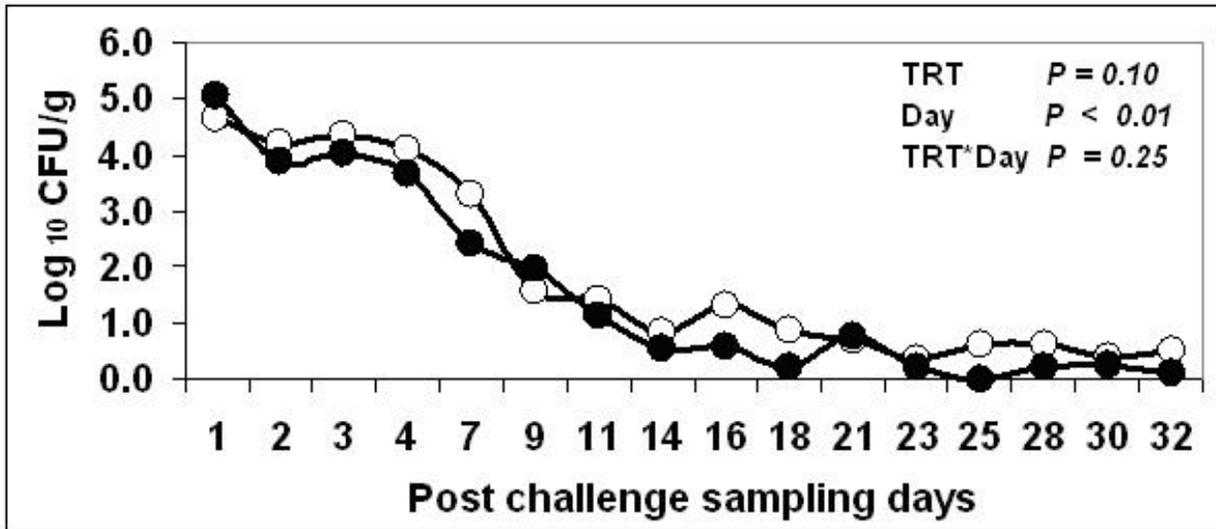


Figure 2-2 Fecal concentrations (\log_{10} CFU/g) of *Escherichia coli* O157 in control (open circles) or vaccinated (closed circles) cattle following oral challenge (day 0) with nalidixic acid-resistant *Escherichia coli* O157.

Typically, after day 9, the mean counts were in the range of 10^1 to 10^2 CFU per g of feces in both groups. On most sampling days, the mean fecal counts of the vaccinated group appeared to be lower than the control group. However, the difference in fecal concentrations between the control and vaccinated group was only a trend with a treatment effect of $P = 0.10$. There was a significant day effect ($P = < 0.01$) but no significant treatment and day interaction ($P = 0.25$). Therefore, the mean counts of *Nal^R E. coli* O157:H7 of all sampling days (days 1 to 32) were compared between the two groups and was 1.87 CFU/g of feces in the control vs. 1.57 CFU/g of feces in the vaccinated group (Figure 2-3).

Figure 2-3 Mean fecal concentration (log₁₀ CFU/g) in the control or vaccinated group.

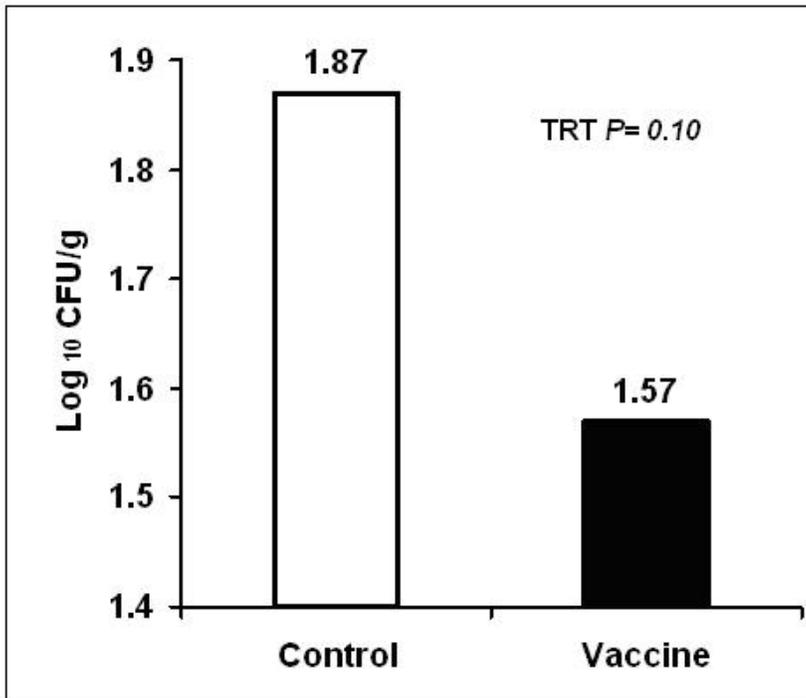


Figure 2-3 Overall average fecal concentration (log₁₀ CFU/g) in the control or vaccinated group.

Overall, the number of cattle culture positive for *NaI^R E. coli* O157:H7, based on fecal sample analysis was lower ($P = 0.05$) in the vaccinated group compared to the control, but the vaccine effect was only a trend ($P = 0.11$), based on RAMS sample data (data not shown). However, the number of cattle culture positive based on fecal or RAMS sample data tended to be lower ($P = 0.07$) for the vaccinated cattle compared to the control cattle (Figure 2-4).

Figure 2-4 The number of cattle culture positive for nalidixic acid-resistant *Escherichia coli* O157

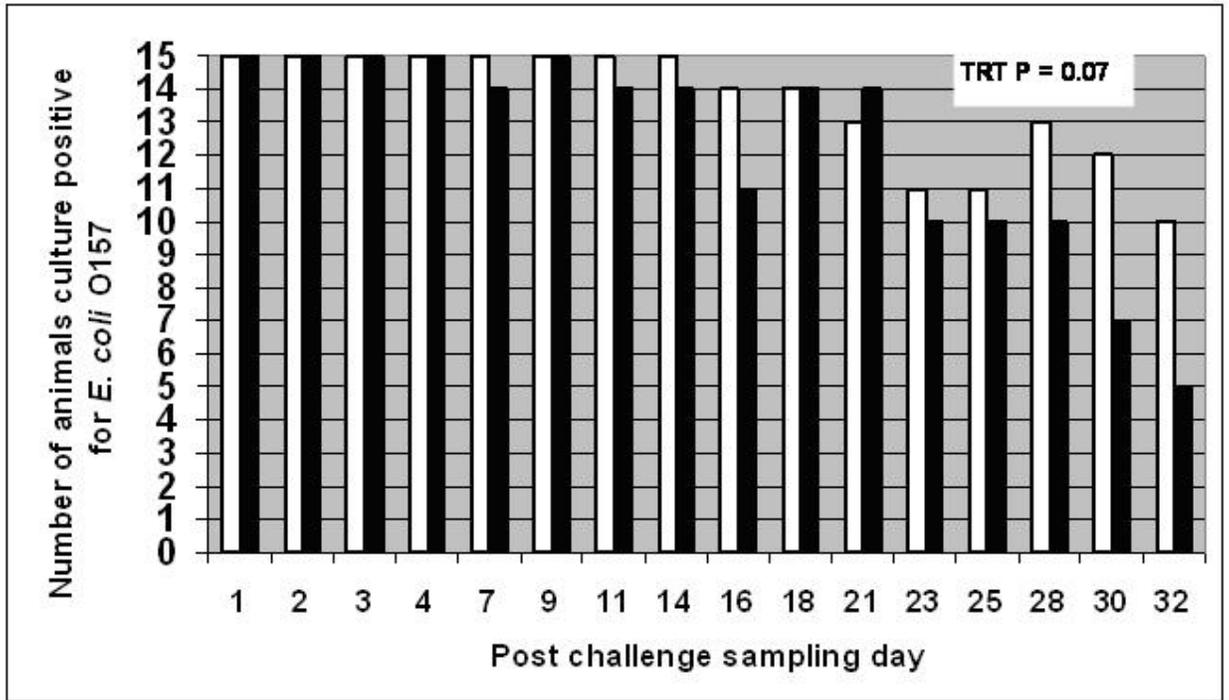
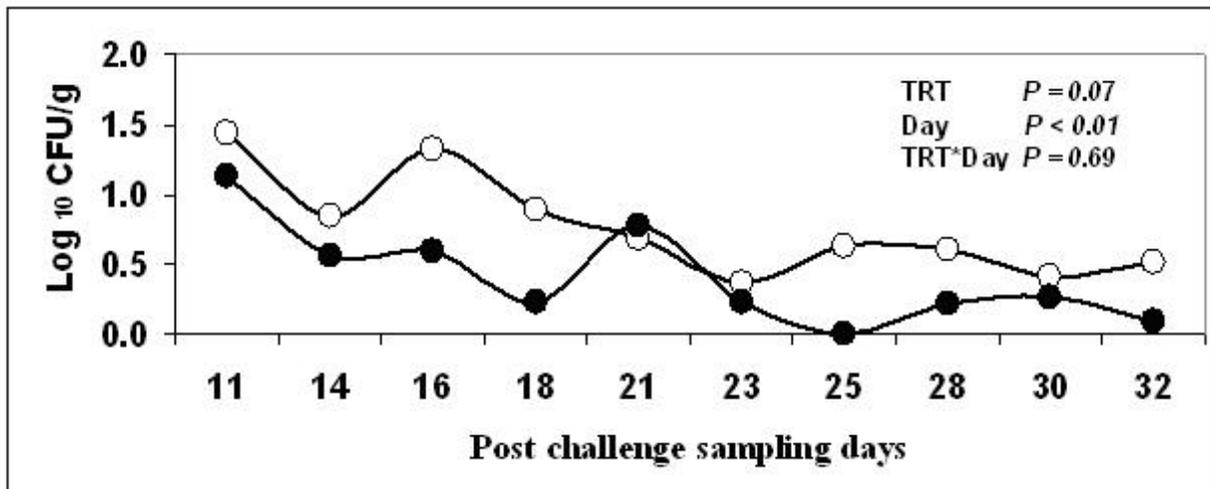


Figure 2-4 The number of cattle culture positive for nalidixic acid-resistant *Escherichia coli* O157 in fecal or rectoanal mucosal swab samples from days 1 to 32 in control (open bars) and vaccinated (closed bars) cattle following oral challenge with nalidixic acid-resistant *Escherichia coli* O157.

Because, the number of cattle culture positive, regardless of fecal or RAMS sample analysis, was 14 or 15 (90 to 100% of cattle) until day 9 of the post challenge period in each group, we statistically analyzed fecal concentrations data from days 11 to 32. The analysis showed that the mean daily fecal concentration in vaccinated cattle tended to be lower ($P = 0.07$) than the control group (Figure 2-5).

Figure 2-5 Fecal concentrations (\log_{10} CFU/g) of *Escherichia coli* O157 from day 11 to 32 following oral challenge with nalidixic acid-resistant *E. coli* O157.



*Figure 2-5 Fecal concentrations (\log_{10} CFU/g) of *Escherichia coli* O157 in control (open circles) or vaccinated (closed circles) cattle following oral challenge with nalidixic acid-resistant *Escherichia coli* O157, from day 11 to 32 following oral challenge with nalidixic acid-resistant *E. coli* O157.*

***E. coli* O157 in necropsy samples in the control or vaccinated group.** Cattle

vaccinated with the SRP *E. coli* O157:H7 vaccine had fewer number of cattle culture positive for *E. coli* O157:H7 in cecal ($P = 0.06$), colon ($P = 0.05$), and rectal contents ($P = 0.06$) compared to the control cattle administered with a placebo (Figure 2-6).

Figure 2-6 The number of cattle culture positive for *Escherichia coli* O157 at necropsy.

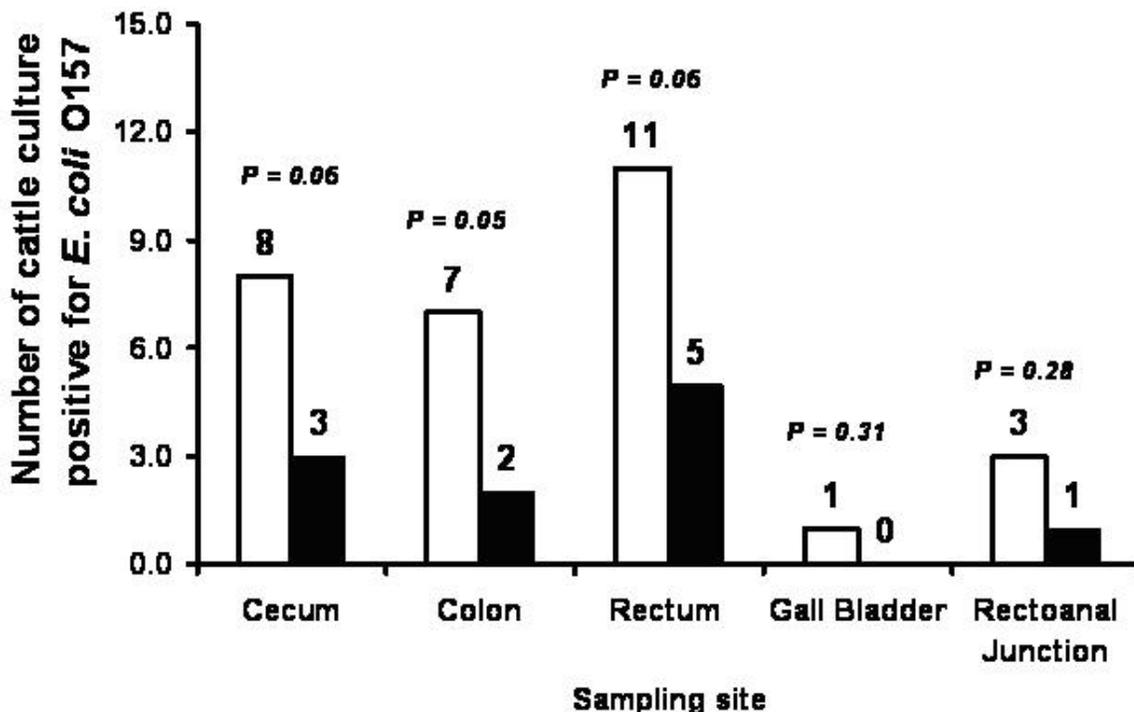


Figure 2-6 The number of cattle culture positive for *Escherichia coli* O157 in gut contents or tissue swab samples collected during necropsy in control (open circles) or vaccinated (closed circles) cattle orally challenged with nalidixic acid-resistant *E. coli* O157.

In the control group, 11 of 15 (73.3%) cattle were culture positive for *E. coli* O157, whereas only 5 of 15 (33.3%) in the vaccinated group were culture positive. There were no statistically significant differences between the number of cattle culture positive for *E. coli* O157:H7 in swabs collected from the rectoanal junction ($P = 0.28$) or the gall bladder mucosa ($P = 0.31$) between the treatment groups. In fact, only one animal in the control group was culture positive for *E. coli* O157 in the gall bladder. The necropsy data also illustrate that vaccination of cattle with SRP *E. coli* O157:H7 significantly decreased the concentration of *E. coli* O157:H7 (Figure

2-7) in cecal ($P = 0.04$), colon ($P = 0.04$) and rectal contents ($P = 0.05$), but had no significant effect on the concentration in the rectoanal junction ($P = 0.15$) or gall bladder mucosa ($P = 0.17$).

Figure 2-7 Concentration of *Escherichia coli* O157 (log₁₀ CFU/g or swab) in gut contents or tissue swab samples at necropsy.

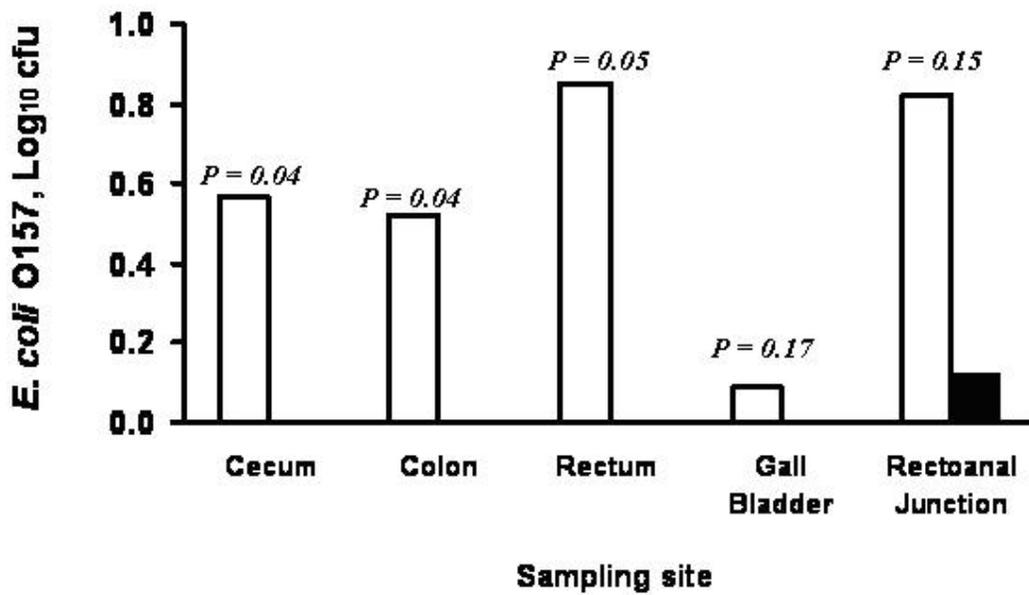


Figure 2-7 Concentration of Escherichia coli O157 (log₁₀ CFU/g or swab) in gut contents or tissue swab samples collected during necropsy in control (open bars) or vaccinated (closed bars) cattle orally challenged with nalidixic acid-resistant E. coli O157.

DISCUSSION

The severity of the *E. coli* O157:H7 infection in children and the elderly, availability of few therapeutic approaches, the general consumer's concern about safety of food products, and cattle being a major reservoir have led to intensive preharvest and postharvest strategies to reduce or eliminate the organism in cattle (Besser et al., 1999; Callaway et al., 2004; Gyles, 2007; Koohmaraie et al., 2005; Lonergan and Brashears, 2005; Lejuene and Wetzel, 2007). Preharvest reduction of *E. coli* O157:H7 in cattle requires targeted intervention strategies, which should reduce carcass contamination (Lonergan and Brashears, 2005) and the intervention strategy will have a distinct advantage over postharvest strategies in reducing potential transfer by direct contact or contamination of the environment including water and produce. Several preharvest interventions have been evaluated and include feed management, prebiotics, direct-fed microbials, bacteriophage therapy, sodium chlorate, vaccination, and neomycin in the feed (Lejune and Wetzel, 2007; Lonergan and Brashears., 2005).

Vaccine technology offers an advantage because of familiarity by cattle producers and ease of acceptance and ready incorporation into existing vaccination practices (Lonergan and Brashears., 2005). The technology offers a challenge , specifically priming the immune system to mount a protective immunity against an organism that is considered a commensal (Lejeune and Wetzel, 2007). Generally, experimental vaccines have targeted against cellular components that are important for bacterial adherence to the intestinal epithelium, such as Type III secreted protein, Tir, (Potter et al., 2004), intimin protein (Dean-Nystrom et al., 2002), and the O157 lipopolysaccharide (Konnadu et al., 1999). The SRP based vaccine that contains outer membrane siderophore-receptors and porin proteins is a novel approach to deprive the cell of

iron, an essential nutrient for all gram negative bacteria, including *E. coli* (Bolin and Jensen, 1987).

This is the first study to report on testing the efficacy of injecting cattle with an *E. coli* O157:H7 SRP vaccine to reduce fecal shedding, fecal concentration, and gut colonization of *E. coli* O157:H7 in cattle. Our method to use cattle orally inoculated with *E. coli* O157 to determine the vaccine efficacy has been used by others (Potter et al., 2004). We assessed culture positive status of calves by both fecal and RAMS analyses. The primary site of *E. coli* O157 colonization in cattle has been shown to be the mucosal epithelium, 1 to 5 cm proximal to the rectoanal mucosal junction (Low et al., 2005; Naylor et al., 2003). This swabbing of this region using a foam-tipped applicator has been shown to be a more sensitive sampling method for detecting *E. coli* O157 in cattle (Davis et al., 2006; Greenquist et al., 2005; Rice et al., 2003). Therefore, inclusion of RAMS technique allowed us to improve detection of culture status of the inoculated calves.

The vaccine was effective in inducing anti-SRP antibodies in the vaccine group. However, the difference in fecal concentration between the vaccine and control groups was only marginally significant ($P=0.10$). In this study, 100% of calves in both groups were shedding *E. coli* O157:H7 in feces for the first 9 days. Naylor *et al* (2003) observed that cattle after oral inoculation shed *E. coli* O157:H7 initially at a high concentration (10^9). In our study, calves were shedding initially at a mean concentration of 10^5 to 10^3 and the wide distribution of data made the treatment effect harder to detect. In instances where no variation in data occurs or data are completely separated, maximum likelihood-based models either fail to converge or when they do, they produce very unstable and imprecise estimates. Therefore, a post proc analysis was performed of data on fecal concentration from 11 days post challenge, which showed a P value

of 0.07. When we compared the number of calves culture positive for *E. coli* O157, regardless of the sampling technique (feces or RAMS), the vaccine group had fewer calves and lower concentrations in gut contents or tissues than the control cattle. The difference in the number of calves between the two groups was also evident in samples collected from gut contents or tissues from necropsied cattle. There were fewer calves culture positive and lower concentration in vaccinated group compared to the control.

Our study showed that the *E. coli* O157:H7 SRP vaccine could be used as an intervention strategy in live cattle to reduce fecal prevalence in cattle. Decreasing the number of cattle contaminated with *E. coli* O157:H7 entering the slaughter house would lead to a decrease in contaminated beef. Further research on the efficacy of the vaccine in a natural prevalence and feedlot operations must be performed to further evaluate the SRP technology.

REFERENCES

1. Bach, S. J., T. A. McAllister, D. M. Veira, V. P. J. Gannon, and R. A. Holley. 2002. Transmission and control of *Escherichia coli* O157-a review. *Can. J. Anim. Sci.* 82:475-490.
2. Besser, R. E., P. M. Griffen, and L. Slutsker. 1999. *Escherichia coli* O157:H7 gastroenteritis and the hemolytic syndrome: an emerging infectious disease. *Ann. Rev. Med.* 50:355-367.
3. Bolin, C. A. and A. E. Jensen. 1987. Passive Immunization with Antibodies Iron-Regulated Outer Membrane Proteins Protects Turkeys from *Escherichia coli* Septicemia. *Infect. Immun.* 55:1239-1242
4. Callaway, T.R., R. C. Anderson, T. S. Edrington, K. J. Genovese, K. M. Bischoff, T. L. Poole, Y. S. Jung, R. B. Harvey, and D. J. Nisbet. 2004. What are we doing about *Escherichia coli* O157 in cattle? *J. Anim. Sci.* 82:E93-E99.
5. Cowan, S. W., T. Schirmer, G. Rummel, M. Steiert, R. Ghosh, R. A. Pauptit, J. N. Jansonius, and J.P. Rosenbusch. 1992. Crystal structures explain functional properties of two *E. coli* porins. *Nature* 358:727-733.

6. Davis M. A., D. H. Rice, H. Sheng, D. D. Hancock, T. E. Besser, R. Cobbold, and C. J. Hovde. 2006. Comparison of cultures from rectoanal-junction mucosal swabs and feces for detection of *Escherichia coli* O157 in dairy heifers. *Appl. Environ. Microbiol.* 72:3766-3770
7. Dean-Nystrom, E. A., L. J. Gansheroff, M. Mills, H. W. Moon, and A. D. O'Brien. 2002. Vaccination of pregnant dams with intimin O157 protects suckling piglets from *Escherichia coli* O157:H7 infection. *Infect. Immun.* 70:2414-2418.
8. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci., USA.* 97: 2999-3003.
9. Emery, D. A., D. E. Straub, R. Huisinga, and B. A. Carlson. November 2000. Active Immunization using a siderophore receptor protein. U.S. Patent 6,027,736.
10. Frensen, P. D., A. Drake, and F. J Angulo. 2005. Economic cost of illness due to *Escherichia coli* O157 infections in the United States. *J. Food Prot.* 68:2623-2630
11. Gansheroff, L. J. and A. D. O'Brein. 2000. *Escherichia coli* O157 in beef cattle presented for slaughter in the U. S.: Higher prevalence rates than previously estimated. *Proc. Natl. Acad. Sci., USA.* 97:2959-2961.

12. Gyles, C. L. 2007. Shiga toxin-producing *Escherichia coli*: An overview. *J. Anim. Sci.*. 85(E Suppl.):E45-E62.
13. Greenquist, M. A., J. S. Drouillard, J. M. Sargeant, B. E. Depenbusch, X. Shi, K. F. Lechtenberg, and T. G. Nagaraja. 2005. Comparison of rectoanal mucosal swab cultures and fecal cultures for determining prevalence of *Escherichia coli* O157:H7 in feedlot cattle. *Appl. Environ. Microbiol.* 71:6431-6433.
14. Grauke, L. J., I. T. Kudva, J. Won Yoon, C. W. Hunt, C. J. Williams, and C. J. Hovde. 2002. Gastrointestinal tract location of *Escherichia coli* O157:H7 in ruminants. *Appl. Environ. Microbiol.* 68:2269-2277.
15. Karmali, M. A. 2005. Use of comparative genomics as a tool to assess the clinical and public health significance of emerging Shiga toxin-producing *Escherichia coli* serotypes. *Meat Sci.* 71:62-71.
16. Kim, J., J. Nietfeldt and A. K. Benson. 1999. Octamer-based genome scanning distinguishes a unique subpopulation of *Escherichia coli* O157:h7 strains in cattle. *Proc. Natl. Acad. Sci., USA.* 23:13288-13293.
17. Konnadu, E., A. Donohue-Rolfe, S. B. Calderwood, Vince Pozsgay, Joseph Shiloach, John B. Robbins and Shousun C. Szu. 1999. Syntheses and immunologic properties of

Escherichia coli O157 O-specific polysaccharide and Shiga toxin 1 B subunit conjugates in mice. *Infect. Immun.* 67:6191-6193.

18. Koohmaraie, M., T. M. Arthur, J. M. Bosilevac, S. D. Shackelford, and T. L. Wheeler. 2005. Post-harvest interventions to reduce/eliminate pathogens in beef. *Meat Sci.* 71:79-91.
19. Lejeune, J.T., and A.N. Wetzel. 2007. Preharvest control of *Escherichia coli* O157 in cattle. *J. Anim. Sci.* 85(E. Suppl.):E73-E80.
20. Lonergan, G H., and M. M. Brasheers, 2005. Pre-harvest interventions to reduce carriage of *E. coli* O157 by harvest-ready feedlot cattle. *Meat Sci.* 71:72-78.
21. Low, J. C., I. J. McKendrick, C. McKenzie, D. Fenlon, S. W. Naylor, C. Currie, D. G. E. Smith, L. Allison, and D. G. Gally. 2005. Rectal carriage of enterohemorrhagic *Escherichia coli* O157 in slaughtered cattle. *Appl. Environ. Microbiol.* 71:93-97.
22. Naylor, S., C. Low, T. Besser, A. Mahajan, G. Gunn, M. Pearce, I. McKendrick, D. Smith, and D. Gally. 2003. Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infect. Immun.* 71:1505-1507.

23. Park, S., R. W. Worobo, and R. A. Durst. 1999. *Escherichia coli* O157:H7 as an emerging food borne pathogen: A literature review. *Crit. Rev. Food Sci. Nutr.* 39:481-502.
24. Potter, A. A., S. Klashinsky, Y. Li, E. Frey, H. Townsend, D. Rogan, G. Erickson, S. Hinkley, T. Klopfenstein, R. A. Moxley, D. R. Smith, and B. B. Finlay. 2004. Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. *Vaccine* 22:362-369.
25. Prescott, L. M., J. P. Harley, and D. A. Klein (6th ed.). 2005. Microbiology. Boston: McGraw Hill Higher Education, p. 101-102.
26. Rassmussen, M. A., and T. A. Casey. 2001. Environmental and food safety aspects of *Escherichia coli* O157:H7 infections in cattle. *Crit. Rev. Microbiol.* 27:57-73.
27. Rice, D. H., H. Q. Sheng, S. A. Wynia, and C. J. Hovde. 2003. Rectoanal mucosal swab culture is more sensitive than fecal culture and distinguishes *Escherichia coli* O157-colonized cattle and those transiently shedding the same organism. *J. Clin. Microbiol.* 41: 4924-4929.
28. Sargeant, J. M., M. W. Sanderson, R. A. Smith, and D. D. Griffen. 2003. *Escherichia coli* O157 in feedlot cattle faeces and water in four major feeder-cattle states in the USA. *Prev. Vet. Med.* 61:127-135.

29. Stevens, E.T., and D.U. Thomson. 2005. Siderophore receptor and porin protein technology for control of *Salmonella* and *E. coli* O157:H7 in cattle. *Bov. Pract.* Salt Lake City, UT., p.25-29.
30. Van Baale, M. J., J. M. Sargeant, D. P. Gnad, B. M. DeBey, K. F. Lechtenberg, and T. G. Nagaraja. 2004. Effect of forage or grain diets with or without monensin on ruminal persistence and fecal *Escherichia coli* O157:H7 in Cattle. *Appl. Environ. Microbiol.* 70:5336-5342
31. Van Donkersgoed, J., D. Hancock, D. Rogan, and A. A. Potter. 2005. *Escherichia coli* O157:H7 vaccine field trial in 9 Feedlots in Alberta and Saskatchewan. *Canadian Vet J.* 46:724-728.

**CHAPTER 3 - Effect of siderophore receptor/porin proteins-based
vaccination on prevalence of *Escherichia coli* O157:H7 in feedlot
cattle**

ABSTRACT

Objective—To evaluate the efficacy of a siderophore receptor/porin proteins-based vaccine in reducing *Escherichia coli* O157:H7 in feedlot cattle.

Animals—1,252 yearling steers and heifers.

Procedures—Cattle were randomly divided into 20 pens, approximately 65 cattle per pen, and were administered either the *E coli* O157 SRP vaccine or a placebo on days 0 and 21. Rectal fecal samples were collected on day 0, and pen floor samples were collected on days 21, 35, and 70. A simulated harvest was performed on day 85 to evaluate prevalence of *E coli* O157:H7 in rectal fecal samples, rectoanal mucosal swab (RAMS) samples, and hide swab samples. Cattle were weighed on days 0, 21, and on the day of simulated harvest (day 85). Amount of feed delivered to each pen on each day was recorded electronically.

Results—There was a treatment effect ($P = 0.04$) on fecal prevalence of *E coli* O157:H7 averaged over time with an overall 54% reduction in prevalence in the SRP vaccinated cattle compared to the control cattle. Vaccination with SRP antigens decreased the prevalence of *E coli* O157 in fecal samples ($P = 0.01$) collected at simulated harvest and tended to decrease the prevalence on hide swab samples ($P = 0.06$). Administration of SRP vaccine had no negative effect on the performance of feeder calves.

Conclusions and Clinical Relevance—The *E coli* O157 SRP vaccine reduced prevalence of *E coli* O157 in feedlot cattle. Because preharvest prevalence is associated with carcass contamination, the vaccine evaluated might be a potential intervention strategy to aid in the control of this pathogen.

INTRODUCTION

Escherichia coli O157 is an on-going and important cause of food-borne illness in the United States (CDC, 2006). Infection with this pathogen leads to hemorrhagic colitis and, in some cases, particularly in children, hemolytic uremic syndrome (Park et al. al. 1999). *Escherichia coli* O157 lives in the intestines of healthy cattle and fecal shedding of this organism is linked to the contamination of unpasteurized milk and fruit juices, ground beef, raw vegetables, and bodies of water, particularly streams and ponds (Rasmussen et al. 2001). Because *E coli* O157 infections in the U.S. are linked to the consumption of contaminated food and water, efforts to develop pre- and post-harvest strategies to control this pathogen in cattle are ongoing (Koohmariae et al., 2005; Loneragan and Brashears, 2005; Lejeune and Wetzel, 2007).

Vaccines that induce a protective immunity against colonization of *E coli* O157 may offer distinct advantages because of likely acceptance by cattle producers and ready incorporation into existing vaccination practices (Loneragan and Brashears, 2005). Generally, experimental *E coli* O157 vaccines have targeted against antigenic components that are important for bacterial adherence to the intestinal epithelium or virulence of the pathogen, such as Type III secreted proteins (Potter et al., 2004), intimin protein (Dean-Nystrom et al., 2002), and the O157 lipopolysaccharide (Konnadu et al., 1999). A novel method that reduces the ability of Gram-negative bacteria to acquire iron (Emery, et al., 2000) may be an effective and practical intervention to reduce infections with Gram negative organisms. Iron is an essential nutrient for the growth and colonization of Gram-negative bacteria and under low iron conditions, bacteria produce a high affinity iron transport system to bring the required nutrient inside the bacterial cell (Neilands et al. 1995). The SRP-based vaccine uses purified siderophore receptor and porin

proteins as antigenic components to induce an immune response in cattle (Emery, et al., 2000). The goal is that immunized animals produce anti-SRP antibodies which bind to siderophore receptors and porins on the outer membrane of the bacterial cell and block iron transport into the cell. Blocking iron transport renders the bacteria at a competitive disadvantage in a mixed microbial environment (Emery et al., 2000).

In a previous study, investigators reported that an SRP vaccination promoted an immune response and significantly reduced fecal shedding of *E. coli* O157:H7 in calves that were orally inoculated with naladixic acid resistant *E. coli* O157:H7 (Thornton et al. 2006). Further application of this vaccine in a feedlot setting was deemed necessary to evaluate the efficacy of this new intervention strategy. Therefore, the objectives of this study were to examine the effects of the SRP vaccination on prevalence in feces and hide contamination of *E. coli* O157:H7 in feedlot cattle.

MATERIALS AND METHODS

Cattle-Twenty pens housing a total of 1,252 of yearling steers and heifers, approximately 60 to 70 cattle per pen, were utilized to examine the effects of SRP *E. coli* vaccination on prevalence of *E. coli* O157:H7 and on cattle performance. The study was conducted in two feedlots located in Central Nebraska. All cattle were processed at the feedlot in which the study was conducted or at a nearby facility prior to the beginning of the study. Cattle were purchased in 10 groups and each group was of the same gender. Purchased groups 1 to 7 were fed in one feedlot and groups 8 to 10 were fed in the second feedlot.

Cattle within each purchased group were randomly assigned to one of two treatments: 1) injected subcutaneously with an SRP *E. coli* O157:H7 vaccine at 1,000µg/dose on days 1 and 21 or 2) injected subcutaneously with a placebo containing physiological saline emulsified with a

commercial adjuvant (Emulsigen^a) on days 1 and 21. Cattle were assigned to treatments by alternately allocating five cattle to one treatment and the next five cattle to the second treatment, as cattle moved through the chute until all cattle in the purchased group were assigned.

Cattle were taken to their home pens which were pipe and cable fenced with concrete fence-line feed bunks, and float-controlled water troughs. All cattle were weighed on days 0, 21 and 85. On day 85, cattle were brought through the processing barn and subjected to simulate harvest. This was considered necessary because the end of the finishing period would have pushed the cattle toward the seasonal change from summer to fall. Therefore, to decrease the risk of having fewer positive cattle, a simulated slaughter was opted to get a more appropriate prevalence.

Cattle had ad libitum access to feed and water during the study. Because cattle were backgrounded and transitioned to a finish diet prior to arrival into the feedlots, all cattle were placed directly on the finish diet on day one of the study. Cattle were fed a typical High Plains finishing diet (74.5% dry matter; 58 % corn, 30 % corn gluten feed, 6.5% alfalfa hay, and 5.5% was a supplement containing minerals, vitamins and antibiotics). Cattle were fed a similar diet in both feedyards. The feed was delivered to the pens in a feed truck with a mixer box mounted on load cells. The amount of feed delivered was recorded at each feeding and feed records for each pen were recorded electronically on a computer to determine feed intake at pen level. All research, feedlot, and laboratory personnel were blinded from treatment assignment throughout the study.

Sampling procedures-Rectal fecal samples were collected on day 0 as cattle were processed and placed in separate, pre-labeled sterile whirlpack bags^b. On days 21, 35, and 70 of

the finishing period, approximately 150 g from each of twenty freshly voided fecal pats were collected from the floor of each pen and placed in separate pre-labeled, sterile whirlpack bags. All samples were placed in coolers on ice and transported to the Preharvest Food Safety Laboratory to be processed.

Samples collected at stimulated slaughter consisted of rectal fecal, RAMS, and hide swab samples. Samples were collected from 30 animals per pen (60 animals per replicate; 600 animals total) and the selection of animals from which samples were collected was dependent on the order in which the cattle entered the chute. The first sample was collected from the second animal of each pen and then every 3rd animal until 30 animals per pen had been sampled. The RAMS samples were collected using a sterile, foam-tipped applicator^b, which was inserted approximately 2 to 5 cm into the anus to gently scrape the mucosal epithelium proximal to the rectoanal junction (Rice et al. 2003; Greenquist et al., 2005). The swabs were placed in culture tubes containing 3 mL of GNccv broth^c. Hide samples were collected using *Nasco* Specisponges^d moistened with 5 mL of GNccv broth. A 1,000 cm² area of hide along the back of each animal was swabbed in a “zig-zag” motion and placed into separate pre-labeled, sterile whirlpack bags (Elder et. al., 2000). Approximately 150 g of fecal material was collected rectally from each animal and placed into separate, pre-labeled sterile whirlpack bags. All samples were placed in coolers on ice and transported to the Preharvest Food Safety Laboratory for analysis.

Isolation of E coli O157-Approximately 1 g of each fecal sample was placed in a tube containing 9 mL of GNccv. The fecal suspension in GNccv broth and RAMS samples (previously placed in 3 mL of GNccv broth) were vortexed for 1 minute and incubated for 6

hours at 37°C. Using a pre-calibrated omnispense^c, 20 mL of sterile GNccv broth was added to each whirlpack bag containing the hide samples. Each bag was sealed and placed in the incubator for 6 hours at 37°C. All samples were then subjected to immunomagnetic separation^f and spread plated onto sorbitol MacConkey agar supplemented with 50 ng/mL cefixime and 2.5 µg/mL potassium tellurite. Plates were incubated overnight (16 to 18 hours) at 37°C and up to six sorbitol negative colonies from each plate were streaked on to blood agar^g. The plates were then incubated overnight at 37°C. Colonies on blood agar plates were tested for indole production and O157 antigen using latex agglutination^h. Species was confirmed by APIⁱ and PCR was used to detect the *eae* (intimin), *stx1* (Shiga toxin 1), *stx2* (shiga toxin 2), *hlyA* (hemolysin), (Fagan et al. 1998) and *fliC* (flagella) (Gannon et al. 1996) genes in each isolate. Genes were determined using TaqMan *E. coli* O157:H7 detection kit^j and the ABI Prism® 7700 Sequence Detection System^l. The amplified DNA fragments were gel electrophoresed using 2% (w/v) agarose^k and stained with ethidium bromide (0.5 µg/mL). The gels were visualized under UV light and genes were detected under a Gel-Doc 2000 fluorescent imager^l.

Statistical analysis- The prevalence of *E. coli* O157:H7 in feces (over sampling days and at simulated slaughter), RAMS, and hide swabs were the outcome variables of interest. For each of these variables, pen-level binomial response variables were created and analyzed using mixed-models logistic regression techniques in a commercially available statistical analysis software package^m. Replicate was included as a random variable in a random intercepts/slopes model. When analyzing pen-floor fecal samples, repeated measures methodology was used to account for within-pen dependency over time. From the various models, least-squares means were computed, back-transformed to normal scale, and converted to risk. From estimates of risk,

relative risk and vaccine efficacy was calculated. Where significant effects were observed in the model, appropriate pair-wise comparisons of means were performed.

RESULTS

The average pen fecal prevalence of *E coli* O157:H7 in the SRP vaccinated and the control group on day 0 (before vaccination) was 12 and 13%, respectively. Fecal prevalence of *E coli* O157 in samples collected after vaccination (days 21, 35, and 70), and at simulated slaughter (day 85) is shown in figure 3-1.

Figure 3-1 Mean prevalence of *Escherichia coli* O157 in cattle feces

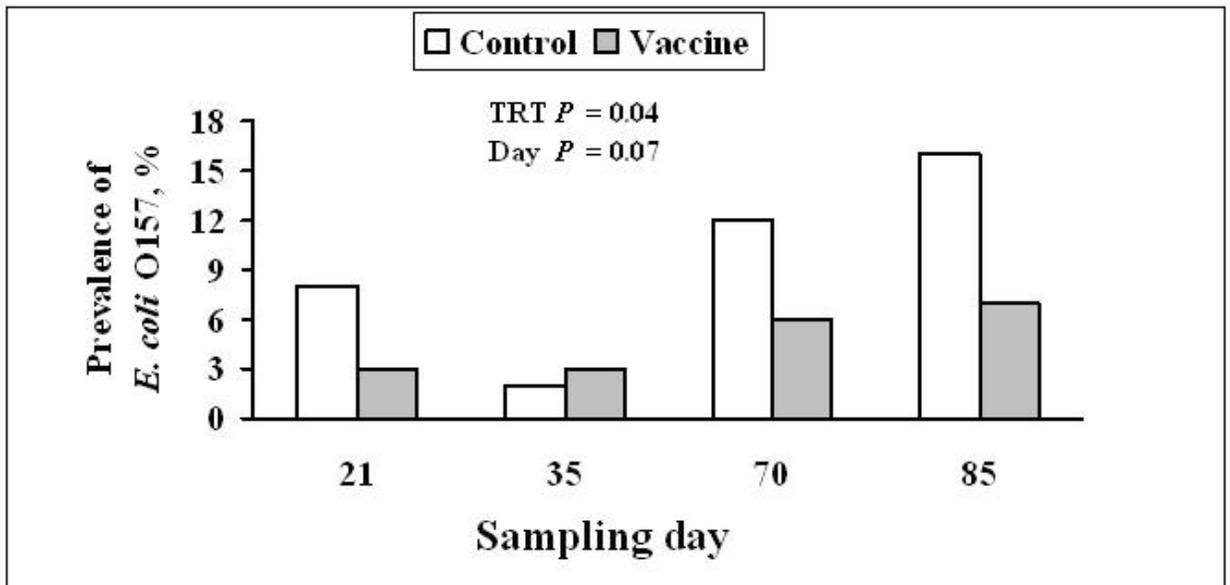


Figure 3-1 Mean prevalence of Escherichia coli O157 in feces (proportion of cattle positive) on days 21, 35, 70 and 85 (after vaccination) in placebo control (white bars) or E coli O157:H7 siderophore receptor and porin proteins-based vaccination in cattle (gray bars). Day 85 was the day of simulated slaughter.

No significant interaction of treatment by time was detected ($P=0.56$). There were a significant treatment effect ($P = 0.04$), but an effect of sampling day was not detected ($P = 0.07$). The

proportion of cattle culture positive for *E coli* O157:H7 in the feces averaged over sampling days was 1.6% for the SRP vaccinated group and 3.4% for the control (Figure 3-2).

Figure 3-2 Mean prevalence (proportions of cattle positive) of *Escherichia coli* O157.

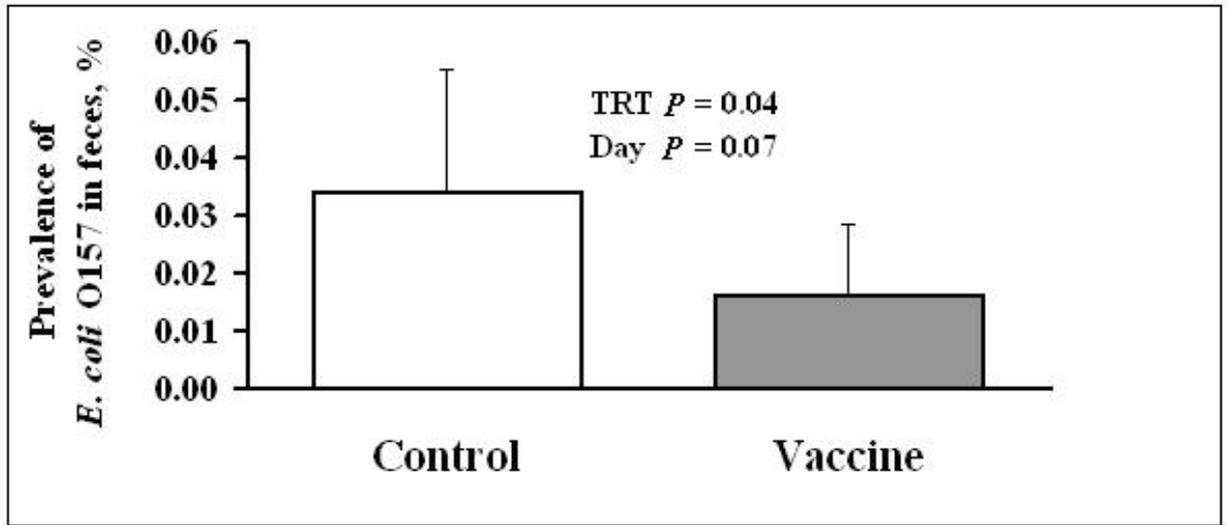


Figure 3-2 Mean overall prevalence (proportions of cattle positive) of *Escherichia coli* O157 in placebo control (white bar) or *E coli* O157:H7 siderophore receptor and porin proteins-based vaccination in cattle (gray bar).

At the time of simulated slaughter (day 85), cattle that received the SRP vaccine had decreased ($P = 0.01$) *E coli* O157:H7 positive fecal samples compared to cattle vaccinated with the placebo (7 vs. 16%, respectively). The reduction in fecal prevalence of *E coli* O157 was 54% in the SRP vaccinated cattle compared to the control cattle injected with the placebo. The mean prevalence of *E coli* O157:H7 in hide samples tended ($P = 0.06$) to be higher in cattle in the placebo group than in the SRP vaccinated group (Figure 3). A difference between the two treatment groups in the likelihood of recovered of *E coli* O157:H7 from rectoanal mucosal swabs was not detected ($P = 0.24$). The total number of cattle positive for *E coli* O157:H7 in at least one sample (feces, RAMS, or hide sample) at simulated harvest was fewer ($P = 0.02$) in the SRP vaccinated group compared to the the placebo group (Figure 3-3); vaccination with SRP and

porin-based antigens reduced the number of cattle culture positive for *E. coli* O157 by 57.8% compared to the control cattle. Out of the 226 *E. coli* O157 isolates, 72% (163/226), 66% (150/226), 70% (159/226) and 75% (170/226) contained the genes *eaeA*, *stx2*, *hlyA*, and *fliC* respectively.

Figure 3-3 Mean overall prevalence of *Escherichia coli* O157 (proportions of cattle positive) at simulated slaughter.

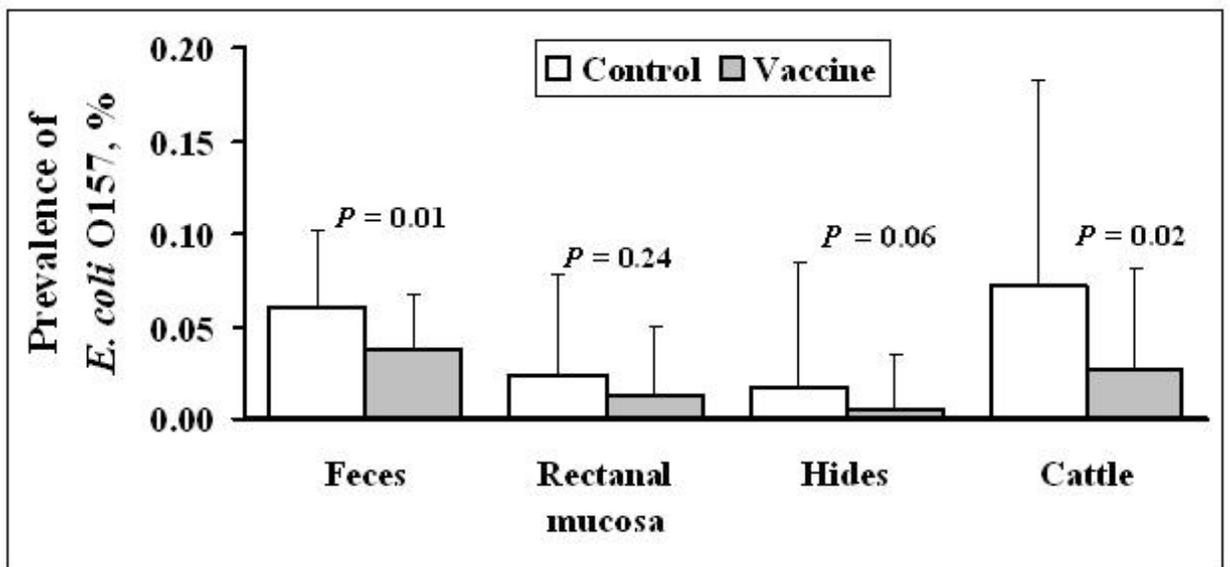


Figure 3-3 Mean overall prevalence of Escherichia coli O157 (proportions of cattle positive) in feces, rectoanal mucosa, hides, and cattle (feces, rectoanal mucosa, or hides) in placebo control (white bars) or E. coli O157:H7 siderophore receptor and porin proteins-based vaccination (gray bars).

The SRP-based *E coli* O157:H7 vaccination had no effect on average daily gain ($P = 0.37$), daily feed intake ($P = 0.29$), and on gain efficiency ($P = 0.18$) for the 85 days on feed (Table 3-1).

Table 3-1 Effects of siderophore receptor and porin proteins (SRP)-based *Escherichia coli* O157:H7 vaccination on the performance of feedlot cattle.

	Treatment		SEM	P value
	Control	SRP vaccination		
Body weight, kg.				
Day 0	350	350	14.8	0.67
Day 21	383	380	9.1	0.18
Day 85	472	469	17.1	0.25
Average daily gain, kg/day				
Days 0 to 21	1.55	1.44	0.42	0.23
Days 0 to 85	1.45	1.42	0.66	0.37
Dry matter intake, kg/day				
Days 0 to 21	8.16	8.30	0.59	0.58
Days 0 to 85	9.21	9.25	0.55	0.29
Gain to feed ratio				
Days 0 to 21	0.086	0.078	0.022	0.18
Days 0 to 85	0.071	0.069	0.001	0.18

DISCUSSION

Several pre- and postharvest exclusion strategies have been researched over the past decade in hopes of reducing or eliminating this microorganism in cattle (Besser et al., 1999, Callaway et al., 2004; Gyles, 2007; Koohmaraie et al., 2005; Loneragan and Brashears, 2005; Lejuene and Wetzel, 2007). Preharvest intervention has a distinct advantage over post-harvest intervention in that it would not only reduce the risk for carcass contamination, but would also reduce the source of potential contamination to the environment. Pre-harvest intervention strategies that are currently being tested include: diet manipulation, direct-fed microbials,

bacteriophage therapy, prebiotics, antibiotics, such as neomycin, sodium chlorate, and vaccination (Loneragan and Brashears, 2005; Lejeune and Wetzel, 2007)

Vaccine approach to reduce preharvest prevalence *E coli* O157:H7 in cattle would be advantageous to producers, because it could easily be incorporated into the existing vaccination practices of most feedlots. However, vaccination against *E coli* O157:H7 can be quite challenging because the targeted organism is a commensal in cattle (Lejeune and Wetzel, 2007). Experimental vaccines for *E coli* O157:H7 have generally targeted the cellular components of the organism, such as the type III secretion protein, Tir, and the intimin protein, both of which would prevent bacterial adherence to the intestinal epithelium (Dean-Nystrom et al., 2002; Potter et al., 2004). Konnadu et al., (1999) have tested a vaccine targeting the O-specific polysaccharide and Stx 1b subunit of *E. coli* O157. This vaccine was intended to inactivate the lipopolysaccharide and further reduce colonization within the intestinal mucosa (Konnadu, et al. 1999). The SRP vaccine is comprised of siderophore-receptors and porin proteins that would provoke an immune response in cattle and deprive *E coli* O157:H7 of iron, an essential nutrient (Bolin and Jensen, 1987). Antibody response in cattle to vaccination was not monitored in this study. However, strong anti-SRP antibody response to vaccination was documented in our previous study (Thornton et al., 2006).

The *E coli* O157:H7 SRP vaccine was previously tested in 4 month-old calves that were orally inoculated with a 5-strain mixture of nalidixic-acid resistant (*Nal^R*) strains of *E coli* O157:H7 (Thornton, et al., 2006). Results indicated that the vaccine was effective in reducing the concentration and number of calves shedding *Nal^R E coli* O157:H7 in the feces. Cattle vaccinated with the SRP *E coli* O157:H7 vaccine also had a fewer number of cattle culture

positive for *Nal^R E coli* O157:H7 in cecal, colon, and rectal contents collected at necropsy compared to the control cattle administered with the placebo.

Data in the current study showed that vaccinating cattle with the SRP *E coli* O157:H7 vaccine reduced the percentage of cattle shedding *E coli* O157:H7 by over 50 %. Other vaccine technologies, such as the vaccine targeting the type III secretion proteins of *E coli* O157:H7, showed mixed results when used in a commercial feedlot environment (Potter et al., 2004; Van Donkersgoed, 2005). Potter et al. (2004) observed a 60% decrease in cattle shedding *E coli* O157:H7 that were vaccinated three times with type III secretion proteins relative to non-vaccinated cattle in a small pen study (24 pens, 2 treatments, 8 head/pen). However, Van Donkergoed et al. (2005) evaluated the effects of the same vaccine in a large scale commercial feedlot setting and observed no difference in *E coli* O157:H7 prevalence in vaccinated vs. the non-vaccinated controls (Van Donkergoed et al., 2005). Pen prevalence was highly variable ranging from 0% to 80% at arrival, to 0% to 87% at revaccination, and 0% to 90% prior to slaughter amongst both treatment groups (Type III vaccinates vs placebo controls) suggesting that the vaccine was not as efficacious in a natural feedlot setting (Van Donkersgoed et al., 2005). The SRP *E coli* O157:H7 vaccine, however, caused a significant decrease in fecal shedding and tended to decrease contamination on hides at simulated slaughter, which has not been documented by any other vaccine efficacy study performed in a commercial feedlot setting.

Hancock et al. (1997) reported that the peak prevalence of *E coli* O157:H7 in North America occurs in the late summer and early fall months. Although this study was conducted when the prevalence was historically highest (late summer), the overall prevalence of *E coli* O157:H7 in this study was quite low. In 2001, prevalence of *E. coli* O157:H7 was evaluated in

29 pens of cattle from five feedyards in the midwest and the percent of cattle shedding the organism ranged from 0.7% to 79.8% with a median prevalence of 17.1% (Smith et al., 2001).

A simulated slaughter was conducted on day 85, which was approximately one to four weeks before the animals were actually slaughtered. This was performed to ensure that the appropriate samples from each group of cattle were collected before the seasonal decline in *E coli* prevalence occurred. *E coli* prevalence has been shown to decrease as the season changes from summer to fall (Bach et al. 2002; Smith et al., 2003).

Simulated slaughter data showed that a treatment effect occurred when evaluating the prevalence of *E coli* O157 in fecal matter and on hides. Hide swab samples were collected along the back region of each animal, which was where site-specific prevalence of *E coli* O157 was shown to be the highest (74%) (Keen et al., 2002). A strong correlation between prevalence of this organism in fecal samples and on hides has been demonstrated (Elder et al., 2000). Fecal prevalence at slaughter for both treatment groups combined was quite low (less than 6%; 1.6% in SRP vaccinated cattle and 4.0% in the control group), which was similar to results reported by Omisakin et al., in 2003, where prevalence at slaughter was 7.5%.

Samples taken from the rectoanal mucosal region did not show significant treatment differences in prevalence of *E coli* O157:H7. Rectoanal mucosa has been shown to be a preferred site of colonization in cattle (Naylor et al., 2003; Low et al., 2005). Rectoanal mucosal swabbing (RAMS) was more a sensitive sampling technique than the fecal culture method when examining the presence of *E coli* O157:H7 (Rice et al., 2003; Greenquist et al., 2005; Davis et al., 2005). Rice et al. (2003) have suggested that when collecting RAMS samples, the swabs must be minimally exposed to fecal material to reduce the risk of obtaining the intestinal natural flora. It is possible that during the simulated slaughter, the rectoanal mucosal swab samples

were contaminated to the animal's fecal matter, which allowed for competitive exclusion of the natural flora, thus eliminating the chance for detection of the organism of interest.

Injection of cattle with the *E coli* O157:H7 SRP vaccine did not have an effect on feeder cattle performance. Some vaccines have been shown to significantly ($P < 0.05$) increase average daily weight gain in animals, which was examined by Barling et al. in 2003 when beef feedlot steers were subcutaneously vaccinated against *Neospora caninum* (Barling et al. 2003). This improvement in performance is generally seen when the targeted organism is a pathogen of the animal receiving the vaccination. *E coli* O157 is not a pathogen of cattle and, therefore, the *E coli* O157:H7 SRP vaccine not affecting cattle performance was not unexpected.

Vaccination of feedlot cattle with the *E coli* O157:H7 SRP vaccine reduced the prevalence of *E coli* O157 and did not affect overall cattle performance. Pre-harvest control of food-borne pathogens decreases the potential for post-harvest contamination of beef products. Use of the SRP *E coli* O157:H7 vaccine could be an appropriate tool to reduce food borne illnesses associated with food or water contaminated with cattle feces, without decreasing the efficiency of beef production.

FOOTNOTES

- a. MVP, Laboratories, Omaha, NE
- b. Fisher, Palatine, IL
- c. VWR International, Buffalo Grove, IL
- d. BD, Franklin Lakes, NJ.
- e. Nasco, Fort Atkinson, WI
- f. Wheaton, Millville, NJ
- g. Dynal, Inc. New Hyde Park, NY
- h. Remel, Lenexa, KS
- i. Oxoid Limited, Basingstoke, Hampshire, England
- j. Biomerieux, Inc., Hazelwood, MO
- k. PE Applied Biosystems, Foster City, CA
- l. Fischer Scientific, Pittsburgh, PA
- m. Bio-Rad, Hercules, CA
- n. SAS, version 9.1.3, SAS Institute Inc, Cary, NC.

ABBREVIATIONS

SRP	Siderophore Receptor/Porin Proteins
RAMS	Rectoanal Mucosal Swab
GNccv	Gram Negative Broth supplemented with cefixime (0.05 mg/L), cefsulodin (10.0 mg/L), and vancomycin (8.0 mg/L)
PCR	Polymerase Chain Reaction

REFERENCES

1. CDC Department of Health and Human Services website. *Escherichia coli* O157:H7 Available at <http://www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacolig.htm> Accessed on October 5, 2006.
2. Park S, Worobo RW, Durst RA. *Escherichia coli* O157:H7 as an emerging foodborne pathogen: A literature review. *Crit Rev Food Sci and Nutr* 1999;39:481-502.
3. Rasmussen MA, Casey TA. Environmental and Food Safety Aspects of *Escherichia coli* O157:H7 Infections in Cattle. *Crit Rev Microbiol* 2001;27:57-73.
4. Koohmaraie M, Arthur TM, Bosilevac JM, et al. Post-harvest interventions to reduce/eliminate pathogens in beef. *Meat Sci* 2005;71:79-91.
5. Lonergan GH, Brasheers MM. Pre-harvest interventions to reduce carriage of *E. coli* O157 by harvest-ready feedlot cattle. *Meat Sci* 2005;71:72-78.
6. Lejeune JT, Wetzel AN. Preharvest control of *Escherichia coli* O157 in cattle. *J. Anim Sci* 2007;85(E. Suppl):E73-E80.
7. Potter AA, Klashinsky SS, Li Y, et al. Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. *Vaccine* 2004;22:362-369.

8. Dean-Nystrom EA, Gansheroff LJ, Mills M, et al. Vaccination of pregnant dams with intimin O157 protects suckling piglets from *Escherichia coli* O157:H7 infection. *Infect Immun* 2002;70:2414-2418.
9. Konnadu EA, Donohue-Rolfe A, Calderwood SB, et al. Syntheses and immunologic properties of *Escherichia coli* O157 O-specific polysaccharide and Shiga toxin 1B subunit conjugates in mice. *Infect Immun* 1999;67:6191-6193.
10. Emery DA, Straub DE, Huisinga R, et al. Active immunization using a siderophore receptor protein United States Patent: 6,027,736. 2000.
11. Neilands JB. Siderophores: Structure and function of microbial iron transport compounds. *J Biol Chem* 1995;270:26723-27726.
12. Thornton AB, Thomson DU, Fox JT, et al. Siderophore receptor/porin protein-based vaccine reduces prevalence and shedding of *Escherichia coli* O157 in cattle. 87th Ann Meeting of the Conference of Research Workers on Animal Diseases 2006;149.
13. Bach SJ, McAllister TA, Veira DM, et al. Transmission and control of *Escherichia coli* O157-a review. *Can J Anim Sci* 2002;82:475-490.

14. Smith DR, Moxley RA, Clowser SL, et al. Use of rope devices to describe and explain the feedlot ecology of *Escherichia coli* O157:H7 by time and place. *Food borne Pathogen Dis* 2003;2:50-60.
15. Rice DH, Sheng HQ, Wynia SA, et al. Rectoanal Mucosal Swab Cultures Is More sensitive than fecal culture and distinguishes *Escherichia coli* O157:H7-colonized cattle and those transiently shedding the same organism. *J Clin Microbiol* 2003;41:4924-4929.
16. Greenquist M A, Drouillard J S, Sargeant J M et al. Comparison of rectoanal mucosal swab cultures and fecal cultures for determining prevalence of *Escherichia coli* O157:H7 in feedlot cattle. *Appl Environ Microbiol.* 2005;71:6431-6433.
17. Elder RO, Siragusa GR, Barkocy-Gallagher GA, et al. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *PNAS* 2000;97:2999-3003.
18. Besser RE, Griffen PM, Slutsker L. *Escherichia coli* O157:H7 gastroenteritis and the hemolytic syndrome: an emerging infectious disease. *Ann Rev Med* 1999;50:355-367.
19. Callaway TR, Anderson RC, Edrington TS, et al. What are we doing about *Escherichia coli* O157 in cattle? *J Anim Sci* 2004;82:E93-E99.

20. Gyles CL. Shiga toxin-producing *Escherichia coli*: An overview. *J Anim Sci* 2007;85(E Suppl.):E45-E62.
21. Bolin CA, Jensen AE. Passive immunization with antibodies iron-regulated outer membrane proteins protects turkeys from *Escherichia coli* septicemia. *Infect Immun* 1987;55:1239-1242.
22. Van Donkersgoed J, Berg J, Potter A, et al. Environmental sources and transmission of *Escherichia coli* O157:H7 in feedlot cattle. *Can Vet J* 2001;42:714-720.
23. Hancock DD, Besser TE, Rice DH, et al. A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. *Epidemiol Infect* 1997;118:193-195.
24. Smith D, Blackford M, Younts S, et al. Ecological Relationships between the Prevalence of Cattle Shedding *Escherichia coli* O157:H7 and Characteristics of the Cattle or Conditions of the Feedlot Pen. *J Food Prot.* 2001;64:1899-1903.
25. Keen JE and Elder RO. Isolation of shiga-toxigenic *Escherichia coli* O157 from hide surfaces and the oral cavity of finished beef feedlot cattle. *JAVMA.* 2002; 220:756-763.
26. Omisakin F, MacRae M, Ogden ID, et al. Concentration and prevalence of *Escherichia coli* O157:H7 in cattle feces at slaughter. *Appl Environ Microbiol.* 2003;69:2444-2447.

27. Naylor S, Low C, Besser T, et al. Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infect. Immun.* 2003;71:1505-1507.
28. Low J C, McKendrick I J, McKenzie C, et al. Rectal carriage of enterohemorrhagic *Escherichia coli* O157 in slaughtered cattle. *Appl Environ Microbiol.* 2005;71:93-97.
29. Davis M A, Rice D H, Sheng H, et al. Comparison of cultures from rectoanal-junction mucosal swabs and feces for detection of *Escherichia coli* O157 in dairy heifers. *Appl Environ Microbiol.* 2006;72:3766-3770.
30. Barling KS, Lunt DK, Graham SL, et al. Evaluation of an inactivated *Neospora caninum* vaccine in beef feedlot steers. *J Am Vet Med Assoc.* 2003;222:624-627.

CONCLUSION

The Center for Disease Control (CDC) continues to report multi-state outbreaks of *Escherichia coli* O157:H7 infections which are consistently identified as being associated with its bovine reservoir. Three separate outbreaks have been reported resulting in over 300 cases of hemorrhagic colitis, 41 cases of hemolytic uremic syndrome (HUS), and 3 deaths within the last two years. This organism has not only caused human illness, but has also created an excessive amount of waste due to the recall of contaminated food products. Best management practices should be implemented to reduce the prevalence of *E. coli* O157:H7 in the environment and reduce its existence in our food chain. It becomes more apparent that the use of multiple forms of intervention must be used in the field to control contamination of beef products with *E. coli* O157:H7.

Our preliminary studies showed that the *E. coli* O157:H7 SRP vaccine could be an effective tool in reducing prevalence and concentration of *E. coli* O157 in cattle. In our challenge study, *E. coli* O157:H7 SRP vaccine was effective in reducing prevalence and fecal concentration of *E. coli* O157 in cattle orally inoculated with *Nal^R E. coli* O157:H7. In our feedlot study, fecal prevalence of *E. coli* O157:H7 averaged over time was reduced by 54% in the SRP vaccinated cattle compared to the control cattle. Vaccination with SRP antigens decreased the prevalence of *E. coli* O157 in fecal samples collected at simulated harvest and tended to decrease hide swab samples prevalence. Administration of SRP vaccine had no effect on the performance of feeder calves.

Vaccination of cattle with the *E. coli* SRP vaccine could be a possible strategy to reducing *E. coli* O157:H7 prevalence within a feedlot setting. By using this strategy with good biosecurity practices, the concentration of the pathogen is greatly reduced both in the gut and in the feces thus reducing the spread of the organism in the environment. Future research on the use of this novel vaccine is necessary to optimize *E. coli* O157:H7 control without decreasing cattle performance.