EPIDEMIOLOGY OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* IN COMMERICIAL FEEDLOT CATTLE

by

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B.S., Kansas State University, 2012
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Shiga toxin-producing *Escherichia coli* serogroups (O26, O45, O103, O111, O121, O145, and O157; STEC-7) are recognized as major food-borne pathogens with outbreaks, human infections, and occasional deaths associated with the consumption of contaminated foods. Cattle are recognized as the primary reservoir for STEC-7 and shed these bacteria in their feces, which are considered a principal source of contamination of cattle hides and carcasses at harvest. Pre-harvest interventions that effectively reduce fecal shedding of STEC-7 have the potential to reduce the public health concerns and economic impact of these bacteria and enhance food safety. In the research presented in this dissertation, distinct study designs were used to evaluate the impact of commercially available pre-harvest interventions and develop a better understanding of the epidemiology of STEC-7 in commercial feedlot cattle. A randomized pen-level trial indicated that a commercially available vaccine significantly reduced the fecal prevalence of STEC O157 and prevalence of high shedders compared to unvaccinated pens. However, there was no evidence of a direct-fed microbial (DFM) effect on either measure of STEC O157 shedding. In a continuum of the efficacy study, the performance and carcass characteristics associated with these pre-harvest interventions were quantified. Results indicated that feeding the DFM to cattle improved performance, whereas the vaccine negatively impacted performance during the intervention period, though most of these attributes were not reflected at the time the animals were harvested. Later, a cross-sectional observational study was used to determine the regional-, feedlot- and pen-level fecal prevalence of enterohemorrhagic *Escherichia coli* (EHEC), a subset of STEC, in commercial feedlot cattle. Results indicated that EHEC serogroup O157 was detected more frequently than non-O157 serogroups of EHEC; however, all feedlots had at least one sample positive for both O157 and non-O157 EHEC.

Further, risk factors associated with non-O157 serogroups of EHEC were identified; further evaluation of these factors as potential control points may enable the ability to positively impact public health concerns and food safety by reducing the pathogen load prior to harvest. Overall, the research described in this dissertation provides an assessment of pre-harvest interventions and multi-level prevalence estimates of STEC-7 in commercial feedlot operations.

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Approved by:

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Table of Contents

List of Figures

List of Tables

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Dedication

I dedicate this dissertation to my son, Carter William Cull, and others affected by rare diseases and medical conditions.

Preface

Although Shiga toxin-producing *Escherichia coli* serogroups (O26, O45, O103, O111, O121, O145, and O157; STEC-7) are major threats to public health and food safety, there are several pre-harvest interventions and epidemiologic aspects of STEC-7 in commercial feedlot cattle that are still unclear. To evaluate the impact of commercially available pre-harvest interventions and develop a better understanding of the epidemiology of STEC-7 in commercial feedlot cattle we used distinct study designs. The studies describe in this dissertation provide an assessment of pre-harvest interventions and multi-level prevalence estimates of STEC-7 in commercial feedlot operations.

My first study, was published in *Vaccine* and evaluated the effects of a commercially available STEC O157 vaccine and direct-fed microbial in commercial feedlot cattle. My second study published, in the *Journal of Animal Science*, quantified the performance and carcass characteristics associated with these pre-harvest interventions. My final study, was a cross-sectional observational study that determined the feedlot- and pen-level fecal prevalence of enterohemorrhagic *Escherichia coli* (EHEC), a subset of STEC, in commercial feedlot cattle, as well as identified potential risk factors associated with non-O157 serogroups of EHEC. Overall, the research described in this dissertation indicates the complex interrelationship among risk factors, targeted interventions, and microbial concentrations that must be considered in order to mitigate the transmission associated with STEC-7 in commercial feedlot cattle.

Chapter 1 - Literature Review on Shiga toxin-producing *Escherichia coli* and Commercial Feedlot Production

Introduction

Seven Shiga toxin-producing *Escherichia coli* (STEC) serogroups (STEC O26, O45, O103, O111, O121, O145, and O157; STEC-7) are recognized as major foodborne pathogens with outbreaks, human illnesses, and occasional deaths associated with the consumption of contaminated foods (USDA-FSIS, 2014; CDC, 2014). Foodborne illnesses of STEC-7 have been linked to a variety of foods commodities in the United States (Scallan et al., 2011; Luna-Gierke et al., 2014). However, ruminants, particularly cattle, are recognized as the primary reservoir of STEC, and intermittently shed these bacteria in their feces. Subsequently, fecal contamination provides a unique opportunity for STEC-7 transfer with the potential for human exposure and contamination of food products by direct- or indirect-contact during harvest (Ferens and Hovde, 2011; Painter et al., 2013). In addition to the major public health concerns, there has been a profound economic impact of STEC-7 on different food industries (NCBA, 2012). Pre-harvest interventions that effectively reduce fecal shedding of STEC-7 and cattle hide contamination may have the potential to reduce the public health concerns and economic impact of these bacteria and enhance food safety. However, further defining the effects of pre-harvest interventions on cattle performance and carcass characteristics are warranted due to potential financial implications for intervention adoption in the industry (Elam et al., 2004; Thomson et al., 2007).

Although *E. coli* O157:H7 (STEC O157) has been the primary serotype studied in human and animal populations for the past three decades, there is a growing body of scientific literature

regarding the epidemiology of non-O157 STEC (STEC O26, O45, O103, O111, O121, and O145) in commercial feedlot operations (Callaway et al., 2009; Smith, 2014). Recent reports have indicated several similarities in the epidemiologic approach of mitigating STEC O157 and non-O157 STEC; however, several differences remain in prevalence frequency, efficacy of pre-harvest interventions, and available data to guide future research along the beef production system. This scientific review on STEC-7 is limited to published studies that were performed at commercial feedlot operations in the United States. Although pertinent information on the pathogenesis and epidemiology of STEC-7 will be discussed, specific biological information is only briefly mentioned. Commercially available pre-harvest interventions will be discussed, with an emphasis on intervention efficacy and economic impacts in commercial feedlot operations. This review of the ecology and epidemiology of STEC-7 in feedlot operations exposes critical data gaps, while providing an introduction for the studies described in subsequent chapters.

STEC in Human Foodborne Illness

Shiga toxin-producing *E. coli* O157 and non-O157 STEC were first recognized as causes of human foodborne illnesses in 1982 and 2007, when outbreaks of hemorrhagic colitis were associated with the consumption of undercooked, contaminated ground beef in the United States (Riley et al., 1983; Wells et al., 1983; USDA-FSIS, 2014). Data trends regarding the frequency of STEC-7 foodborne illnesses, since this time, indicate the importance of these pathogens as serious public health risks due to the frequency and severity of cases. Shiga toxin-producing *E. coli*-7 are estimated to cause approximately 175,000 foodborne illnesses and 20 deaths each year in the United States (Scallan et al., 2011), while less than 40% of the STEC-7 foodborne illness

cases were associated with the consumption of contaminated ground beef or non-intact beef products (Withee et al., 2009). In 2013, there were approximately 1.7 cases of STEC-7 per 100,000 people (i.e. 1.15 cases of STEC O157 per 100,000 people and 1.18 cases of non-O157 STEC per 100,000 people) in the United States (CDC, 2013), with the highest incidence occurring in children less than five years of age (4.2 cases per 100,000 people). Further, the annual incidence of STEC-7 has decreased by 30% compared to initial surveillance data from 1996 to 1998, yet there is no difference in incidence from 2006 to 2008 (CDC, 2013). Although human STEC-7 illnesses are relatively uncommon compared to other foodborne pathogens (i.e. *Campylobacter*, *Listeria, Salmonella, Shigella*), the severity of STEC-7 infections justifies their classification as major foodborne pathogens (CDC, 2014).

Although the clinical presentation and severity of STEC-7 infections may vary between human populations, life-threatening complications tend to occur in children (< 5 years old), elderly, and immunocompromised individuals (Griffin and Tauxe, 1991; CDC, 2014). These complications are typically associated with hemorrhagic colitis (i.e. bloody diarrhea) and hemolytic-uremic syndrome (HUS), as 90% and 8% of all the STEC-7 infections in the United States lead to hemorrhagic colitis and HUS (CDC, 2014). Hemolytic-uremic syndrome, a dangerous complication of STEC-7, is the most common cause of acute renal failure in children (Coia et al., 1998). In 2012, the incidence of HUS in children less than 18 years of age was 0.56 cases per 100,000 people, with the highest incidence (1.27 cases per 100,000 people) in children less than 5 years of age (Crim et al., 2014). Recently, the overall incidence of HUS from STEC-7 infections has decreased by 30% compare to surveillance data from 2006 to 2008 (Crim et al., 2014), while the overall estimated case fatality rate is un-changed at 3 to 5% (Coia et al., 1998; CDC, 2010). Although premature death rarely occurs among STEC-7 infected individuals (1 per

1000 cases), the economic impact of these cases account for approximately 95% of the total estimated human health-associated cost (i.e. $478 million) in the United States (Frenze et al., 2005; USDA-ERS, 2011). As a result, there has been an increase in industry and regulatory actions to improve pre- and post-harvest methods to prevent beef contamination, and other foodborne risks, due to the public health concerns and severity of STEC-7 foodborne illnesses.

Epidemiology of STEC in Cattle

Although the body of scientific literature on STEC-7 pathogens was limited following the first reported foodborne outbreaks, pathogenic and non-pathogenic *E. coli* have been studies for decades. *Escherichia coli* are commonly differentiated based on three surface antigens: capsular (K), flagellar (H), and somatic (O) (Gyles, 2007; Meng et al., 2007). Although numerous *E. coli* serotypes are considered non-pathogenic (i.e. commensal bacteria in human and animal gastrointestinal tracts), the pathogenicity of diarrheagenic *E. coli* are further categorized into six major groups: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffuse-adhering *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), and enterohemorrhagic *E. coli* (EHEC) (Kaper, 2004; Meng et al., 2007). Shiga toxin-producing *E. coli* (STEC) belong to the diarrheagenic class known as EHEC; STEC are described by their ability to produce Shiga toxins (Moxley, 2004; Meng et al., 2007). The potent cytotoxin, Shiga toxin, plays a key role in inducing vascular lesions and virulence for more than 200 pathogenic *E. coli* serotypes (Nataro and Kaper, 1998; Paton and Paton, 1998; Karmali et al., 2010; CDC, 2014). Of the pathogenic *E. coli* serotypes, STEC-7 foodborne pathogens have been linked to beef and are associated with human illnesses in the United States and elsewhere in the world (Bettelheim, 2007; Dambrosio *et al*., 2007; Cobbold *et al*., 2008; Ethelberg *et al*., 2009; CDC,

2014). Consequently, these pathogens were declared adulterants in ground beef and non-intact beef products in the US (USDA-FSIS, 2014); this declaration provided the framework for additional STEC-7 research in beef production systems and public health.

Shiga toxin-producing *E. coli*-7 have been discovered in many food commodities with the first known produce associated outbreak occurring in the early 1990s (Rangel et al., 2005). Human foodborne illness due to STEC-7 have been associated with a variety of produce, including leafy greens, vegetables, and fruits or nuts (Besser et al., 1993; Bilborn et al., 1999; Breuer et al., 2001; Rangel et al., 2005; Cobbold et al., 2008; Smith, 2014). While other outbreaks have been associated with water sources, including drinking water, lake water, and ponds (Licence et al., 2001; CDC, 2014; Smith, 2014). In addition, there are cattle-associated products which have been linked to raw milk, cheese, ground beef, and non-intact beef (Rangel et al., 2005; Cobbold et al., 2008; USDA-FSIS, 2014). Although the public health risk for STEC-7 includes many food commodities and production systems, the pathway within commercial feedlot operations is the primary purpose for this review.

The evidence to date suggests that ruminants, particularly cattle, are recognized as the primary reservoir of STEC-7 (CDC, 2014; USDA-FSIS, 2014). Colonization of STEC occurs primarily in the distal rectum of cattle. Cattle are colonized by these bacteria primarily in the distal rectum (Nataro and Kaper, 1998; Kaper et al., 2004). Although cattle are asymptomatic (i.e. does not result in clinical signs of illness) carriers of STEC-7, they intermediately shed these pathogens in their feces for different periods of time and at different concentrations (Low et al., 2005; Cobbold et al., 2007; Cernicchiaro et al., 2014; CDC, 2014). One study reported that approximately 65% of individual cattle shed for less than 30 days (Besser et al., 1997), while others indicated a potential relationship between the duration and concertation of fecal shedding

(Low et al., 2005; Cobbold et al., 2007; Chase-Topping et al., 2008). Further, fecal shedding of STEC appears to vary based on O serogroup, with reports indicating a lower prevalence of non-O157 STEC in non-intact beef products, raw ground beef, and cattle feces relative to STEC O157 (Renter et al., 2005; Renter et al., 2007; Bosilevac et al., 2007; Bosilevac and Koohmaraie, 2011; Fratamico et al., 2011; Cernicchiaro et al., 2014). More specifically, these studies indicated that STEC O157 was greater than 5 times more frequently detected than non-O157 STEC. However, the published literature has reported wide fecal prevalence estimates: ranging from 0.0 to < 20.0% for non-O157 STEC (Cernicchiaro et al., 2013 and 2014; Baltasar et al., 2014; Ekiri et al., 2014; Paddock et al., 2014; Dewsbury et al., 2015) and 0.0 to 100.0% for STEC O157 (Dargatz et al., 1997; Laegreid et al., 1999; Elder et al., 2000; Smith et al., 2001; Reinstein et al., 2009).

Furthermore, seasonality and geographic location are thought to affect fecal prevalence of STEC-7. Recent studies have reported an increase of STEC-7 shedding in cattle feces during the summer months relative to the winter months (Chapman et al., 1997; Van Donkersgoed et al., 2001; Renter et al., 2008; Dewsbury et al., 2015), while others have hypothesized potential prevalence differences when comparing commercial feedlot operations in southern states to northern states (Hancock et al., 1997; Callaway et al., 2009; Smith, 2014). Although the exact reason for this phenomena is yet to be identified, a few studies have indicated that day length, pen condition, and temperature may effect fecal prevalence of STEC-7 in cattle (Smith et al., 2001; Sargeant et al., 2004; Edrington et al., 2006; Renter et al., 2007; Baltasar et al., 2014; Ekiri et al., 2014; Dewsbury et al., 2015). While the majority of these studies have focused on STEC O157, additional prevalence estimates are required at different hierarchical levels to data gaps on non-O157 STEC and enable the building quantitative risk assessment models of STEC-7 along

the beef chain. Further, the ability to correctly identify and managed other potential risk factors, such as cattle source, management, biosecurity, diet and cattle handling, against STEC-7 shedding may enable positive impacts on food safety, as fecal shedding has been positively associated with hide and beef carcass contamination at harvest (Elder et al., 2002; Fox et al., 2008; Jacob et al., 2010; Stromberg et al., 2015). Some propose that cattle shedding higher concentrations of STEC-7 may pose a greater risk of hide and carcass contamination (Arthur et al., 2009; Smith, 2014; Stromberg et al., 2015). Hence, studies of the effects of pre-harvest control strategies may need to be investigated for both prevalence and concentration of STEC-7 in commercial feedlot cattle.

High shedders (i.e. super shedders) have been identified as cattle shedding at greater than 10^4 CFU STEC-7/g of feces, while lower concentrations ($<10^4$ CFU STEC-7/g of feces) are often simply defined as fecal shedding (Chase-Topping et al., 2008). Some reports propose that mitigation efforts should target high-shedding cattle since these animals contribute the highest potential fecal load for hide and carcass contaminations at harvest (Loneragan and Brashears, 2005; Matthews et al., 2006; Cobbold et al., 2007; Fox et al., 2008; Stephens et al., 2009). As a result, additional research is required to determine the ecology and epidemiology of high shedders, as the proportion of high shedders within a cattle cohort may be important to reduce the risk of STEC-7 transfer and contamination (Matthews et al., 2006). Regardless, high prevalence of STEC-7 in cattle feces at either concentration (i.e. fecal shedding or high shedders) is likely to contribute to the risk of contamination during slaughter (Loneragan and Brashears, 2005). Thus, epidemiologic studies properly designed to identify the prevalence and fecal concertation of STEC-7 at different hierarchical levels in the beef production systems is extremely important to help guide future control efforts.

Although there are multiple factors effecting the transmission of STEC-7 within cohorts of cattle from the commercial feedlot operation to harvest, hides are the most likely imminent source of carcass contamination due to STEC-7 (Loneragan and Brashears, 2005; Woerner et al., 2006). Elder et al., (2002) indicated a significant correlations between hide prevalence and carcass contamination, while another report indicated a difference in hide prevalence of STEC O157 when comparing pens with greater than 20% fecal prevalence versus less than 20% (Ransom et al., 2003). In addition, research indicates that the prevalence of STEC-7 from sampling cattle upon entry into the plant to final product reduces at each step (e.g., hides, pre-evisceration, post-evisceration, in-cooler samples); thus illustrating the effectiveness of post-harvest intervention against STEC-7 (Elder et al., 2002; Stromberg et al., 2015). However, additional animal- and pen-level data are required to accurately describe the entire relationship for prevalence and concertation of STEC-7 among fecal, hide, and carcass samples. Yet, it is reasonable to hypothesize that reducing the STEC-7 fecal load prior to harvest should in turn reduce the likelihood of hide prevalence and subsequently carcass contamination at harvest (Loneragan and Brashears, 2005).

Pre-harvest Interventions

Over the past three decades, the beef industry has focused a lot of time and resources on mitigating STEC-7 contamination at harvest by incorporating specific trainings procedures, programs (i.e. Hazard Analysis and Critical Control Points), interventions, and diagnostic tests within packing plants. Although effective, researchers have proposed that reducing the STEC-7 fecal shedding load before harvest may increase the capabilities of post-harvest procedures (Callaway et al., 2004; Loneragan and Brashears 2005; Callaway et al., 2009; Smith 2014). In

addition to reducing the fecal shedding of STEC-7 in cattle, pre-harvest interventions may have the potential to reduce the economic impact and public health concerns of these bacteria and enhance food safety (Hynes and Wachsmuth, 2000). As a result, this opportunity has led to the development and research of many pre-harvest interventions (i.e. antibiotics, bacteriophages, diets, prebiotics, probiotics, and vaccines). However, there are currently only two commercially available pre-harvest products (i.e. a vaccine and a direct-fed microbial) approved for reducing STEC O157 fecal shedding in the United States (Callaway et al., 2009; Smith 2014; Wisener et al., 2014).

The use of antibiotics to specifically control fecal shedding of STEC in cattle is controversial. However, reports have indicated that the use of neomycin, an aminoglycoside antibiotic, in the feed significantly reduces fecal shedding of STEC O157 in cattle (Elder et al., 2002; Callaway et al., 2009). Despite these findings, the beef industry has not adopted antibiotic treatment as a pre-harvest intervention strategy due to the potential concern of antimicrobial resistance (Loneragan and Brashears, 2005; Callaway et al., 2009). In addition, a different class of feed grade antibiotics (i.e. ionophores) was tested for its effectiveness to reduce STEC O157 shedding, as ionophores are not used in human medicine. Although the issue of resistance may not be as critical with ionophores as with other antibiotics, research has indicated a lack of efficacy for ionophores against STEC O157 shedding in cattle feces (Edrington et al., 2006; Callaway et al., 2009). However, ionophores are still approved and used for growth performance and coccidia control in commercial feedlot operations.

Bacteriophages or phages, viruses that have the ability to specifically target certain organisms, use self-replication to adapt with bacteria and are reported to be harmless to animals (Sheng 2006; Sillankorva et al., 2012). *In vitro* studies have indicated exceptional efficacy of

phages against STEC O157 (Tanji et al., 2005; Sheng et al., 2006), while initial in *vivo* studies indicated a reduction of STEC O157 when applying phages in drinking water or the recotanal junction (Sheng et al., 2006; Rozema et al., 2009). However, other live animal studies have indicated mixed results, seemingly due to incorrect uses, doses, or strains of phages (Raya et al., 2006; Sheng et al., 2006; Niu et al., 2008; Standford et al., 2010). Still the unique ability to incorporate phages in production systems is intriguing due to the ease of administration (i.e. water, feed, spray), yet additional trials are required before incorporating phages in commercial feedlot operations (Rozema et al., 2009; Sillankorva et al., 2012). However, phages are approved for commercial uses as a spray application to hides at packing plants.

Scientists also have investigated sodium chlorate as a potential pre-harvest intervention (Loneragan and Brashears, 2005; Callaway et al., 2014). *Enterobacteriaceae* (e.g. STEC -7) are facultative anaerobes that have the ability to use oxygen for aerobic respiration as well as anaerobic fermentation. More specifically, STEC have the nitrate reductase enzyme that allows respiration and converts chlorate to a cytotoxic chlorite inside of STEC pathogens. Therefore, the use of sodium chlorate may be considered a selective microbial product with the ability to target STEC-7 due to occurrence of the nitrate reductase enzyme. Research has suggested that oral administration of sodium chlorate reduced STEC O157 shedding in cattle feces by more than two logs (Callaway et al., 2002; Anderson et al., 2005). However, to date sodium chlorate is not commercially available as a pre-harvest intervention in commercial feedlot cattle, as additional data may need to be generated for approval.

Scientists also have proposed that diets may be an important contributing factor to the gastrointestinal flora and pathogen populations of STEC in cattle (Fox et al., 2007; Jacob et al., 2008; Reinstein et al., 2009). Feeding cattle a high energy diet has been shown to positively

impact the STEC population in the lower gastrointestinal tract of cattle (Callaway et al., 2009). There have been mixed results regarding the impact of forage levels on STEC population, however, with some reports indicating a decrease (Diez-Gonzalez et al., 1998; Tkalcic et al., 2000), no change (Zhang et al., 2010), or increase (Van Baale et al., 2004) in shedding of STEC O157 in cattle feces when compared to grain based diets. Similarly, studies have indicated a higher fecal prevalence of STEC O157 when cattle are fed greater than 25% distiller's grains compared to diets with less than 25% distiller's grains (Jacob et al., 2008; Wells et al., 2009; Jacob et al., 2010). In addition, the type of grain and processing methods may significantly impact the STEC population within cattle's gastrointestinal tract (Fox et al., 2007). Research has indicated an increased fecal shedding of STEC O157 in cattle fed a barley-based diet compared to a corn-based diet (Dargatz et al., 1997; Buchko et al., 2000; Berg et al., 2004). Further, studies on the differences in processing method of grains (i.e. corn and barley) have shown a greater STEC O157 burden in cattle receiving a steam-rolled or steam-flaked grain compared to dry-rolled grain diet (Fox et al., 2007; Depenbusch et al., 2008; Callaway et al., 2009). Although researchers have proposed multiple hypotheses for increased STEC shedding due to specific diet ingredients, additional data are required to determine the exact physiological factors that affect shedding. Further, it seems unlikely that commercial feedlot operations are going to alter cattle diets based on fecal shedding of STEC due to the potential financial implications (i.e. potential loss in cattle performance and carcass characteristics) of adopting unique diets combinations.

Administration of prebiotics is currently being tested for the ability to alter STEC shedding in cattle. Prebiotics are non-digestible organic compounds, such as oligosaccharides, trisaccharide and dietary fiber, which cannot be directly utilized by animals, but have the ability to be digested by specific populations of the microflora (Houdijk et al., 1998; Willard et al.,

2000). Some researchers have hypothesized that beneficial bacteria are able to outperform the pathogens by utilizing prebiotics (Schrezenmeir et al., 2001), while others believe that prebiotics have the potential to target specific segments of the microbial population by competitive exclusion (Zopf and Roth, 1996; Baines et al., 2011). To date, it is unlikely that commercial feedlot operations are willing to implement prebiotics as a pre-harvest intervention due to their expense, limited data, and the ability of ruminal microorganism to degrade a range of prebiotic compounds.

Direct-fed microbials are another pre-harvest intervention that is being explored for their ability to control STEC shedding. Traditionally, direct-fed microbials have been fed in cattle diets to enhance performance (Elam et al., 2003; Callaway et al., 2014). Direct-fed microbials utilize commensal microbial cultures to beneficially affect the microflora of the gastrointestinal tract through either the potential upregulation of desirable microbial populations or by physically attaching to the gastrointestinal epithelium to prevent harmful pathogens (e.g. STEC) from thriving (Zhao et al., 1998; Kim et all., 2008; Wisener et al., 2014). By far the most studied direct-fed microbial products include a *Lactobacillus*-based strain (Wisener et al., 2014). Studies have reported significant reductions of STEC O157 shedding in cattle fed a DFM comprising of *L. acidophilus* (Brashears et al., 2003; Elam et al., 2003; Vasconcelos et al., 2008; Hanford et al., 2011). Further studies have indicated efficacy of a modified direct-fed microbial culture, which includes the *L. acidophilus* and *Propionibacterium freudenreichii* strains, with an overall STEC O157 fecal shedding reduction between 20% to 75% (Younts-Dahl et al., 2004; Stephens et al., 2007; Arthur et al., 2010; Cernicchiaro et al., 2010). However, the published literature has indicated multiple dosing volumes (e.g., high versus low) for some of the DFM products due to the potential differential efficacy and performance affects at either a high or low dose (Wisener

et al., 2014). However, a recent study indicated no evidence for a difference in EHEC based on a high versus low dose of a DFM product (i.e., Bovamine®) (Luedtke et al., 2016). To date, the use of DFM has become a relatively common practice in commercial feedlot operations due to their potential ability to improve cattle performance and reduce pathogens (NAHMS, 2013; Callaway et al., 2014). However, additional large commercial feedlot trials are required to quantify both the pre-harvest food-safety (i.e. STEC-7 shedding) and performance impacts associated with the implementation of direct-fed microbial feeding, as the current literature includes studies with key differences in study design, statistical power, and pen size (Wisener et al., 2014).

Along with other pre-harvest interventions, vaccines have been tested for their ability to reduce STEC O157 shedding. Although most vaccines are utilized to stimulate the immune system of animals to protect against disease, the Siderophore Receptor and Porin protein (SRP) and the Type III secretory proteins (Type III) vaccines are uniquely produced to target different physiological aspect of STEC O157 due to the natural exposure and commensal, asymptomatic nature of the organism in cattle (Callaway et al., 2014; Smith, 2014). More specifically, the SRP based vaccine disrupts iron uptake by the bacteria, effectively starving STEC of iron which leads to cell lysis (Emery et al., 2000; Thornton et al., 2009), while the antibody production against the Type III prevents villi adherence and colonization of STEC in the gastrointestinal tract of cattle (Dziva et al., 2007; Moxley et al., 2009). Recently, there were two systematic reviews indicating the efficacy of the SRP and Type III vaccines against STEC O157 shedding in cattle feces (Snedeker et al., 2012; Varela et al., 2012) with research indicating greater than 25% reduction of STEC O157 shedding in cattle administered the SRP vaccine (Thornton et al., 2009; Thomson et al., 2009; Wileman et al., 2010) or the Type III vaccine (Potter et al., 2004; Peterson et al.,

2007; Smith et al., 2009). Further, these studies reported no adverse cattle performance effects for vaccinated versus non-vaccinated animals. However, in the reported studies, cattle in the control groups were re-handled and administered a placebo, and research on other cattle vaccines have indicated negative cattle performance effects due to immunization or handling effects of cattle (Voisinet et al., 1997; Rodrigues et al., 2015). Currently, the three dose regimen of the SRP vaccine is the only commercially available vaccine approved in the United States for reducing STEC O157 shedding in cattle feces, and the three dose regimen of the Type III vaccine is no longer being produce. However, the published literature has not reported a wide-spread implementation of the SRP vaccine in commercial feedlot operations. Limited use of the SRP vaccine in commercial feedlot operations may be due to the lack of economic incentives, lack of data on the economic feasibility of these products, ease of product incorporation into existing protocols, or the potential performance impacts for the implementation of pre-harvest interventions (Snedeker et al., 2011; Callaway et al., 2014; Smith, 2014).

Conclusion

This review of the scientific literature regarding the epidemiology of STEC-7 in commercial feedlot operations exposes critical knowledge gaps in pre-harvest intervention efficacy and performance impacts, as well as a general lack of prevalence data for non-O157 STEC at different hierarchical levels in feedlot production systems. In order to identify and validate pre-harvest interventions that may significantly reduce the fecal load prior to harvest and subsequent risk of food contamination, additional research is required to determine the important risk factors of STEC-7 shedding in cattle feces. Understanding the transfer of STEC-7 along the

fecal to hide to carcass pathways likely depends on both prevalence and concentration along the beef production systems. Previous research indicates that pre-harvest interventions for STEC O157 may have the ability to compliment post-harvest interventions and decrease the risk of STEC transfer to beef at harvest; however, there are limited data illustrating the impact of pre-harvest interventions on STEC-7 prevalence at each step along the beef production system. Although there is a growing body of literature for pre-harvest interventions, additional data are required to determine the efficacy of these products in commercial feedlot operations from different geographic locations with different management practices. In addition, there is a need to determine the effects of using multiple pre-harvest interventions at different time points along the beef production system. Data regarding the concentration, prevalence, and transmission of STEC-7 within cohorts of cattle are necessary to improve the knowledge on the epidemiology of STEC-7. Further, there is a need for a more comprehensive understanding of the economic impacts of implementing pre-harvest interventions in commercial feedlot operations.

References

Anderson RC, Carr MA, Miller RK, King DA, Carstens GE, Genovese KJ, Callaway TR, Edrington TS, et al. Effects of experimental chlorate preparations as feed and water supplements on *Escherichia coli* colonization and contamination of beef cattle and carcasses. *Food Microbiol* 2005;22:439-447.

Arthur TM, Bosilevac JM, Kalchayanand N, Wells JE, Shackelford SD, Wheeler TL and Koohmaraie M. Evaluation of a direct-fed microbial product effect on the prevalence and load of *Escherichia coli* O157:H7 in feedlot cattle. *J Food Prot* 2010;73:366-371.

Arthur TM, Keen JE, Bosilevac JM, Brichta-Harhay DM, Kalchayanand N, Shackelford SD, Wheeler TL, Nou XW and Koohmaraie M. Longitudinal study of *Escherichia coli* O157:H7 in a beef cattle feedlot and role of high-level shedders in hide contamination. *Appl Environ Microbiol* 2009;75:6515-6523.

Bai J, Shi X, Nagaraja TG. A multiplex PCR procedure for detection of six major virulence genes in *Escherichia coli* O157:H7. *J Microbiol Methods* 2010;82:85-89.

Bai J, Paddock ZD, Shi X, Li S, An B, Nagaraja TG. Applicability of a multiplex PCR to detect the seven major Shiga toxin-producing *Escherichia coli* based on genes that code for serogroup-specific O-antigens and major virulence factors in cattle feces. *Foodborne Pathog Dis* 2012;9:541-548.

Baines D, Erb S, Lowe R, Turkington K, Sabau E, Kuldau G, Juba J, Masson L, Mazza A, and Roberts R. A prebiotic, Celmanax, decreases *Escherichia coli* O157:H7 colonization of bovine cells and feed-associated cytotoxicity in vitro. *BMC Res* 2011;4:110-118.

Baltasar P, Milton S, Swecker W Jr, Elvinger F, Ponder M. Shiga toxin-producing *Escherichia coli* distribution and characterization in a pasture-based cow-calf production system. *J Food Prot* 2014;77:722-731.

Berg J, McAllister T, Bach S, Stilborn R, Hancock D, and LeJeune J. *Escherichia coli* O157:H7 excretion by commercial feedlot cattle fed either barley- or corn-based finishing diets. *J Food Prot* 2004;67:666-671.

Besser RE, Lett SM, Weber JT, Doyle MP, Barrett TJ, Wells JG and Griffin PM. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *J Am Med Assoc* 1993; 269:2217-2220.

Besser TE, Hancock DD, Pritchett LC, McRae EM, Rice DH and Tarr PI. Duration of detection of fecal excretion of *Escherichia coli* O157:H7 in cattle. *J Infect Dis* 1997;175:726-729.

Bettelheim KA. The non-O157 shiga-toxigenic (verocytotoxigenic) *Escherichia coli*; under-rated pathogens. *Crit. Rev. Microbiol.* 2007;33:67-87.

Bosilevac JM, Guerine MN, Brichta-Harhay DM, Arthur TM, Koohmaraie M. Microbiological characterization of imported and domestic boneless beef trim used for ground beef. *J Food Prot* 2007;70:440–449.

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

Brashears MM, Galyean ML, Loneragan GH, Mann JE, Killinger-Mann K. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given Lactobacillus direct-fed microbials. *J Food Prot* 2003;66(5):748-54.

Breuer T, Benkel DH, Shapiro RL, Hall WN, Winnett MM, Linn MJ, Neimann J, Barrett TJ, Dietrich S, Downes FP, Toney DM, Pearson JL, Rolka H, Slutsker L and Griffin PM. A multistate outbreak of *Escherichia coli* O157:H7 infections linked to alfalfa sprouts grown from contaminated seeds. *Emerg Infect Dis* 2001;7:977.

Buchko SJ, Holley RA, Olson WO, Gannon VPJ, and Veira DM.The effect of different grain diets on fecal shedding of *Escherichia coli* O157:H7 by steers. *J Food Prot* 2000;63:1467-1474.

Callaway TR, Anderson RC, Edrington TS, Genovese KJ, Harvey RB, Poole TL and Nisbet DJ. Recent pre-harvest supplementation strategies to reduce carriage and shedding of zoonotic enteric bacterial pathogens in food animals. *Anim Health Res Rev* 2004;5:35-47

Callaway TR, Anderson RC, Genovese KJ, Poole TL, Anderson TJ, Byrd JA, Kubena LF, and Nisbet DJ. Sodium chlorate supplementation reduces *E. coli* O157:H7 populations in cattle. *J Anim Sci* 2002;80:1683-1689.

Callaway TR, Carr MA, Edrington TS, Anderson RC, and Nisbet DJ. Diet, Escherichia coli O157:H7, and cattle: A review after 10 years. *Curr Issues Mol Biol* 2009;11:67–80.

Callaway TR, Edrington TS and Nisbet DJ. Meat Science and Muscle Biology Symposium: Ecological and dietary impactors of foodborne pathogens and methods to reduce fecal shedding in cattle. *J Anim Sci* 2014; 92:1356-1365.

Centers for Disease Control and Prevention (CDC). Preliminary FoodNet data on the incidence of infections with pathogens transmitted commonly through food--10 states. 2010. *Morb Mortal Wkly Rep* 59: 418-422.

Centers for Disease Control and Prevention (CDC). *E. coli* (*Escherichia coli*). 2014. Available at: http://www.cdc.gov/ecoli/general/index.html/, accessed November 30, 2015.

Centers for Disease Control and Prevention (CDC). Foodborne Disease Active Surveillance Network (FoodNet) 2013 Annual Report. 2013. Available at: http://www.cdc.gov/foodnet/reports/annual-reports-2013.html , accessed November 30, 2015.

Cernicchiaro N, Cull CA, Paddock ZD, Shi X, Bai J, Nagaraja TG, Renter DG. Prevalence of Shiga toxin-producing *Escherichia coli* and associated virulence genes in feces of commercial feedlot cattle. *Foodborne Pathog Dis* 2013;10:835-841.

Cernicchiaro N, Renter DG, Cull CA, Paddock ZD, Shi X, Nagaraja TG. Fecal shedding of non-O157 serogroups of Shiga toxin-producing *Escherichia coli* in feedlot cattle vaccinated with an *Escherichia coli* O157:H7 SRP vaccine or fed a Lactobacillus-based direct-fed microbial. *J Food Prot* 2014; 77:732-7.

Cernicchiaro N, Pearl DL, McEwen SA, Zerby HN, Fluharty FL, Loerch SC, Kauffman MD, Bard JL, and Lejeune JT. A randomized controlled trial to assess the impact of dietary energy sources, feed supplements, and the presence of super-shedders on the detection of *Escherichia coli* O157:H7 in feedlot cattle using different diagnostic procedures. *Foodborne Path Dis* 2010;7:1071-1081.

Chapman PA, Siddons CA, Cerdan Malo AT, Harkin MAA. 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. *Epidemiol Infect* 1997;119:245-250.

Chase-Topping M, Gally D, Low C, Matthews L and Woolhouse M. Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. *Nat Rev Microbiol* 2008;6:904-912.

Cobbold RN, Davis MA, Rice DH, Szymanski M, Tarr PI, Besser TE and Hancock DD. Associations between bovine, human, and raw milk, and beef isolates of non-O157 Shiga toxigenic *Escherichia co*li within a restricted geographic area of the United States. *J Food Prot* 2008;71:1023-1027.

Coia JE. Clinical, microbiological and epidemiological aspects of *Escherichia coli* O157 infection. *FEMS Immunol Med Microbiol* 1998; 20:1-9.

Crim SM, Griffin PM, Tauxe R, Marder EP, Gilliss D, Cronquist AB. Preliminary incidence and trends of infection with pathogens transmitted commonly through food—Foodborne Diseases Active Surveillance Network, 10 US sites, 2006–2014. *MMWR Morb Mortal Wkly Rep* 2015;64:495–9.

Dambrosio A, Lorusso V, Quaglia NC, Parisi A, La Salandra G, Virgilio S, Mula G, Lucifora G, Celano GV and Normanno G. *Escherichia coli* O26 in minced beef: Prevalence, characterization and antimicrobial resistance pattern. *Int J Food Microbiol* 2007; 118:218-222

Dargatz DA, Wells SJ, Thomas LA, Hancock DD and Garber LP. Factors associated with the presence of *Escherichia coli* O157 in feces of feedlot cattle. *J Food Prot* 1997;60:466-470.

Dargatz DA, Bai J, Lubbers BV, Kopral CA, An B, Anderson GA. Prevalence of *Escherichia coli* O-types and Shiga-toxin genes in fecal samples from feedlot cattle. *Foodborne Pathog Dis* 2013;10:392-396.

Depenbusch BE, Nagaraja TG , Sargeant JM, Drouillard JS, Loe ER, Corrigan ME. Influence of processed grains on fecal pH, starch concentration, and shedding of *Escherichia coli* O157 in feedlot cattle. *J Anim Sci* 2008;86:632–39

Dewsbury DM, Renter DG, Shridhar PB, Noll LW, Shi X, Nagaraja TG, and Cernicchiaro N. Summer and Winter Prevalence of Shiga Toxin-Producing *Escherichia coli* (STEC) O26, O45, O103, O111, O121, O145, and O157 in Feces of Feedlot Cattle. *Foodborne Pathog Dis* 2015; 12:726-32.

Diez-Gonzalez F, Callaway TR, Kizoulis MG, and Russell JB. Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. *Science* 1998;281:1666-1668.

Dohoo I, Martine S, and Stryhyn H. Measures of Association. In *Veterinary Epidemiologic Research*. Charlottetown: AVC, 2009, pp 121-138.

Dziva F, Vlisidou I, and Crepin V. 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* 2007;123:254-261.

Edrington TS, Callaway TR, Ives SE, Engler MJ, Looper ML, Anderson RC, Nisbet DJ. Seasonal shedding of *Escherichia coli* O157:H7 in ruminants: A new hypothesis. *Foodborne Pathog Dis* 2006;3:413–421.

Ekiri AB, Landblom D, Doetkott D, Olet S, Shelver WL, Khaitsa ML. Isolation and characterization of Shiga toxin-producing *Escherichia coli* serogroups O26, O45, O103, O111, O113, O121, O145, and O157 shed from range and feedlot cattle from postweaning to slaughter. *J Food Prot* 2014;77:1052-1061.

Elam NA, Gleghorn, JF, Rivera JD, Galyean ML, Defoor PJ, Brashears MM, and Younts-Dahl SM. 2003. Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium fruendenreichii* on performance, carcass, and intestinal characteristics, and *Escherichia coli* strain O157 shedding of finishing beef steers. *J Anim Sci* 81:2686–2698.

Elder RO, Keen JE, Siragusa GR, Barkocy-Gallagher GA, Koohmaraie M and Laegreid WW. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc Natl Acad Sci* 2000; 97:2999-3003.

Elder RO, Keen JE, Wittum TE, Callaway TR, Edrington TS, Anderson RC, and Nisbet DJ. Intervention to reduce fecal shedding of enterohemorrhagic *Escherichia coli* O157:H7 in naturally infected cattle using neomycin sulfate. *J Anim Sci* 2002;80(1):15.

Emery DA, Straub DE, Huisinga R, and Carlson BA. Active Immunization using a siderophore receptor protein. U.S. Patent 6,027,736. 2000.

Ethelberg S, Smith B, Torpdahl M, Lisby M, Boel J, Jensen T, Nielsen EM and Molbak K. Outbreak of non-O157 Shiga toxin-producing *Escherichia coli* infection from consumption of beef sausage. *Clin Infect Dis* 2009; 48:78-81.

Ferens WA and Hovde CJ. *Escherichia coli* O157:H7: Animal Reservoir and Sources of Human Infection. *Foodborne Pathog Dis* 2011; 8:465-487.

Fox JT, Renter DG, Sanderson MW, Nutsch AL, Shi X, Nagaraja TG. Associations between the presence and magnitude of *Escherichia coli* O157 in feces at harvest and contamination of preintervention beef carcasses. *J Food Prot* 2008;71(9):1761-7.

Fox JT, Renter DG, Sanderson MW, Nutsch AL, Shi X, Nagaraja TG. Associations between the presence and magnitude of *Escherichia coli* O157 in feces at harvest and contamination of preintervention beef carcasses. *J Food Protect* 2008;71:1761-1767.

Fox JT, Thomson DU, Drouillard JS, Thornton AB, Burkhardt DT, Emery DA, et al. Efficacy of *Escherichia coli* O157:H7 siderophore receptor/porin proteins–based vaccine in feedlot cattle naturally shedding *E. coli* O157. *Foodborne Pathog Dis* 2009;6(7):893-9.

Fratamico PM, Bagi LK, Cray WC, Narang N, Yan X, Medina M, Liu Y. Detection by multiplex real time polymerase chain reaction assays and isolation of Shiga toxin-producing *Escherichia coli* serogroups O26, O145, O103, O111, O121, and O145 in ground beef. *Foodborne Pathog Dis* 2011;8:601–607.

Frenzen PD, Drake A and Angulo FJ. Economic cost of illness due to *Escherichia coli* O157 infections in the United States. *J Food Prot* 2005; 68:2623.

Gould LH, Mody RK, Ong KL, Clogher P, Cronquist AB, Garman KN, Lathrop S, Medus C, Spina NL, Webb TH, White PL, Wymore K, Gierke RE, Mahon BE, Griffin PM. Increased recognition of non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States during 2000-2010: Epidemiologic features and comparison with *E. coli* O157 infections. *Foodborne Pathog Dis* 2013;10:453-460.

Griffin PM and Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol Rev* 1991; 13:60-98.

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

Hanford KJ, Kreikemeier WM, Ware DR. The effect of Bovamine on feedlot performance of finishing cattle: A meta-analysis. Proceedings of the American Dairy Science Association and American Society of Animal Science Conference; 2011 July 10-14; New Orleans, Louisiana. *J Anim Sci* 2011;89(1):25

Hancock DD, Rice DH, Thomas LA, Dargatz DA and Besser TE. Epidemiology of *Escherichia coli* O157 in feedlot cattle. *J Food Prot* 1997;60:462-465.

Houdijk JG, Bosch MW, Verstegen MW, Berenpas HJ. Effects of dietary oligosaccharides on the growth performance and faecal characteristics of young growing pigs. *Ani Feed Sci Tech* 1998;71(1):35-48.

Jacob ME, Fox JT, Drouillard JS, Renter DG, and Nagaraja TG. Effects of dried distillers' grain on fecal prevalence and growth of *Escherichia coli* O157 in batch culture fermentations from cattle. *Appl Environ Microbiol* 2008;74:38-43.

Jacob ME, Paddock ZD, Renter DG, Lechtenberg KF, Nagaraja TG. Inclusion of dried or wet distillers' grains at different levels in diets of feedlot cattle affects fecal shedding of *Escherichia coli* O157:H7. *Appl Environ Microbiol* 2010;76(21):7238-42.

Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. *Nat Rev Microbiol* 2004;2:123–140.

Karmali, MA, Gannon V, and Sargeant JM. Verocytotoxin-producing *Escherichia coli* (VTEC). *Vet Microbiol* 2010;140:360-370.

Kim GB, Seo YM, Kim CH, and Paik IK. Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. *Poult Sci* 2011;90:75–82.

Laegreid WW, Elder RO, and Keen JE. Prevalence of *Escherichia coli* O157:H7 in range beef calves at weaning. *Epi and Inf* 1999;23: 291–298.

Loneragan GH and Brashears MM. Pre-harvest interventions to reduce carriage of *E. coli* O157 by harvest-ready feedlot cattle. *Meat Science* 2005;71(1):72-8.

LeJeune JT, Hancock DD, Besser TE. Sensitivity of *Escherichia coli* O157 detection in bovine feces assessed by broth enrichment followed by immunomagnetic separation and direct plating methodologies. *J Clin Microbio*l 2006;44:872-875.

Licence K, Oates KR, Synge BA and Reid TMS. An outbreak of *E. coli* O157 infection with evidence of spread from animals to man through contamination of a private water supply. *Epidemiol Infect* 2001;126:135-138.

Low JC, McKendrick IJ, McKechnie C, Fenlon D, Naylor SW, Currie C, Smith DE, Allison L and Gally DL. Rectal carriage of enterohemorrhagic *Escherichia coli* O157 in slaughtered cattle. *Appl Environ Microbiol* 2005;71:93-97.

Luna-Gierke RE, Griffin PM, Gould LH, Herman K, Bopp CA, Strockbine N, Mody RK. Outbreaks of non-O157 Shiga toxin-producing *Escherichia coli* infection: USA. *Epidemiol Infect* 2014;142:2270-2280.

Luedtke BE, Bosilevac JM, Harhay DM, and Arthur TM. Effect of Direct-Fed Microbial Dosage on the Fecal Concentrations of Enterohemorrhagic *Escherichia coli* in Feedlot Cattle. *Foodborne Pathog Dis* 2016;13(4):190-195.

Matthews L, Low JC, Gally DL, Pearce MC, Mellor DJ, Heesterbeek JAP, Chase-Topping M, Naylor SW, Shaw DJ, Reid SWJ, Gunn GJ, and Woolhouse MEJ. Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control. *Proc Natl Acad Sci* 2006;103:547-552.

Meng J, Doyle MP, Zhao T, and Zhao S. Enterohemorrhagic *Escherichia coli*. Food Microbiology: Fundamentals and Frontiers. Washington, D.C., ASM Press: 2007;249-269.

Moxley, RA. *Escherichia coli* O157:H7: an update on intestinal colonization and virulence mechanisms. *Animal Health Research Reviews* 2004;5(1):15-33.

Moxley RA, Smith DR , Luebbe M, Erickson GE, Klopfenstein TJ, and Rogan D. *Escherichia coli* O157:H7 vaccine dose-effect in feedlot cattle. *Foodborne Pathog Dis* 2009;6:879-84

Nataro JP and Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 1998:11(1):142-201.

National Animal Health Monitoring System (NAHMS), USDA APHIS. Feedlot 2011, Part III: Trends in Health and Management Practice on U.S. Feedlots, 1994-2011. 2013. http://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_dr_Part%20III.pdf. (Accessed 30 December 2015)

National Cattleman's Beef Association (NCBA). 2012. Facts for beef producers: a basic look at *E. coli* O157. Available at: http://www.beefresearch.org/CMDocs/BeefResearch/Safety_Fact_Sheets/E.Coli_Final.pdf , assessed December 29, 2015.

Niu YD, Xu Y, McAllister TA, Rozema EA, Stephens TP, Bach SJ, Johnson TP, and Stanford K. Comparison of fecal versus rectoanal mucosal swab sampling for detecting *Escherichia coli* O157:H7 in experimentally inoculated cattle used in assessing bacteriophage as a mitigation strategy. *J Food Prot* 2008;71:691-8.

Noll LW, Baumgartner WC, Shridhar PB, Cull CA, Dewsbury DM, Shi X, Cernicchiaro N., Renter DG, and Nagaraja TG. Pooling of Immuunomagnetic Separation Beads Does Not Affect Detection Sensitivity of Six Major Serogroups of Shiga Toxin–Producing *Escherichia coli* in Cattle Feces. *J Food Prot* 2016;7:59-65.

Omisakin F, MacRae M, Ogden ID, Strachan NJC. Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. *Appl Environ Microbiol* 2003;69:2444-2447.

Paddock Z, Shi X, Bai J, Nagaraja TG. Applicability of a multiplex PCR to detect O26, O45, O103, O111, O121, O145, and O157 serogroups of *Escherichia coli* in cattle feces. *Vet Microbiol* 2012;156:381-388.

Painter JA, Hoekstra RM, Ayers T, Tauxe RV, Braden CR, Angula FJ, Griffin PM. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. *Emerg Infect Dis* 2013;19:407-415.

Paton JC and Paton AW. Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infection. *Clin Microbiol Rev* 1998:11:450–479.

Peterson RE, Klopfenstein TJ, Erickson GE, Folmer J, Hinkley S, Moxley RA, and Smith DR. Effect of *Lactobacillus acidophilus* Strain NP51 on *Escherichia coli* O157:H7 fecal shedding and finishing performance in beef feedlot cattle. *J Food Prot* 2007;70:287–291.

Possé B, De Zutter L, Heyndrickx M, Herman L. Novel differential and confirmation plating media for Shiga toxin-producing *Escherichia coli* serotypes O26, O103, O111, O145 and sorbitol-positive and –negative O157. *FEMS Microbiol Lett* 2008;282:124-131.

Potter AA, Klashinsky S, Li Y, Frey E, Townsend H , et al. Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. *Vaccine* 2004;22:362-69.

Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg Infect Dis* 2005;11:603-609.

Raya RR, Varey P, Oot RA, Dyen MR, Callaway TR, Edrington TS, Kutter EM, and Brabban AD. Isolation and characterization of a new T-even bacteriophage, CEV1, and determination of its potential to reduce *Escherichia coli* O157:H7 levels in sheep. *Appl Environ Microbiol* 2006;72:6405-6410.

Reinstein S, Fox JT, Shi X, Alam MJ, Renter D G, Nagaraja TG. Prevalence of *Escherichia coli* O157:H7 in organically and naturally raised beef cattle. *Appl Environ Microbiol* 2009;75:5421–23

Renter DG, Sargeant JM, Hygnstrom SE, Hoffman JD and Gillespie JR. *Escherichia coli* O157:H7 in free-ranging deer in Nebraska. *J Wildlife Dis* 2001;37:755–760.

Renter DG, Morris JG, Sargeant JM, Hungerford LL, Berezowski J, NGO T, Williams K, and Acheson DWK. Prevalence, risk factors, O serogroups, and virulence profiles of Shiga toxin-producing bacteria from cattle production environments. *J Food Prot* 2005;68:1556-1565.

Renter DG, Bohaychuk V, Van Donkersgoed J, King R. Presence of non-O157 Shiga toxin-producing *Escherichia coli* in feces from feedlot cattle in Alberta and absence on corresponding beef carcasses. *Can J Vet Res* 2007;71:230–235.

Renter DG, Smith DR, King R, Stilborn R, Berg J, Berezowski J and McFall M. Detection and determinants of *Escherichia coli* O157:H7 in Alberta feedlot pens immediately prior to slaughter. *Can J Vet Res* 2008;72:217-227.

Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, and Hargrett NT. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N Engl J Med* 1983;308:681.

Rodrigues MC, Cooke RF, Marques RS, Cappellozza BI, Arispe SA, Keisler DH, Bohnert DW. Effects of vaccination against respiratory pathogens on feed intake, metabolic, and inflammatory responses in beef heifers. *J Ani Sci*. 2015;93(9):4443-52.

Rozema EA, Stephens TP, Bach SJ, Okine EK, Johnson RP, Stanford K, and McAllister TA. Oral and rectal administration of bacteriophages for control of *Escherichia coli* O157:H7 in feedlot cattle. *J Food Prot* 2009;72:241-250.

Sargeant, JM, Sanderson M., Griffin DD, and Smith RA. Factors associated with the presence of *Escherichia coli* O157 in feedlot-cattle water and feed in the Midwestern USA. *Prev Vet Med* 2004;66:207-237.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis* 2011;17:7-15.

Schrezenmeir J and De Vrese ME. Probiotics, prebiotics, and synbiotics Approaching a definition. *Am J Clin Nutr* 2001;73:354-361.

Sheng H, Knecht HJ, Kudva IT, and Hovde CJ. Application of bacteriophages to control intestinal *Escherichia coli* O157:H7 levels in ruminants. *Appl Environ Microbiol* 2006;72:5359-5366.

Sillankorva SM, Oliveira H, and Azeredo J. Bacteriophages and Their Role in Food Safety. *Int J Micro* 2012. doi:10.1155/2012/863945

Smith DR. Cattle Production Systems: Ecology of Existing and Emerging *Escherichia coli* Types Related to Foodborne Illness. *Annual Review of Animal Biosciences*, 2014;2:445-468.

Smith D, Blackford M, Younts S, Moxley R, Gray J, Hungerford L, Milton T and Klopfenstein T. Ecological relationship between the prevalence of cattle shedding *Escherichia coli* O157:H7 and characteristics of the cattle or conditions of the feedlot pens. *J Food Prot* 2001;64:1899-1903.

Smith DR, Moxley RA, Clowser SL, Folmer JD, Hinkley S, Erickson GE, Klopfenstein TJ. Use of rope devices to describe and explain the feedlot ecology of *Escherichia coli* O157:H7 by time and place. *Foodborne Pathog Dis* 2005;2:50-60.

Smith DR, Moxley R A, Peterson RE, Klopfenstein TJ, Erickson G E, et al. A two-dose regimen of a vaccine against type III secreted proteins reduced *Escherichia coli* O157:H7 colonization of the terminal rectum in beef cattle in commercial feedlots. *Foodborne Pathog Dis* 2009;6:155-161

Snedeker KG, Campbell M, Sargeant JM. A systematic review of vaccinations to reduce the shedding of *Escherichia coli* O157 in the feces of domestic ruminants. *Zoonoses Public Health* 2011 Jun;59(2):126-38.

Stanford K, McAllister TA, Niu YD, Stephens TP, Mazzocco A, Waddell TE, and Johnson RP. Oral Delivery Systems for Encapsulated Bacteriophages Targeted *Escherichia coli* O157: H7 in Feedlot Cattle. *J Food Prot* 2010;73:1304-1312.

Stephens TP, McAllister TA and Stanford K. Perineal swabs reveal effect of super shedders on the transmission of *Escherichia coli* O157:H7 in commercial feedlots. *J Anim Sci* 2009; 87:4151-4160.

Stromberg ZR, Baumann NW, Lewis GL, Sevart NJ, Cernicchiaro N, Renter DG, Marx DB, Phebus RK, Moxley RA. Prevalence of Enterohemorrhagic *Escherichia coli* O26, O45, O103, O111, O121, O145, and O157 on Hides and Preintervention Carcass Surfaces of Feedlot Cattle at Harvest. *Foodborne Path Dis* 2015;12(7):631-8.

Tanji Y, Shimada T, Fukudomi H, Miyanaga K, Nakai Y, and Unno H. Therapeutic use of phage cocktail for controlling *Escherichia coli* O157:H7 in gastrointestinal tract of mice. *J Biosci Bioeng* 2005;100:280-287.

Thomson DU, Loneragan GH, Thornton AB, Lechtenberg KF, Emery DA, Burkhardt DT, and Nagaraja TG. Use of a siderophore receptor and porin proteins based vaccine to control the burden of *Escherichia coli* O157:H7 in feedlot cattle. *Foodborne Pathog Dis* 2009;6:871-877.

Thornton AB, Thomson DU, Loneragan GH, Fox JT, Burkhardt DT, Emery DA, et al. Effects of a siderophore receptor and porin proteins based vaccination on fecal shedding of *Escherichia coli* O157:H7 in experimentally inoculated cattle. *J Food Prot* 2009;72(4):866-9.

Tkalcic S, Zhao T, Harmon BG, Doyle MP, Brown CA, and Zhao P. Fecal shedding of enterohemorrhagic *Escherichia coli* in weaned calves following treatment with probiotic *Escherichia coli*. *J Food Prot* 2003;66:1184-1189.

U.S. Department of Agriculture, Economic Research Service (USDA-ERS). Foodborne illness cost calculator: STEC O157:H7 [updated 2011 June 24; cited 2011 Nov 15]. Available from: http://www.ers.usda.gov/Data/FoodBorneIllness/ecoli_intro.asp

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). Pre-harvest management controls and intervention options for reducing *Escherichia coli* O157:H7 shedding in cattle. 2014. Available at: http://www.fsis.usda.gov/wps/wcm/connect/d3fdd61d-924a-4dd9-a363-a45b42b83163/2012-0038-A.pdf?MOD=AJPERES , accessed March 01, 2016.

Van Baale MJ, Sargeant JM, Gnad DP, DeBey BM, Lechtenberg KF, and Nagaraja TG. Effect of forage or grain diets with or without monensin on ruminal persistence and fecal *Escherichia coli* O157:H7 in cattle. *Appl Environ Microbiol* 2004;70:5336-5342

Van Donkersgoed J, Berg J, Potter A, Hancock D, Besser Rice D, LeJeune J and Klashinsky S. Environmental sources and transmission of *Escherichia coli* O157 in feedlot cattle. *Can Vet J* 2001;42:714-720.

Varela NP, Dick P, Wilson J. 2012. Assessing the existing information on the efficacy of bovine vaccination against *Escherichia coli* O157:H7—a systematic review and meta-analysis. *Zoonoses Public Health* 2012;60:253-68

Vasconcelos JT, Elam NA, Brashears MM, and Galyean ML. Effects of increasing dose of live cultures of *Lactobacillus acidophilus* (Strain NP 51) combined with a single dose of *Propionibacterium freudenreichii* (Strain NP 24) on performance and carcass characteristics of finishing beef steers. *J Anim Sci* 2008;86:756–762

Voisinet BD, Grandin T, O'Connor SF, Tatum JD, and Deesing MJ. Bos indicus cross feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. *Meat Sci* 1997;46:367–377.

Wells JG, Davis BR, Wachsmuth IK, Riley LW, Remis RS, Sokolow R and Morris GK. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *J Clin Microbiol* 1983;18:512-520.

Wileman BW, Thomson DU, Olson KC, Jaeger JR, Pacheco LA, Bolte J, et al. *Escherichia coli* O157:H7 shedding in vaccinated beef calves born to cows vaccinated prepartum with *Escherichia coli* O157:H7 SRP vaccine. *J Food Prot* 2011 Oct;74(10):1599-604.

Willard MD, Simpson RB, Cohen ND, and Clancy JD. Effects of dietary fructooligosaccharide on selected bacterial populations in feces of dogs. *Amer J Vet Res* 2000;61:820-825

Wisener LV, Sargeant JM, O'Connor AM, Faires MC, Glass-Kaastra SK. The Use of Direct-Fed Microbials to Reduce Shedding of *Escherichia coli* O157 in Beef Cattle: A Systematic Review and Meta-analysis. *Zoonoses and public health* 2015;62(2):75-89.

Withee J, Williams M, Disney T, Schlosser W, Bauer N and Ebel E. Streamlined analysis for evaluating the use of preharvest interventions intended to prevent *Escherichia coli* O157:H7 illness in humans. *Foodborne Pathog Dis* 2009;6:817-825.

Woerner DR, Ransom JR, Sofos JN, Dewell GA, Smith GC, Salman MD and Belk KE. Determining the prevalence of *Escherichia coli* O157 in cattle and beef from the feedlot to the cooler. *J Food Prot* 2006;69:2824-2827.

Younts-Dahl SM, Galyean ML, Loneragan GH, Elam NA, Brashears MM. Dietary supplementation with Lactobacillus- and Propionibacterium-based direct-fed microbials and prevalence of *Escherichia coli* O157 in beef feedlot cattle and on hides at harvest. *J Food Prot* 2004 May;67(5):889-93.

Zhang J, Kraft BL, Pan Y, Wall SK, Saez AC, and Ebner PD. Development of an anti-*Salmonella* phage cocktail with increased host range. *Foodborne Pathog Dis* 2010;7:1415-1419.

Zhao T, Tkalcic S, Doyle MP, Harmon BG, Brown CA, and Zhao P. Pathogenicity of enterohemorrhagic *Escherichia coli* in neonatal calves and evaluation of fecal shedding by treatment with probiotic *Escherichia coli*. *J Food Prot* 2003;66:924–930

Zopf D and Roth S. Oligosaccharide anti-infective agents. *Lancet* 1996;347:1017-1021.

Chapter 2 - Efficacy of a vaccine and a direct-fed microbial against fecal shedding of *Escherichia coli* O157:H7 in a randomized pen-level field trial of commercial feedlot cattle

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Abstract

Our primary objective was to determine the efficacy of a siderophore receptor and porin proteins-based vaccine (VAC) and a *Lactobacillus acidophilus*-based direct-fed microbial (DFM) against fecal shedding of *E. coli* O157:H7 in commercial feedlot cattle fed a corn grain-based diet with 25% distiller's grains. Cattle projected to be on a finishing diet during the summer were randomly allocated into 40 study pens within ten blocks based on allocation dates. Blocks were complete; each of the four pens within a block was randomly assigned one treatment: control, VAC, DFM, or VAC+DFM. The DFM was fed (10^6 CFU/animal/day of *Lactobacillus*) throughout the study periods (84 - 88 days) and cattle were vaccinated at enrollment and again three weeks later. Fresh fecal samples (30/pen) from pen floors were

collected weekly for four consecutive weeks (study days 52 to 77). Two concurrent culture procedures were used to enable estimates of *E. coli* O157:H7 shedding prevalence and prevalence of high shedders. From 4,800 total samples, 1,522 (31.7%) were positive for *E. coli* O157:H7 and 169 (3.5%) were considered high shedders. Pen-level linear mixed models were used for data analyses. There were no significant interactions among treatments and time of sampling. However, vaccinated pens had lower ($P < 0.01$) overall prevalence of *E. coli* O157:H7 (model-adjusted mean ± SEM = 17.4 ± 3.95%) and lower ($P < 0.01$) prevalence of high shedders (0.95 ±0.26%) than unvaccinated pens (37.0±6.32% and 4.19±0.81%, respectively). There was no evidence of a DFM effect on either measure of *E. coli* O157:H7 shedding. Results indicate that a two-dose regimen of the vaccine significantly reduces fecal prevalence of *E. coli* O157:H7 (vaccine efficacy of 53.0%) and prevalence of *E. coli* O157:H7 high shedders (vaccine efficacy of 77.3%) in commercial feedlot cattle reared in the summer on a finishing diet with 25% distiller's grains.

Abbreviations

ADG – average (mean) daily weight gain
DFM – direct fed microbial
DG – distiller's grains
F:G – ratio of feed weight to gained weight of cattle
IMS – immunomagnetic separation
SRP – siderophore receptor and porin proteins-based vaccine
VAC – vaccinated group

Introduction

Escherichia coli O157:H7 is an important cause of food-borne illness [1]. In addition to public health concerns, the economic impact of *E. coli* O157:H7 has been severe [2]. Pre-harvest interventions that reduce fecal shedding of these bacteria in cattle have the potential to enhance food safety and reduce economic impacts of *E. coli* O157:H7. It has been proposed that beef processors extend their food safety plans to the pre-harvest phase by purchasing cattle from producers who implement *E. coli* O157:H7 control programs [3]. However, most pre-harvest interventions have not been validated for the diverse production settings in the beef industry [3-5]. Both prevalence and concentration of *E. coli* O157:H7 in cattle feces are associated with beef contamination; occasionally cattle shed *E. coli* O157:H7 at high concentrations (e.g., > 10^4 CFU/g of feces; hereafter "high shedders") [6-8]. Although few factors associated with shedding have been consistently observed, cattle shed more *E. coli* O157:H7 in summer than winter months [4,9,10]. Dietary components also influence fecal shedding [4,9]. For instance, diets containing distillers grains (DG), a co-product of the ethanol industry, can increase *E. coli* O157:H7 fecal shedding [9,11,12]. Since efficacy of pre-harvest interventions is most important during periods of high fecal shedding [13], data from studies of cattle fed DG-supplemented diets in the summer months are important.

Two interventions that are commercially available in the United States and have demonstrated efficacy for reducing *E. coli* O157:H7 shedding in cattle are a siderophore receptor and porin (SRP) proteins-based vaccine and a *Lactobacillus acidophilus*-based direct-fed microbial (DFM) [5,14]. This DFM includes a strain of *Lactobacillus acidophilus* (NP51) shown to have inhibitory effects on *E. coli* O157:H7 [10]. The vaccine uses SRP proteins as antigens so immunized animals produce anti-SRP antibodies that bind to outer membrane

proteins of bacterial cells and block iron transport [15]. Although literature indicates potential benefits of these products, there is a need for additional data on efficacy in commercial settings [5,14]. Further, there are no data on concurrent use of these interventions. Therefore, our primary objective was to determine the efficacy of intervention programs including the SRP vaccine, the DFM, or both products against fecal shedding of *E. coli* O157:H7 in pens of commercial feedlot cattle fed a DG-supplemented finishing diet during the summer. A secondary objective was to evaluate impacts of intervention programs on cattle health and performance outcomes as compared to control cattle reared using standard practices.

Materials and methods

A commercial feedlot in Nebraska, USA was identified based on criteria that included: capacity to fill 40 pens with cattle on a finishing diet during summer, use of a finishing diet that included $\geq$ 25% DG, ability to feed the DFM, willingness to vaccinate cattle according to protocol, and ability to perform research. Individual cattle were eligible for inclusion if projected to be on a finishing diet during summer; with this feedlot's management system, cattle had to be enrolled approximately 100 days prior to harvest of the first subset. Following a brief transition period, cattle were fed a finishing diet which included (dry matter basis): 46.4% high moisture corn, 25.0% wet DG, 17.0% corn gluten, 7.1% silage, 2.5% steep, and 2.0% micro/minerals mix (including 280 mg of monensin/animal/day and 90 mg of tylosin/animal/day (Elanco Animal Health, Greenfield, IN, USA)). The feedlot's standard operating procedures were followed for cattle care and management; sprinklers were used as needed to reduce heat stress risks. Kansas State University (KSU) Institutional Animal Care and Use Committee approved the study (#2723).

The study was designed as a randomized complete block with a 2 x 2 factorial treatment structure. *A priori* sample size estimates were generated by data simulation and power calculations; assumptions included: 40% mean control group prevalence of *E. coli* O157:H7 [16], 25% mean prevalence in pens receiving an intervention, and no interaction among interventions. Forty pens (10/treatment) and 120 samples (30/week for four weeks) per pen were considered sufficient for 80% statistical power to detect expected treatment differences with a 5% Type 1 error. Individual cattle were randomly allocated to 40 pens grouped in 10 blocks (defined based on allocation dates; March 31 through May 14, 2011). Within block, one pen each was randomly allocated to one treatment: control, administered vaccine (VAC), fed DFM (DFM), or both VAC and DFM (VAC + DFM). Cattle in VAC and VAC+DFM groups were administered a 2 mL dose of the vaccine subcutaneously (SC, 1 ½ inch needle) in the left lower neck on study day 0 and again three weeks later (*E. coli* SRP® vaccine, Pfizer Animal Health, New York, NY, USA; lot # 840-0006, expiration August 19, 2011). Cattle allocated to DFM or control groups never received a placebo and were not re-handled three weeks following enrollment. The DFM, labeled for 10^6 CFU/animal/day of *Lactobacillus acidophilus* and 10^9 CFU/animal/day of *Propionibacterium freudenreichii*, was fed throughout the study periods (Bovamine®, Nutrition Physiology Corp., Guymon, OK, USA). On study day 0, all cattle received a herpes virus vaccine (Pyramid IBR, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO, USA; 2 mL, SC) and a growth promoting implant (Synovex Choice, Pfizer Animal Health, New York, NY, USA; SC in the left ear).

The feedlot's computer system randomly allocated animals to treatment groups as they were handled on study day 0. For each block, four contiguous pens within the feedlot were identified and pen locations for treatment groups within blocks were then randomly allocated

using the computer's randomization algorithm. The primary study outcome was within-pen *E. coli* O157:H7 prevalence, whereas within-pen prevalence of high shedding animals was considered a secondary outcome. Thus, each sample was classified twice (independently) as positive or negative to: 1) a culture procedure including immunomagnetic bead separation (IMS) to assess fecal shedding, and 2) a direct plating culture procedure to assess high shedding. Laboratory personnel were blinded to treatment: samples were tracked only by sequential numbers. Cumulative (study period) risks of cattle mortality and culling, and cattle performance measures also were considered secondary outcomes and were collected by feedlot staff blinded to treatment group; personnel administering treatments did not collect health or performance data. Average (mean) daily weight gain (ADG) and feed conversions (F:G; ratio of feed weight to gained weight of cattle) were calculated as:

ADG = Total Weight Gain of Cattle (out-weight for cattle finishing the trial + out-weight of cattle culled + out-weight of dead cattle – total enrollment weight) ÷ Total Cattle Days

F:G = Total Dry Matter Weight of Feed ÷ Total Weight Gain (as defined above)

Feedlot personnel performed daily health monitoring following standardized procedures. Animals were weighed individually at the beginning and end of the study. Fresh fecal samples (30/pen) from animals observed defecating were collected from separate pats in multiple areas throughout the pen. Care was taken to avoid ground contamination. Pens were sampled weekly for four consecutive weeks prior to study end-dates for each block. Samples (approximately 30 g) were placed in sterile bags, stored in coolers, and transported to KSU for refrigeration (4°C) until the following morning. Samples were cultured for *E. coli* O157:H7 using IMS and direct plating methods previously described [7,8]. Confirmation included a multiplex PCR for

identifying the *rfb*E (O157), *eae* (intimin), *stx*1 (Shiga toxin 1), *stx*2 (Shiga toxin 2), *hly*A (hemolysin), and *fli*C (H7) genes [17].

Pen-level general and generalized linear mixed models (LMM and GLMM, respectively) were used to assess potential treatment effects. For response variables recorded as pen-level proportions, data were fit using a GLMM with a binomial distribution and a logit link. Prevalence outcomes were the proportion of samples positive of the total samples collected within the pen at each sampling. Mortality and culling risks were proportions based on the number of animals that died or were culled, respectively, during the study period out of the total number of animals enrolled within the pen. Data on ADG and F:G were modeled using LMM that assume a Gaussian distribution. For all models, random effects were fitted to recognize block as the clustering factor and pen as the experimental unit for treatment. For *E. coli* data, additional random effects were used to account for pen-specific repeated measures over time. Independent variables included treatments (VAC, DFM, VAC x DFM interaction), and for *E. coli* data, effects of time and time-by-treatment interaction. Model diagnostics were based on studentized residuals (LMM) and functions of the Pearson χ^2 statistic (GLMM). P values < 0.05 were considered significant. Model-adjusted means (lsmeans back transformed to original scale) and SE were reported, and used to estimate vaccine efficacy using standard formula [18].

Results

Study pens were filled with 17,148 steers. Pen sizes ranged between 398 and 464 steers (mean = 430.0). Mean weight at enrollment was 378.4 kg with no significant difference among treatment groups. Projected finishing days were re-assessed by feedlot personnel during the study and determined to be 14 days earlier than expected. Resulting end-dates for study blocks

ranged between June 20 and August 3, 2011; thus, days on study ranged between 84 and 88 (mean = 86.6 days) across blocks. Sampling began approximately five weeks prior to projected study-end for each block, resulting in samples collected (for four consecutive weeks) between study days 52 to 56 (week one), 59 to 63 (week two), 66 to 70 (week three), and 73 to 77 (week four).

From 4,800 total samples, 1,522 (31.7%) were positive for *E. coli* O157:H7 and 169 (3.5%) were considered high shedders; percentages by week of sampling are provided in Figure 1. Isolates considered *E. coli* O157:H7 were positive for the *rfb*E (100%), *eae* (99.8%), *stx*1 (66.2%), *stx*2 (99.5%), *hly*A (99.7%), and *fli*C (99.8%) genes. *Escherichia coli* O157:H7 were isolated at least once from all pens (100%) and 34 pens (85%) had at least one high shedder. Within pens, unadjusted cumulative prevalence of shedding (across sampling times) ranged between 1.7% and 66.7% and high shedder prevalence ranged between 0% and 12.5%.

Analysis of within-pen prevalence of *E. coli* O157:H7 shedding data indicated no significant two- or three-way interactions among treatments and time of sampling. There also was no significant main effect of DFM (Table 1). However, a main effect of VAC was apparent, such that VAC decreased prevalence of fecal shedding (Table 2). Figure 2 illustrates estimated efficacy (53.0%) of vaccination for reducing fecal prevalence of *E. coli* O157:H7 and means for the contrast between vaccinated and non-vaccinated pens ($P < 0.01$). A main effect of sampling time on fecal shedding was also apparent ($P = 0.02$), whereby mean prevalence on sampling week two differed from prevalence on week four; no other week-to-week differences were detected. Means (SEM) were 24.6% (5.07), 20.7% (4.53), 27.2% (5.39) and 32.4% (5.92) for sampling weeks one through four, respectively.

Regarding high shedder prevalence, results indicated no significant two- or three-way interactions among treatments and time of sampling, and no significant main effects of DFM (Table 1) or sampling week. However, a significant effect of VAC was identified, whereby vaccination decreased the prevalence of high shedders (Table 2). Figure 2 illustrates the difference in means for vaccinated and non-vaccinated pens ($P < 0.01$) and the estimated vaccine efficacy (77.3%) for reducing prevalence of *E. coli* O157:H7 high shedders.

Effects of treatment were apparent on both ADG and F:G, but there were no significant interactions between VAC and DFM. For ADG, there was no significant DFM effect (Table 1), but the VAC effect was significant (Table 2). For F:G, effects of DFM (Table 1) and VAC (Table 2) were both statistically significant. There was no evidence of VAC and DFM interactions, main effect of DFM (Table 1), or main effect of VAC (Table 2) on either mortality or culling risks.

Discussion

An important finding of this study is that two doses of the SRP® vaccine applied in a commercial feedlot reduced *E. coli* O157:H7 shedding by more than 50% and reduced high shedders by more than 75%. These results from a cattle population with relatively high levels of *E. coli* O157:H7 have important practical implications since efficacy of pre-harvest interventions is most important when prevalence is high [13]. Another important finding is that the commercial DFM (Bovamine®) had no effect on *E. coli* O157:H7 fecal shedding. These results also have practical significance since end-users of pre-harvest interventions may wonder whether these commercially available products – the SRP® vaccine and the Bovamine® DFM – are equally efficacious. Results also indicate that DFM-fed cattle may have improved performance

whereas cattle in vaccinated pens had relatively poorer performance. Performance effects need to be further quantified since cattle performance affects beef production costs, and the adoption of pre-harvest control programs will be affected by all costs associated with implementation.

Study cattle were fed a diet with 25% DG during the summer; thus, the interventions were tested in a situation when fecal shedding of *E. coli* O157:H7 was expected to be high. Feeding DG to cattle can increase fecal shedding of *E. coli* O157:H7 approximately two- to three-fold [9,11,12]. Seasonal effects associated with *E. coli* O157:H7 shedding (higher in the summer) also has been well documented; study data (Figure 1) demonstrate a well-described seasonal pattern [4,16,19]. The sample-level prevalence for high shedders (3.5%) and overall fecal shedding (31.7%) were relatively high, but numerically similar to estimates from comparable populations. Reports on summer-harvested cattle included prevalence estimates for high shedders of 3.7% [7] and 3.3% [8]. Recent estimates of overall fecal prevalence in summer-fed feedlot cattle have ranged between 37% and 10%, but within-pen prevalence is highly variable [16,20,21]. Thus, the range in cumulative within-pen prevalence (1.7% to 66.7%) reported in this current study is consistent with previous reports. While diagnostic sensitivity and specificity of culture methods used in this study are not perfect for identifying fecal shedding and high shedding [22], any misclassification would be expected to be non-differential with respect to treatments. Further, these methods have previously provided useful data on fecal shedding relative to important food safety parameters such as *E. coli* O157:H7 carcass and hide prevalence [7,8]. Gene profiles of isolates recovered in this study are similar to those previously reported; indicating that the *E. coli* O157:H7 isolates have potential for human virulence [23,24].

This is the first published study demonstrating efficacy of a 2-dose regimen of the commercially available SRP® vaccine for reducing both the prevalence of *E. coli* O157:H7 shedding and high shedding in a large-pen commercial feedlot. Although vaccine efficacy has been demonstrated previously [15,25,26], key features differ between previous studies and the study reported here. The SRP® vaccine was first shown to reduce fecal shedding in young calves orally inoculated with *E. coli* O157:H7 [28]. Cattle that were naturally shedding *E. coli* O157:H7 in a research feedlot were used to show that 3 mL doses of vaccine reduced fecal shedding; a dose-response trend was also observed [25]. In one feedlot study, a 2-dose regimen of the vaccine reduced fecal prevalence, and in another study, a 3-dose regimen reduced fecal concentration [26]. A cow-calf study found no significant vaccine effects, but cattle were vaccinated at much different production phases [27]. In addition to differing study designs, vaccine dosages, or study populations, this commercial feedlot study reported here utilized very large pens while others used smaller pens ($\leq$ 70 animals/pen) [15,25,26]. A recent systematic review indicating efficacy of the SRP® vaccine suggested that further studies in commercial settings were needed [14].

No evidence for any DFM (Bovamine®) effect on *E. coli* O157:H7 fecal shedding was observed, contradicting some results of empirical studies and a systematic review indicating efficacy of this *Lactobacillus acidophilus* strain (NP51) [5,10,28-32]. Possibly larger pen sizes and a lower dose of product in the current study compared to previous studies could explain seemingly contradictory results. This commercial feedlot study utilized large pens while many other studies used much smaller ($\leq$ 10 animals/pen) pens [28-32]. Further, efficacy of this DFM for reducing *E. coli* O157:H7 may be improved at a higher dose [10,29,32]. The commercial low-dose Bovamine® product (10^6 CFU/head/d of *Lactobacillus*) was utilized in the current study

because of the perception that this product can reduce fecal shedding and also improve cattle performance. Indeed, there are important practical implications if a pre-harvest control program could reduce *E. coli* O157:H7 fecal shedding while improving animal performance.

A meta-analysis demonstrated that this DFM can improve feedlot cattle performance [33]; reported summary effects were similar to effects reported here. However, results indicating lower weight gain per day and less efficient feed conversion for vaccinated versus unvaccinated pens are novel. Previous feedlot studies with this vaccine did not detect significant differences in cattle performance [15]. However, in previous studies both vaccine and control groups were handled on re-vaccination days and controls were given a placebo. Further, previous studies had much smaller sample sizes to detect differences with half as many pens (20) and much fewer animals overall (< 1,300) than the current study (40 and > 17,000, respectively). For the current study, controls were not re-handled or given a placebo during vaccinations times because the feedlot would not normally do these procedures; the intent of assessing performance was to evaluate the total program (not just the vaccine) as compared to normal production practices. Since production costs must be considered for implementation of a vaccination program, further research specifically designed for evaluating performance effects may be warranted.

Conclusions

We found the overall fecal prevalence of *E. coli* O157:H7 and prevalence of high shedders in this large commercial feedlot population were relatively high as expected for summer-fed cattle supplemented with distiller's grains. We conclude that this DFM, Bovamine® (labeled for 10^6 CFU/head/d of *Lactobacillus*), administered alone or in combination with the SRP® vaccine, does not significantly affect fecal shedding. However, the SRP® vaccine

significantly reduces fecal prevalence of *E. coli* O157:H7 and prevalence of high shedders, and therefore may be an effective intervention for *E. coli* O157:H7 control in commercial feedlots.

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References

[1] Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. Emerg Infect Dis 2005 Apr;11(4):603-9.

[2] U.S. Department of Agriculture, Economic Research Service. Foodborne illness cost calculator: STEC O157:H7 [updated 2011 June 24; cited 2011 Nov 15]; [p.1]. Available from: http://www.ers.usda.gov/Data/FoodBorneIllness/ecoli_intro.asp

[3] U.S. Department of Agriculture, Food Safety and Inspection Service. Pre-harvest management controls and intervention options for reducing *Escherichia coli* O157:H7 shedding in cattle. 2010 May [cited 2011 Nov 15]; [p. 28]. Available from: http://www.fsis.usda.gov/pdf/reducing_ecoli_shedding_in_cattle_0510.pdf

[4] Renter DG, Sargeant JM. Enterohemorrhagic *Escherichia coli* O157: epidemiology and ecology in bovine production environments. Animal Health Research Reviews 2002;3(02):83-94.

[5] Sargeant JM, Amezcua MR, Rajic A, Waddell L. Pre-harvest interventions to reduce the shedding of *E. coli* O157 in the feces of weaned domestic ruminants: A systematic review. Zoonoses Public Health 2007;54(6-7):260-77.

[6] Elder RO, Keen JE, Siragusa GR, Barkocy-Gallagher GA, Koohmaraie M, Laegreid WW. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc Natl Acad Sci USA 2000 Mar 28;97(7): 2999-03.

[7] Fox JT, Renter DG, Sanderson MW, Nutsch AL, Shi X, Nagaraja TG. Associations between the presence and magnitude of *Escherichia coli* O157 in feces at harvest and contamination of preintervention beef carcasses. J Food Prot 2008;71(9):1761-7.

[8] Jacob ME, Renter DG, Nagaraja TG. Animal- and truckload-level associations between *Escherichia coli* O157:H7 in feces and on hides at harvest and contamination of preevisceration beef carcasses. J Food Prot 2010;73(6):1030-7.

[9] Jacob ME, Callaway TR, Nagaraja TG. Dietary interactions and interventions affecting *Escherichia coli* O157 colonization and shedding in cattle. Foodborne Pathog Dis 2009 Sep;6(7):785-92.

[10] Loneragan GH, Brashears MM. Pre-harvest interventions to reduce carriage of *E. coli* O157 by harvest-ready feedlot cattle. Meat Science 2005;71(1):72-8.

[11] Jacob ME, Paddock ZD, Renter DG, Lechtenberg KF, Nagaraja TG. Inclusion of dried or wet distillers' grains at different levels in diets of feedlot cattle affects fecal shedding of *Escherichia coli* O157:H7. Appl Environ Microbiol 2010 Nov;76(21):7238-42.

[12] Wells JE, Shackelford SD, Berry ED, Kalchayanand N, Guerini MN, Varel VH, et al. Prevalence and level of *Escherichia coli* O157:H7 in feces and on hides of feedlot steers fed diets with or without wet distillers grains with solubles. J Food Prot 2009 Aug;72(8):1624-33.

[13] Dodd CC, Sanderson MW, Jacob ME, Renter DG. Modeling preharvest and harvest interventions for *Escherichia coli* O157 contamination of beef cattle carcasses. J Food Prot 2011;74(9):1422-33.

[14] Snedeker KG, Campbell M, Sargeant JM. A systematic review of vaccinations to reduce the shedding of *Escherichia coli* O157 in the feces of domestic ruminants. Zoonoses Public Health 2011 Jun;59(2):126-38.

[15] Thomson DU, Loneragan GH, Thornton AB, Lechtenberg KF, Emery DA, Burkhardt DT, et al. Use of a siderophore receptor and porin proteins-based vaccine to control the burden of *Escherichia coli* O157:H7 in feedlot cattle. Foodborne Pathog Dis 2009; 6(7):871-7.

[16] Renter DG, Smith DR, King R, Stilborn R, Berg J, Berezowski J, et al. Detection and determinants of *Escherichia coli* O157:H7 in Alberta feedlot pens immediately prior to slaughter. Can J Vet Res 2008 Apr;72(3):217-27.

[17] Bai J, Shi X, Nagaraja TG. A multiplex PCR procedure for the detection of six major virulence genes in *Escherichia coli* O157:H7. J Microbiol Methods 2010 Jul;82(1):85-9.

18] Dohoo IR, Martin SW, Stryhn H, editors. Measures of association. In: Veterinary Epidemiologic Research. Charlottetown: AVC, Inc., PEI; 2003:121-38.

[19] Williams MS, Withee JL, Ebel ED, Bauer NE, Jr., Schlosser WD, Disney WT, et al. Determining relationships between the seasonal occurrence of *Escherichia coli* O157:H7 in live cattle, ground beef, and humans. Foodborne Pathog Dis 2010 Oct;7(10):1247-54.

[20] Smith D, Blackford M, Younts S, Moxley R, Gray J, Hungerford L, 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 Dec;64(12):1899-903.

[21] Dewell GA, Ransom JR, Dewell RD, McCurdy K, Gardner IA, Hill AE, et al. Prevalence of and risk factors for *Escherichia coli* O157 in market-ready beef cattle from 12 U.S. feedlots. Foodborne Pathog Dis 2005 Spring;2(1):70-6.

[22] Fox JT, Renter DG, Sanderson MW, Thomson DU, Lechtenberg KF, Nagaraja TG. Evaluation of culture methods to identify bovine feces with high concentrations of *Escherichia coli* O157. Appl Environ Microbiol 2007 Aug;73(16):5253-60.

[23] Paddock ZD, Walker CE, Drouillard JS, Nagaraja TG. Dietary monensin level, supplemental urea, and ractopamine on fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle. J Anim Sci 2011 Sep;89(9):2829-35.

[24] Renter DG, Sargeant JM, Oberst RD, Samadpour M. Diversity, frequency, and persistence of *Escherichia coli* O157 strains from range cattle environments. Appl Environ Microbiol 2003 Jan;69(1):542-7.

[25] Fox JT, Thomson DU, Drouillard JS, Thornton AB, Burkhardt DT, Emery DA, et al. Efficacy of *Escherichia coli* O157:H7 siderophore receptor/porin proteins–based vaccine in feedlot cattle naturally shedding *E. coli* O157. Foodborne Pathog Dis 2009;6(7):893-9.

[26] Thornton AB, Thomson DU, Loneragan GH, Fox JT, Burkhardt DT, Emery DA, et al. Effects of a siderophore receptor and porin proteins based vaccination on fecal shedding of *Escherichia coli* O157:H7 in experimentally inoculated cattle. J Food Prot 2009;72(4):866-9.

[27] Wileman BW, Thomson DU, Olson KC, Jaeger JR, Pacheco LA, Bolte J, et al. *Escherichia coli* O157:H7 shedding in vaccinated beef calves born to cows vaccinated prepartum with *Escherichia coli* O157:H7 SRP vaccine. J Food Prot 2011 Oct;74(10):1599-604.

[28] Younts-Dahl SM, Galyean ML, Loneragan GH, Elam NA, Brashears MM. Dietary supplementation with Lactobacillus- and Propionibacterium-based direct-fed microbials and prevalence of *Escherichia coli* O157 in beef feedlot cattle and on hides at harvest. J Food Prot 2004 May;67(5):889-93.

[29] Younts-Dahl SM, Osborn GD, Galyean ML, Rivera JD, Loneragan GH, Brashears MM. Reduction of *Escherichia coli* O157 in finishing beef cattle by various doses of Lactobacillus acidophilus in direct-fed microbials. J Food Prot 2005 Jan;68(1):6-10.

[30] Brashears MM, Galyean ML, Loneragan GH, Mann JE, Killinger-Mann K. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given Lactobacillus direct-fed microbials. J Food Prot 2003 May;66(5):748-54.

[31] Stephens TP, Loneragan GH, Chichester LM, Brashears MM. Prevalence and enumeration of *Escherichia coli* O157 in steers receiving various strains of Lactobacillus-based direct-fed microbials. J Food Prot 2007 May;70(5):1252-5.

[32] Stephens TP, Loneragan GH, Karunasena E, Brashears MM. Reduction of *Escherichia coli* O157 and *Salmonella* in feces and on hides of feedlot cattle using various doses of a direct-fed microbial. J Food Prot 2007;70(10):2386-91.

[33] Hanford KJ, Kreikemeier WM, Ware DR. The effect of Bovamine on feedlot performance of finishing cattle: A meta-analysis. Proceedings of the American Dairy Science Association and American Society of Animal Science Conference; 2011 July 10-14; New Orleans, Louisiana. J Anim Sci 2011;89(1): p. 258.

Table 2.1 Model-adjusted[1] means, standard errors (SEM), 95% confidence intervals (CI), and *P* values for comparisons of outcome measures between pens of feedlot cattle that were fed a direct-fed microbial[2] (DFM) containing *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* (n = 20) and pens that were not fed the DFM (n = 20).

Outcome measures	Fed DFM			No DFM			
	Mean	SEM	CI	Mean	SEM	CI	*P* value
Fecal prevalence, %	25.8	5.24	16.6 – 37.9	26.2	5.26	16.9 – 38.3	0.94
High shedder prevalence, %	2.16	0.49	1.36 – 3.42	1.87	0.47	1.12 – 3.11	0.59
Average daily weight gain, kg	1.48	0.02	1.43 – 1.53	1.46	0.02	1.40 – 1.51	0.09
Feed to gain ratio	6.01	0.08	5.84 – 6.17	6.14	0.08	5.99 – 6.31	0.03
Mortality risk, %	1.14	0.11	0.93 – 1.40	1.08	0.11	0.87 – 1.34	0.70
Culling risk, %	0.42	0.15	0.21 – 0.86	0.41	0.14	0.21 – 0.86	0.78

[1]From linear mixed models accounting for allocation of pens within blocks (and repeated measures on pens over time for fecal and high shedder prevalence)

[2]Bovamine® (labeled for 10^6 CFU/animal/day of *Lactobacillus acidophilus* and 10^9 CFU/animal/day of *Propionibacterium freudenreichii*), Nutrition Physiology Corp., Guymon, OK, USA)

Table 2.2 Model-adjusted[1] means, standard errors (SEM), 95% confidence intervals (CI), and *P* values for comparisons of outcome measures between pens of feedlot cattle that were vaccinated twice with a siderophore receptor and porin proteins-based vaccine[2] (n = 20) and pens that were not vaccinated (n = 20).

	Vaccinated			Non-Vaccinated			
Outcome measures	Mean	SEM	CI	Mean	SEM	CI	*P* value
Fecal prevalence, %	17.4	3.95	10.7 – 27.0	37.0	6.32	25.2 – 50.6	< 0.01
High shedder prevalence, %	0.95	0.26	0.54 – 1.67	4.19	0.81	2.82 – 6.20	< 0.01
Average daily weight gain, kg	1.45	0.02	1.39 – 1.50	1.49	0.02	1.44 – 1.54	0.01
Feed to gain ratio	6.14	0.08	5.99 – 6.31	6.01	0.08	5.85 – 6.18	0.04
Mortality risk, %	1.14	0.11	0.93 – 1.40	1.08	0.11	0.87 – 1.34	0.70
Culling risk, %	0.41	0.14	0.20 – 0.83	0.43	0.15	0.21 – 0.87	0.78

[1]From linear mixed models accounting for allocation of pens within blocks (and repeated measures on pens over time for fecal and high shedder prevalence)

[2]*E. coli* SRP® vaccine, Pfizer Animal Health, New York, NY, USA

Figure 2.1 Descriptive data on fecal samples from feedlot cattle that tested positive for *E. coli* O157:H7 shedding and *E. coli* O157:H7 high shedding for each week during the sampling period. Data are summarized across all pens (treatments and blocks) sampled for each week.

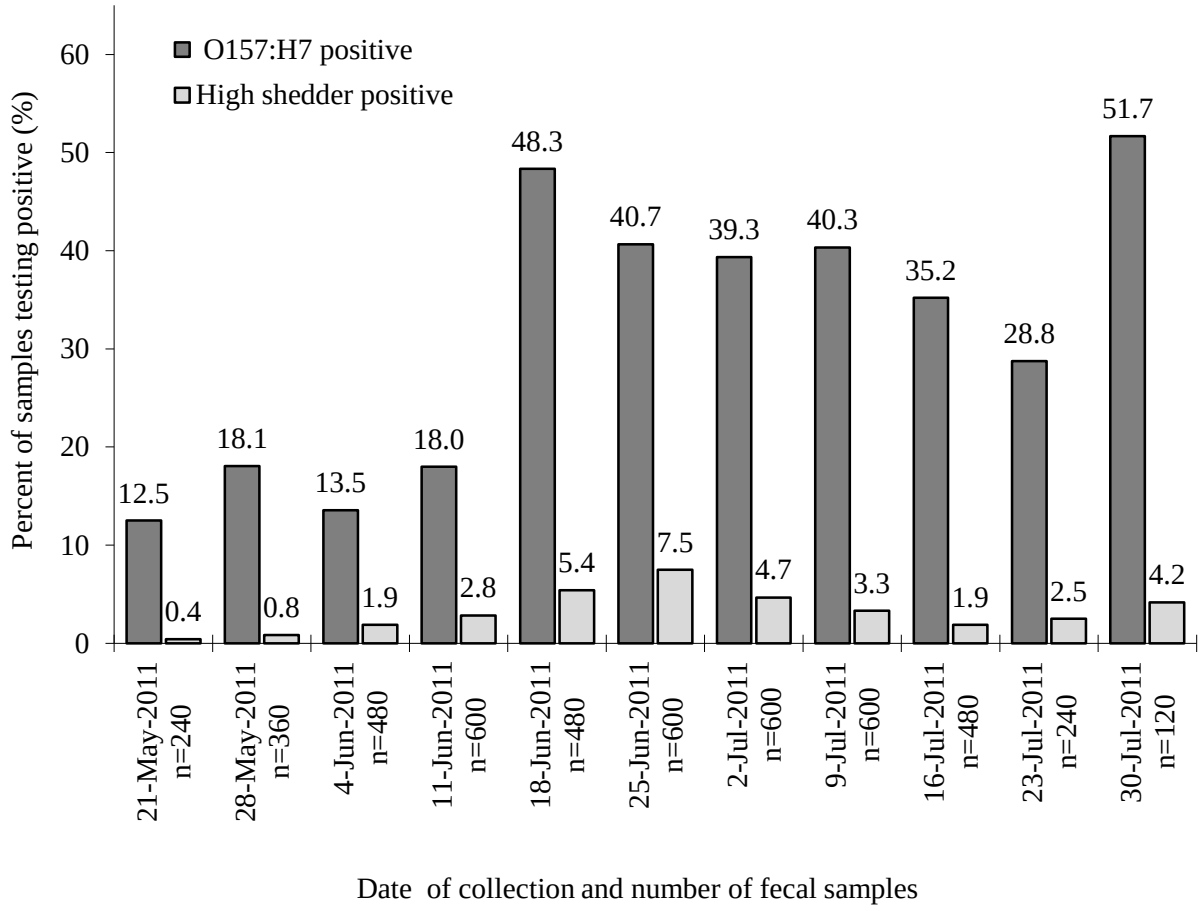
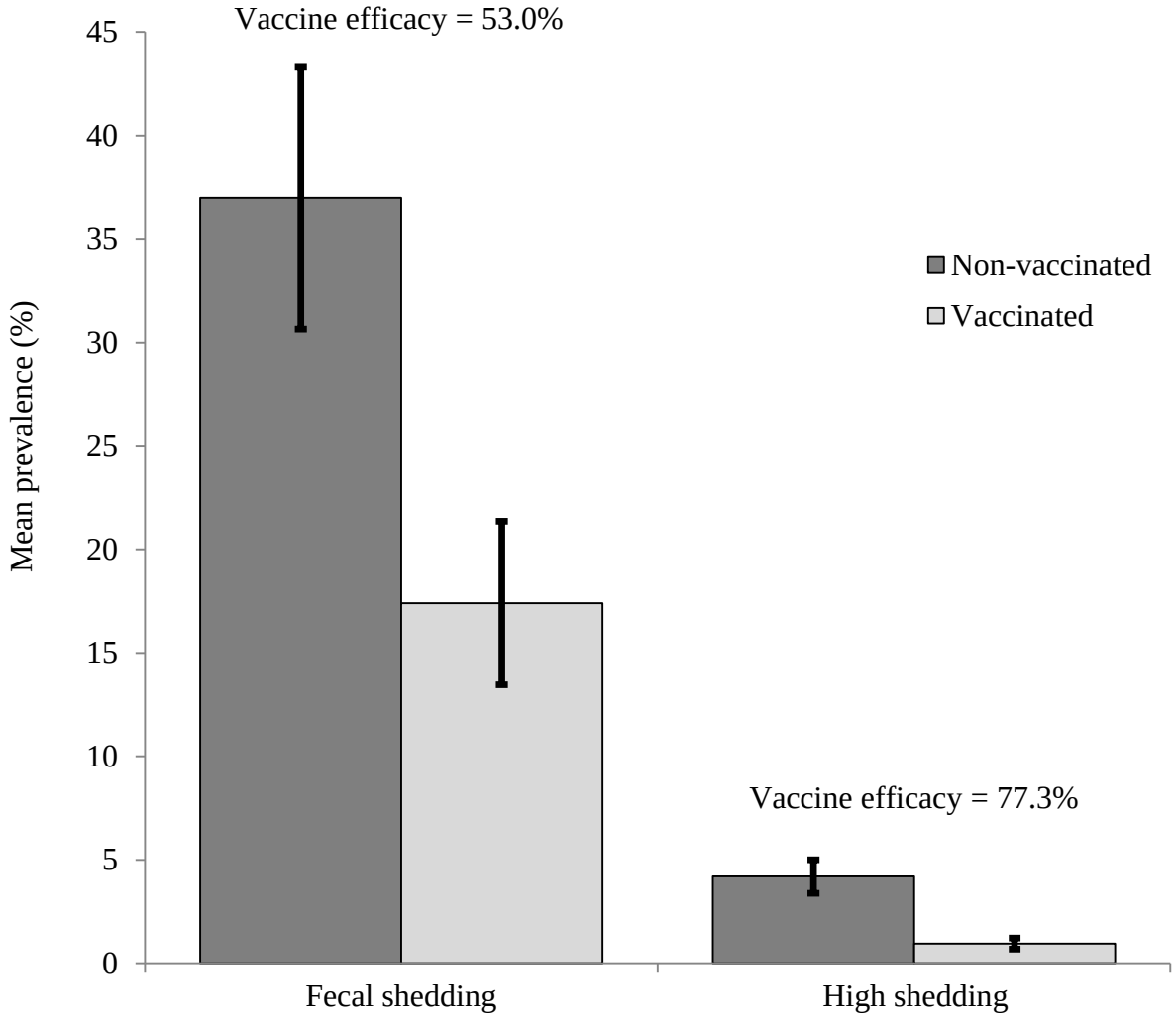


Figure 2.2 Vaccine efficacy estimates and corresponding model-adjusted[1] means (with standard error bars) demonstrating the effects a siderophore receptor and porin proteins-based vaccine for controlling overall fecal prevalence of *E. coli* O157:H7 and prevalence of *E. coli* O157:H7 high shedders in pens of commercial feedlot cattle. A significant vaccine effect was demonstrated for both measures of *E. coli* O157:H7 prevalence (*P* values < 0.01)[1].



[1]From generalized linear mixed models accounting for allocation of pens within blocks and repeated measures on pens over time.

Chapter 3 - Performance and carcass characteristics of commercial feedlot cattle from a study of vaccine and direct-fed microbial effects on *E. coli* O157:H7 fecal shedding

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Abstract

The objective of this study was to quantify cattle performance and carcass characteristics associated with administration of a siderophore receptor and porin proteins-based vaccine (VAC) and a direct-fed microbial (DFM) which were originally evaluated for their impact on *E. coli* O157:H7 fecal shedding in a commercial feedlot population. Cattle (n=17,148) were randomly allocated into 40 pens grouped by allocation dates into 10 complete blocks; pens within block were randomly allocated to control, VAC, DFM, or VAC+DFM treatment groups in a 2x2 factorial design. The DFM (Bovamine) was fed at the labeled dose of 10^6 CFU/animal/day of

Lactobacillus acidophilus for the duration of the intervention period (mean=86.6 d). The VAC cattle were vaccinated on days 0 and 21 whereas unvaccinated cattle were not given a placebo or re-handled on day 21. Data were analyzed using general and generalized linear mixed models that accounted for the study design. Main effects of DFM and VAC are reported as there were no significant treatment interactions for any of the outcomes evaluated. Vaccinated cattle had lower total weight gain ($P < 0.01$), ADG ($P = 0.03$), and cumulative DMI during the intervention period ($P < 0.01$) as compared to unvaccinated cattle, while the DFM increased total weight gain ($P = 0.03$) and G:F ($P = 0.05$) during the intervention period. Daily DMI was decreased ($P < 0.01$) in vaccinated pens as compared to unvaccinated pens during a five day period immediately following revaccination. After the intervention period was completed, cattle were sorted following the standard operating procedure for the feedlot and all cattle were fed the DFM from that point until harvest. Each steer was individually identified through harvest. At harvest, vaccinated cattle had more total days on feed ($P < 0.01$) with a larger HCW ($P = 0.01$) than non-vaccinated cattle, whereas cattle not fed the DFM during the intervention period had a significantly larger HCW ($P < 0.01$) than those fed the DFM during the intervention period. We conclude that the use of these direct-fed microbial and vaccine products have differential and independent effects on cattle performance and carcass characteristics in a commercial feedlot setting. Although the magnitude of these effects may vary among production systems, a more comprehensive understanding of the potential production costs of pre-harvest food safety pathogen control programs is essential if such programs are to be fully adopted in the industry.

Introduction

For the past two decades, *Escherichia coli* O157:H7 has been recognized as a major foodborne pathogen that is economically important to the beef industry (NCBA, 2003). As emphasis increases for implementation of pre-harvest food safety interventions, the lack of data on potential live and carcass performance effects associated with the pre-harvest interventions has become more apparent. These effects need to be further quantified since cattle performance directly affects beef production costs and subsequent business decisions. Thus, the adoption of pre-harvest control programs will likely be affected by all costs and benefits associated with implementation.

Previously, we evaluated the efficacy of two pre-harvest interventions, a siderophore receptor and porin (SRP) proteins-based vaccine (VAC) and a *Lactobacillus acidophilus*-based direct-fed microbial (DFM; Bovamine), on *E. coli* O157:H7 fecal shedding in feedlot cattle (Cull et al., 2012). A preliminary assessment of cattle weight gain and feed conversion during the study period indicated potential differences in performance among treatment groups (Cull et al., 2012). Previous literature on the vaccine demonstrated no significant effects on feedlot cattle performance; however, control cattle in these studies were handled and given a placebo which would not be a typical production practice (Thomson et al., 2009). Previous literature on the DFM demonstrates positive or neutral effects on performance outcomes; generally, a lower dose of the DFM was thought to increase performance as compared to animals fed a higher dose or no DFM (Loneragan and Brashears, 2005). Although literature indicates potential food safety or performance benefits of these products, there is a need for more comprehensive performance data in a commercial feedlot setting (Cull, 2012). Therefore, our objective was to further

quantify the live and carcass performance of cattle administered VAC, DFM, or both in a large-scale commercial feedlot system.

Materials and Methods

Study Population

Previously, Cull et al. (2012) described a commercial feedlot study in the central USA that took place during the summer months and included 17,148 head of steers randomly allocated to 40 pens grouped into 10 blocks. Within each block, a pen was randomly allocated to one treatment group: control (CON), vaccine (VAC), direct-fed microbial (DFM) or both VAC and DFM (VAC + DFM). Cattle in the VAC and VAC + DFM groups were vaccinated at allocation on day 0 and re-vaccinated 21 days later with a SRP vaccine (*E. coli* SRP vaccine, Zoetis Animal Health, New York, NY, USA); re-vaccination occurred pen-side with the entire pen of cattle processed within seventy minutes or less. Cattle allocated to CON or DFM groups never received a placebo vaccine and were not re-handled at the re-vaccination time. Cattle allocated to the DFM or VAC + DFM were fed a product with *Lactobacillus acidophilus* (NP51) and *Propionibacterium freudenreichii* at a dose of 10^6 CFU/head/day and10^9 CFU/animal/day, respectively, throughout the study period (Bovamine, Nutrition Physiology Corp., Guymon, OK, USA). All animals were weighed upon allocation to the study with the mean weight at enrollment of 377.6 kg (range 350.2 – 403.9 kg); study animals had previously been on feed for 60-90 days before allocation. After a brief step-up period (< one week), cattle were fed twice daily a finishing diet which included (dry matter basis): 46.4% high moisture corn, 25.0% wet distillers grain, 17.0% corn gluten, 7.1% silage, 2.5% steep, and 2.0% micro/minerals mix [including 280 mg of monensin/animal/day and 90 mg of tylosin/animal/day (Elanco Animal

Health, Greenfield, IN, USA)]. Standard operating procedures at the feedlot were followed for cattle management and care; sprinklers were used as needed to reduce heat stress risks. Standard bunk management procedures of the feedlot were followed, which allowed feed to be available to cattle at all times. Feedlot personnel who were responsible for bunk management were blinded to treatment allocation throughout the study. Bunk calls were made each morning prior to feeding, and feed was added or taken away on a per head basis according to the bunk call to maintain a target amount of feed left in the bunk the next morning. The Kansas State University Institutional Animal Care and Use Committee approved the study.

Intervention Period

The intervention period of the study lasted an average of 86.6 days (range 84 – 88 d) as the allocation period for study blocks ranged from March 31 through May 14, 2011 and the end-dates of the intervention period ranged between June 20 and August 3, 2011 (Cull et al., 2012). Feedlot personnel individually weighed all animals at the beginning and conclusion of the study while total feed delivered (dry matter basis) was weighed and recorded daily at the pen level. Feedlot personnel administering treatments were not the same personnel as those collecting health, feeding, and performance data throughout the study; daily health monitoring and performance collection followed standardized procedures of the feedlot. Response variables of interest to evaluate live performance impacts during the study period were: cumulative dry matter intake (cDMI), daily dry matter intake (dDMI), cumulative ADG and G:F. The DMI values were based on feed delivery records for the feedlot. The dDMI was recorded continuously over the entire intervention period; however, we determined *a priori* to analyze these data in three different periods; the initial allocation, treatment and step-up feeding period (d

0 – 15); the re-vaccination period (d 16 – 30); and the final duration of the intervention period (d ≥ 31). The cumulative calculations for cDMI, ADG and G:F were computed on a pen-level basis at the end of the intervention period; however, dDMI was calculated for each pen daily. Calculations were:

Mean cumulative DMI (cDMI) = total dry matter delivered to pen ÷ total head days (total number of animals within home pen x days on feed)

Mean daily DMI (dDMI) = total dry matter delivered to pen ÷ total number of animals within the home pen on that day

ADG = total weight gain for cattle finishing the intervention period (total out-weight–total in-weight) ÷ total head days for cattle finishing the intervention period

G:F = total weight gain (as defined above) ÷ total feed delivered (dry matter weight delivered to the home pen)

Intervention to Harvest

Following the intervention period, a terminal sort program that targets carcass goals was followed. Cattle were sorted and either shipped for harvest within the week, or re-allocated to pens based on their projected finishing time (using the feedlot's confidential formula which includes data on weight, hip height, and other parameters). All sorting was completed using a process completely independent of previous interventions (i.e. blinding to treatment groups). Cattle that were re-allocated for further finishing were not maintained in the initial pens based on the previous intervention assignment; however, all steers were individually identified with identity maintained through harvest, and all cattle (regardless of initial treatment group) were managed identically following resorting. After the intervention period, the DFM was fed to all

cattle until harvest. Cattle were sent to a commercial packing plant where all animals were humanely harvested and processed according to standardized procedures. Individual carcasses were tracked by animal electronic identification tags and the plant's carcass identification system. Plant personnel collected individual carcass data including: HCW, yield grade, and quality grade. Individual carcasses received yield grades of 1, 2, 3, 4 or 5 and quality grades of Prime, Choice, Certified Angus Beef, Select, Standard, Commercial, or Dark Cutter. Other calculated response variables of interest included days on feed [DOF; enrollment date into the study (d 0) – harvest date] and enrollment weight/head [total pen weight at enrollment ÷ total head count at enrollment (pen level)]. Dressing percentage and live weight at harvest (and thus cumulative ADG from allocation until harvest) were not measured on an individual animal basis and thus were not utilized to evaluate potential treatment effects.

Data Analysis

Pen-level general and generalized linear mixed models (LMM and GLMM, respectively; Proc Glimmix, SAS, Version 9.2, Cary, NC) were used to assess potential treatment effects on responses of interest. Cattle performance (i.e. ADG, DMI, and G:F) data were modeled using LMM that assume a Gaussian distribution of the data. Carcass yield grade and quality grade data were modeled using a GLMM with a cumulative link function and a proportional odds assumption (Osterstock et al., 2010) on the ordered categorical nature of the data. A likelihood ratio test was used to evaluate the proportional odds assumption against a multinomial model fitted with a generalized logit link function of quality grade and yield grade data from harvest. For all LMM and GLMM models, the random effects of block (and block-by-treatment for GLMM) were fitted in order to recognize block as the clustering factor and pen as the

178 experimental unit for treatment. For dDMI data, additional random effects were used to account
179 for repeated pen data over time (using a first order autoregressive term). Fixed effects in the
180 model included treatment effects (i.e. VAC and DFM) and time as appropriate for each response;
181 further, all applicable 2- and 3-way interactions. Model diagnostics were assessed based on
182 studentized residuals (LMM) and functions of the maximum-likelihood based Pearson χ^2
183 statistics for overdispersion (GLMM). Comparisons with *P*-values ≤ 0.05 were considered
184 statistically significant. Treatment comparisons were based on tailor-built contrasts. The Tukey-
185 Kramer or Bonferroni procedures, as appropriate in each case, were used to prevent inflation of
186 Type I error due to multiple comparisons. Kenward-Rogers' method was used to estimate
187 degrees of freedom and make corresponding adjustments to estimated standard errors. Estimated
188 model-adjusted means (lsmeans; back transformed to original scale for GLMMs) and
189 corresponding standard errors were reported.
190
191 **Results**
192 In a previous study of this population of steers, Cull et al. (2012) reported no significant
193 differences in mean pen size, mortality risk, or culling risk. However, vaccination (but not DFM
194 feeding) was found to significantly reduce *E. coli* O157:H7 fecal shedding. Further, preliminary
195 assessments of cattle performance (after removing mortalities and culled steers) indicated that
196 DFM was associated with improved F:G during the intervention period and that vaccination was
197 associated with poorer ADG and F:G during the intervention period (Cull et al., 2012).
198 In this current study, we provided a much more in-depth assessment of cattle
199 performance during the intervention period. More specifically, steer weights and performance
200 indicators are reported separately and marginalized relative to each other for DFM (Table 1) and

VAC (Table 2), because no significant interactions between treatments were observed ($P > 0.05$). Overall mean enrollment weight was 377.6 kg (range 350.2 to 403.9 kg) with no significant differences among treatments. Descriptive statistics on weight and performance indicators (mean; range) were: out-weight (534.3kg; 489 to 534.3kg), total gain (129.2 kg; 109.0 to 144.3kg), cDMI (8.9 kg/h/d; 8.4 to 9.2kg/h/d), ADG (1.12 kg; 0.65 to 1.45kg), and G:F (1.27 x 10^{-2}; 0.70 x 10^{-2} to 1.62 x 10^{-2}). The out weight and cumulative DMI were significantly lower for VAC pens (Table 2), but no significant effect of DFM was observed for this response (Table 1). Total weight gain was significantly increased in pens fed the DFM (Table 1), whereas total gain was significantly lower in vaccinated pens as compared to those that were not vaccinated (Table 2). Vaccinated pens (Table 2) had a significant reduction of ADG compared to non-vaccinated pens, while DFM-fed pens (Table 1) had significantly increased G:F compared to non-DFM pens. However, no evidence for any effects of DFM on ADG (Table 1) or of VAC on G:F were apparent (Table 2).

The overall daily DMI means for the three study periods analyzed were: 8.26 kg/head/d for days 0 – 15, 8.64 kg/head/d for days 16 – 30 and 9.17 kg/head/d for days 31 – 85. During the step-up and initial feeding period (d 0 – 15) there was no evidence of significant effects of treatment or treatment by time; however, time was significantly associated with dDMI ($P < 0.01$). For period 2 (d 16 – 30), there was a significant treatment by time interaction ($P < 0.01$). More specifically, vaccinated pens showed a temporary decrease in dDMI relative to those non-vaccinated and this effect was apparent from 2 to 6 days following re-vaccination (i.e. days 23, 24, 25, 26 and 27 of the study; Figure 1). There was no evidence for any DFM effects ($P = 0.94$) during this study period. For final feeding period (d 31 – 85), there was an effect of time ($P <$

223 0.01) on dDMI, but there was no evidence for effects of treatment (P=0.69) or a treatment by
224 time (P=0.98) interaction.
225 Of the initial study population (n=17,148 animals), 96.15% (n=16,488) were harvested,
226 2.96% (n=508) died, 0.56% were culled (n=96) and 0.32% (n=56) were missing from the feedlot
227 database. Complete carcass data were available for 92.85% (15,309/16,488) of harvested steers,
228 and an additional 1.95% (322/16,488) of the animals had data on HCW, yield grade, and quality
229 grade. The remaining 5.20% (857/16,488) of the animals were considered missing data as cattle
230 were sent to harvest, but no carcass data were received. Estimates of carcass performance are
231 reported separately for DFM (Table 3) and VAC (Table 4), as no significant interactions were
232 apparent for any of the corresponding outcomes ($P > 0.05$).
233 At harvest, there were no significant effects of DFM or VAC on number of cattle
234 harvested or enrollment weight (Tables 3 and 4). However, vaccinated pens had increased DOF
235 and HCW compared to non-vaccinated pens (Table 4). Feeding the DFM during the intervention
236 period had no significant effect on total DOF (Table 3). Furthermore, pens fed the DFM during
237 the intervention period had decreased HCW as compared to pens that were not fed the DFM
238 during the intervention period (Table 3).
239 Descriptive statistics on the count (percentage) of cattle in each quality and yield grade
240 category are provided in Table 5. There was no evidence for any effect of treatment on the
241 distribution of response categories for quality grade (P = 0.63) and yield grade (P = 0.49).
242

Discussion

The results from this study demonstrate that the live animal performance of commercial feedlot cattle was significantly affected by the use of the SRP vaccine and DFM products which were originally administered to evaluate their effects on fecal shedding of *E. coli* O157:H7. In addition, the evaluations of these products during a relatively short-term intervention period (mean=86.6 d) resulted in subsequent differences in HCW despite the fact that all cattle were enrolled in a terminal sorting program after the intervention period and then were managed identically as they were fed for an average of approximately 80 additional days until harvest. Although the actual magnitude of the effects of the vaccine and DFM, as well as associated costs or financial benefits, may differ across production systems, this study demonstrated that impacts on production need to be considered when implementing pre-harvest interventions for food safety pathogens. The need to consider both direct and indirect production costs or benefits associated with pre-harvest food safety programs has been discussed previously (Loneragan and Brashears, 2005; Thomson et al., 2009; Cull et al., 2012), but this study is the most comprehensive assessment of live and carcass performance outcomes following interventions in commercial feedlot cattle.

The current study is a continuum from a previous study demonstrating that a two dose regimen of the commercially available SRP vaccine in large commercial feedlot pens yielded a significant reduction in *E. coli* O157:H7 shedding, but also had a potentially negative impact on cattle weight gains (Cull et al., 2012). However, that study used relatively unique performance formulae, did not evaluate live or carcass performance impacts in detail, and found no evidence for vaccine-associated differences in feed conversions. Feed conversions over the same time period for the same data were significantly different in this current study; the discrepancy

between the previous and current studies can be attributed to calculation differences as the previous study utilized the production systems' formula that added the estimated body weight of cattle that died or were culled during the study intervention period (Cull et al., 2012). In turn, the current study utilized the total measured cattle weight for each pen at both study initiation and at the end of the intervention period (i.e. pen in-weight to pen out-weight).

In the current study, the performance effects that were observed from study initiation to harvest indicated significant increases in DOF, by approximately 3 days, for vaccinated pens which is likely associated with the significantly larger HCW in vaccinated pens. Although previous studies with this vaccine have reported no evidence for any vaccination effects in pen-level cattle performance (Thomson et al., 2009; Fox et al., 2009; Thornton et al., 2009; Rezac et al., 2012), there were critically different study design features in the current versus previous studies. In previous studies, both vaccinated and control pens were handled at re-vaccination and controls were given a placebo. While this practice is common for research protocols, it is not necessarily representative of commercial scenarios. Additionally, those studies had half as many pens (20), multiple feedlot sites (Rezac et al., 2012) and much fewer animals overall (< 5,000) compared to the current study.

To our knowledge, this is the first published study to report a difference in DMI and other performance indicators between vaccinated (two dose regimen of SRP vaccine) and non-vaccinated pens that were neither handled nor given a placebo at re-vaccination. The significant difference in DMI for vaccinated pens during the re-vaccination period (d 16 – 30), more specifically for the days immediately following revaccination (Figure 1), may be attributed to the biological response associated with the vaccine and/or the effects of handling cattle for revaccination. Although previous studies with this vaccine have demonstrated no evidence for

differences in cattle DMI or performance (Thomson et al., 2009; Fox et al., 2009; Thornton et al., 2009; Rezac et al., 2012), decreased performance effects associated with vaccination have been reported in studies of clostridial vaccines; where injection location and type of vaccine have been associated with reductions in animal performance (Chirase et al., 2001). Previous studies, unrelated to vaccination, also have reported a decrease in DMI when comparing handled cattle to non-handled controls (Voisinet et al., 1997; Franisco et al., 2012), as well as an increased stress response and temperament differences in handled cattle (Grandin, 1997). Although we demonstrated a difference in DMI and related performance indicators, the specific reason for the effect (i.e. vaccine response versus handling) cannot be determined in our study due to the study design. Indeed, an additional control group that was re-handled and given a placebo would have been needed to sort out the effect of vaccination from that of handling. Regardless of mechanism, the rationale for the experimental design on our current study is based on management practices that are representative of commercial feedlot settings, whereby handling alone, without any revaccination, would not be likely in the case that a vaccination program was implemented. For such a commercial setting, our data indicate the potential for negative effects of handling and vaccination on cattle performance.

The use of direct-fed microbials, particularly strains of *Lactobacillus acidophilus*, has become a relatively common practice in the cattle feeding industry (NAHMS, 2013). While many potential positive benefits have been described, effects on live cattle or carcass performance have not been demonstrated on a consistent basis across different commercial feedlot settings. The commercial low-dose Bovamine product (10^6 CFU/head/d of *Lactobacillus* NP51) was utilized in the current study because of the perception that this product can improve cattle performance while reducing fecal shedding of *E. coli* O157:H7 (Loneragan and Brashears,

2005; Cull et al., 2012). Pens receiving the DFM during the intervention period had a significant increase in total gain and G:F, which is in agreement with multiple other studies (Rust et al., 2000; Vasconcelos et al., 2008; Hanford et al., 2011). In contrast, other studies have found no significant differences associated with DFM feeding (Brashears et al., 2003; Younts-Dahl et al., 2005; Peterson et al., 2007). In the current study, pens fed, versus those not fed the DFM during the intervention period had significantly lower HCW at harvest; however, it is worth noting that all pens were fed the DFM after the intervention period. Some previous DFM studies have reported no significant differences in HCW (Rust et al., 2000; Elam et al., 2003) while other primary studies and a meta-analysis demonstrated increased HCW (Galyean et al., 2000; Peterson et al., 2007; Hanford et al., 2011). Finally, our study and others reported no evidence for a significant difference in ADG relative to DFM feeding (Elam et al., 2003; Peterson et al., 2007), while other previous studies demonstrated an increase in ADG (Ware et al., 1988; Swinney-Floyd et al., 1999; Hanford et al., 2011). Some of these seemingly contradictory results are likely due to important differences among studies in their study design, statistical power, pen size, feeding period, diet, cattle type, or product dose. Further, DFM strains have differed among studies with some using strain NP51 (Elam, 2003 et al.; Younts-Dahl et al., 2005; Peterson et al., 2007; Hanford et al., 2011; Cull et al., 2012) and others using strain NP45 (Rust et al., 2000) or a combination of NP51 and NP45 (Vasconcelos et al., 2008). In addition, our study is unique as the DFM was fed or not fed to half the pens during the intervention period, but all cattle remaining on feed following the post-intervention period terminal sort were fed the DFM until harvest.

The distributions of carcass quality and yield grades were typical for the type of steers and production management system (Table 5) and were in agreement with results from other

studies on the DFM (Swinney-Flyod et al., 1999; Galyean et al., 2000; Elam et al., 2003) and the SRP vaccine (Rezac et al., 2012) in which no significant treatment effects were observed. It is perhaps not surprising that the distributions of quality and yield grades were not significantly different amongst treatments given the management and terminal sort program of the production system and the goal to target common carcass end-points from all individual animals regardless of their prior history.

Based on the collective results of this study, we conclude that the use of these direct-fed microbial and vaccine products have differential and independent effects on cattle performance and carcass characteristics in a commercial feedlot setting. The re-vaccination process resulted in a temporary but significant decrease in DMI, which likely led to the lower weight gains for vaccinated cattle during the intervention period, and the subsequent increase in DOF until harvest. The negative impact of utilizing this vaccine on cattle performance, from vaccine administration through harvest, had not been demonstrated previously. Although the actual costs of vaccination were not estimated for this study, a recently published paper estimated direct costs to producers of $8.35/hd to $14.99/hd depending on whether there were cattle performance effects of vaccination or not (Tonsor and Schroeder, 2015). Interestingly, these authors showed that if a pre-harvest *E. coli* vaccine led to reduction of retail or packer costs of 1.2% to 3.9%, producers would be economically neutral to adoption (Tonsor and Schroeder, 2015). Feeding the DFM increased total weight gain and improved feed conversion during the intervention period, though these positive attributes were not reflected at harvest. However, all cattle were fed the DFM following the terminal sort that took place after the intervention period. We acknowledge that the observed magnitude of the intervention effects may differ among production systems, particularly when cattle types, feeding programs, and other management

attributes differ. However, it is clear that further insight into the effects of pre-harvest food safety pathogen control programs on cattle performance is warranted due to potential financial implications for program adoption in the industry.

References

Brashears MM, Galyean ML, Loneragan GH, Mann JE, and Killinger-Mann K. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. *J Food Prot* 2003;66:748–754.

Chirase N, Greene KLW, Graham GD, and Avampato JM. Influence of clostridial vaccines and injection sites on performance, feeding behavior, and lesion size scores of beef steers. *J Anim Sci* 2001;79:1409–1415.

Cull CA, Paddock ZD, Nagaraja TG, Bello NM, Babcock AH, and Renter DG. Efficacy of a vaccine and a direct-fed microbial against fecal shedding of *Escherichia coli* O157:H7 in a randomized pen-level field trial of commercial feedlot cattle. *Vaccine* 2012;30:6210–6215.

Elam NA, Gleghorn JF, Rivera JD, Galyean ML, Defoor PJ, Brashears MM, and Younts-Dahl SM. Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium fruendenreichii* on performance, carcass, and intestinal characteristics, and *Escherichia coli* strain O157 shedding of finishing beef steers. *J Anim Sci* 2003;81:2686–2698.

Fox JT, Thomson DU, Drouillard JS, Thornton AB, Burkhardt DT, Emery DA, and Nagaraja TG. Efficacy of *Escherichia coli* O157:H7 siderophore receptor/porin proteins–based vaccine in feedlot cattle naturally shedding *E. coli* O157. *Foodborne Pathog Dis* 2009;6:893–899.

Francisco C, Cooke RF, Marques R, Cooke FT, Guarnieri Fiho TA, and Bohnert DW. Effects of acclimation to human handling on temperament, physiological responses, and performance of beef steers during feedlot receiving. *Proc. West. Sec. Amer. Soc. Anim. Sci* 2012 http://oregonstate.edu/dept/eoarc/sites/default/files/publication/735.pdf. 63:134–137.

Galyean ML, Nunnery GA, Defoor PJ, Salyer GB, and Parsons CH. Effects of live cultures of *Lactobacillus acidophilus* (Strains 45 and 51) and *Propionibacterium freudenreichii* PF-24 on performance and carcass characteristics of finishing beef steers. Burnett Center Internet Progress Report. No. 8. 2000. http://www.depts.ttu.edu/afs/burnett_center/progress_reports/bc8.pdf. (Assessed 16 March 2014.)

Grandin T. Assessment of stress during handling and transport. *J Anim Sci* 1997;4:249–257.

Hanford KJ, Kreikemeier WM, and Ware DR. The effect of Bovamine on feedlot performance of finishing cattle: A meta-analysis. *Proc Amer Dairy Sci Association and Amer Soc of Anim Sci* New Orleans, LA. 2011;89:258.

Loneragan GH and Brashears MM. Pre-harvest interventions to reduce the carriage of *E. coli* O157 by harvest-ready feedlot cattle. *Meat Sci* 2005;71:72–78.

National Animal Health Monitoring System (NAHMS), USDA APHIS. Feedlot 2011, Part III: Trends in Health and Management Practice on U.S. Feedlots, 1994-2011. 2013. http://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_dr_Part%20III.pdf. (Accessed 22 July 2014.)

National Cattleman's Beef Association. Facts for beef producers: a basic look at *E. coli* O157. 2012. http://www.beefresearch.org/CMDocs/BeefResearch/Safety_Fact_Sheets/E.Coli_Final.pdf. (Assessed 11 March 2014.)

Osterstock JB, MacDonald JC, Boggess MM, and Brown MS. Technical note: Analysis of ordinal outcomes from carcass data in beef cattle research. *J Anim Sci* 2010;88:3384–3389

Peterson RE, Klopfenstein TJ, Erickson GE, Folmer J, Hinkley S, Moxley RA, and Smith DR. Effect of *Lactobacillus acidophilus* Strain NP51 on *Escherichia coli* O157:H7 fecal shedding and finishing performance in beef feedlot cattle. *J Food Prot* 2007;70:287–291.

Rezac DJ, Thomson DU, Butler BA, Wileman BW, and Reinhardt CD. Vaccinating with SRP *E. coli* does not affect feeder cattle performance, health, or carcass characteristics. In: Proc. Cattleman's Day. Manhattan, KS. 2012;19–20.

Rust SR, Metz K, and Ware DR. Effects of Bovamine™ rumen culture on the performance and carcass characteristics of feedlot steers. *Michigan Agric Exp Stn Beef Cattle, Sheep and Forage Sys. Res Dem Rep* 2000;569:22–26.

Swinney-Floyd D, Gardner BA, Owens FN, Rehberger T, and Parrot T. Effect of inoculation with either *Propionibacterium* strain P-63 alone or combined with *Lactobacillus acidophilus* strain LA53545 on performance of feedlot cattle. *J Anim Sci* 1999;77(Suppl. 1):77.

Thomson DU, Loneragan GH, Thornton AB, Lechtenberg KF, Emery DA, Burkhardt DT, and Nagaraja TG. Use of a siderophore receptor and porin proteins-based vaccine to control the burden of *Escherichia coli* O157:H7 in feedlot cattle. *Foodborne Pathog. Dis.* 2009;6:871–877.

Thornton AB, Thomson DU, Loneragan GH, Fox JT, Burkhardt DT, Emery DA, and Nagaraja TG. Effects of a siderophore receptor and porin proteins based vaccination on fecal shedding of *Escherichia coli* O157:H7 in experimentally inoculated cattle. *J Food Prot* 2009;72:866–869.

Tonsor GT and Schroeder TC. Market impacts of *E. coli* vaccination in U.S. Feedlot cattle. *J Agric Resour Econ* 2015;3:7.

Vasconcelos JT, Elam NA, Brashears MM, and Galyean ML. Effects of increasing dose of live cultures of *Lactobacillus acidophilus* (Strain NP 51) combined with a single dose of *Propionibacterium freudenreichii* (Strain NP 24) on performance and carcass characteristics of finishing beef steers. *J Anim Sci* 2008;86:756–762.

Voisinet BD, Grandin T, O'Connor SF, Tatum JD, and Deesing MJ. Bos indicus cross feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. *Meat Sci* 1997;46:367–377.

444 Ware DR, Read PL, and Manfredi ET. Pooled summary of eight feedlot trials evaluating
445 performance and carcass characteristics of steers fed *Lactobacillus acidophilus* strain
446 BT1386. *J Anim Sci* 1988;66(Suppl. 1):436.
447 Wileman BW, Thomson DU, Olson KC, Jaeger JR, Pacheco LA, Bolte J, Burkhardt DT, Emery
448 DA, and Straub D. *Escherichia coli* O157:H7 shedding in vaccinated beef calves born to
449 cows vaccinated prepartum with *Escherichia coli* O157:H7 SRP vaccine. *J Food Prot*
450 2011;74:1599–604.
451 Younts-Dahl SM, Osborn GD, Galyean ML, Rivera JD, Loneragan GH, and Brashears MM.
452 Reduction of *Escherichia coli* O157 in finishing beef cattle by various
453 doses of *Lactobacillus acidophilus* in direct-fed microbials. *J Food Prot* 2005;68:6–10.

Table 3.1 Model estimated[1] means, standard errors, and corresponding *P*-values for pair-wise comparisons of performance data during the intervention period for study pens that received, or did not receive, the direct-fed microbial (DFM)[2]

	Treatment		
Item	Fed DFM	No-DFM	*P*-value
Cattle (pens)	8,575 (20)	8,573 (20)	-
Enrollment weight/head, kg (SE)	377.25 (4.98)	377.87 (4.98)	0.06
Out-weight/head, kg (SE)	507.78 (4.51)	505.78 (4.51)	0.11
Total Gain/head, kg (SE)	130.53 (2.67)	127.91 (2.67)	0.03
DMI, kg/h/d (SE)	8.85 (0.06)	8.87 (0.06)	0.55
ADG, kg (SE)	1.43 (0.03)	1.40 (0.03)	0.13
Gain:Feed (SE) ($x10^{-1}$)	1.61 (0.02)	1.57 (0.02)	0.05

[1] Least square mean estimates for marginal means of DFM

[2]Bovamine (10^6 CFU/animal/day of *Lactobacillus acidophilus* and 10^9 CFU/animal/day of *Propionibacterium freudenreichii*), Nutrition Physiology Corp., Guymon, OK, USA

Table 3.2 Model estimated[1] means, standard errors, and corresponding *P*-values for pair-wise comparisons of performance data during the intervention for study pens that received, or did not receive, the vaccine[2]

	Treatment		
Item	Vaccinated	Non-Vaccinated	*P*-value
Cattle (pens)	8,575 (20)	8,573 (20)	-
Enrollment weight/head, kg (SE)	377.52 (4.98)	377.59 (4.98)	0.82
Out-weight/head, kg (SE)	505.02 (4.51)	508.54 (4.51)	< 0.01
Total Gain/head, kg (SE)	127.50 (2.67)	130.94 (2.67)	< 0.01
DMI, kg/h/d (SE)	8.79 (0.06)	8.93 (0.06)	< 0.01
ADG, kg (SE)	1.39 (0.03)	1.43 (0.03)	0.03
Gain:Feed (SE) ($x10^{-1}$)	1.58 (0.02)	1.60 (0.02)	0.30

[1] Least square mean estimates for marginal means of vaccination

[2] *E. coli* SRP vaccine, Zoetis Animal Health, New York, NY, USA

Figure 3.1 Model estimated[1] means for daily DMI during the period (d 16 – 30) when the pens were, or were not, given the second dose of the vaccine[2]. Re-vaccination occurred on day 21 represented by the star symbol (✦). Means for days 23, 24, 25, 26 and 27 were significantly different ($P \leq 0.05$) between groups as indicated by an asterisk (*). The lower standard error bars for vaccinated and upper standard error bars for non-vaccinated pens are displayed.

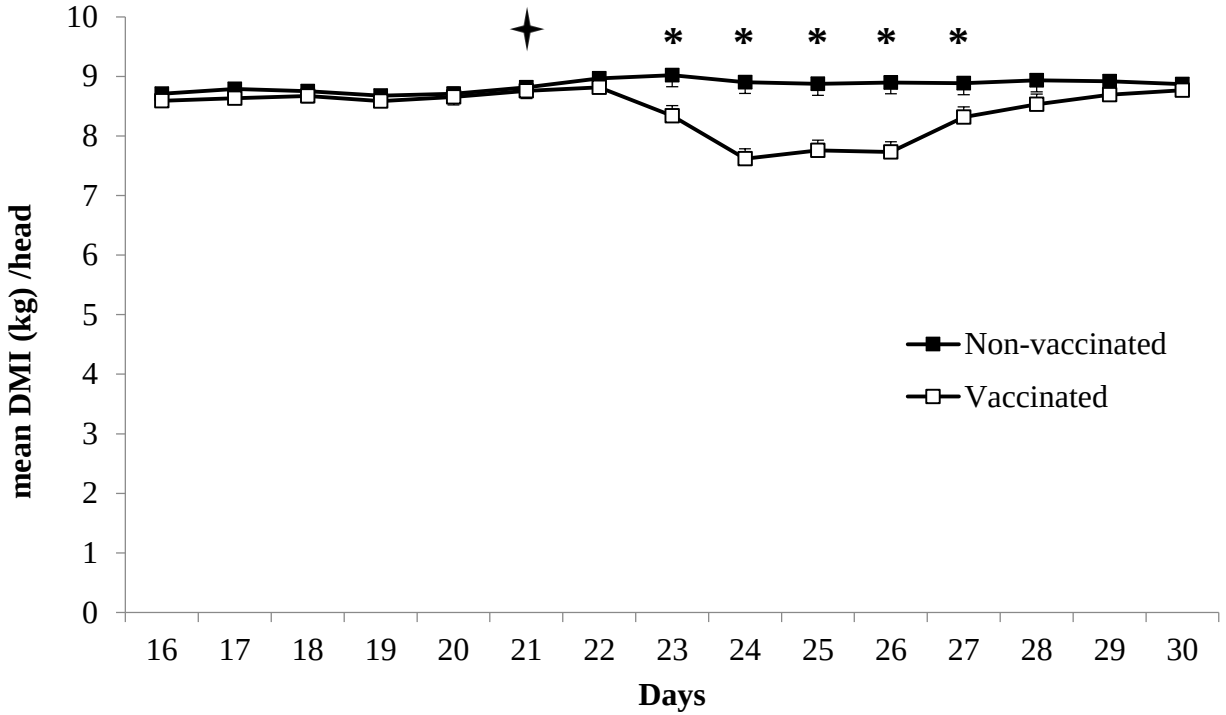

Table 3.3 Model estimated[1] means, standard errors, and corresponding *P*-values for pair-wise comparisons of performance data for the intervention to harvest period for cattle that received, or did not receive, the direct-fed microbial (DFM)[2] during the intervention period.

Item	Population	Treatment		*P*-value
		Fed DFM	No-DFM (during intervention period)[3]	
Days on Feed (SE)	N = 16,203	165.03 (4.10)	166.26 (4.10)	0.16
Enrollment weight, kg (SE)	N = 16,488	395.42 (5.14)	395.96 (5.14)	0.14
HCW, kg (SE)	N = 15,631	408.00 (1.41)	409.50 (1.41)	< 0.01

[1] Least square mean estimates for marginal means of DFM

[2]Bovamine (10^6 CFU/animal/day of *Lactobacillus acidophilus* and 10^9 CFU/animal/day of *Propionibacterium freudenreichii*), Nutrition Physiology Corp., Guymon, OK, USA)

[3]Cattle that were re-allocated for further finishing following the intervention period were not maintained in the initial pens based on the previous intervention assignment; however, all steers were individually identified with identity maintained through harvest, and all cattle (regardless of initial treatment group) were fed DFM and managed identically following resorting through harvest.

Table 3.4 Model estimated[1] means, standard errors, and corresponding *P*-values for pair-wise comparisons of performance data for the intervention to harvest period for cattle that received, or did not receive, the vaccine[2]

		Treatment		
Item	Population	Vaccinated	Non-Vaccinated	*P*-value
Days on feed (SE)	N = 16,203	167.23 (4.10)	164.07 (4.10)	< 0.01
Enrollment weight, kg (SE)	N = 16,488	395.64 (5.14)	395.74 (5.14)	0.77
HCW, kg (SE)	N = 15,631	409.49 (1.41)	408.10 (1.41)	0.01

[1] Least square mean estimates for marginal means of vaccination

[2]*E. coli* SRP vaccine, Zoetis Animal Health, New York, NY, USA

Table 3.5 Descriptive statistics on the count (percentage) of cattle in each quality and yield grade category by treatment group; there was no evidence of treatment effects during the intervention period on quality grade (*P* = 0.63) or yield grade (*P* = 0.49) at harvest.

Item	Population (Pen)	Treatment			
		Control	VAC	DFM	VAC+DFM
Quality Grade	N = 15,631 (40)				
Prime (%)		41 (1.0)	36 (0.9)	31 (0.8)	47 (1.2)
CAB - Choice (%)		2338 (59.2)	2272 (57.9)	2252 (58.0)	2223 (57.4)
Select (%)		1314 (33.2)	1381 (35.2)	1372 (35.3)	1357 (35.1)
Standard (%)		160 (4.0)	146 (3.7)	145 (3.7)	149 (3.8)
Outs (%)[3]		101 (2.6)	89 (2.3)	82 (2.2)	95 (2.5)
Total		3954 (100.0)	3924 (100.0)	3882 (100.0)	3871 (100.0)
Yield Grade	N = 15,631 (40)				
1 (%)		747 (18.9)	744 (19.0)	710 (18.3)	714 (18.4)
2 (%)		1553 (39.2)	1617 (41.2)	1590 (41.0)	1574 (40.7)
3 (%)		1304 (33.0)	1239 (31.6)	1263 (32.5)	1275 (32.9)
4 (%)		312 (7.9)	298 (7.5)	295 (7.6)	281 (7.3)
5 (%)		38 (1.0)	26 (0.7)	24 (0.6)	27 (0.7)
Total		3954 (100.0)	3924 (100.0)	3882 (100.0)	3871 (100.0)

[1]*E. coli* SRP vaccine (VAC), Zoetis Animal Health, New York, NY, USA

[2]Bovamine (DFM; 10^6 CFU/animal/day of *Lactobacillus acidophilus* and 10^9 CFU/animal/day of *Propionibacterium freudenreichii*), Nutrition Physiology Corp., Guymon, OK, USA

[3]Outs included individual carcasses that received a quality grade of Commercial, Standard or Dark Cutter

Chapter 4 - Feedlot- and pen-level prevalence of Enterohemorrhagic *Escherichia coli* in feces of commercial feedlot cattle in two major U.S. cattle feeding areas

Abstract

The objective of the study was to determine feedlot- and pen-level fecal prevalence of seven Enterohemorrhagic *Escherichia coli* (EHEC) serogroups (O26, O45, O103, O111, O121, O145, and O157, or EHEC-7) and their associated virulence genes (*stx*1, *stx*2, and *eae*) in feces of commercial feedlot cattle in two feeding areas in United States. Cattle pens from four commercial feedlots in each of the two major cattle feeding areas, study areas A and B, were sampled. Up to 16 pen-floor fecal samples were collected from each of 4 to 6 pens per feedlot, per monthly visit, for a total of three visits from June to August, 2014. Culture-based procedures including fecal enrichment in *E. coli* broth, immunomagnetic separation with individual beads for O157 and two sets of pooled beads for the six non-O157 serogroups, and plating on selective medium, followed by molecular-based detection methods for EHEC confirmation were conducted. Generalized linear mixed models were fitted to estimate feedlot-, pen-, and sample-level fecal prevalence of EHEC-7 and to evaluate associations between potential demographic and management risk factors with feedlot prevalence of EHEC-7. All study feedlots and 31.0% of the study pens had at least one fecal sample that tested positive for non-O157 EHEC, whereas 61.9% of pens tested positive for EHEC O157; sample-level prevalence estimates ranged from 0.0% for EHEC O121 to 18.7% for EHEC O157. Within-pen prevalence of EHEC O157 varied significantly by month of sampling; similarly within-pen prevalence of non-O157 EHEC varied significantly by month as well as by the sex composition of the pen (i.e., heifer, steer or mixed).

Feedlot management factors, however, were not significantly associated with fecal prevalence of EHEC-7 serogroups. Intraclass correlation coefficients for EHEC-7 models indicated that most of the variation occurred between pens, rather than within pens, or between feedlots. Hence, the potential combination of pre-harvest interventions and pen-level management strategies (e.g., sex segregation/exclusion) may have positive food safety impacts downstream along the beef chain.

Introduction

For the past three decades, studies of Shiga toxin-producing *Escherichia coli* (STEC) in commercial feedlot systems have focused primarily on *E. coli* O157:H7 (STEC O157), despite the fact that non-O157 STEC are estimated to cause twice as many human illnesses (e.g., diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome) in the United States (Scallan et al., 2011). In fact, it has been estimated that STEC O26, O45, O103, O111, O121, and O145 (non-O157 STEC) represented 83% of the confirmed non-O157 STEC human infections from 2000 to 2010 (Gould et al., 2013). Although a variety of food commodities (e.g., leafy vegetables, game meat, beef, dairy, pork, and fruits or nuts) are linked to these non-O157 STEC outbreaks and human infections (Luna-Gierke et al., 2014), cattle are recognized as the primary reservoir for STEC and intermittently shed these bacteria in their feces (CDC, 2014; USDA-FSIS, 2014). As a result, the fecal to hide to carcass pathway (i.e., contamination pathway) provides a unique opportunity for STEC transfer with the potential for contamination of beef and other food products during the harvest process (Ferens and Hovde, 2011; Painter et al., 2013). In addition to the major public health concerns, the economic impact of STEC on the beef industry has been profound (NCBA, 2012); indicating the need for effective pre- and post-harvest interventions against STEC-7 pathogens along the beef system.

Although the majority of research has focused on STEC O157, there is a growing body of scientific literature on the effects of non-O157 STEC colonization, prevalence, and transmission in commercial feedlot operations. Shiga toxin-producing *E. coli* are characterized by the presence of an O antigen and Shiga toxin virulence genes (*stx*1 and/or *stx*2). Enterohemorrhagic *Escherichia coli* or EHEC, are a subset of STEC that also possess the intimin gene (*eae*). Recently, published literature has reported wide prevalence estimates of non-O157 STEC and EHEC, ranging from 0.0 to 16.9% and 0.0 to 10.5%, respectively, in cattle feces (Cernicchiaro et al., 2013 and 2014; Baltasar et al., 2014; Ekiri et al., 2014; Paddock et al., 2014; Dewsbury et al., 2015). However, these studies were limited to reporting prevalence estimates of non-O157 STEC from range cattle or feedlot cattle in a single operation. Although one study indicated a high pen to pen variability of non-O157 STEC shedding in cattle feces (Dewsbury et al., 2015), additional studies are needed for effective interventions to reduce the fecal load non-O157 STEC in cohorts of cattle. Processors have successfully applied post-harvest interventions to hides and carcasses towards significantly reducing foodborne pathogens in beef. Understanding sources of variability and drivers of STEC shedding in cattle production environments will help the identification and implementation of needed pre-harvest approaches. The objective of this study was to determine the feedlot- and pen-level prevalence of seven serogroups of EHEC (O26, O45, O103, O111, O121, O145 and O157) and their associated virulence genes (*stx*1, *stx*2, and *eae*) in feces of commercial feedlot cattle in two major cattle feeding areas of the United States.

Materials and Methods

Study population and sampling

579 Samples were collected from eight commercial feedlot operations in two major U.S.
580 cattle feeding areas from June to August, 2014. Cattle were managed following standard
581 operating procedures of the respective study feedlot operations. This observational study
582 followed a repeated cross-sectional design. Study area A consisted of four feedlots within a 150-
583 mile area in northwest Texas, whereas study area B consisted of four feedlots within a 100-mile
584 area in central Nebraska. Feedlot eligibility criteria were determined based on: close proximity
585 (within 150 miles of each other within each area) to study areas (A and B), having cattle on a
586 finishing diet during the summer months, and willingness to complete a questionnaire to gather
587 demographic and management data in each visit. A priori sample size estimates were generated
588 by data simulation and power calculations; assumptions included: 10% prevalence of EHEC
589 O157 and differences of up to 5% prevalence of non-O157 EHEC between pens, 20% Type II
590 error and 5% Type I error. Feedlots were visited once per month for three months (three visits
591 per feedlot). Up to 16 pen-floor fecal samples from each of four to six pens per feedlot, per visit,
592 were obtained. Samples (~30 g) of freshly defecated individual fecal pats were collected from
593 multiple areas throughout the pen; care was taken to avoid ground contamination. Each week,
594 the total number of pens sampled was determined based on the availability of pre-harvest pens
595 (approximately two weeks before cattle were harvested) within each feedlot, as pens were not re-
596 sampled throughout the study. Samples were placed in sterile bags and transported on ice packs
597 to the Pre-harvest Food Safety Laboratory (College of Veterinary Medicine, Kansas State
598 University, in Manhattan, Kansas) where they were refrigerated (4°C) for 14-16 h, followed by
599 processing and testing.
600
601 ***Laboratory protocols for detection of EHEC***

Approximately 2 g of feces were mixed into 18 mL of *E. coli* broth (EC; Difco, ThermoFisher, Waltham, MA) and incubated at 40°C for 6 h (Paddock et al., 2012). A 980-µL sample of enriched fecal suspension was subjected to an immunomagnetic separation (IMS) procedure using Abraxis® beads (Abraxis LLC, Warminster, PA). The IMS procedure consisted of three separate IMS runs, one for individual O157 beads and two using separate sets of pooled non-O157 serogroup beads with pools including the following serogroups: 1) O26, O45, and O111, and 2) O103, O121, and O145 serogroup beads (Noll et al., 2016). A 50-µL aliquot of the O157 bead suspension was spread-plated onto sorbitol MacConkey agar with cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L) (CT-SMAC; Bai et al., 2010), and 50 µL of bead suspension for pooled non-O157 serogroups was spread-plated onto a modified chromogenic Possé medium (MP; Possé et al., 2008; Noll et al., 2016) for each of the two pooled procedures. Plates were incubated for 20-24 h at 37°C. Six sorbitol-negative colonies from CT-SMAC and 10 chromogenic colonies from each MP plate were randomly picked and individually streaked onto blood agar plates, then incubated for 18-24 h at 37°C. Isolates obtained from CT-SMAC plates were tested for the presence of the O157 antigen using latex agglutination (Oxoid Ltd., Basingstoke, UK); if positive, isolates were then tested for indole production. Confirmation of EHEC O157 isolates included a 6-gene multiplex PCR assay for identification of the *rfb*E (O157), *eae* (intimin), *stx*1 (Shiga toxin 1), *stx*2 (Shiga toxin 2), *ehx*A (hemolysin), and *fli*C (H7) genes (Bai et al., 2010). Ten isolates from each non-O157 IMS procedure were pooled and tested by an 11-gene multiplex PCR assay that detects seven serogroups (O26, O45, O103, O111, O121, O145, and O157) and four major virulence genes (*stx*1, *stx*2, *eae*, and *ehx*A) as a screening procedure (Bai et al., 2012). If pooled colonies were positive for an O serogroup and *stx* gene, then each isolate (total of 10 isolates/MP plate) was subjected to an additional 11-gene

multiplex PCR assay to confirm the presence of O serogroup and virulence genes at the colony level. A sample was defined as EHEC positive if at least one individual colony tested positive for an O serogroup, *stx* gene (*stx*1 and/or *stx*2), and *eae* gene.

Questionnaire

A questionnaire was pre-tested in four of the eligible feedlot managers prior to the start of the study. Modifications to the questionnaire were made based on their feedback to eliminate confusing or misleading questions.

The questionnaire was written in English and consisted of open-ended (e.g., fill in the blank) and closed-ended (e.g., multiple choice) questions to obtain information on demographic and management variables at the feedlot- and pen-levels. The questionnaire was administered in person to feedlot managers at the time of each sampling visit. Information collected on demographic and management factors at the feedlot-level included: distillers percentage (ration percentage of distillers: <10%, 10-30%, and >30% on an as-fed basis), feedlot capacity (number of animals per feedlot), implementation of pre-harvest interventions (yes or no, name of the product(s), and dose), and manure storage management (on site: pen-floor fecal material stored at the feedlot, or off site: pen-floor fecal material stored outside the feedlot). Information gathered on demographic factors at the pen-level included: days on feed (DOF), sex composition of the pen (heifers, steers, or both), pen size (number of animals per pen), sampling month (June, July, or August), and type of fed cattle (beef, dairy, or both).

Statistical analysis

Proportion positives for *E. coli* and EHEC O serogroups were calculated at the sample-level, across all pens, as the number of samples that tested positive for an *E. coli* O serogroup (O26, O45, O103, O121, O145, and O157) and EHEC serogroup (O serogroup and *stx*1 and/or *stx*2 and *eae*) divided by the total number of samples collected. Cumulative pen- and feedlot-level percent positives were calculated as the number of pens or feedlots that had at least one positive sample for each EHEC serogroup of interest, divided by the total number of pens or feedlots, respectively.

Model-adjusted cumulative within-pen prevalence estimates and their 95% confidence intervals were evaluated from model intercepts via generalized linear mixed models (GLMMs) that included random effects of sampling week and feedlot within study area. Outcome variables, modeled using a binomial distribution, were modeled as the number of test-positive samples within each pen divided by the number of samples collected per pen. Independent models were fitted for each of the seven EHEC serogroups, and for all non-O157 EHEC. Fixed effects for feedlot and study area were added, in independent models, to estimate mean within-pen prevalence of each of the EHEC-7 serogroups in each of the study feedlots and study areas: random effects for sampling week or feedlot were included, respectively. Models were fitted using Proc Glimmix (SAS Version 9.3, SAS Institute Inc., Cary, NC) using a logit link function, restricted pseudo-likelihood estimation, and Kenward-Rogers degrees of freedom approximation.

Similar GLMMs were used to determine associations between potential risk factors (pen- and feedlot-level factors) with the within-pen or within-feedlot fecal prevalence of EHEC. Initially, all independent variables were examined in a univariable screen. Independent variables included into the univariable screening included: DOF (<100, 100-200, 200-300, and >300 days on feed/pen), sex of the pen (heifer, steer, or both), pen size (<100, 100-200, 200-300, and >300

670 animals/pen), sampling month (June, July, and August), and type of fed cattle (beef and dairy) at
671 the pen level. Feedlot-level explanatory variables tested included: use of a direct-fed microbial
672 (yes vs. no), ration percentage of distiller's (<10%, 10-30%, and >30%), feedlot capacity (<
673 25,000 and $\geq$ 25,000 animals), and manure storage management (on site or off site). The
674 linearity assumption between continuous predictors and the outcome (on a logit scale) was
675 assessed graphically. If there was evidence of non-linearity, independent variables were
676 categorized based on quartiles (Dohoo et al., 2009). The Spearman's rank correlation statistic
677 was used to identify possible collinearity between variables; if the value of the correlation was
678 greater than |0.8|, then only one of the variables was selected for inclusion in the multivariable
679 model (Dohoo et al., 2009). All independent variables that were significantly associated with the
680 outcome at $P < 0.20$ were considered for inclusion in a main effects model. These variables were
681 then fitted in a multivariable model where a manual backward elimination procedure was
682 conducted until only statistically significant ($P < 0.05$) variables and confounders were kept.
683 Model diagnostics were assessed based upon visual examination of best linear unbiased
684 predictors and residual plots. The Tukey-Kramer procedure was used to prevent inflation of
685 Type I error due to multiple comparisons. Model-adjusted means and corresponding 95%
686 confidence intervals were reported.
687 Covariance parameters estimates for pen and feedlot were evaluated from model
688 intercepts via GLMMs. The outcome variables included the number of samples that tested
689 positive for EHEC O157 or for all non-O157 EHEC serogroups, in independent models. Non-
690 O157 EHEC were collapsed due to problems of convergence due to the small number of
691 positives for some of the non-O157 EHEC serogroups. Intraclass correlation coefficients (ICC)

for EHEC O157 and non-O157 EHEC were computed for pens and feedlots (Dohoo et al., 2009), and calculated using the latent variable technique (Dohoo et al., 2009) as follows:

$$ICC\ for\ cattle\ within\ a\ pen = \frac{\sigma_p^2}{\sigma_p^2 + \sigma_f^2 + \pi^2/3}$$

$$ICC\ for\ cattle\ pens\ within\ a\ feedlot = \frac{\sigma_f^2}{\sigma_p^2 + \sigma_f^2 + \pi^2/3}$$

where σ_p^2 corresponds to the variance component for pen, σ_f^2 the variance component for feedlot, and $\pi^2/3$ corresponds to the value at which the error variance is fixed (Dohoo et al., 2009).

Results

Study population and sample collection

The overall feedlot capacity and pen size of the study feedlots ranged from 8,000 to 85,000 animals and 30 to 582 cattle/pen, respectively (Table 1). Most of the study feedlots fed beef cattle only (n = 5), one fed cull dairy only, and two fed both beef and dairy cattle. Seventy-five percent (95/126) of the study pens consisted of fed beef and 24.6% (31/126) fed dairy cattle. The number of days on feed of cattle in the study pens ranged from 11 to 382 days (Table 1). Cattle were fed a corn based finishing diet, representing 34 to 65% of the as-fed ration, with 87.5% (7/8) of the feedlots utilizing a wet distiller's grain within their ration. Direct fed microbials were fed to cattle, from arrival to harvest, in 75% (6/8) of the feedlots (Table 1). Pre-harvest food safety vaccines for *E. coli* were not used in any of the study feedlots.

A total of 1,886 samples were collected from a total of 126 pens (62 pens from study area A and 64 pens from study area B) in 8 feedlots (4 feedlots in each of the study areas) between

June and August, 2014. Of the total number of samples (n = 1,886), 100% (1,886) of samples that were tested for non-O157 EHEC and 73.9% (1,393) of samples that were tested for EHEC O157 were included in the analysis. The remaining 26.1% (493) of samples, including one entire pen, that were tested for EHEC O157, were not utilized in the analysis due to extensive microbial overgrowth on the media plates that rendered them unreadable.

Sample-level percent positives for E. coli O serogroups and EHEC

Out of the total number of fecal samples, 20.4% (384/1,886) tested positive for at least one of the six non-O157 serogroups. Serogroup-specific proportion of positives are as follows: 4.6% (86/1,886) for O26, 4.7% (88/1,886) for O45, 14.1% (266/1,886) for O103, 0.4% (7/1,886) for O111, 1.8% (34/1,886) for O121, and 1.3% (25/1,886) for O145. Overall, EHEC O157 was the most frequently isolated EHEC serogroup (18.7%) whereas fewer samples (3.3%) tested positive for non-O157 EHEC (Table 2). Among EHEC O157 positive samples, 0.0% (0/260), 56.5% (147/260), and 43.5% (113/260) tested positive for *stx*1, *stx*2, and both *stx*1 and *stx*2, respectively, while for non-O157 EHEC test-positive samples 79.4% (50/63), 11.1% (7/63), and 9.5% (6/63) tested positive for *stx*1, *stx*2, and both *stx*1 and *stx*2 virulence genes, respectively.

Crude and model-adjusted pen-level prevalence

At the pen-level, 61.9% (78/125) and 31.0% (39/126) of the pens had at least one sample testing positive for EHEC O157 and non-O157 EHEC, respectively (Table 2). The crude cumulative prevalence varied greatly between pens. More specifically, the percentage of positive samples within a pen ranged from 0.0 to 12.5% for EHEC O26, 0.0 to 6.5% for EHEC O45, 0.0 to 68.7% for EHEC O103, 0.0 to 33.3% for EHEC O111, 0.0 to 0.0% for EHEC O121, 0.0 to

41.7% for EHEC O145, and 0.0 to 83.3% for EHEC O157. Cumulative model-adjusted (95% confidence intervals) within-pen prevalence estimates of EHEC serogroups were as follows: 0.1% (0.0 - 4.5%) for EHEC O26, 0.1% (0.0 - 4.5%) for EHEC O45, 1.5% (0.0 - 4.5%) for EHEC O103, 0.1% (0.0 - 5.6%) for EHEC O111, 0.0% (0.0 - 0.0%) for EHEC O121, 0.5% (0.0 - 3.8%) for EHEC O145, and 17.2% (14.5 - 19.9%) for O157 (Figure 1).

Crude and model-adjusted feedlot-level prevalence

All study feedlots (n = 8) had at least one fecal sample testing positive for EHEC O157 or non-O157 EHEC. EHEC serogroup-specific crude prevalence percentages are as follows: 0.0% for EHEC O121; 25.0% for EHEC O26, O45, and O111; 62.5% for EHEC O145; and 100.0% for EHEC O103 and O157 (Table 2). Model-adjusted mean within-pen prevalence estimates for EHEC O157 varied significantly ($P < 0.01$) by feedlot; however, no significant differences ($P > 0.05$) were observed for prevalence of non-O157 EHEC serogroups among feedlots (Table 3).

Crude and model-adjusted study area prevalence

Each study area had at least one fecal sample testing positive for EHEC O157 or non-O157 EHEC. Cumulative model-adjusted (95% confidence intervals) within-pen prevalence estimates by study area are as follows: 16.2% (9.1 - 27.2%) for EHEC O157 and 2.7% (1.2 - 6.1%) for non-O157 EHEC in study area A, whereas study area B prevalence estimates were 18.5% (10.6 - 30.3%) for EHEC O157 and 2.1% (1.0 - 4.9%) for non-O157 EHEC. However, there were no significant differences in the prevalence of EHEC O157 ($P = 0.71$) and non-O157 EHEC ($P = 0.56$) between study areas.

Evaluation of pen- and feedlot-level risk factors

Pen-level variables significantly associated with the within-pen prevalence estimates of EHEC (EHEC O157 and non-O157 EHEC [all non-O157 grouped]) based on univariable analyses ($P < 0.20$) included sampling month, size of the pen, and sex composition of the pen for EHEC O157, and sampling month and sex composition of the pen for the non-EHEC O157 outcome (Table 4). Only sampling month remained significantly associated ($P < 0.01$) with the within-pen prevalence of EHEC O157 in the final model. Cumulative model-adjusted within-pen prevalence estimates (95% confidence intervals) for EHEC O157 were 35.4% (22.1 - 51.4%) for June, 11.4% (6.3 - 19.8%) for July, and 18.6% (10.7 - 30.4%) for August, with significant within-pen prevalence differences between June and July ($P < 0.01$), June and August ($P < 0.01$), and July and August ($P < 0.01$). Sampling month ($P < 0.01$) and sex composition of the pen ($P = 0.02$) were significantly associated with the within-pen prevalence of non-O157 EHEC. Model-adjusted within-pen prevalence estimates (95% confidence intervals) for non-O157 EHEC were 2.8% (1.1 - 7.1%) for June, 1.5% (0.6 - 3.7%) for July, and 0.6% (0.1 - 1.8%) for August, with significant within-pen prevalence differences between June and July ($P = 0.03$), June and August ($P < 0.01$), and July and August ($P = 0.04$). Model-adjusted within-pen prevalence estimates (95% confidence intervals) for non-O157 EHEC were 1.1% (0.3 - 3.8%) for heifer-based pens, 3.1% (1.3 - 7.1%) for steer-based pens, and 0.5% (0.2 - 2.8%) for mixed pens (i.e., both steers and heifers), with significant within-pen prevalence differences between steer and mixed pens ($P = 0.02$).

With non-O157 EHEC, separate models were fitted for EHEC O103 and O145. Model-adjusted mean within-pen prevalence varied significantly by month for EHEC O103 ($P = 0.03$) and EHEC O145 ($P < 0.01$). Within-pen prevalence estimates (95% confidence intervals) for

EHEC O103 were 0.1% (0.0 - 1.7%) for June, 2.1% (1.0 - 4.4%) for July, and 2.4% (1.1 - 5.4%) for August, with significant within-pen prevalence differences between June and August (P = 0.01) and between July and August (P = 0.02). Within-pen prevalence estimates (95% confidence intervals) for EHEC O145 were 1.5% (0.0 - 4.7%) for June, 0.2% (0.0 - 1.0%) for July, and 0.2% (0.0 - 1.6%) for August, with a significant difference in within-pen prevalence between June and July ($P < 0.01$).

None of the feedlot-level demographic or management variables (direct-fed microbial, distiller's percentage, feedlot capacity, and manure management) were significantly associated with the cumulative within-pen prevalence of EHEC O157 and non-O157 EHEC by feedlot in a univariable analyses (all P values > 0.20).

Intraclass correlation coefficient

The intraclass correlation coefficient (ICCs) describing the correlation between fecal samples within pens for EHEC O157 was 0.26, and was 0.31for non-O157 EHEC, indicating that most of the variability occurred between pens rather than within pens. The ICCs for EHEC O157 (0.08) and non-O157 EHEC (0.04) for samples within feedlots indicate that most of the variability occurred within rather than between feedlots. Intraclass correlation coefficients for individual non-O157 EHEC serogroups (O26, O103, O111, O121, and O145) were not computed due to lack of model convergence.

Discussion

Our findings provide insight into the regional-, feedlot-, and pen-level differences of EHEC-7 shedding in cattle feces, which enhances our knowledge of the epidemiology and

806 distribution of EHEC and provides a better understanding of the level at which pre-harvest
807 interventions could be effective. By lowering the pathogen load prior to harvest, transmission
808 along the contamination pathway (i.e., fecal to hide to carcass pathway) may be mitigated. In the
809 present study, all study feedlots had at least one sample positive for EHEC O157 and non-O157.
810 Furthermore, approximately 31% of the study pens had one or more fecal samples that tested
811 positive for non-O157 EHEC, whereas 62% of pens tested positive for EHEC O157. Although
812 multiple studies have reported non-O157 EHEC fecal prevalence estimates in cattle
813 (Cernicchiaro et al., 2013; Baltasar et al., 2014; Ekiri et al., 2014; Paddock et al., 2014;
814 Dewsbury et al., 2015), the current study provides the most comprehensive assessment of
815 feedlot- and pen-level prevalence of EHEC-7 serogroups in feces of commercial feedlot cattle,
816 prior to harvest, from several feedlots in two main U.S. cattle feeding areas.
817 Although there slight difference between demographic and management characteristics of
818 study feedlot operations, we believe these operations are fairly representative of the other
819 commercial feedlot operations in the region. The low prevalence of non-O157 EHEC found in
820 our study pens is consistent with findings from recent reports on summer-fed commercial feedlot
821 cattle, despite key differences in diagnostic methods, case definitions, and study populations
822 (Cernicchiaro et al., 2013; Dargatz et al., 2013; Baltasar et al., 2014; Ekiri et al., 2014; Dewsbury
823 et al., 2015). To date, the published literature has indicated well-established diagnostic methods
824 for detection of EHEC O157 (Osmisakin et al., 2003; LeJeune et al., 2006; Fox et al., 2008);
825 however, given the lack of a reference method of detection for non-O157 EHEC, several
826 detection methods are used which makes the comparison of prevalence estimates across studies
827 challenging (Bettelheim, 2007; Cernicchiaro et al., 2013; Baltasar et al., 2014; Ekiri et al., 2014;
828 Dewsbury et al., 2015; Noll et al., 2016). In the current study we used pooled IMS beads for

non-O157 EHEC serogroup detection. As reported by Noll et al. (2016), pooling IMS beads resulted in higher throughput of samples, and a less labor-intensive procedure which has shown to have similar detection capabilities than single IMS bead procedures.

To our knowledge, there are few studies that have reported prevalence estimates for EHEC-7 from several commercial feedlot operations in the United States. The current study demonstrated that the overall frequency of detection of non-O157 EHEC was three times lower than EHEC O157 among pens. Moreover, as also seen in previous studies (Cernicchiaro et al., 2013; Dargatz et al., 2013; Baltasar et al., 2014; Ekiri et al., 2014; Dewsbury et al., 2015), prevalence varied greatly between pens in all study feedlots for both non-O157 EHEC and EHEC O157. Although the Dargatz et al., (2013) multi-feedlot (n = 21) and multi-state (n = 4) study reported fecal prevalence of EHEC-7, prevalence was based on pooled samples from up to 40 animals prior to PCR-only confirmation. In our study, we utilized individual fecal pats that were later confirmed for the presence of EHEC-7 by both culture and PCR methods.

Despite the fact that multiple risk factors for STEC O157 prevalence have been identified in commercial feedlot cattle (Sargeant et al., 2004; Renter et al., 2005; Rangel et al., 2005; Smith et al., 2005; Fox et al., 2008; Callaway et al., 2014; Smith, 2014), associations between those risk factors with non-O157 STEC prevalence have not been yet demonstrated (Smith, 2014). In the current study, significant differences in fecal prevalence between June, July, and August were identified for EHEC O157, all non-O157 EHEC, EHEC O103 and EHEC O145. Although variation in prevalence of STEC O157 has been reported in weeks within a season (Renter et al., 2005; Smith et al., 2005), the reason behind this phenomenon is still unknown. The temporal effects of EHEC shedding in cattle cohorts may be due to animal housing, pen condition, precipitation, and temperature at time of sample collection. Additionally, our study found

852 significant differences in the overall non-O157 EHEC prevalence based on the sex composition
853 of the study pens. Potential environmental disturbances (e.g., stress, behavioral dynamics,
854 dietary differences) affecting cattle in pens of mixed sex composition may modify conditions of
855 their lower gastrointestinal tract allowing non-O157 EHEC to out-compete other organisms. In
856 contrast, feedlot management factors were not significantly associated with the prevalence of
857 EHEC-7 serogroups, likely due to either the small number of feedlots sampled (n = 8) or perhaps
858 because most of the variability occurred within versus between feedlots which prevented us to
859 detect significant differences.
860 Variance components indicated a higher variability between than within pens for both
861 fecal prevalence of non-O157 EHEC and EHEC O157; for feedlots, the variability was higher
862 within than between feedlots. Hence, pre-harvest interventions targeted at the pen-level would
863 likely have higher impact on EHEC-7 fecal shedding compared to those targeted at the feedlot-
864 level. Although Sargeant et al. (2004) reported similar results for pen and feedlot of STEC
865 O157, the effects of non-O157 STEC have not been reported.
866 We found a widespread distribution of EHEC-7 in feces from cohorts of finishing cattle
867 fed during the summer months, with EHEC O157, O103, and O145 being the serogroups most
868 frequently detected in this study population. Further, sampling month and sex composition of
869 the pen were identified as potential risk factors for non-O157 EHEC and EHEC O157. Thus, the
870 potential combination of pre-harvest interventions and pen-level management strategies may
871 have positive food safety impacts. Moreover, data on pre-harvest fecal prevalence at different
872 hierarchical levels provide necessary estimates to input into quantitative risk assessment models
873 to assess potential human risks associated with EHEC-7 along the beef chain.
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References

Bai J, Shi X, Nagaraja TG. A multiplex PCR procedure for detection of six major virulence genes in *Escherichia coli* O157:H7. *J Microbiol Methods* 2010;82:85-89.

Bai J, Paddock ZD, Shi X, Li S, An B, Nagaraja TG. Applicability of a multiplex PCR to detect the seven major Shiga toxin-producing *Escherichia coli* based on genes that code for serogroup-specific O-antigens and major virulence factors in cattle feces. *Foodborne Pathog Dis* 2012;9:541-548.

Baltasar P, Milton S, Swecker W Jr, Elvinger F, Ponder M. Shiga toxin-producing *Escherichia coli* distribution and characterization in a pasture-based cow-calf production system. *J Food Prot* 2014;77:722-731.

Bettelheim KA. (2007). The non-O157 shiga-toxigenic (verocytotoxigenic) *Escherichia coli*; under-rated pathogens. *Crit. Rev. Microbiol.* 2007;33:67-87.

Callaway TR, Edrington TS and Nisbet DJ. Meat Science and Muscle Biology Symposium: Ecological and dietary impactors of foodborne pathogens and methods to reduce fecal shedding in cattle. *J Anim Sci* 2014; 92:1356-1365.

Centers for Disease Control and Prevention (CDC). *E. coli* (*Escherichia coli*). 2014. Available at: http://www.cdc.gov/ecoli/general/index.html/, accessed November 30, 2015.

Cernicchiaro N, Cull CA, Paddock ZD, Shi X, Bai J, Nagaraja TG, Renter DG. Prevalence of Shiga toxin-producing *Escherichia coli* and associated virulence genes in feces of commercial feedlot cattle. *Foodborne Pathog Dis* 2013;10:835-841.

Cernicchiaro N, Renter DG, Cull CA, Paddock ZD, Shi X and Nagaraja TG. Fecal shedding of non-O157 serogroups of Shiga toxin-producing *Escherichia coli* in feedlot cattle vaccinated with an *Escherichia coli* O157:H7 SRP vaccine or fed a Lactobacillus-based direct-fed microbial. *J Food Prot* 2014; 77:732-7.

Dargatz DA, Bai J, Lubbers BV, Kopral CA, An B, Anderson GA. Prevalence of *Escherichia coli* O-types and Shiga-toxin genes in fecal samples from feedlot cattle. *Foodborne Pathog Dis* 2013;10:392-396.

Dewsbury DM, Renter DG, Shridhar PB, Noll LW, Shi X, Nagaraja TG, and Cernicchiaro, N. Summer and Winter Prevalence of Shiga Toxin-Producing *Escherichia coli* (STEC) O26, O45, O103, O111, O121, O145, and O157 in Feces of Feedlot Cattle. *Foodborne Pathog Dis* 2015; 12:726-32.

Dohoo I, Martine S and Stryhyn H. Measures of Association. In *Veterinary Epidemiologic Research*. Charlottetown: AVC, 2009, pp 121-138.

Ekiri AB, Landblom D, Doetkott D, Olet S, Shelver WL, Khaitsa ML. Isolation and characterization of Shiga toxin-producing *Escherichia coli* serogroups O26, O45, O103, O111, O113, O121, O145, and O157 shed from range and feedlot cattle from postweaning to slaughter. *J Food Prot* 2014;77:1052-1061.

Ferens WA and Hovde CJ. *Escherichia coli* O157:H7: Animal Reservoir and Sources of Human Infection. *Foodborne Pathog Dis* 2011; 8:465-487.

Fox JT, Renter DG, Sanderson MW, Nutsch AL, Shi X, Nagaraja TG. Associations between the presence and magnitude of *Escherichia coli* O157 in feces at harvest and contamination of preintervention beef carcasses. *J Food Protect* 2008;71:1761-1767.

Gould LH, Mody RK, Ong KL, Clogher P, Cronquist AB, Garman KN, Lathrop S, Medus C, Spina NL, Webb TH, White PL, Wymore K, Gierke RE, Mahon BE, Griffin PM. Increased recognition of non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States during 2000-2010: Epidemiologic features and comparison with *E. coli* O157 infections. *Foodborne Pathog Dis* 2013;10:453-460.

LeJeune JT, Hancock DD, Besser TE. Sensitivity of *Escherichia coli* O157 detection in bovine feces assessed by broth enrichment followed by immunomagnetic separation and direct plating methodologies. J Clin Microbiol 2006;44:872-875.

Luna-Gierke RE, Griffin PM, Gould LH, Herman K, Bopp CA, Strockbine N, Mody RK. Outbreaks of non-O157 Shiga toxin-producing *Escherichia coli* infection: USA. *Epidemiol Infect* 2014;142:2270-2280.

National Cattleman's Beef Association (NCBA). 2012. Facts for beef producers: a basic look at *E. coli* O157. Available at: http://www.beefresearch.org/CMDocs/BeefResearch/Safety_Fact_Sheets/E.Coli_Final.pdf , assessed December 29, 2015.

Noll LW, Baumgartner WC, Shridhar PB, Cull CA, Dewsbury DM, Shi X, Cernicchiaro N., Renter DG, and Nagaraja TG. Pooling of Immuunomagnetic Separation Beads Does Not

Affect Detection Sensitivity of Six Major Serogroups of Shiga Toxin–Producing *Escherichia coli* in Cattle Feces. *J Food Prot* 2016;7:59-65.

Omisakin F, MacRae M, Ogden ID, Strachan NJC. Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. *Appl Environ Microbiol* 2003;69: 2444-2447.

Paddock Z, Shi X, Bai J, Nagaraja TG. Applicability of a multiplex PCR to detect O26, O45, O103, O111, O121, O145, and O157 serogroups of *Escherichia coli* in cattle feces. *Vet Microbiol* 2012;156:381-388.

Painter JA, Hoekstra RM, Ayers T, Tauxe RV, Braden CR, Angula FJ, Griffin PM. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. *Emerg Infect Dis* 2013;19:407-415.

Possé B, De Zutter L, Heyndrickx M, Herman L. Novel differential and confirmation plating media for Shiga toxin-producing *Escherichia coli* serotypes O26, O103, O111, O145 and sorbitol-positive and –negative O157. *FEMS Microbiol Lett* 2008;282:124-131.

Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg Infect Dis* 2005;11:603-609.

Renter DG, Morris JG, Sargeant JM, Hungerford LL, Berezowski J, NGO T, Williams K, and Acheson DWK. Prevalence, risk factors, O serogroups, and virulence profiles of Shiga toxin-producing bacteria from cattle production environments. *J Food Prot* 2005;68:1556-1565.

Sargeant JM, Sanderson MW, Griffin DD and Smith RA. Factors associated with the presence of *Escherichia coli* O157 in feedlot-cattle water and feed in the Midwestern USA. Prev Vet Med 2004; 66:207-237.

964 Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin
965 PM. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis*
966 2011;17:7-15.
967 Smith DR, Moxley RA, Clowser SL, Folmer JD, Hinkley S, Erickson GE, Klopfenstein TJ. Use
968 of rope devices to describe and explain the feedlot ecology of *Escherichia coli* O157:H7
969 by time and place. *Foodborne Pathog Dis* 2005; 2:50-60.
970 Smith DR. Cattle Production Systems: Ecology of Existing and Emerging *Escherichia coli*
971 Types Related to Foodborne Illness. Annual Review of Animal Biosciences, Vol 2 2014;
972 2:445-468.
973 U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). Pre-harvest
974 management controls and intervention options for reducing *Escherichia coli* O157:H7
975 shedding in cattle. 2014. Available at:
976 http://www.fsis.usda.gov/wps/wcm/connect/d3fdd61d-924a-4dd9-a363-
977 a45b42b83163/2012-0038-A.pdf?MOD=AJPERES , accessed March 01, 2016.
978

Table 4.1 Demographic and management characteristics of study feedlots operation.

	Study Area A				Study Area B			
	Feedlots				Feedlots			
Variable, units	1	2	3	4	A	B	C	D
Feedlot capacity, animals	50,000	25,000	50,000	11,500	85,000	12,000	8,000	14,000
Average pen size (range), animals	179.7 (68-234)	134.9 (55-283)	184.9 (98-279)	66.4 (30-113)	341.1 (235-582)	56.2 (39-119)	196.7 (50-297)	142.8 (60-225)
Average days on feed (range), days	319.1 (283-368)	278.1 (146-364)	151.8 (94-230)	174.7 (135-279)	55.2 (28-110)	317.4 (116-382)	68.1 (11-157)	125.1 (79-179)
Type of cattle[1]	Dairy	Beef	Beef	Both	Beef	Both	Beef	Beef
Manure Storage[2]	On site	Off site	On site	On site	Off site	On site	Off site	On site
Ration percentage of distillers, %	10-30	<10	10-30	10-30	10-30	>30	10-30	>30
Fed DFM product[3]	No	Yes	No	Yes	Yes	Yes	Yes	Yes

[1] Type of Fed cattle: Dairy = Holstein, Beef = Cross-bred cattle, or Both = Dairy and Beef

[2] Manure storage: on site (pen-floor fecal material stored at the feedlot) and off site (pen-floor fecal material is not stored at the feedlot)

[3] Cattle were fed a direct fed microbial (DFM) product as reported by the feedlot manager at each visit: Yes (animals received a DFM product) and No (indicates no DFM was fed)

Table 4.2 Cumulative sample-, pen-, and feedlot-level percent positive of EHEC O26, O45, O103, O111, O121, O145, and O157, per study area[1] and overall

EHEC	EHEC positives, n (%)								
	Sample-level[2]			Pen-level[3]			Feedlot-level[4]		
	Study Area A (n=926)	Study Area B (n=960)	Overall (n=1,886)	Study Area A (n=62)	Study Area B (n=64)	Overall (n=126)	Study Area A (n=4)	Study Area B (n=4)	Overall (n=8)
O26	1 (0.1)	3 (0.3)	4 (0.2)	1 (1.6)	2 (3.1)	3 (2.4)	1 (25.0)	1 (25.0)	2 (25.0)
O45	0 (0.0)	4 (0.4)	4 (0.2)	0 (0.0)	4 (6.3)	4 (3.2)	0 (0.0)	2 (50.0)	2 (25.0)
O103	26 (2.8)	12 (1.3)	38 (2.0)	12 (19.4)	11 (17.2)	23 (18.3)	4 (100.0)	4 (100.0)	8 (100.0)
O111	4 (0.4)	2 (0.2)	6 (0.3)	1 (1.6)	1 (1.6)	2 (1.6)	1 (25.0)	1 (25.0)	2 (25.0)
O121	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
O145	3 (0.3)	8 (0.8)	11 (0.6)	3 (4.8)	4 (6.3)	7 (5.6)	3 (75.0)	2 (50.0)	5 (62.5)
EHEC	Sample-level[2]			Pen-level[3]			Feedlot-level[4]		
	Study Area A (n=683)	Study Area B (n=710)	Overall (n=1,393)	Study Area A (n=61)	Study Area B (n=64)	Overall (n=125)	Study Area A (n=4)	Study Area B (n=4)	Overall (n=8)
O157	117 (17.1)	143 (20.1)	260 (18.7)	33 (54.1)	45 (70.3)	78 (62.4)	4 (100.0)	4 (100.0)	8 (100.0)

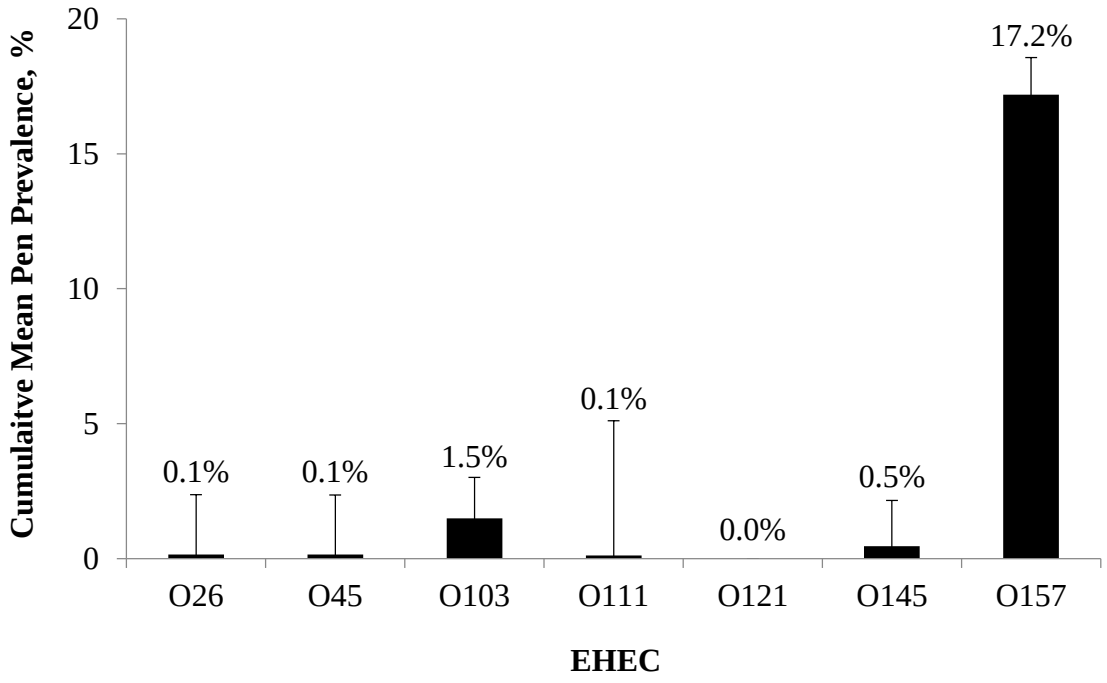
[1] Study Area A comprised 4 feedlots in a 150-mile area in northwest Texas and Study Area B comprised 4 feedlots in a 100-mile area in central Nebraska

[2] Cumulative sample-level percent positives was calculated as the number of samples that tested positive for each EHEC, divided by the total number of samples tested

[3] Cumulative pen-level percent positives was calculated as the number of pens that had at least one positive sample for each EHEC, divided by the total number of pens sampled

[4] Cumulative feedlot-level percent positives was calculated as the number of feedlots that had at least one positive sample for each EHEC, divided by the total number of study feedlots

Figure 4.1 Cumulative model-adjusted[1] within-pen prevalence estimates of EHEC O26, O45, O103, O111, O121, O145, and O157 in cattle feces. Error bars indicate the upper limit of the 95% confidence interval of model-adjusted prevalence mean estimates.



[1] From generalized linear mixed models using a binomial distribution, logit link and random intercepts for sampling week and feedlot within study area

Table 4.3 Model-adjusted mean within-pen prevalence of EHEC O26, O45, O103, O111, O121, O145, and O157 in cattle feces, by feedlot operation and study area

	EHEC Prevalence, % (SEM)[1]							
	Study Area A				Study Area B			
	Feedlots				Feedlots			
EHEC	1	2	3	4	A	B	C	D
O26	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.4 (0.4)	0.0 (0.0)	0.0 (0.0)	1.3 (0.7)	0.0 (0.0)
O45	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.3 (0.7)	0.4 (0.4)
O103	4.5 (3.0)	1.1 (0.9)	1.7 (1.3)	1.6 (1.3)	0.4 (0.5)	0.3 (0.3)	1.6 (1.3)	2.0 (1.5)
O111	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.7 (0.8)	0.0 (0.0)	0.0 (0.0)	0.8 (0.6)	0.0 (0.0)
O121	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
O145	0.0 (0.0)	0.5 (0.5)	0.4 (0.4)	0.4 (0.4)	0.0 (0.0)	0.0 (0.0)	1.3 (0.7)	2.1 (0.9)
O157	10.1 (5.0)	7.7 (4.4)	25.8 (10.0)	23.5 (9.5)	15.8 (7.1)	25.6 (9.9)	5.4 (3.2)	39.1 (12.5)

[1] From generalized linear mixed models using a binomial distribution, logit link, fixed effect for feedlot and random effect for sampling week,

SEM: Standard error of the mean

Table 4.4 Cumulative model-adjusted[1] mean within-pen fecal prevalence estimates of EHEC O157 and non-O157 EHEC for pen-level demographic variables tested in univariable models

	EHEC O157		Non-O157 EHEC	
Variable	Prevalence % (95% CI)	*P*-value	Prevalence % (95% CI)	*P*-value
Sampling month[2]		< 0.01		< 0.01
June	35.4 (22.1-51.4)		5.0 (2.2-10.8)	
July	11.4 (6.3-19.8)		2.8 (1.3-6.1)	
August	18.6 (10.7-30.4)		1.1 (0.4-2.8)	
Sex composition of pen[3]		0.11		< 0.01
Heifer	13.6 (6.4-26.6)		0.7 (0.1-3.1)	
Steer	17.0 (9.0-29.7)		2.9 (1.0-8.2)	
Mixed	23.3 (11.8-40.7)		0.5 (0.1-2.3)	
Pen Size (animals/pen)[4]		0.07	-	-
< 100	14.5 (7.3-26.7)			
100-200	22.2 (12.1-37.0)			
> 200-300	15.2 (7.9-27.4)			
> 300	19.3 (7.5-41.3)			

[1] From generalized linear mixed models (GLMM) using a binomial distribution and logit link

[2] GLMM included a fixed effect for month and a random intercept for feedlot within study area

[3] GLMM included a fixed effect for sex composition of the pen and random intercepts for sampling week and feedlot within study area

[4] GLMM included a fixed effect for pen size and random intercepts for sampling week and feedlot within study area

Chapter 5 - Conclusions

Several conclusions on the epidemiology of Shiga toxin-producing *Escherichia coli* serogroups (O26, O45, O103, O111, O121, O145, and O157; STEC-7) in commercial feedlot cattle can be made based on the literature review and the subsequent research described within this dissertation. Previous research has focused more on the epidemiology of STEC O157 than non-O157 STEC in commercial feedlot cattle, yet both pose a public health risk due to the potential transmission along the fecal to hide to carcass pathway. Further, understanding and mitigation of this transmission pathway requires knowledge of both the STEC concentration and prevalence at the time and place of sampling along this pathway. In general, post-harvest interventions seem to be effective at reducing STEC-7 from plant entry to final product, yet previous research suggests that pre-harvest interventions for STEC O157 could be important in reducing the pathogen load prior to harvest. In addition, data regarding the effectiveness of pre-harvest interventions against fecal shedding of STEC-7 and subsequent performance impacts required additional evaluation in commercial feedlot operations. Review of the literature indicates that additional cattle studies are needed to develop a better understanding of the epidemiology of STEC-7 and evaluate potential risk factors associated with shedding of STEC-7 in cattle feces.

We used two distinct study designs to evaluate the impact of commercially available pre-harvest interventions and develop a better understanding of the epidemiology of STEC-7 in commercial feedlot cattle. The first study design was an experimental randomized pen-level field trail in a large commercial feedlot, which allowed us to evaluate the efficacy of pre-harvest interventions against fecal shedding of STEC O157 (first study in this dissertation) and quantify

the corresponding impacts of pre-harvest interventions on cattle performance (second study in this dissertation was a continuum of the efficacy study). Overall, we indicated that there were no significant interactions between the commercially available vaccine (SRP® vaccine) and the direct-fed microbial (DFM; Bovamine®) in either study. This is important because it indicates that beef producers (or their clients) can't expect added benefits of using both products. However, we believe that we were the first to demonstrate that the two dose regimen of the SRP vaccine reduced fecal prevalence of STEC O157 and prevalence of high shedders, but negatively impacted performance (e.g., dry matter intake and weight gain) when compared to non-vaccinated pens (e.g., cattle were not re-handled or given a placebo). The low dose DFM had no effect on fecal prevalence of STEC O157 and prevalence of high shedders, but positively impacted performance (e.g., increased total gain and gain to feed ratio). Although these commercially available products, SRP® vaccine and Bovamine®, indicated differential effects for efficacy and cattle performance, these results should provide practical information for end-users (e.g. feedlot and packing plant operators) to utilize prior to making operational decisions for pre-harvest implementation. Yet, our work was built upon the idea that pre-harvest interventions may not only complement post-harvest interventions, but further decrease the public health concerns and costly beef recalls by reducing the overall STEC O157 fecal load prior to plant entry. However, we understand that the ecology and epidemiology of STEC O157 in cattle is complex and additional information is warranted, due to the multiple risk factors impacting the transmission, environmental survival, and widespread shedding differences among cattle, pens, feedlots, and regions. Additionally, continual education, research and surveillance are required to enhance our knowledge, detection, and reduction methods of STEC O157. However, the beef

industry has the opportunity to strive to reduce human illnesses and beef recalls caused by these foodborne pathogens through pre-harvest interventions.

The second study design was an observational cross-sectional study that occurred in eight commercial feedlot operations from two distinct cattle feeding areas (e.g., Texas and Nebraska) during the summer months. In this study, we reported a widespread distribution of EHEC-7 in cattle feces, with EHEC O157, O103, and O145 being the serogroups most frequently detected in this study population. Although EHEC O157 was much more frequently detected, both EHEC O157 and non-O157 EHEC were present in all feedlot operations. Fecal shedding of EHEC O157 and non-O157 EHEC varied over-time, but there was no evidence for a difference between study areas. In addition, fecal shedding was not associated with feedlot-level management factors. However, we found that most of the variability in prevalence occurred at the pen-level. Thus, the potential combination of pre-harvest interventions and management strategies applied at the pen-level may have the greatest potential impacts on EHEC in the beef chain. Moreover, our findings provide insight into the regional-, feedlot-, and pen-level differences of EHEC-7 shedding in cattle feces, which enhances our knowledge of the epidemiology and distribution of EHEC and provides a better understanding of the level at which pre-harvest interventions could be effective. By lowering the pathogen load prior to harvest, transmission along the fecal to hide to carcass pathway could be mitigated. However, additional studies are required to further evaluate the relationship of fecal prevalence with hide contamination and subsequent carcass contamination at the cohort levels. Further, the ability to identify risk factors as potential control points may enable the ability to positively impact public health concerns and food safety by reducing the fecal load in the feedlot production environment.

Overall, the research described in this dissertation provides an assessment of the efficacy and production impacts of commercially available pre-harvest interventions and provides multi-level prevalence estimates of STEC-7 in commercial feedlot operations. Previous research indicates that pre-harvest interventions for STEC O157 may have the ability to complement post-harvest interventions and decrease the risk of STEC transfer to beef at harvest; however, there are limited data illustrating the impact of a single pre-harvest intervention or the combination of pre-harvest interventions on STEC-7 prevalence at each step (e.g., fecal shedding, hide contamination, carcass contamination, and beef at the grocery store) along the beef production system. Although there is a growing body of literature for pre-harvest interventions, additional data is required for several of these interventions to determine the efficacy of these products in commercial feedlot operations with different attributes, including differences in biosecurity, cattle source, cattle handling practice, diet, geographic location, and season. Data regarding the concentration, prevalence, and transmission of STEC-7 within cohorts of cattle are necessary to improve the knowledge on the epidemiology of STEC-7. Finally, there is a need for a more comprehensive understanding of the economic impacts of implementing pre-harvest interventions in commercial feedlot operations, as there are currently no well-defined mechanisms (e.g., government policies, cattle marketing incentives, and/or consumer demands) to mitigate the cattle producers financial burden associated with implementing pre-harvest interventions.