EPIDEMIOLOGY OF *SALMONELLA* AND *E. COLI* O157 IN BEEF CATTLE PRODUCTION SYSTEMS

by

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B.S., University of Missouri, 1988
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Food Science

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Abstract

Salmonella and *Escherichia coli* O157 are important causes of foodborne illness in humans and have been associated with the consumption of undercooked, contaminated beef. Individual feedlot cattle may shed these organisms in their feces and subsequently contaminate cattle hides and carcasses at harvest. Preharvest and harvest interventions may significantly decrease the risk of beef contamination and subsequent risk of human illness. Previous research suggests that preharvest interventions for *Salmonella* or *E. coli* O157 may compliment harvest interventions and reduce the risk of carcass contamination. In my research, I used diverse study designs to develop a better understanding of the epidemiology of *Salmonella* and *E. coli* O157 and evaluate the impact of specific preharvest interventions in commercial feedlot cattle. A randomized controlled trial indicated that a commercially available vaccine did not affect the fecal prevalence of *Salmonella*, or health and performance of cohorts of feedlot cattle. However, the fecal prevalence of *Salmonella* varied by cohort, suggesting cattle source as a risk factor. In a repeated cross-sectional study, the fecal prevalence of *Salmonella* in cattle at feedlot arrival was not associated with the prevalence immediately prior to harvest, yet specific *Salmonella* subtypes, as defined by pulsed-field gel electrophoresis (PFGE), persisted throughout the feeding period. Another of my studies defined and compared PFGE subtypes of *E. coli* O157 isolated from cattle feces and carcass samples at harvest to determine relationships between fecal shedding and carcass contamination. Truckload appeared to be an important factor, and feces from cattle shedding both high- and low-concentrations of *E. coli* O157 posed a risk for carcass contamination. A stochastic Monte-Carlo modeling framework was later used to assess the impact of seasonal fecal prevalence and combinations of preharvest interventions on the risk of carcass contamination with *E. coli* O157. Results indicated that it may be important to incorporate multiple preharvest interventions, especially during periods of high fecal prevalence of *E. coli* O157. Overall, the research described in this dissertation demonstrates that multiple risk factors and interventions at the cohort level must be considered in order to mitigate the risks associated with *Salmonella* and *E. coli* O157 in beef production systems.

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David G. Renter
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Table of Contents

List of Figures

List of Tables

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Preface

Salmonella and *Escherichia coli* O157 are significant threats to public health. Several epidemiologic aspects of *Salmonella* and *E. coli* O157 in beef cattle production systems are unclear, and some preharvest interventions for these bacteria have not been evaluated in commercial feedlot settings. We purposefully used diverse study designs to develop a better understanding of the epidemiology of *Salmonella* and *E. coli* O157, evaluate preharvest interventions, and provide a unique and relatively broad research experience. The studies described in this dissertation provided training in veterinary epidemiology, food microbiology, and risk analysis. One study was a randomized controlled trial of a potential preharvest intervention for *Salmonella*. Two of the four studies enhanced previously collected data and employed pulsed-field gel electrophoresis (PFGE) to determine the genetic relatedness of specific strains of *E. coli* O157 and *Salmonella* across time or sample type. One study used parameters derived from a systematic search of scientific literature to build a Monte-Carlo model to predict risk of cattle carcass contamination with *E. coli* O157 at harvest. These diverse scientific approaches ensured a comprehensive training experience while providing valuable epidemiologic information regarding *E. coli* O157 and *Salmonella* in beef cattle production systems.

My first study, a randomized controlled trial, was an ideal study design for investigating the effects of a commercially available *Salmonella* vaccine in commercial feedlot cattle. My second study was a repeated cross-sectional study that investigated associations among fecal prevalence of *Salmonella* in cattle at feedlot arrival and immediately prior to harvest. The persistence of specific *Salmonella* subtypes within cohorts during the feeding period, as well as cattle heath and performance, were also evaluated. My third study was a cross-sectional study that determined the genetic relatedness of *E. coli* O157 isolates from cattle feces and carcass samples at harvest to determine relationships between fecal shedding and carcass contamination within truckload. My final study used a stochastic Monte-Carlo modeling framework to assess the impact of seasonal fecal prevalence and combinations of preharvest interventions on the risk of cattle carcass contamination with *E. coli* O157. Overall, the research described in this dissertation demonstrates the complex interrelationships among cattle management factors (including cattle source and transport groups), targeted interventions and microbial persistence

that must be considered in order to mitigate the risks associated with *Salmonella* and *E. coli* O157 in beef production systems.

CHAPTER 1 - Review of the Epidemiology of *Salmonella* and *Escherichia coli* O157 in Beef Production Systems from Feedlot to Harvest

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Introduction

Salmonella enterica spp. *enterica* (hereafter *Salmonella*) and *Escherichia coli* O157:H7 (hereafter *E. coli* O157) are important causes of foodborne illness in humans and have been associated with the consumption of undercooked, contaminated beef (Riley *et al.*, 1983; Wells *et al.*, 1983; Rodrigue *et al.*, 1995; Dechet *et al.*, 2006; McLaughlin *et al.*, 2006). *Salmonella* and *E. coli* O157 have been found in several food production environments (Michino *et al.*, 1999; Maki, 2009), including at various stages within beef production systems (Rodrigue *et al.*, 1995; Barkocy-Gallagher *et al.*, 2003). Individual feedlot cattle may shed these organisms in feces and subsequently contaminate hides and carcasses within a cohort of cattle at harvest (Beach *et al.*, 2002; Woerner *et al.*, 2006). Preharvest and harvest interventions for either organism may significantly decrease the risk of beef contamination and subsequent risk of foodborne illness in people (Losinger *et al.*, 1997; House *et al.*, 1998; Berry *et al.*, 2010). Generally, if preharvest interventions for *Salmonella* and/or *E. coli* O157 are effective, pathogen-load thresholds of harvest interventions may not be exceeded and the risk of carcass contamination may be reduced (Brichta-Harhay *et al.*, 2008; Arthur *et al.*, 2010). Single and multiple preharvest interventions may compliment harvest interventions and reduce foodborne illness risks associated with *Salmonella* and *E. coli* O157 in beef production systems.

There is a growing body of scientific literature regarding the epidemiology of *Salmonella* or *E. coli* O157, and several observational studies have investigated these organisms within the context of commercial feedlot cattle environments (Van Donkersgoed *et al.*, 1999; Barham *et al.*, 2002; Kalchayanand *et al.*, 2009). There are several similarities in the epidemiologic approach of mitigating *Salmonella* and *E. coli* O157 in beef cattle production systems, yet there remain several differences in available interventions, proportional attribution of respective foodborne illness to beef products, regulatory surveillance, and available data to guide future research and/or develop effective control strategies. This review of scientific literature on *Salmonella* and *E. coli* O157 in beef cattle production systems is primarily limited to observational studies and randomized controlled trials (RCTs) that were conducted within commercial feedlot and harvest settings. Although multiple serotypes of *Salmonella* will be discussed, other non-O157 serotypes of Shiga toxin-producing *E. coli* are only briefly mentioned. Preharvest interventions for both organisms are discussed, yet harvest and post-harvest interventions are not emphasized. Pertinent pathogenesis is discussed, while virulence factors, microbial physiology, and detection methods are not addressed. This overview of the ecology and epidemiology of *Salmonella* and *E. coli* O157 in beef cattle production systems exposes critical information gaps and sets the stage for the studies described in subsequent chapters.

E. coli O157 in Human Foodborne Illness

Esherichia coli O157 was recognized as a cause of human foodborne illness in 1982, when two outbreaks of hemorrhagic colitis were associated with the consumption of undercooked, contaminated ground beef (Riley *et al.*, 1983; Wells *et al.*, 1983). Trends in data regarding the frequency of *E. coli* O157 foodborne illness since this time reveal that *E. coli* O157 remains a serious public health risk because of both the severity and frequency of cases; these trends may have been impacted by changes in surveillance (e.g., sensitivity of detection methods). Over ten years ago, *E. coli* O157 was reported to cause approximately 73,480 human illnesses each year in the US, including 2,168 hospitalizations and 61 deaths (Mead *et al*., 1999). From 1982 to 2002, 47% of 183 *E. coli* O157 foodborne illness outbreaks, which represented 44% of 5,269 *E. coli* O157 foodborne illness cases, were associated with the consumption of beef products (Rangel *et al*., 2005). Most of these beef-related *E. coli* O157 outbreaks (87.2%) and cases (75.8%) were associated with ground beef. In 2006, there were 27 recognized

outbreaks of foodborne illness due to *E. coli* O157, which was similar to the annual number (mean = 24) of outbreaks from 2001 – 2005 (CDC, 2009). In 2009, there was approximately one (mean = 0.99) human case of *E. coli* O157 from any source per 100,000 people annually in the US (CDC, 2010). Incidence was highest in children less than four years of age (3.84/100,000). The incidence of *E. coli* O157 foodborne illness decreased 41% (95% CI 27 – 52%) from 1998 to 2009 (CDC, 2010). Although the frequency of foodborne illness due to all sources of *E. coli* O157 appears to have decreased, the severity of these infections justifies the classification of *E. coli* O157 as a relatively high public health risk.

Although the frequency of *E. coli* O157 infections is lower than some other foodborne illnesses (e.g., *Campylobacter*, *Salmonella*, and *Shigella*), complications from *E. coli* O157 can be severe (CDC, 2010). As reviewed by Griffin et al. (1991), hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), and hemorrhagic colitis (HC) are complications of *E. coli* O157 infections in humans (Griffin *et al.*, 1991). Approximately 90% of *E. coli* O157 infections may result in HC (Slutsker *et al.*, 1998), while the more serious HUS occurs in approximately 7% of *E. coli* O157 infections, usually about five to ten days after the onset of symptoms (Coia, 1998). Hemolytic uremic syndrome, which is a leading cause of acute renal failure in children, causes death in approximately 5% of HUS patients (Coia, 1998). Surveillance data indicate that there is a higher risk of complications attributed to *E. coli* O157 infection in young (less than five years of age), elderly, and immunocompromised individuals (Griffin *et al.*, 1991). In 2009, there were 1.4 cases of HUS from any cause per 100,000 US children less than five years of age (CDC, 2010). The proportion of *E. coli* O157 cases that were hospitalized was highest (59.4%) among people who were 50 years of age or older (CDC, 2010). The case fatality risk in the overall US population was reported by Coia (1998) to be approximately 0.03, and preliminary FoodNet data for 10 US states in 2009 revealed that the case fatality risk in persons aged 50 years or greater (0.015) was higher than other age groups (Coia, 1998; CDC, 2010). In 2003, the estimated health-associated cost of *E. coli* O157 in the US was $405 million (Frenzen *et al.*, 2005). *Escherichia coli* O157 remains a frequent and severe cause of human foodborne illness.

Salmonella in Human Foodborne Illness

When reviewed by Mead et al. over ten years ago, there were an estimated 1.3 million foodborne illnesses and over 500 deaths attributed to *Salmonella* in the US each year (Mead *et al*., 1999). *Salmonella* were the cause of over 30% of all bacterial food-related deaths in the United States (Mead *et al*., 1999). In 2007, there were approximately 14.92 cases of salmonellosis per 100,000 people (CDC, 2008). In 2009, there were an average of 15.19 reported human cases of salmonellosis per 100,000 people, which was approximately 10 times the annual incidence of *E. coli* O157 (0.99 cases per 100,000 people) (CDC, 2010). Annual incidence of salmonellosis was highest (72.93/100,000) in children less than four years of age (CDC, 2010). Attribution data are limited and the relative proportion of salmonellosis cases attributed to beef products versus other products is not clear (Batz *et al.*, 2005; Pires *et al.*, 2009). In Denmark during 2000-2001, only 0.9% of human salmonellosis cases were confirmed to be associated with the consumption of beef products, while 37.6% were attributed to egg consumption (Hald *et al.*, 2007). Guo et al. (2007) used an attribution calculation model based upon this same work by Hald et al. (2007) and estimated 19% of human salmonellosis cases from 1998-2003 in the US could be attributed to ground beef products, while 41% could not be attributed to any of the meat or egg categories in their model (Guo *et al.*, 2007). Generally, human salmonellosis remains one the most common bacterial foodborne illnesses and the consumption of contaminated beef remains an important contributor.

The clinical signs of foodborne illness due to *Salmonella* range from moderate to severe. Although most cases entail a self-limiting enterocolitis, some infected individuals need to be hospitalized, and further complications can occur, such as aseptic reactive arthritis and Reiter's syndrome (D'Aoust *et al.*, 2007). Antimicrobial resistance also is an important concern in managing *Salmonella* infections. Multidrug-resistant *Salmonella* infections have been associated with increased frequency of sepsis and hospitalization (Lee *et al.*, 1994; Varma *et al.*, 2005). Multidrug-resistant *Salmonella* also have been found in cattle feces (Kunze *et al.*, 2008; Alam *et al.*, 2009) and beef products (White *et al.*, 2001); multidrug-resistant *Salmonella* also have been associated with beef-related outbreaks (Zansky *et al.*, 2002; Dechet *et al.*, 2006; CDC, 2006_a). Based on hospitalization and fatality risks of 0.20 and 0.006, respectively, and extrapolating for unreported cases of human salmonellosis, Voetsch et al. estimated that *Salmonella* caused 14,860 hospitalizations and 415 deaths annually during 1996 – 1999 in the US (Voetsch *et al.*, 2004). The proportion of *Salmonella* cases that were hospitalized was higher (45.2%) among people

who were older than 50 years of age (CDC, 2010). The case fatality risk in the overall US population was approximately 0.006 during 1996-1999 (Kennedy *et al.*, 2004), and preliminary FoodNet data for 10 US states in 2009 revealed that the case fatality risk in persons aged 50 years or greater (0.0121) was higher than other age groups (CDC, 2010). The incidence of *Salmonella* foodborne illness decreased 10% (95% CI 3-16%) from 1998 to 2009 (CDC, 2010). These data suggest that *Salmonella* foodborne illnesses create considerable burden because of their frequency and severity.

Ecology and Epidemiology of *E. coli* O157

The body of scientific literature regarding the microbiology, ecology and epidemiology of *E. coli* O157 was limited following the first *E. coli* O157 foodborne outbreak in 1982; however, pathogenic and nonpathogenic *E. coli* bacteria have been studied for decades. Many *E. coli* bacteria are considered non-pathogenic, commensal bacteria in the human gastrointestinal tract. *Escherichia coli* are commonly serologically differentiated by three surface antigens: somatic (O), flagellar (H), and capsular (K) (Meng *et al*., 2007). Diarrheagenic *E. coli* are categorized into several pathotypes, including enteropathogenic, enterotoxigenic, enteroinvasive, diffuse-adhering, enteroaggregative, and enterohemorrhagic *E. coli* (Meng *et al*., 2007). *Escherichia coli* O157 is classified as enterohemorrhagic *E. coli* and is often described by the ability to produce Shiga toxin; hence, *E. coli* O157 is one of several Shiga toxin-producing *E. coli* (STEC) serotypes. Shiga toxin is largely responsible for the virulence of *E. coli* O157 and several pathogenic serotypes of STEC (e.g., *Esherichia coli* serotypes O26, O45, O103, O111, O12, and O145) (Brooks *et al.*, 2005). Many non-O157 Shiga toxin-producing *E. coli* foodborne pathogens have been linked to beef and associated with human foodborne illness (Bettelheim, 2007; Dambrosio *et al*., 2007; Cobbold *et al*., 2008; Ethelberg *et al*., 2009). The scientific literature now includes several studies of the epidemiology of non-*E. coli* O157 STECs (Bettelheim, 2007; Renter *et al.*, 2007; Hedican *et al.*, 2009); future interventions and regulatory actions also may target these *E. coli* serotypes.

Escherichia coli O157 has been found in several food production systems. Produce-associated outbreaks were first recognized in 1991 (Rangel *et al.*, 2005). Foodborne illness outbreaks have been associated with several vegetable products, including alfalfa sprouts (Breuer *et al*., 2001), spinach (CDC, 2006$_b$), lettuces (Hilborn *et al*., 1999), apple cider and juice (Besser

et al., 1993), and coleslaw (Rangel *et al*., 2005). Other outbreaks have been associated with water sources, including lake water (Bruce *et al*., 2003) and drinking water (Swerdlow *et al*., 1992; Licence *et al*., 2001). Cattle-associated products have been linked to several *E. coli* O157 outbreaks, including from unpasteurized milk (Keene *et al*., 1997; Bhat *et al*., 2007), cheese and butter from unpasteurized milk (Rangel *et al*., 2005), and dry fermented salami (Tilden *et al*., 1996). In particular, several outbreaks have been linked to ground beef (Bell *et al.*, 1994; Slutsker *et al.*, 1998; Rangel *et al.*, 2005). Although the public health risk for *E. coli* O157 may include several food production systems, the pathway within beef production systems is of primary importance in this review.

The epidemiology of *E. coli* O157 in cattle is relatively unique; cattle appear to become colonized, particularly in the terminal rectum, yet do not exhibit clinical signs (Low *et al.*, 2005; Sheng *et al.*, 2006). Individual asymptomatic cattle shed *E. coli* O157 in their feces for different periods of time and at different concentrations. One study showed that 63% of individual cattle shed for less than one month (Besser *et al*., 1997). More recent studies have indicated there may be a relationship between the duration and concentration of fecal shedding, and that there may be differences in the level of colonization in the animals' terminal rectum (Low *et al.*, 2005; Cobbold *et al.*, 2007; Chase-Topping *et al.*, 2008). Furthermore, fecal shedding trends at the cohort level may be seasonal, with higher fecal shedding prevalence peaks occurring in the warmer seasons (Barkocy-Gallagher *et al.*, 2003; Renter *et al.*, 2008; Stephens *et al.*, 2009). Fecal shedding of *E. coli* O157 has been positively associated with contamination of cattle hides and carcasses at harvest (Fox *et al.*, 2008; Jacob *et al.*, 2010), and cattle shedding higher concentrations of *E. coli* O157 in the feces likely pose a higher risk of hide and carcass contamination (Omisakin *et al.*, 2003; Arthur *et al.*, 2009). Hence, studies of the effects of preharvest control strategies may need to investigate both prevalence of shedding cattle as well as the prevalence of cattle shedding *E. coli* O157 at higher concentrations.

Super-shedders or high-shedders have been defined as cattle shedding at least 10^4 CFU *E. coli* O157/g feces (Chase-Topping *et al*., 2008). Some researchers propose that mitigation efforts should target high-shedding cattle since they may contribute the most to hide and carcass contamination risk (Matthews *et al*., 2006; Cobbold *et al*., 2007; Fox *et al*., 2008; Stephens *et al*., 2009). A particular animal may remain a high-shedder for an unknown period, yet the presence or proportion of high-shedders within a cohort may be important in minimizing the risk of

carcass contamination at harvest (Matthews *et al.*, 2006). Regardless, high prevalence of *E. coli* O157 in feces within a cattle cohort and the presence of individual cattle shedding at high concentrations are likely both important contributors to the risk of hide and carcass contamination at slaughter. Further studies to clarify the relationship between fecal prevalence and fecal concentration of *E. coli* O157 at the cohort-level will help guide future control efforts. The best mitigation strategy(s) for the beef industry is not clear; the industry could primarily attempt to decrease preharvest fecal prevalence of a cohort, or identify and manage high-shedders prior to harvest, or both. Epidemiologic studies properly designed to trace the transmission of *E. coli* O157 from cattle feces to hides to carcasses within a cohort may guide mitigation efforts.

The transport-to-harvest and lairage phases of beef production systems also are important in the transmission of *E. coli* O157 within cohorts of cattle. Some studies have shown an apparent decrease in fecal and hide prevalence from feedlot to harvest (Dewell *et al.*, 2008; Fegan *et al.*, 2009), while some have shown an increase (Childs *et al*., 2006; Woerner *et al*., 2006). Arthur et al. (2007) reported an 87% increase in positive hides from pre-transport to hide removal at harvest. Interestingly, only 29% of *E. coli* O157 hide isolates obtained at harvest matched, as defined by pulsed-field gel electrophoresis (PFGE), isolates obtained before transport in this study (Arthur *et al.*, 2007). This finding suggests that transport and lairage environments may provide additional sources of *E. coli* O157 that contribute to hide contamination at harvest. Regardless of pathogen source, effective interventions for *E. coli* O157 may exist for the transport and lairage phases, yet more data are needed to understand these effects.

Hides are the most likely proximate source of carcass contamination with *E. coli* O157 (Loneragan *et al*., 2005). Hide interventions (e.g., hide washing, hide removal procedures) appear to mitigate some of the hide to carcass transfer (Elder *et al*., 2000), yet the relationship of hide concentration and hide prevalence in this transfer is unclear. The hide prevalence to carcass prevalence ratio derived from studies of commercial harvested feedlot cattle in the US has ranged from 0.5 to 0.84 (Woerner *et al*., 2006; Fegan *et al*., 2009), yet these estimates are based upon overall mean prevalence. Jacob et al. (2010) found a cohort (truck-load) level hide to carcass prevalence ratio of 0.26 (range 0 – 1.25) (Jacob *et al.*, 2010). Another study calculated a hide to carcass transfer of *E. coli* O157 based upon changes in microbial concentration, but these

data were derived from measuring aerobic plate counts from cull-cow samples at harvest (Brichta-Harhay *et al.*, 2008). More individual animal- and cohort-level data are needed to accurately describe the relationship among fecal prevalence, fecal concentration, hide prevalence, hide concentration, carcass prevalence, and carcass concentration of *E. coli* O157.

Ecology, and Epidemiology of *Salmonella*

Although a causative agent was unknown in the early 19th century, French doctors observed cases of gastrointestinal disease in humans caused by a bacillus; this condition later became known as typhoid fever (D'Aoust *et al.*, 2007). In 1885, Salmon and Smith isolated *Salmonella enterica* serotype Choleraesuis from swine with hog cholera (Le Minor, 1981). Today, there are over 2,500 recognized serotypes of *Salmonella* (Popoff *et al.*, 2004), and some are considered to be host adapted (Uzzau *et al.*, 2000). For example, *Salmonella* serotype Typhi is a putative human-adapted species and not transmitted by animals, while other *Salmonella enterica* serotypes are often transmitted by animals. *Salmonella* serotype Dublin is the only serotype considered to be host adapted to cattle (Uzzau *et al.*, 2000), yet many other *Salmonella* serotypes have been isolated from cattle (Fedorka-Cray *et al.*, 1998; Dargatz *et al.*, 2003; Callaway *et al.*, 2008).

There are over 1,504 serotypes within *Salmonella enterica* subsp. *enterica*, but relatively few are commonly isolated from animals and humans (D'Aoust *et al.*, 2007). As reviewed by Callaway et al. (2008), the most frequent *Salmonella* serotypes isolated from ground beef, according to the US Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS), were Montevideo, Anatum, Muenster, Newport, and Mbandaka (Callaway *et al.*, 2008). According to the 2006 *Salmonella* Annual Summary of the Centers for Disease Control and Prevention (CDC), the most common *Salmonella* serotypes isolated from both healthy and clinically ill cattle (in order of descending frequency) were Newport, Orion var. 15+ 34+, Dublin, Montevideo, Typhimurium, Agona, and Anatum (CDC, 2008). In 2006, *Salmonella* serotypes Typhimurium and Newport were, respectively, the first and third most frequent *Salmonella* serotypes isolated from human cases (CDC, 2008). These data only represent isolates that were obtained from human samples submitted to state public health laboratories or animal (both healthy and clinically ill) samples submitted to the USDA Animal and Plant Health Inspection Services (APHIS) National Veterinary Services Laboratories (NVSL). *Salmonella*

serotypes that are common in both cattle and human infections are likely important to public health, although the reasons for commonality are unknown.

Salmonella are ubiquitous and have been found in several food production systems including vegetables, poultry, and beef. Produce-associated outbreaks include alfalfa sprouts (Van Beneden *et al.*, 1999), tomatoes (Cummings *et al.*, 2001), cantaloupe (Mohle-Boetani *et al.*, 1999), unpasteurized orange juice (Cook *et al.*, 1998), mangos (Sivapalasingam *et al.*, 2003), peanut butter (CDC, 2008), spinach (CDC, 2006b), lettuce (Horby *et al.*, 2003) and peppers (CDC, 2008). *Salmonella* serotype Enteritidis has primarily been associated with poultry meat and eggs (Louis, 1988; Mishu *et al.*, 1994) and is one of the most common serotypes in poultry (Antunes *et al.*, 2003), but also has been found in raw almonds, mixed salad, peanut sauce, and orange juice (D'Aoust *et al.*, 2007). *Salmonella* have been found in cattle feces (Barkocy-Gallagher *et al.*, 2003; Rodriguez *et al.*, 2006; Kunze *et al.*, 2008; Cummings *et al.*, 2009) and several outbreaks of salmonellosis in people have been linked to the consumption of beef products (CDC, $1995_{a;}$ CDC, $1995_{b;}$ Dechet *et al.*, 2006; McLaughlin *et al.*, 2006; CDC, 2006_{a}). The ubiquitous nature of *Salmonella* and large variety of associated foods make it difficult to assess the probability of human illness from the consumption of beef products; however, recent beef-related outbreaks justify the examination of interventions in beef production systems.

The contamination pathway of *Salmonella* in beef production systems is similar to that of *E. coli* O157. Infected cattle shed *Salmonella* in their feces, which can subsequently contaminate hides and carcasses within a cattle cohort (Beach *et al.*, 2002; Sorensen *et al.*, 2002; Fegan *et al.*, 2005; Brichta-Harhay *et al.*, 2008). Little is known about fecal shedding dynamics of *Salmonella* in commercial feedlot cattle; counts of *Salmonella* in grass-fed and feedlot cattle feces taken at harvest in Australia ranged from <3 MPN g^{-1} to 3 x 10^3 MPN g^{-1} (Fegan *et al.*, 2004). The fecal prevalence in feedlot cattle may be higher in warmer months (Barkocy-Gallagher *et al.*, 2003), and the fecal prevalence of *Salmonella* may be lower in feedlot cattle that have been on feed longer (Galland *et al.*, 2000). However, national data from the National Animal Health Monitoring System (NAHMS) Cattle on Feed Evaluation (COFE) revealed that 5.5% of 4,977 fecal samples from 100 feedlots were positive for *Salmonella,* and the fecal prevalence in feedlot cattle that had been on feed longer (7.4%) was significantly higher than the prevalence in cattle that had been on feed for a shorter time (3.5%) (Fedorka-Cray et al., 1998). More studies are needed to determine the distribution of *Salmonella* prevalence and

concentration in feedlot cattle feces, the persistence of *Salmonella* within cohorts of cattle, transport and lairage dynamics, and associations with hide and carcass contamination.

Comparative Epidemiology of *Salmonella* and *E. coli* O157

There are several notable differences and similarities in the epidemiology of *Salmonella* and *E. coli* O157. The human foodborne illness risk of these two bacteria may be difficult to compare, especially risk that can be attributed to beef products. The 2009 annual incidence of reported *Salmonella* infections in people in the US was approximately 15/100,000 people, compared to *E. coli* O157 infections, which was approximately 1/100,000 people (CDC, 2010). However, characterization of the risk should also convey the aforementioned severity of the disease and proportion of cases attributable to beef products. Although *E. coli* O157 infections are less frequent overall, the relatively higher attribution to beef-associated products and complications such as HUS have justified considerable focus on risk mitigation efforts for *E. coli* O157 in the beef industry. *Escherichia coli* O157 has been more frequently associated with beef recalls than *Salmonella* (FSIS, 2010). However, antimicrobial resistance in *Salmonella* can add to the severity of human cases of salmonellosis (Lee *et al.*, 1994). Assessing the comparative risk of *E. coli O157* and *Salmonella*, particularly in beef products, is difficult; both are critical threats to public health.

Antimicrobial resistance in human *E. coli* O157 infections has been documented, but it has not been a large concern (Schroeder *et al.*, 2002). The use of antibiotics in humans infected with *E. coli* O157 is often not recommended, since bacteriocidal antibiotics may lyse the cell walls and increase the release of Shiga toxins. In a prospective cohort study of children infected with *E. coli* O157, children who were treated with antibiotics were approximately 17 times more likely (RR = 17.3, CI = 2.2 – 137) to acquire HUS than those who did not, although a majority of study patients (87.3%) did not receive antibiotic therapy (Wong *et al.*, 2000).

Contrary to management of *E. coli* O157 infections, antimicrobial therapy and antimicrobial resistance are important for the management of human salmonellosis. As reviewed by Alcaine et al. (2007), foodborne illness cases and outbreaks due to multidrug-resistant *Salmonella* are a growing concern (Alcaine *et al.*, 2007). *Salmonella* serotypes such as Newport and Typhimurium, common in both cattle and human *Salmonella* infections, have expressed multidrug resistance (Zhao *et al.*, 2003; Dechet *et al.*, 2006). Several serotypes of *Salmonella*

that have been isolated from cattle have exhibited antimicrobial resistance; particularly to tetracycline, sulfamethoxazole and other common antibiotics (Dargatz *et al.*, 2002; Dargatz *et al.*, 2003). Davis et al. (2007) found that *Salmonella* Dublin isolates from beef cattle had less resistance to ampicillin, ceftazidime, kanamycin, neomycin, streptomycin, and tetracycline than isolates from dairy cattle; they suggested their finding may have been due to a higher use of antibiotics in dairy production systems (Davis *et al.*, 2007). Although antimicrobial resistance may be a lesser concern with *E. coli* O157, antimicrobial resistance appears to be a driving force in public health concerns with *Salmonella* in beef products.

While *E. coli* O157 bacteria are generally considered non-pathogenic in cattle (Cray *et al.*, 1995; Dean-Nystrom *et al.*, 1997), several *Salmonella* serotypes are considered pathogens and capable of causing gastroenteritis, abortion, and other clinical manifestations in cattle (Gay *et al.*, 1993; House *et al.*, 1998; Alam *et al.*, 2009; Cummings *et al.*, 2009). Particular *Salmonella* serogroups or serotypes may be associated with increased pathogenicity in cattle (e.g., serogroup B), but more data are needed to understand these relationships (Alam *et al.*, 2009). Salmonellosis may contribute to poor health and performance in dairy cattle systems (Cummings *et al.*, 2009; Cummings *et al.*, 2010), although few data exist to support this association in commercial feedlot cattle (Alam *et al*., 2009). *Salmonella* are also likely contributors to morbidity and mortality in feedlot cattle (House *et al.*, 1998; Alam *et al.*, 2009, Losinger *et al.*, 1997). The health and performance impact of *Salmonella* in cattle may provide a direct economic incentive for the industry to expend intervention resources, while the preharvest control of *E. coli* O157 may not garner the direct financial incentives to producers unless beef processors reward the use of preharvest interventions.

The comparative persistence of *Salmonella* and *E. coli* O157 within the cattle feedlot environment is difficult to assess because of the ubiquitous nature and multiple sources of both bacteria in cattle production systems. *Salmonella* were able to survive over 150 days in experimentally inoculated cattle feces (Sinton *et al.*, 2007), and Franz et al. (2005) determined that *Salmonella* Typhimurium could survive over 133 days in cattle feces (Franz *et al.*, 2005). *E. coli* O157 can also survive in experimentally inoculated feces for up to 70 days (Wang *et al.*, 1996) and in manure slurry for up to 21 months (Kudva *et al.*, 1998). Water troughs and cattle feed also are likely sources of *Salmonella* and *E. coli* O157 within the feedlot environment (Hancock *et al.*, 1998; LeJeune *et al.*, 2001; Van Donkersgoed *et al.*, 2001; Davis *et al.*, 2003;

Dodd *et al.*, 2003; Sargeant *et al.*, 2003). Wildlife and flies also may be important vectors for *E. coli* O157 and *Salmonella*, especially as mechanisms for persistence, in cattle production environments (Hancock *et al.*, 1998; Kobayashi *et al.*, 1999; Olsen *et al.*, 2000; Kirk *et al.*, 2002; Renter *et al.*, 2006). Since feedlot cattle can defecate approximately 15 kg of feces per day (Barker *et al.*, 2002) and shed concentrations over 10^3 or 10^4 CFU *E. coli* O157 or *Salmonella*/g feces (Fegan *et al.*, 2004; Fegan *et al.*, 2005; Arthur *et al.*, 2009), non-cattle pathogen sources are likely to contribute relatively less to feedlot environment contamination. More data are needed to better understand the persistence of *E. coli* O157 and *Salmonella* within cohorts of feedlot cattle.

Some cross-sectional studies have shown a negative association between the fecal prevalence of *Salmonella* and *E. coli* O157 within cohorts of cattle. Smith et al. (2005) noticed this apparent inverse relationship when manila rope devices were used to assess the presence of *Salmonella* and *E. coli* O157 within feedlot cattle cohorts (Smith *et al.*, 2005; Smith *et al.*, 2005). Cohorts of cattle were less likely (OR = 0.58, CI 0.41 – 0.83) to be positive for *E. coli* O157 when they were positive for *Salmonella*. This negative association should not imply a specific cause; the effect could be attributed to a microbiological interaction between the two organisms within individual animals, microbial competition in samples during the enrichment phase of the isolation protocol, or different risk factors for these organisms at the cohort level. A randomized controlled trial designed to determine the effects of feeding distiller's grains and dry-rolled corn on the pen-floor fecal prevalence of *E. coli* O157 and *Salmonella* in feedlot cattle did not reveal any association between the prevalence of these organisms (Jacob *et al.*, 2009), but low pathogen prevalence and small sample size may have provided low statistical power to detect such an association had it existed. Future studies that measure the fecal prevalence and concentration of both organisms at individual- and cohort-levels are needed to investigate potential associations between *Salmonella* and *E. coli* O157 in beef production systems.

Preharvest and Harvest Interventions for *E. coli* O157 and *Salmonella*

Several preharvest interventions may have similar effects on the fecal shedding of *Salmonella* and *E. coli* O157 in feedlot cattle. Since both genera are facultative anaerobic gram-negative bacteria of the family Enterobacteriaceae, effective non-specific preharvest control strategies that target both organisms may be plausible. Improvements in cattle pen hygiene,

including water troughs and feed bunks, and biosecurity measures could impact the prevalence of both *Salmonella* and *E. coli* O157; yet data showing significant effects of environmental measures in cattle feedlots are limited (Smith *et al.*, 1997). Furthermore, cattle feedlots have limited, realistic capabilities for preventing the introduction of pathogens and controlling exposure to pathogens (Brandt *et al.*, 2008). Some have argued that preharvest interventions could be negated by cross contamination that occurs during transport and lairage (Koohmaraie *et al.*, 2007), yet some studies have reported a lower prevalence of *E. coli* O157 on cattle hides (Fegan *et al.*, 2009) and feces (Dewell *et al.*, 2008) at harvest compared to pre-transport to harvest. Some dietary preharvest interventions that target facultative anaerobic bacteria, such as feeding sodium chlorate, may be effective in controlling both *Salmonella* and *E. coli* O157 in cattle (Anderson *et al.*, 2000), but further studies are needed to assess these effects. Preharvest interventions that are effective in mitigating the risks of both *E. coli* O157 and *Salmonella* may help further prevent foodborne illnesses associated with consumption of contaminated beef products.

Current dietary interventions for *E. coli* O157 in cattle have been reviewed (Jacob *et al.*, 2009; Berry *et al.*, 2010), but relatively less is known regarding dietary interactions or interventions for *Salmonella* in cattle (Losinger *et al.*, 1997). The colonization of cattle with *E. coli* O157 may be affected by diet-related conditions (e.g., volatile fatty acid concentration and pH) within the gastrointestinal tract (Depenbusch *et al.*, 2008). Potential dietary interventions for *E. coli* O157 in cattle include modifications in grain type (Buchko *et al.*, 2000; Jacob *et al.*, 2009) and concentrate:forage ratios (Tkalcic *et al.*, 2000), feeding seaweed products (e.g., *Ascophyllum nodosum)* (Braden *et al.*, 2004), and phenolic compounds (Jacob *et al.*, 2009). However, feeding 2% *Ascophyllum nodosum* did not appear to lower the fecal prevalence of *Salmonella* in feedlot cattle (Braden *et al.*, 2004). More studies are needed to develop a better understanding of the impact of dietary interactions and interventions on *E. coli* O157 and *Salmonella* in beef cattle production systems.

The effect of direct-fed microbials (DFMs) in reducing the preharvest fecal prevalence of *E. coli* O157 in cattle has been reviewed (Callaway *et al.*, 2004), but few data on the effect of DFMs on *Salmonella* exist in the scientific literature. Direct-fed microbials are cattle feed additives that contain viable microorganisms, commonly *Lactobacillus* spp. bacteria, and are considered to potentially improve animal performance and reduce fecal shedding of *E. coli* O157

in feedlot cattle (Brashears *et al.*, 2003). Reported efficacy estimates from studies in commercial feedlot settings range from 21% to 74% reduction in fecal shedding of *E. coli* O157 (Brashears *et al.*, 2003; Elam *et al.*, 2003; Younts-Dahl *et al.*, 2004; Woerner *et al.*, 2006; Peterson *et al.*, 2007; Stephens *et al.*, 2007; Tabe *et al.*, 2008; Arthur *et al.*, 2010; Cernicchiaro *et al.*, 2010). Recent studies have not shown an effect of DFMs on the fecal shedding of *Salmonella* in cattle (Stephens *et al.*, 2007; Tabe *et al.*, 2008). Existing data on the effects of DFMs in the preharvest control of *E. coli* O157 in feedlot cattle support the use of these products, but further evaluations of DFMs in a variety of commercial feedlot settings may be needed.

Bacteriophages are bacteriocidal viruses and obligate parasites of their host bacteria. Natural bacteriophages that target *E. coli* O157 have been found in feedlot cattle feces, manure, and water troughs (Callaway *et al.*, 2006; Niu *et al.*, 2009). Applying bacteriophage to the rectoanal junction as well as in drinking water has been shown to reduce *E. coli* O157 shedding in cattle (Sheng *et al.*, 2006). Oral administration of bacteriophage may be more effective and practical than rectal administration (Rozema *et al.*, 2009). Since there may be differences in the lytic ability of specific bacteriophages, combinations (cocktails) may be effective preharvest control strategies for *E. coli* O157 in cattle but further research is needed (Niu *et al.*, 2009). The potential for bacteriophage as a preharvest intervention for *Salmonella* in feedlot cattle remains unclear, but bacteriophages have shown effectiveness in reducing *Salmonella* colonization in broiler chickens (Atterbury *et al.*, 2007). If further studies provide evidence of significant efficacy of these bacteriocidal products, bacteriophages may become commercially available.

As reviewed by others, sodium chlorate has been investigated as a preharvest intervention for *E. coli* O157 in feedlot cattle (Loneragan *et al.*, 2005; LeJeune *et al.*, 2007), and also has potential effects in controlling *Salmonella* (Anderson *et al.*, 2000). Enterobacteriaceae are facultative anaerobes that use oxygen for aerobic respiration, but undergo fermentation during anaerobic conditions. However, the respiratory nitrate reductase of *E. coli* O157 and *Salmonella* can allow respiration and covert chlorate to cytotoxic chlorite ions inside the bacteria. Since many other anaerobes do not have nitrate reductase, sodium chlorate is considered a selectively bactericidal product that can target *E. coli* O157 and *Salmonella* without affecting these other anaerobes. In a small *in vivo* experiment, chlorate treatment reduced *E. coli* O157 in cattle without altering total anaerobic bacteria counts in feces (Callaway *et al.*, 2002). An *in vitro* study using bovine rumen fluid suggested that chlorate was bactericidal to *E. coli* O157 and

Salmonella Typhimurium DT104 but not other anaerobic bacteria (Anderson *et al.*, 2000). Although an acidified solution of sodium chlorate is approved for use in poultry processing (CFR, 2010), it is not yet commercially available as a cattle feed additive. The commercial availability of feed-grade sodium chlorate as a preharvest intervention in feedlot cattle may depend on the results of future studies.

The use of antibiotics in the preharvest control of *E. coli* O157 and *Salmonella* remains controversial. Although salmonellosis in individual cattle may be treated with antibiotics, concerns over antimicrobial-resistant *Salmonella* bacteria preclude the prophylactic use of antibiotics to control the prevalence of *Salmonella* in asymptomatic cattle (House *et al.*, 1998). Neomycin sulfate can decrease the fecal shedding of *E. coli* O157 in cattle (Elder *et al.*, 2002; Woerner *et al.*, 2006); however, neomycin sulfate is an aminoglycoside antibiotic that is closely related to several antibiotics frequently used in human medicine. Hence, it is approved to treat bacterial gastrointestinal infections in cattle, but not to control *E. coli* O157. Because of the risk of contributing to antimicrobial resistance in livestock and human foodborne pathogens, antimicrobials for preharvest control of *Salmonella* and *E. coli* O157 is not likely a viable consideration.

Vaccines are commercially available for the control of *E. coli* O157 and *Salmonella* in cattle. There are two types of vaccines for *E. coli* O157 in cattle; one vaccine technology targets the siderophore receptor and porin protein (SRP®) and another targets the type III secreted protein mechanisms of *E. coli* O157 bacteria. The *E. coli* O157 SRP® vaccine stimulates cattle to produce antibodies against the siderophore receptors and porin proteins of *E. coli* O157 bacteria, which are needed to transport iron into *E. coli* O157 cells. Without an active iron transport mechanism, these cells die from lack of iron because iron is required for normal bacterial cell metabolism. The vaccine has been shown to reduce fecal prevalence and concentration of *E. coli* O157 in inoculated calves (Thornton *et al*., 2009), but has not been evaluated extensively in commercial feedlot settings (Fox *et al.*, 2009; Thomson *et al.*, 2009). The Type III secreted protein vaccine works on the principle that Type III secreted proteins are required for *E. coli* O157 colonization of the terminal rectum in cattle. In previous studies, vaccinated cattle were less likely to shed *E. coli* O157 in the feces and less likely to be colonized in the terminal rectum (Peterson *et al.*, 2007; Moxley *et al.*, 2009; Smith *et al.*, 2009; Smith *et al.*, 2009). The SRP® technology also has been used to produce a vaccine for *Salmonella*

Newport in cattle. This *Salmonella* Newport SRP® vaccine may generate cross protection from other *Salmonella* serotypes and one study showed a decrease in the fecal prevalence of *Salmonella* in cull dairy cows (Loneragan *et al.*, 2009). Two other studies demonstrated no significant effect of the *Salmonella* Newport SRP® vaccine on fecal shedding of *Salmonella* in dairy cattle (Heider *et al.*, 2008; Hermesch *et al.*, 2008). However, one of these studies did demonstrate higher milk production and lower somatic cell counts during the first 30 days of lactation in cattle administered the *Salmonella* Newport SRP® vaccine than in cattle not administered the vaccine (Hermesch *et al.*, 2008). The reported efficacy of *E. coli* O157 vaccines also has varied, ranging from no significant effect (Van Donkersgoed *et al.*, 2005) to studies with significant effects and vaccine efficacy up to 85% (Potter *et al.*, 2004; Peterson *et al.*, 2007; Moxley *et al.*, 2009; Smith *et al.*, 2009; Thomson *et al.*, 2009). Further investigations evaluating the effects of the *Salmonella* Newport SRP® and *E. coli* O157 vaccines are needed to determine the impact of these vaccines in the preharvest control of *Salmonella* and *E. coli* O157 in beef production systems.

Several harvest-level interventions for *Salmonella* and *E. coli* O157 appear to be effective in reducing the risk of beef contamination. Although seldom considered a contamination intervention, the Humane Slaughter Act of 1958 mandates slaughter methodology that is further detailed in the Code of Federal Regulations (2005). Food Safety and Inspection Service inspectors prevent non-ambulatory or sick animals from entering the harvest process, and cattle in lairage and movement corridors are kept calm and ensured safe footing. These steps help minimize hide contamination and safeguard cattle welfare. Several good manufacturing processes (GMPs) are endorsed by regulatory agencies and the beef processing industry that provide specific guidance in preventing the contamination of beef products throughout the harvest process. As reviewed by Fung et al. (2008), established guidelines for proper stunning, exsanguination, hide removal, bung tying, evisceration, and carcass handling/storage are critical points in preventing carcass contamination (Fung *et al.*, 2008). Harvest interventions to prevent or mitigate contamination of cattle carcasses include hide washing, knife trimming, vacuuming, steam pasteurization, carcass rinsing, and spray chilling (Nutsch *et al.*, 1997; Phebus *et al.*, 1997; Huffman, 2002; Fung *et al.*, 2008). Although harvest interventions are not the focus of this literature review, these methods and technologies are critical in reducing the risk of carcass contamination.

Substantial research has focused on cattle hide washing and carcass rinsing as harvest interventions for microbial pathogens. Most hide wash systems involve cabinets that deliver a high volume of high-pressure water for a specified amount of time as the hide-on carcass moves through the processing line (Bosilevac *et al.*, 2005; Arthur *et al.*, 2007). Studies in commercial harvest facilities have reported a significant hide wash effect on reducing *E. coli* O157 on hides; reported hide wash efficacy has ranged from 39 to 62% reduction in the prevalence of *E. coli* O157 on cattle hides (Bosilevac *et al.*, 2004; Bosilevac *et al.*, 2005; Arthur *et al.*, 2007). Carcass rinsing generally involves spraying carcasses with hot water, organic acids or quaternary ammonium compounds (e.g., cetylpyridinium chloride, lactic acid). Although carcass rinse studies often use different rinses and methods, reported carcass rinse efficacies range from 35% to 98% in reducing the prevalence of *E. coli* O157 (Elder *et al.*, 2000; Arthur *et al.*, 2004; Bosilevac *et al.*, 2006). *Escherichia coli* O157 appears to be a common model for hide wash or carcass rinse intervention studies. Few studies have examined the effects of harvest interventions on *Salmonella* prevalence on hides and carcasses, but these interventions are likely to have similar effects on these gram negative organisms. An *in vitro* hide wash study showed that aerobic plate counts and Enterobacteriaceae counts were reduced by 2.1 and 3.4 log CFU/100 cm^2, respectively, following a simulated hide wash using sodium hydroxide and a proprietary surfactant (Bosilevac *et al.*, 2005). Bosilevac et al. (2004) demonstrated a 1.1 log CFU/100 cm^2 reduction in Enterobacteriaceae counts on carcasses following hide wash using 1% cetylpyidinium chloride in a commercial beef processing plant (Bosilevac *et al.*, 2004). Future studies need to quantify effects of multiple intervention hurdles upon the risk of carcass contamination, measured by both prevalence and concentration, given various cattle hide pathogen loads of *Salmonella* and *E. coli* O157 before harvest. Improvements in cattle hide washing and carcass rinsing may significantly reduce the risk of contamination of beef products.

The feasibility of single or multiple preharvest interventions for *E. coli* O157 and *Salmonella* may depend on their ability to reduce the colonization of cattle so that the effective thresholds of harvest interventions are not exceeded. Little is known about the impact of preharvest interventions on risk of contamination of carcasses in conjunction with harvest interventions. Factorial study designs to assess preharvest intervention combinations may not be parsimonious approaches to elucidate multiple-hurdle preharvest control strategies. Mathematical simulation modeling may be another method to assess the impacts of multiple

interventions within a complex system and may clarify assumptions about empirical data (Hethcote, 2009). Simulation models of preharvest and harvest interventions for *E. coli* O157 in beef production systems have been published (Wood *et al.*, 2007; Signorini *et al.*, 2009; Signorini *et al.*, 2010). Ayscue et al. (2009) examined the population dynamics of *E. coli* O157 within feedlot cattle pens based upon modeled *E. coli* O157 habitats in cattle, water, feed, and the remaining feedlot pen environment (Ayscue *et al.*, 2009). Another study used economic simulation modeling to assess risk of *E. coli* O157 foodborne illness in humans and associated costs following vaccination of feedlot cattle for *E. coli* O157 (Withee *et al.*, 2009). A recent study in Argentina utilized Monte-Carlo risk analysis techniques to assess the risk of human illness from STEC based on postharvest interventions (Signorini *et al.*, 2009). Models of these complex biological systems can provide important information, yet their usefulness can be limited by model framework and input parameter selection. Regardless, mathematical models may be a feasible way to investigate complex relationships in the epidemiology of *Salmonella* and *E. coli* O157 in beef production systems.

Regulatory Agency Efforts to Mitigate Risk of *E. coli* O157 and *Salmonella* within Beef Production Systems

Federal regulation of the beef industry promotes reduction in contamination risk in retail beef products. Regulation merely oversees industry practices to mitigate this risk; regulatory agencies are not exclusively responsible for risk outcomes (Taylor *et al.*, 2001). Inherently, regulatory agencies don't assume responsibility; rather, they allocate responsibility to the industry (Dodd *et al.*, 2009). Regulatory agencies invoke industry action by sampling and testing products, providing guidance of approved processing practices, and allowing financial and marketing consequences through public reporting of foodborne pathogen testing (Withee *et al.*, 2008). In response, often preemptively, beef processors conduct their own foodborne pathogen testing and implement multiple processing-level interventions.

Although the USDA FSIS does not have the authority to demand product recalls when a product has been contaminated with a foodborne pathogen such as *Salmonella* or *E. coli* O157, FSIS has achieved industry action by requesting voluntary product recalls as a means to protect public health (FSIS, 2006). Typically, beef processors incur high costs during a recall; consumer risk awareness is heightened and demand for beef products often declines (Marsh *et al.*, 2004).

Hence, meat recalls not only provide incentive to improve manufacturing processes and prevent contamination of beef, but they also provide a reasonable method to protect consumers from high contamination risk. The proper communication of this risk is paramount to creating realistic expectations of regulatory agencies by consumers, retailers, and other stakeholders (Dodd *et al.*, 2009).

Ground beef processing inherently increases the risk of contamination by increasing the surface area of the product available to contamination. *Escherichia coli* O157 was declared an adulterant in ground beef in 1994, allowing beef processors and retailers to be held legally liable for the sale of beef products contaminated with *E. coli* O157. Although *Salmonella* has not been declared an adulterant in beef, FSIS still establishes performance standards and tests for the pathogen in randomly selected beef products. Regardless of adulterant declaration status, the presence of either organism in beef products can constitute immediate action, including discard or recall of potentially contaminated products. The Food Safety and Inspection Service has conducted a formal risk assessment for *E. coli* O157 but not for *Salmonella* in ground beef (FSIS, 2001). The risk assessment justifies the need for regulatory oversight and reviews the scientific literature to characterize the risk of *E. coli* O157 in ground beef. Formal risk assessments also may be needed for *Salmonella* and non-O157 STEC in ground beef in order to guide regulatory policies concerning these risks.

Conclusion

This review of the scientific literature regarding the epidemiology of *Salmonella* and *Escherichia coli* O157 in the preharvest phase of beef cattle production exposes critical knowledge gaps in intervention efficacy and the resulting relationship among fecal, hide, and carcass prevalence and concentration of these pathogens. In order to identify and validate preharvest and harvest interventions that may significantly decrease the risk of beef contamination and subsequent risk of human illness, future research needs to determine the important contributors to these risks. Understanding the transfer of *E. coli* O157 and *Salmonella* from cattle feces to hides to carcasses likely depends on knowledge of both pathogen prevalence and concentration at each step in the contamination pathway. Previous research suggests that preharvest interventions for *Salmonella* or *E. coli* O157 may compliment harvest interventions and reduce the risk of carcass contamination, yet data are limited on the impact of preharvest

interventions on the prevalence and concentration of these organisms throughout the contamination pathway. Several prehavest interventions have not been sufficiently evaluated in commercial feedlot settings with different geographical, animal source, hygiene, and seasonal variables. Furthermore, combinations of preharvest interventions used as multiple hurdles in the contamination pathway have not been evaluated. Data regarding the transmission and persistence of *E. coli* O157 and *Salmonella* within cohorts of cattle are sparse. Further cohort-level studies are needed to develop a better understanding of the epidemiology of *Salmonella* and *E. coli* O157 and evaluate the impact of specific preharvest interventions in commercial feedlot cattle. Since observational studies and randomized controlled trials studies of multiple variables are often not feasible, stochastic Monte-Carlo modeling also may be needed to assess the impact of combinations of preharvest interventions on the risk of cattle carcass contamination. This epidemiologic research may facilitate significant improvements in the preharvest control of *E. coli* O157 and *Salmonella* in beef production systems and ultimately reduce the risk of human foodborne illness.

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CHAPTER 2 - Evaluation of the Effects of a Commercially Available *Salmonella* Newport Siderophore Receptor and Porin Protein Vaccine on Fecal Shedding of *Salmonella* Bacteria and Health and Performance of Feedlot Cattle

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Abstract

Objective—To evaluate the effects of a *Salmonella* Newport siderophore receptor and porin protein (SRP) vaccine on cattle health and performance and on the prevalence of fecal shedding of *Salmonella* bacteria in commercial feedlot cattle.

Animals—1,591 beef cattle.

Procedures—Cattle were randomly allocated within a replicate (n = 10 replicates [20 total pens]), administered 2 mL of a *Salmonella* Newport SRP vaccine (n = 795 cattle) or a placebo (796), and revaccinated approximately 21 days after the first administration. Health and performance data were recorded by trained feedlot personnel who were blinded to treatment. Fresh fecal samples (n = 25) were collected from pen floors on days 0, 60, 120, and within 24 hours of harvest and were subjected to selective *Salmonella* spp culture and serotyping by laboratory personnel who were blinded to treatment. Pen-level mixed models were used to analyze data.

Results—Significant differences in fecal prevalence of *Salmonella* bacteria or any health and performance variables were not detected between vaccinated and control cattle. *Salmonella* bacteria were recovered from all 10 replicates, and cumulative prevalence estimates ranged from

1.5% to 22%. Overall prevalence of fecal shedding of *Salmonella* bacteria was 10.2% and 10.9% in vaccinated and control cattle, respectively. Overall morbidity was 34.8% for both vaccinated and control cattle. Overall mortality risks were 1.9% and 1.1% for vaccinated and control cattle, respectively.

Conclusions and Clinical Relevance—In this setting, administration of the *Salmonella* Newport SRP vaccine in feedlot cattle had no effect on fecal prevalence of *Salmonella* bacteria or cattle health and performance.

Abbreviations

ADG	Average daily gain
CI	Confidence interval
CG	Cost of gain
F:G ratio	Feed-to-gain ratio
SRP	Siderophore receptor and porin protein

Introduction

Salmonella bacteria are ubiquitous in several food production systems and environments (Rodriguez *et al.*, 2006). Certain *Salmonella* serotypes are a significant cause of foodborne illness in humans and cause over 30% of foodborne deaths associated with bacterial infection in the United States (Mead *et al*., 1999). *Salmonella* bacteria are acquired by the fecal-oral route of transmission, and a few major human outbreaks have been associated with consumption of *Salmonella*-contaminated beef (CDC, 1995a; CDC, 1995b; CDC, 2003; CDC, 2006a). *Salmonella* bacteria can be found on cattle hides and in the feces of cattle (Barham *et al*., 2002; Beach *et al*., 2002; Fegan *et al.*, 2005), which may lead to the contamination of beef products during the harvest process.

Infection with *Salmonella* bacteria can cause clinical or subclinical illness in cattle that may increase production costs. A paucity of evidence exists for the clinical or subclinical effects of *Salmonella* infections in feedlot production systems (Wray *et al.*, 2000; Smith, 2002; Alam *et al.*, 2009). Infected animals may be asymptomatic, yet still shed *Salmonella* bacteria in their feces; therefore, asymptomatic cattle may serve as a reservoir for transmission to uninfected cattle (or other mammals) (Smith, 2002). Preharvest intervention programs that would reduce

the prevalence of *Salmonella* bacteria in the feedlot environment may enhance the safety of beef and, in addition, may improve the health and performance of cattle (Smith *et al.*, 1997). While production management factors may affect the prevalence of *Salmonella* bacteria in the feces of cattle (Losinger *et al.*, 1997; Smith *et al.*, 1997), it is critical that new strategies are developed to effectively minimize the risks associated with this genus of bacteria (Callaway *et al.*, 2008). A novel vaccine technology[a] that uses siderophore receptors and porin proteins of *Salmonella* bacteria was recently described (Stevens *et al.*, 2005) and has the potential to control the prevalence of *Salmonella* bacteria in several animal species.

This novel vaccine technology makes use of an iron transport mechanism of gram-negative bacteria, which is unique to certain bacterial species but is potentially conserved among *Salmonella* serotypes (Payne *et al.*, 1978; Neilands, 1995). The method of action of the vaccine is to induce the production of antibodies against siderophore receptors and porin proteins that are located on the outer membrane of certain gram negative bacteria. Once anti-siderophore receptor and anti-porin protein antibodies bind to the corresponding outer membrane proteins, bacteria will be unable to transport iron across the cell membrane. Because iron is critically important for cell homeostasis, bacteria will die as a result of a lack of iron caused by the inhibition of iron transport mechanisms (Kingsley *et al.*, 1995).

A commercially available vaccine[a] that has the SRP technology incorporated into its formulation (*Salmonella* Newport SRP vaccine) is approved for use in cattle for the control of fecal shedding and disease associated with *Salmonella enterica* subsp. *enterica* serovar Newport. Previous authors (Heider *et al.*, 2008; Hermesch *et al.*, 2008) have discussed anecdotal reports suggesting the use of this vaccine is effective for the control of clinical salmonellosis in dairy cattle. One study (Loneragan *et al.*, 2009) has indicated that the prevalence of *Salmonella* bacteria in the feces of cull dairy cows that were administered the *Salmonella* Newport SRP vaccine (7.6%) was significantly lower than that of the prevalence in cull dairy cows that were not administered the vaccine (39.2%). However, another study (Heider *et al.*, 2008) demonstrated no significant effect of the administration of the *Salmonella* Newport SRP vaccine on subclinical shedding of *Salmonella* bacteria in the feces of dairy cattle. Another study (Hermesch *et al.*, 2008) in dairy cattle did not demonstrate a significant effect of the administration of the *Salmonella* Newport SRP vaccine on fecal shedding, but did demonstrate a production-enhancing effect in cattle administered this vaccine; milk production was 3% higher

and somatic cell counts were 30% lower during the first 30 days of lactation in cattle administered the vaccine than in cattle not administered the vaccine. In addition, SRP technology has been used to reduce the shedding of *Escherichia coli* O157:H7 in the feces of cattle (Thornton *et al.*, 2009).

To the authors' knowledge, studies investigating the effects of the administration of the *Salmonella* Newport SRP vaccine in beef cattle housed in feedlot production environments do not exist. The purpose of the randomized and blinded trial reported here was to evaluate the effects of the *Salmonella* Newport SRP vaccine[a] on cattle health and performance and prevalence of shedding of *Salmonella* bacteria in the feces of vaccinated feedlot cattle.

Materials and Methods

Cattle

An approximately 30,000-head capacity commercial feedlot in south central Kansas was selected for the location of the study. A sufficient number of cattle (n = 1,591) to fill 20 pens was purchased for inclusion in the study. Feeder calves that had mean weight of 227 to 250 kg were procured through typical industry means used by the participating feedlot and arrived in 16 truckloads to the feedlot between October 16, 2008 and October 25, 2008. Cattle originated from livestock markets and ranches located in Kansas, Oklahoma, South Dakota, and Texas. All cattle were managed according to the feedlot's standard health, feeding, and management protocols that were developed and applied at the discretion of trained feedlot personnel and the consulting veterinarian and nutritionist. Cattle were fed a ration from a series of 4 step-up diets, which were primarily comprised of alfalfa hay, distillers grains, and steam-flaked corn, from the time of entry (receiving) to harvest (finishing); the 4 sequential step-up diets had roughage-concentrate proportions of 46%-54%, 31%-69%, 12%-88%, and 6%-94%, respectively. Individual animal weights were measured and recorded on days 0 and 21. Pen weights were collected at feedlot arrival and preharvest (within 24 hours of transport to the slaughter facility). This study was approved by the Institutional Animal Care and Use Committee at Kansas State University.

Sample size determination

Sample size estimates were based on the ability to determine a difference in fecal shedding prevalence of *Salmonella* bacteria at the time of preharvest sample collection. On the basis of our preliminary data and other reports (Dargatz *et al.*, 2003; Kunze *et al.*, 2008; Alam *et al.*, 2009), we estimated the mean apparent prevalence of *Salmonella* bacteria at the time of summer preharvest sample collection to be 40% (range, 0%–80%) for cattle not vaccinated with the *Salmonella* Newport SRP vaccine. We wanted to detect a reduction in apparent prevalence of *Salmonella* bacteria such that mean prevalence in pens of cattle vaccinated with the *Salmonella* Newport SRP vaccine was 25%. Sample size estimates were generated by use of simulation and linear mixed models.[b] We simulated pen prevalence data as appropriate for the study design, varied the number of pens and samples collected per pen, and analyzed these data by use of mixed models; *P* values for each simulation-analysis were used to generate a graphical output displaying the power to detect hypothesized differences and the sample size where the total number of samples and the number of pens for each treatment were displayed. We estimated that 20 pens and 25 samples per pen (at the time of preharvest sampling) would be sufficient to detect a difference, as described, with a type I error rate ≤ 0.05 and a type II error rate < 0.20.

Study design

On arrival to the feedlot, cattle were allocated to pairs (replicates) of study pens. Cattle within each arrival lot were systematically allocated by groups of 3 animals into 2 holding pens until each holding pen held the appropriate number of cattle to fill the corresponding study pens. Then, pen weights were obtained and cattle were moved to permanent study pens. The allocation process continued until 20 total pens (10 pens/treatment) were filled. Replicates of study pens were adjacently located and the characteristics (eg, open-air and dirt-floor) of all pens were typical for the industry standard. For allocation to treatment groups, 1 pen from each replicate was randomly selected by coin toss to be administered the *Salmonella* Newport SRP vaccine[a] (vaccinated pen) and the other pen was selected by default to be administered a placebo[c] (control pen).

Cattle were processed by individual pen within 48 hours after arrival to the feedlot. On the initial processing day (day 0), all cattle were administered a dose of a modified-live

respiratory virus vaccine,[d] *Mannheimia haemolytica* toxoid,[e] ivermectin,[f] tilmicosin phosphate,[g] and 2 mL of the *Salmonella* Newport SRP vaccine[a] or placebo[c] (SC in the right neck), according to manufacturers' recommendations. On day 21, cattle in the vaccinated pens or control pens were administered a second dose of the *Salmonella* Newport SRP vaccine[a] or the placebo,[c] respectively; in addition, cattle were administered the first of 2 hormone implants[h] and a second modified-live respiratory virus vaccine.[i] In order to blind feedlot processing personnel to the assignment of cattle to treatment group, labels of the *Salmonella* Newport SRP vaccine and placebo vials were covered and coded as vaccine A and vaccine B, respectively. Furthermore, these products had the same fluid color and consistency and were both contained in 100-mL vials. In addition, personnel that administered the treatment were not the same personnel that were responsible for assessing cattle health and performance during the study; personnel responsible for assessing cattle health and performance also were blinded to treatment group. At approximately 80 days prior to harvest, cattle were administered a third modified-live respiratory virus vaccine,[j] an external parasiticide,[k] and a second hormone implant.[h]

Fecal sample collection

Freshly voided fecal samples (n = 25/ pen) were collected from the pen floor on days 0, 60, 120, and preharvest. Each sample was collected by hand by use of a clean rectal sleeve, and appropriate precautions were observed to avoid samples potentially being contaminated by other feces or pen floor material. After each sample was collected, the rectal sleeve was inverted and tied, labeled, and placed in a refrigerated (4°C) cooler until processing at a laboratory.[l] Laboratory personnel were blinded to treatment groups during the entire study period.

Bacterial isolation and serotyping

A previously reported (Barkocy-Gallagher *et al.*, 2002) standard isolation protocol was used to detect *Salmonella* bacteria in fecal samples. Ten grams of feces was enriched in 90 mL of tryptic soy broth[m] in 532-mL stand-up sample bags.[n] The stand-up sample bags then were incubated at 25°C for 2 hours, 42°C for 6 hours, and at 4°C overnight. Samples were agitated and 10 mL of fecal slurry from each sample bag was added to a culture solution that contained 90 mL of tetrathionate broth[m] and 1.8 mL of iodine.[o] The culture solution then was incubated for 24 hours at 37°C. After incubation, 1 mL of the culture solution was subjected to immunomagnetic separation with anti-*Salmonella* magnetic beads.[p] The immunomagnetic

separation product was adjusted to a final volume of 100 µl with PBS,[q] transferred into 10 mL of Rappaport-Vassiliadis broth,[l] and incubated at 42°C for 16 to 18 hours. The Rappaport-Vassiliadis cultures were vortexed and 50 µl of each culture was spread plated onto Hektoen Enteric agar plates[m] and then incubated at 37°C for 24 hours. Three colonies that had morphology consistent with *Salmonella* spp were streaked onto blood agar[r] and incubated for 24 hours at 37°C. At least 1 isolate from each sample was tested for the *Salmonella* polyvalent O antigen, as well as serogroups B, C1, C2, D1, D2, and E, by slide agglutination.[m] Isolates, which were presumed to be *Salmonella* spp based on colony morphology observed on Hektoen Enteric agar and agglutination with polyvalent O antisera, were stored at –80°C on cryoprotection beads[s]. One isolate from each sample was sent to a reference laboratory[t] for serotyping.

Statistical analysis

All pen-level cattle health and performance data were collected via the feedlot's operational database. Study data were recorded and descriptive analyses were performed by use of a commercially available spreadsheet program.[u] Exact 95% binomial CIs were calculated for proportions by use of a function included in the spreadsheet program[u] that returns the inverse of the cumulative β probability density function for a specified β distribution. All multivariable analyses of fecal shedding and cattle health and performance data were performed by use of a commercial software program[v] via general and generalized linear mixed models as appropriate for normal and binomial distributions (Dohoo *et al.*, 2003). Logistic regression models were used to assess dichotomous outcomes (eg, morbidity, mortality, and fecal shedding of *Salmonella* bacteria) among vaccinated and control pens, while including pen within replicate as a random effect. A categorical variable representing sampling times (days 0, 60, 120, and preharvest) was used when assessing repeated pen-measures of fecal prevalence at all 4 sampling periods to allow the investigation of potential time-dependent effects of the vaccine. A first-order autoregressive correlation structure was used, which is a standard approach for repeated measures over equal time periods that allows for power decay of correlations (Dohoo *et al.*, 2003). General linear mixed models were used to compare cumulative data for pen-level continuous outcomes (eg, ADG, F:G ratio, and treatment costs) among vaccinated and control pens, while controlling for the lack of independence within a replicate by use of a random intercept model. A value of $P < 0.05$ was used to indicate significance in all analyses. Fit of a

model was assessed by evaluating plots of residuals, and for logistic models, the ratio of the deviance of the model to the degrees of freedom of the model was also assessed.

Results

Mean weight of cattle on arrival was 256 kg (n = 1,591). Cattle were allocated to the vaccinated (n = 795) and control (796) groups with 10 pens for each treatment group. Data for health and performance of vaccinated and control cattle were summarized (Table 2.1). There was no significant ($P = 0.80$) difference in mean weight on arrival between treatment groups within replicates. The number of cattle per study pen ranged from 61 to 105 (mean, 79.5; median, 68) and 61 to 105 (mean, 79.6; median, 67) for the vaccinated and control pens, respectively. Mean number of days on feed for the vaccinated and control pens were 228.8 (median, 229) and 228.9 (median, 229) days, respectively, and did not differ statistically.

Dates of the 4 fecal sampling periods for pairs of pens were October 17 and 24, 2008 (day 0), December 12, 2008 (day 60), February 13 and 20, 2009 (day 120), and May 28 and June 18, 2009 (preharvest). The single sampling date for day 60 was to accommodate closure of the participating laboratory for a holiday. The within-pen fecal prevalence of *Salmonella* bacteria following randomization after arrival (day 0; Figure 2.1) ranged from 0% (0/25) to 48% (12/25) and differed significantly ($P < 0.01$) among replicates, but did not differ significantly ($P = 0.73$) between control and vaccinated pens. Overall prevalence of fecal shedding of *Salmonella* bacteria across all sampling times and treatment groups was 10.6% (211/2,000). Of the 211 *Salmonella* isolates characterized, most were from serogroups E (n = 166), C1 (20), and C2 (9; Table 2.2). Predominant serotypes recovered were Anatum (n = 133), Lexington var 15+ (22), Lille (11), Newport (8), and Senftenberg (6).

Salmonella bacteria were recovered from all 10 replicates of pens for both treatment groups, and cumulative prevalence estimates across all sampling times ranged from 1.5% to 22%. Unadjusted cumulative prevalence of fecal shedding was 10.2% (95% binomial CI; 8.3% to12.1%) and 10.9% (95% binomial CI; 9.0% to 12.1%) for vaccinated and control pens, respectively. Crude prevalence estimates for each sequential sampling time across all pens were 10.0% (95% binomial CI; 7.5% to 13.0%), 2.4% (95% binomial CI; 1.3% to 4.2%), 29.4% (95% binomial CI; 25.4% to 33.6%), and 0.4% (95% binomial CI; 0.1% to 1.4%). Multivariable analysis indicated significant ($P < 0.01$) differences in the prevalence of *Salmonella* bacteria

among sampling times. However, there was no significance ($P = 0.89$) difference between treatment groups and no significant ($P = 0.12$) treatment by sampling time interaction. These effects, or lack thereof, were evident in the display of the raw data for the fecal prevalence of *Salmonella* bacteria (Figure 2.1).

Unadjusted summary data of common feedlot cattle health and performance indices were summarized (Table 2.1). Furthermore, model-adjusted estimates for cumulative incidence risks of adverse health outcomes for all vaccinated and control cattle were calculated (Figure 2.2). On the basis of multivariable models accounting for replicates, outcomes did not differ significantly between vaccinated and control pens (Table 2.1). Overall morbidity in study cattle was 34.8% and ranged from 15.9% to 58.7% within pens; however, there was no significant difference among pens (within replicates) in different treatment groups. Illness in the study population was primarily caused by respiratory tract disease and lameness; furthermore, there were no suspected or confirmed cases of salmonellosis. Overall, only 2.1% of the vaccinated cattle and 1.9% of the control cattle were treated for illness on > 1 occasion. No significant difference was detected among pens in different treatment groups for the number of cattle requiring treatment on > 1 occasion. Overall mean treatment (medication) costs in vaccinated and control cattle were $5.91 and $5.85 per head, respectively, and no significant difference in mean treatment costs were detected between pens in different treatment groups. During the study, 13 cattle were culled because of illness, and no significant difference in culling was detected among pens in different treatment groups. Overall mortality in the study population was 1.5% and ranged from 0% to 4.9% within pens. Overall mortality in pens of vaccinated and control cattle did not differ significantly.

Significant differences for any of the standard measures of feedlot performance were not detected among pens of vaccinated and control cattle (Table 2.1). On the basis of analysis of pens within replicate, ADG for pens of vaccinated and control cattle did not differ significantly. When adjustments were made for losses related to dead and culled cattle, ADG still did not differ significantly among pens between treatment groups. The F:G ratio, which is calculated as the amount of feed (kg) delivered to the amount of weight gain (kg), and the adjusted F:G ratio for vaccinated and control cattle did not differ significantly. Overall CG and adjusted CG also did not differ significantly between treatment groups. On further multivariable analysis of pen-level

data, mean hot carcass weight, carcass yield, and carcass price adjustment for pens of vaccinated and control cattle did not differ significantly.

Discussion

Our study of feeder cattle in a commercial feedlot production system revealed no significant differences between cohorts of vaccinated and control cattle in the prevalence of fecal shedding of *Salmonella* bacteria or cattle health and performance variables. These findings may have been caused by several factors. First, there may have been a lack of efficacy of the *Salmonella* Newport SRP vaccine in cattle located in this type of a production setting. Second, there may have been an insufficient number of *Salmonella* bacteria in this environment which may have reduced the ability to detect differences between the treatment groups. Last, the use of the *Salmonella* Newport SRP vaccine in a subset of the population (10 pens) may have reduced the overall exposure of cattle to *Salmonella* bacteria in the portion of the feedlot where the pens included in this study were located; thus, all cattle in these study pens may not have been exposed to a sufficient number of *Salmonella* bacteria to demonstrate vaccine efficacy.

The last explanation, which also may be formally characterized as herd immunity, was suggested by investigators of another study (Hermesch *et al.*, 2008) as a plausible explanation for low prevalence of *Salmonella* bacteria in vaccinated and control cattle in a dairy production system (Hermesch *et al.*, 2008). A significant herd immunity effect on the fecal shedding of *E. coli* O157 in feedlot cattle also has been described (Peterson *et al.*, 2007). In that study, unvaccinated feedlot cattle were 59% less likely to have detectable levels of *E. coli* O157 in their feces when housed with cattle that were vaccinated for *E. coli* O157 (Peterson *et al.*, 2007); although this study of *E. coli* O157 was not a pen-level investigation of the shedding of *Salmonella* bacteria, it suggests that herd immunity may be an important factor when evaluating the effect of a vaccine on fecal bacteria in feedlot production systems. Therefore, it may now be evident that an evaluation of vaccinated and control cattle located in adjacent pens within a single segment of a feedlot is not an ideal study design for assessing the efficacy of vaccines for *E. coli* O157 or *Salmonella* bacteria.

The results of a recent observational study (Loneragan *et al.*, 2009) indicated that the administration of the *Salmonella* Newport SRP vaccine may reduce the shedding of *Salmonella* bacteria in the feces of cull dairy cows. However, results of the study reported here

demonstrated similar levels of shedding of *Salmonella* bacteria in vaccinated and control cattle and was consistent with the results of 2 randomized controlled trials (Heider *et al.*, 2008; Hermesch *et al.*, 2008) conducted in dairy cattle. Investigators of 1 of these trials (Hermesch *et al.*, 2008) administered the *Salmonella* Newport SRP vaccine to 75 cows within a 1,200-cow dairy herd and did not detect significant differences in fecal shedding of *Salmonella* bacteria between treatment groups; yet, they detected significantly higher milk production and lower cumulative somatic cell counts in vaccinated cows. Investigators of the other trial (Heider *et al.*, 2008) administered the *Salmonella* Newport SRP vaccine to 25% of the mature dairy cows within two herds that had a history of salmonellosis and did not detect significant differences in fecal shedding of *Salmonella* bacteria between vaccinated and control cattle; health and performance variables were not assessed (Heider *et al.*, 2008). In both trials (Heider *et al.*, 2008; Hermesch *et al.*, 2008), an inability to detect a difference in fecal shedding may have been affected by herd immunity or the relatively small proportion of cattle vaccinated within herds and variability of shedding of *Salmonella* bacteria in the feces that contributed to a small effective sample size for the potential to detect differences. However, results of these trials (Heider *et al.*, 2008; Hermesch *et al.*, 2008) also may indicate a lack of efficacy of the *Salmonella* Newport SRP vaccine for the reduction of fecal shedding of the diverse *Salmonella* serotypes found in bovine production systems. In the study reported here, *Salmonella* Anatum was predominantly detected while other serotypes, which included *Salmonella* Newport for which the *Salmonella* Newport SRP vaccine has labeled indications, were detected infrequently or rarely. The distribution and diversity of serotypes that we detected in the present study are similar to the findings of other studies (Callaway *et al.*, 2008; Kunze *et al.*, 2008; Alam *et al.*, 2009; Cummings *et al.*, 2009) of the shedding of *Salmonella* bacteria in the feces of cattle; however, this diversity in *Salmonella* bacteria may have affected our ability to detect significant vaccine effects.

Fecal prevalence of *Salmonella* bacteria in the present study was much lower than we expected for feedlot cattle in this region, particularly at the time of the preharvest sampling (prevalence of < 1%). Our previous study (Alam *et al.*, 2009) and those of other investigators (Dargatz *et al.*, 2003; Loneragan *et al.*, 2005; Kunze *et al.*, 2008) have demonstrated much higher fecal prevalence of *Salmonella* bacteria in feedlot cattle, and thus, our sample size calculations were based on an expected prevalence of 40% in non-vaccinated cattle. The lower

prevalence of *Salmonella* bacteria observed in the present study combined with the extreme variability in prevalence among replicates and within replicates over time would have adversely affected our ability to detect significant vaccine effects. However, the 120-day prevalence (29.4%) in the study reported here was not low and all but 1 replicate had *Salmonella*-positive fecal samples, which indicates that cattle were broadly exposed to *Salmonella* bacteria at some level during the study period. Within-pen prevalence of *Salmonella* bacteria at the time of that sampling (day 120) ranged between 0% (0/25) and 80% (20/25), but prevalence estimates among pens within replicates were similar. Given the paired-pen allocation of cattle on arrival, data suggest that shedding of *Salmonella* bacteria is largely affected by cattle source or factors associated with arrival at the feedlot, even after the cattle have been in the feedlot for several months (Figure 2.1).

As it was for many other field studies of *Salmonella* spp. (Barham *et al.*, 2002; Beach *et al.*, 2002; Dargatz *et al.*, 2003; Alam *et al.*, 2009), including those studies that evaluated the efficacy of the *Salmonella* Newport SRP vaccine (Heider *et al.*, 2008; Hermesch *et al.*, 2008; Loneragan *et al.*, 2009), evaluating the prevalence of fecal shedding of *Salmonella* bacteria was a primary aim of the study reported here. Although concentrations of *Salmonella* organisms within positive fecal samples and prevalence (and concentration) of *Salmonella* bacteria on the hides of cattle may be important indicators of preharvest food safety (Brichta-Harhay *et al.*, 2008), we did not measure these indicators in the study reported here. There may have been a significant difference in the concentration of *Salmonella* bacteria in positive fecal samples even though there were no significant differences in prevalence between vaccinated and control pens. Determination of the presence or concentration of *Salmonella* bacteria on cattle hides post-transport to the slaughter facility also may have revealed differences between cohorts of cattle as evidence suggests hide prevalence increases during transport (Barham *et al.*, 2002; Reicks *et al.*, 2007). Surprisingly, only 0.4% (2/500) of samples were positive across all pens immediately prior to harvest, arguably the most important potential food safety indicator that we measured. Given those prevalence results, it is extremely unlikely that determining the concentration of *Salmonella* bacteria within these positive fecal samples (n = 2) would have provided additional useful information for the evaluation of vaccine efficacy. This extremely low prevalence, following the much higher 120-day prevalence, again may be perceived as the potential effect of herd immunity and reduced overall exposure in the study environment or simply may be because

of time-dependent effects that were not measured. Preharvest samples were collected in the present study during the summer months, which is presumably the time of year when the shedding of *Salmonella* bacteria is most common in feedlots (Dargatz *et al.*, 2003).

Health and performance indicators for cattle included in the present study were typical for this type of cattle and production system. All performance measures were very similar among pens in different treatment groups, suggesting that there were no significant vaccine effects. We failed to demonstrate any evidence that the *Salmonella* Newport SRP vaccine affected health and performance variables despite the fact that we analyzed multiple outcome variables. To prevent further multiplicity in the analysis, we assessed potential carcass effects by use of a pen-level mean carcass price adjustment, which is an economic index representing carcass premiums and discounts associated with USDA quality grade, yield grade, and several other carcass variables assessed post-harvest. If the *Salmonella* Newport SRP vaccine was considered an effective preharvest food safety intervention, it would be important to demonstrate no adverse effects on cattle health and performance. In addition, preharvest interventions that would enhance the safety of beef by reducing the prevalence of *Salmonella* bacteria in the feedlot environment could theoretically improve cattle health and performance (Smith, 2002).

In general, adverse health outcomes were not rare in this study population; thus, potential health effects of the vaccine could have been demonstrated had they existed. However, there were no reported clinical effects consistent with salmonellosis in the cattle of the present study. As described previously (Alam *et al.*, 2009), the lack of health and performance effects could be because of an insufficient challenge dose of *Salmonella* bacteria or diversity among *Salmonella* serotypes. Subclinical shedding of *Salmonella* bacteria previously has been associated with some adverse health outcomes in feedlot cattle, such as lot-level measures of hospital pen mortality and retreatment risks, as well as individual animal case fatality risk for cattle with bovine respiratory tract disease (Alam *et al.*, 2009). However, the effect on case fatality risk was only demonstrated for cattle shedding serogroup B *Salmonella* spp and was not associated with overall shedding of *Salmonella* bacteria. In addition, that study (Alam *et al.*, 2009) failed to show associations between shedding of *Salmonella* bacteria and several other common health variables measured in feedlots; the authors suggested that some *Salmonella* serotypes might be considered commensal bacteria in feedlot cattle.

To our knowledge, this is the first study to evaluate the effects of the *Salmonella* Newport SRP vaccine in cattle maintained in a commercial feedlot production setting. Although we did not detect any effects of vaccination with the *Salmonella* Newport SRP vaccine on the fecal prevalence of *Salmonella* bacteria or cattle health and performance, we recognize that further investigation of this vaccine in different cattle production settings could provide evidence of vaccine efficacy. We also recognize that long-term herd vaccination strategies may need to be considered in order to affect subclinical fecal shedding in cattle. This maybe why a recent observational study (Loneragan *et al.*, 2009) demonstrated a lower fecal prevalence of *Salmonella* bacteria in cull cows from dairies that had been administered the *Salmonella* Newport SRP vaccine, compared with cull cows in dairies that did not administer the vaccine, yet 2 previous experimental trials (Heider *et al.*, 2008; Hermesch *et al.*, 2008) that allocated individual dairy cows to receive this same vaccine detected no effect of vaccine administration on fecal shedding of *Salmonella* bacteria. We also recognize that the lower than expected prevalence of *Salmonella* bacteria in feces combined with the extreme variability in prevalence among replicates and within replicates over time may have adversely affected the ability to detect significant vaccine effects in the study reported here. Furthermore, the present study was conducted in only 1 commercial feedlot, and prevalence and serotypes of *Salmonella* bacteria vary among feedlots and regions (Dargatz *et al.*, 2003). Because the control of *Salmonella* bacteria in commercial feedlot production systems may enhance food safety and potentially cattle health and performance, further studies are necessary to validate control methods.

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Endnotes

a. *Salmonella* Newport bacterial extract SRP, distributed by AgriLaboratories, Ltd, St Joseph, Mo.

b. SAS, version 9.1, SAS Institute Inc, Cary, NC.

c. Emulsigen, MVP Laboratories, Omaha, Neb.

d. Bovishield Gold 5, Pfizer Animal Health, New York, NY.

e. One Shot, Pfizer Animal Health, New York, NY.

f. Ivomec, Merial Limited, Duluth, Ga.

g. Micotil, Elanco, Greenfield, Ind.

h. Component TE-S, Vetlife, West Des Moines, Iowa.

i. Pyramid 2 + Type II BVDV, Fort Dodge Animal Health, Fort Dodge, Iowa.

j. Titanium 3, AgriLaboratories Ltd, St Joseph, Mo.

k. Exile, Agripharm, Westlake, Tex.

l. Preharvest Food Safety Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, Kan.

m. Becton Dickinson, Sparks, Md.

n. Whirl-pak, Nasco, Ft Atkinson, Wis.

o. 1%, Fisher Scientific, Fairlawn, NJ.

p. Dynal Inc, New Hyde Park, NY.

q. Sigma-Aldrich, St Louis, Mo.

r. Remel, Lenexa, Kan.

s. CryoBeads, Hardy Diagnostics, Santa Maria, Calif.

t. National Veterinary Services Laboratory, Ames, Iowa.

u. Microsoft Excel 2007, Microsoft, Redmond, Wash.

v. Stata, version 10, StataCorp LP, College Station, Tex.

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Table 2.1 Unadjusted summary data of health and performance outcomes for vaccinated and control cattle.*

Variable	Vaccinated pens*(n=10)	Control pens* (n=10)	*P* value†
Cattle (No.) ‡	795	796	—
Entry weight (kg)§	255.6 ± 11.08	256.5 ± 11.90	0.80
Cattle morbidity‖¶	277 (34.8)	277 (34.8)	0.99
Cattle retreated‖¶	17(2.1)	15(1.9)	0.72
Cattle culled for health reasons‖¶	9 (1.1)	4 (0.5)	0.23
Cattle mortality‖¶	15 (1.9)	9 (1.1)	0.22
Case fatality risk (%)#	5.4	3.3	0.16
Treatment cost/head ($)	5.91 ± 3.78	5.85 ± 2.46	0.93
ADG (kg)	1.38 ± 0.12	1.40 ± 0.12	0.13
F:G ratio	5.58 ± 0.16	5.57 ± 0.18	0.74
CG ($)	1.56 ± 0.03	1.58 ± 0.05	0.56
Adjusted ADG (kg)§**	1.41 ± 0.12	1.42 ± 0.15	0.88
Adjusted F:G ratio§**	5.46 ± 0.19	5.47 ± 0.27	0.71
Adjusted CG ($)§**	1.54 ± 0.04	1.54 ± 0.07	0.89
Hot carcass weight (kg)	375.6 ± 12.42	378.3 ± 14.78	0.38
Carcass yield (%)	64.7 ± 0.53	64.9 ± 0.39	0.12
Carcass price adjustment ($/45.5 kg of carcass weight)††	0.95 ± 0.62	1.05 ± 1.07	0.70

*Cattle in the vaccinated and control pens were administered the *Salmonella* Newport SRP vaccine[a] or placebo[c], respectively, according to manufacturer's recommendations.

†Within a row, *P* values demonstrate the lack of significant ($P < 0.05$) vaccine effects on each outcome and were determined by use of multivariable logistical and linear models, which accounted for the paired pen (replicate) study design.

‡Cattle were systematically allocated by groups of 3 animals into 2 pens until 20 pens (10 /treatment) were filled.

§Values are reported as mean ± SD.

‖Value is reported as the No. (%).

¶Cattle morbidity, retreatment, culling and mortality values are for all causes and for reasons primarily associated with respiratory disease and lameness; furthermore, there were no suspected or confirmed cases of salmonellosis.

#Value is based on all causes of morbidity and subsequent death.

**Value is adjusted for dead and culled cattle.

††Carcass price is an economic index representing carcass premiums and discounts associated with USDA quality grade, yield grade, and several other carcass variables assessed post-harvest. Values of premiums and discounts are based on carcass characteristics at the time of harvest.

— = Not determined.

Table 2.2 Summary of *Salmonella* serotypes isolated from fecal samples of vaccinated and control cattle.*

		No. of isolates		
Serotype†	Serogroup	Vaccinated pens	Control pens	Total‡
Anatum§	E	67	66	133 (63.0%)
Lexington var 15+	E	11	11	22 (10.4%)
Lille	C1	3	8	11 (5.2%)
Newport	C2	4	4	8 (3.8%)
Senftenberg	E	1	5	6 (2.8%)
3,15:z10:–	—	5	0	5 (2.4%)
6,7:–:1,5	E	1	3	4 (1.9%)
Tennessee	C1	4	0	4 (1.9%)
Enteritidis	D1	0	3	3 (1.4%)
Others and non-typeable	—	6	9	15 (7.1%)
Total	—	102	109	211 (100%)

†Only 1 isolate from each sample was sent to a laboratory[t] for serotyping of the *Salmonella* isolates.

‡Within a row, values are reported as total No. (proportion of the column total [%]).

§Serotype designation includes all variants (n = 2) of Anatum 15+. *See* Table 2.1 for remainder of key.

Figure 2.1 Fecal prevalence of *Salmonella* bacteria in each vaccinated (n = 10, gray bars) and control pen (10, black bars) within a replicate on days 0, 60 and 120 and preharvest. Cattle in the vaccinated and control pens were administered 2 mL of the *Salmonella* Newport SRP vaccine or placebo, respectively, according to manufacturer's recommendations. Error bars represent the exact 95% CIs for proportions. Multivariable logistic regression analysis of these data, which was used to account for the paired-pen (replicate) study design, indicated significant differences in prevalence of *Salmonella* bacteria among sampling times, but no significant difference between treatment groups or time by treatment interaction.

[Next Page]

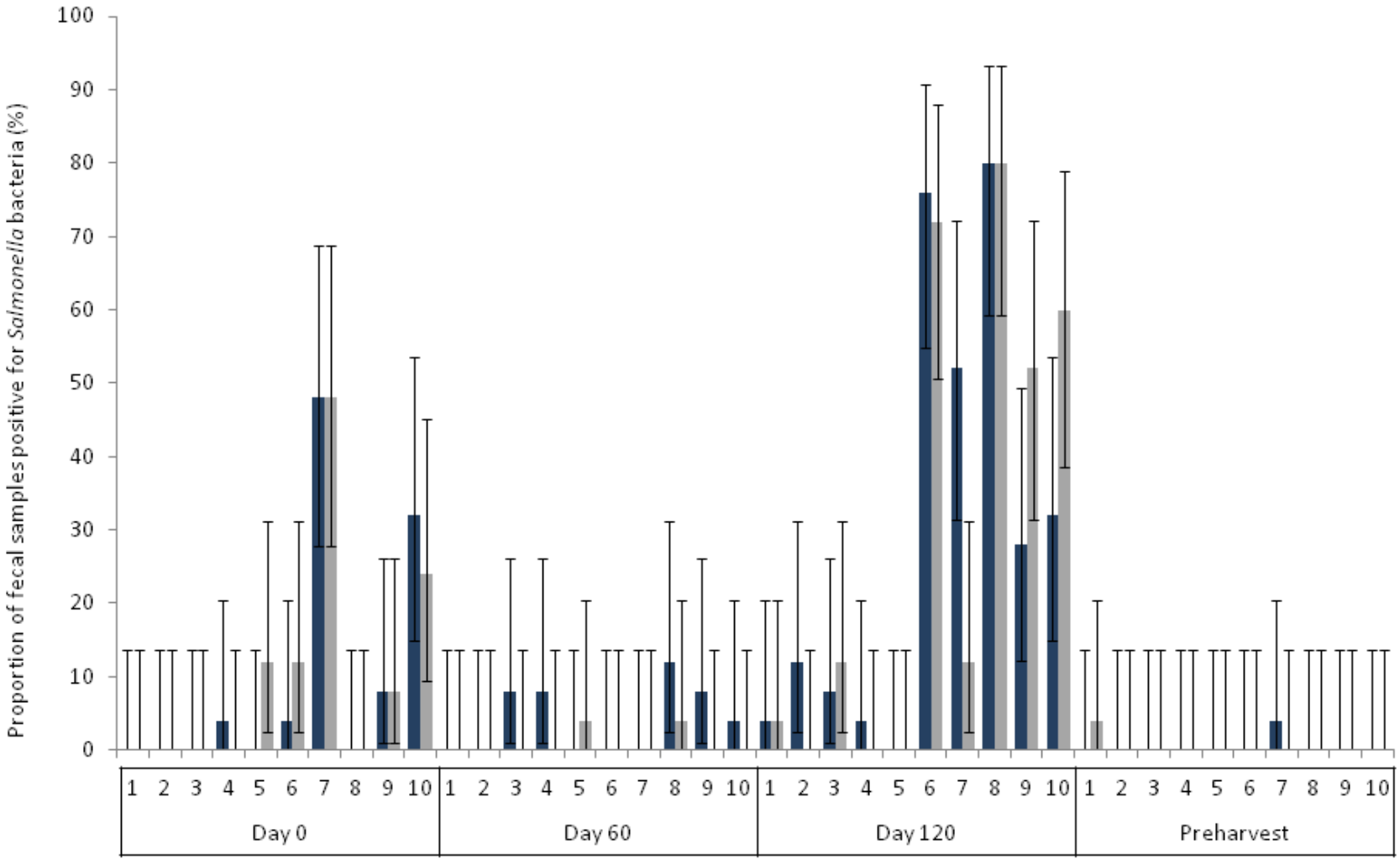
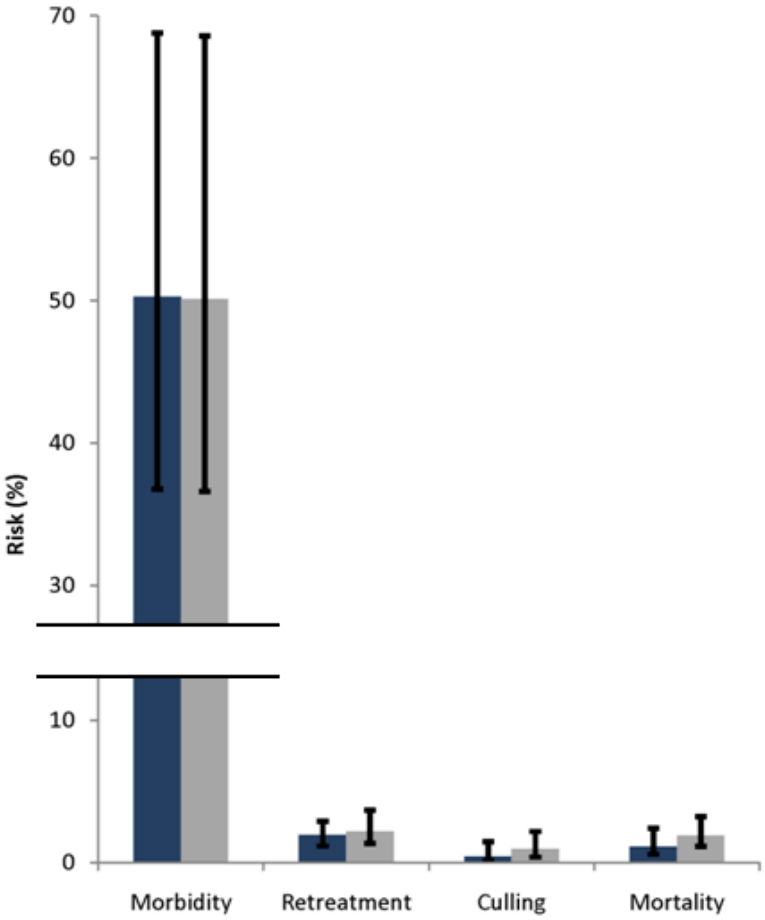

Proportion of fecal samples positive for *Salmonella* bacteria (%)
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10
Day 0
1 2 3 4 5 6 7 8 9 10
Day 60
1 2 3 4 5 6 7 8 9 10
Day 120
1 2 3 4 5 6 7 8 9 10
Preharvest

Figure 2.2 Cumulative incidence risks of adverse health outcomes (morbidity, treatment, culling, and mortality) for all vaccinated (n = 10, gray bars) and control pens of cattle (10, black bars). Cattle in the vaccinated and control pens were administered 2 mL of the *Salmonella* Newport SRP vaccine or placebo, respectively, according to manufacturer's recommendations. Risks and corresponding 95% CIs are model-adjusted estimates calculated via logistic regression models that accounted for the paired-pen study design; outcomes did not differ significantly between treatment groups.



CHAPTER 3 - Prevalence and Persistence of *Salmonella* in Cohorts of Feedlot Cattle

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(In Review, *Foodborne Pathogens and Disease*)

Abstract

Our objective was to determine factors associated with fecal prevalence of *Salmonella* at feedlot entry and within 24 hrs of harvest (preharvest), and to assess potential persistence of *Salmonella* strains within cattle populations. This repeated cross-sectional study followed 5,559 beef cattle within 30 feedlot cohorts. Samples (n = 30) of fresh feces were collected from the pen floor of each cohort at feedlot entry and preharvest. Samples were subjected to a selective *Salmonella* isolation protocol and serotypes were determined for *Salmonella* isolates. Genetic similarity of a subset of isolates was determined using pulsed-field gel electrophoresis (PFGE). Cattle health and performance data were recorded electronically by feedlot personnel. Cohort-level generalized linear mixed models were used to assess bivariable associations. Fecal prevalence of *Salmonella* within a cohort at feedlot entry (mean = 64.7%) was not associated with preharvest prevalence (mean = 72.6%). Prevalence at feedlot entry was negatively associated with mean entry weight ($P = 0.02$). Preharvest prevalence was positively associated with the number of days in the feedlot ($P = 0.02$), cumulative morbidity ($P = 0.01$), and cumulative mortality ($P = 0.03$). We recovered *Salmonella* isolates with identical PFGE profiles both at feedlot entry and preharvest from 14 cohorts of cattle. Fecal prevalence of *Salmonella* immediately prior to harvest may be higher in subsets of the feedlot population, but does not appear to be affected by prevalence at feedlot entry. However, PFGE subtypes of *Salmonella* appear to persist within and among feedlot cohorts throughout the feeding period.

Introduction

Salmonella spp. cause gastrointestinal illness in both livestock and humans (Mead *et al.*, 1999; Callaway *et al.*, 2008). These ubiquitous bacteria can be found in several food production environments, including beef production systems (Barkocy-Gallagher *et al.*, 2003; Rodriguez *et al.*, 2006; Cummings *et al.*, 2009). Several recent U.S. outbreaks of human salmonellosis have been associated with eating contaminated beef (CDC, $1995_{a;}$ CDC, $1995_{b;}$ CDC, 2003; Dechet *et al.*, 2006; McLaughlin *et al.*, 2006). Beef may become contaminated during the harvest process from *Salmonella* present in cattle feces or on hides (Beach *et al.*, 2002).

Pre- and post-harvest interventions may be important in reducing *Salmonella* contamination of beef products, as well as potentially improving cattle health and performance (Losinger *et al.*, 1997; House *et al.*, 1998; Fegan *et al.*, 2005; Brichta-Harhay *et al.*, 2008; Alam *et al.*, 2009). Contamination of cattle hides may occur during transport and lairage (Barham *et al.*, 2002; Beach *et al.*, 2002; Dewell *et al.*, 2008), and *Salmonella* strains can be found at various stages of transport to harvest (Arthur *et al.*, 2008). The source, transmission, and persistence of *Salmonella* within cohorts of beef cattle are not well understood (Fedorka-Cray *et al.*, 1998; Davis *et al.*, 2003).

Several risk factors may be associated with the fecal prevalence of *Salmonella* and *Salmonella*-associated morbidity or mortality in cattle (Losinger *et al.*, 1997; House *et al.*, 1998; Alam *et al.*, 2009). Preharvest interventions that mitigate *Salmonella* risks may improve cattle health and performance, reduce the presence and concentrations of *Salmonella* in cattle feces and on hides, and subsequently reduce contamination of beef. Assessments of longitudinal data on the frequency and persistence of *Salmonella* strains within cohorts of feedlot cattle may enable the identification of effective preharvest interventions. The objectives of this study were to identify factors associated with the prevalence of *Salmonella* at feedlot entry and immediately prior to harvest, and to determine if specific *Salmonella* strains present in feces of newly arrived cattle persist until cattle are shipped for harvest.

Materials and Methods

Cattle allocation, management, and sampling

A commercial feedlot in Texas with a capacity of approximately 70,000 animals

participated in the study. Feeder calves were procured through normal industry channels of the participating feedlot. A convenience sample of 30 cohorts of incoming cattle was enrolled in the study. Cohorts were defined as groups of cattle that arrived to and were shipped from the feedlot together. Cattle within a cohort were not comingled with cattle from other cohorts. Cattle were managed according to the feedlot's standard health, feeding and management protocols that were developed and applied at the discretion of feedlot personnel and their consulting staff. Upon arrival to the feedlot, cohorts of cattle were identified as "low" or "high" risk for bovine respiratory disease (BRD) in accordance with standard operating procedures of the feedlot. Cattle in cohorts designated as "high risk" all received tulathromycin for metaphylaxis according to label directions (Pfizer Animal Health, New York, New York). Cohort-level health and performance data that were collected by feedlot personnel were retrieved through the feedlot's operational database. Cattle were fed a series of diets of increasing concentrate level from receiving to finishing; diets and individual components were similar among study cohorts.

Sample size estimates were based on detecting a 10% difference in mean *Salmonella* fecal prevalence among cattle cohorts with different health or performance parameters, assuming a base-line cohort-level prevalence of 50% and type I and type II error rates of 0.05 and 0.20, respectively. For each cohort, samples (n = 30) of individual fresh fecal pats were collected from the pen floor (while avoiding contact with soil or other feces on the pen floor) within 24 hrs after feedlot arrival (entry) and again within 24 hrs prior to transport to harvest (preharvest). Each sample was collected with a new plastic sleeve; then the sleeve was tied, labeled, and packed in a cooler with frozen ice packs. Coolers were shipped via overnight courier to the Preharvest Food Safety Laboratory, College of Veterinary Medicine, Kansas State University.

Isolation protocol

A standard protocol, modified from previously described methods, was used to isolate *Salmonella* in fecal samples (Barkocy-Gallagher *et al.*, 2002). For each sample, 10 g of feces was mixed with 90 ml tryptic soy broth (Becton Dickinson, Sparks, Maryland) for enrichment and incubated at 25°C for 2 hrs, 42°C for 6 hrs, then overnight at 4°C. Ten ml of the resulting fecal enrichment culture was added to 90 ml tetrathionate broth (Becton Dickinson) with 1.8 ml iodine (1%, Fisher Scientific, Fairlawn, New Jersey) and incubated for 18 hrs at 37°C. One ml was then subjected to immunomagnetic separation (IMS) with anti-*Salmonella* magnetic beads

(Dynal Inc., New Hyde Park, New York). One hundred µl of phosphate buffered saline (Sigma-Aldrich, St. Louis, Missouri) was added to the final IMS product and the resulting cell concentrate was transferred into 10 ml of Rappaport-Vassiliadis (RV) broth (Becton Dickinson) for 16-18 hrs of incubation at 42°C. Cultures were then vortexed; 50 µl was spread plated onto Hektoen Enteric (HE) agar (Becton Dickinson) and incubated at 37°C for 24 hrs. Up to three suspect colonies with morphology consistent with *Salmonella* spp. were streaked onto blood agar (Remel, Lenexa, Kansas) and incubated for 24 hrs at 37°C. Isolates from blood agar plates were subjected to slide agglutination (Becton Dickinson) for the *Salmonella* polyvalent O antigen and *Salmonella* serogroups B, C1, C2, D1, D2, and E. One isolate from each sample, confirmed as *Salmonella* based on hydrogen sulfide production on HE agar and agglutination with polyvalent O antisera, was stored at -80°C on cryo-protection beads (Hardy Diagnostics, Santa Maria, California), and later serotyped at the National Veterinary Services Laboratories (Ames, Iowa).

Pulsed-field gel electrophoresis protocol

Isolates that had matching serotypes within a cohort at both sampling times were differentiated by pulsed-field gel electrophoresis (PFGE) separation of *Xba*1-digested genomic DNA in accordance with the PulseNet Protocol (Ribot *et al.*, 2006). Briefly, stored isolates were transferred onto blood agar and incubated at 37°C for 18 hrs. Colonies were added to cell suspension buffer (100 mM Tris:100 mM EDTA, pH8.0) at room temperature and concentration was adjusted to an optical density of 1.3 to 1.4 at 610 nm. Suspensions were incorporated into gel plugs by mixing with TE buffer (10 mM Tris:1 mM EDTA, pH 8.0) consisting of 1% SeaKem Gold® agarose, 1% SDS (BioWhittaker Molecular Applications, Rockland, Maine), and Proteinase K (Fisher Scientific, Fair Lawn, New Jersey). Cast gel plugs were then subjected to cell lysis buffer (50 mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosyl) containing Proteinase K. Following washing in reagent grade, type-1 water and TE buffer, extracted DNA samples underwent restriction digestion with the *Xba*1 enzyme (Promega Corporation, Madison, Wisconsin). Along with a *Salmonella* Braenderup H9812 standard, each sample was loaded into wells. Restricted plug slices were subjected to electrophoresis, stained with an ethidium bromide solution, and viewed under UV light. Gel imaging software (Quantity One®, Bio Rad, Hercules, CA and BioNumerics®, Applied Maths, Austin, TX) was used to digitize, normalize, and assign bands for each isolate image. Band-sharing similarity coefficients were generated from the DNA

fragments in the 20- to 1150-kb range. Dendrograms were constructed to provide a visual representation of the relationship among *Salmonella* isolates. Isolates were grouped based on banding pattern similarities where types and subtypes were defined as isolates having PFGE patterns of > 95% or 100% Dice similarity (no band differences), respectively.

Data analysis

Data were recorded and descriptive analyses were performed using Microsoft Excel® 2007 (Microsoft, Redmond, Washington). Prevalence of *Salmonella* was estimated as the proportion of positive samples within a cohort at a specific sampling time. Persistence of *Salmonella* subtypes was defined as the detection of at least one identical PFGE subtype at both sampling times within a cohort. Exact binomial confidence intervals (95% CI) for proportions were calculated using the beta inverse function of Excel®, and are reported in parentheses in the text. The Spearman rank correlation test was used to compare within-cohort prevalence estimates of *Salmonella* between the different sampling times. Cattle health and performance data were evaluated for potential associations with 1) the prevalence of *Salmonella* at feedlot entry, 2) prevalence immediately prior to harvest, and 3) persistence of ≥1 *Salmonella* subtype within a cohort. Logistic regression models in STATA Version 10 (StataCorp, LP, College Station, Texas) were used to assess bivariable associations with *Salmonella* prevalence and persistence. Within-pen prevalence of *Salmonella* was modeled with sample-level generalized linear mixed models including a random effect for cohort; an exchangeable covariance structure was used to adjust for within-cohort correlations among cohort-level prevalence outcomes. Persistence of at least one *Salmonella* strain within a cohort was modeled using cohort-level generalized linear models. For all analyses, cohort-level independent variables were mean entry weight, BRD risk category, gender, month of enrollment, days on feed, mean daily weight gain, and cumulative crude morbidity and mortality. We also evaluated cohort prevalence estimates as independent variables in the analysis of factors associated with persistence. Continuous independent variables did not meet assumptions of linearity and were categorized by graphing the data, determining meaningful cut points, and using hierarchical selection and combination of ordinal categories (Walter *et al.*, 1987). Independent variables that were highly correlated with other independent variable(s) were assessed separately. Statistical significance was determined using a *p*-value of 0.05 for all analyses.

Results

Cattle (n = 5,559) enrolled in the study originated from livestock markets and ranches in the Midwest and High Plains U.S. Within cohort mean entry body weights ranged from 209.1 to 364.5 kg and the mean cohort size was 185 (standard deviation (SD): 91.7). There were 23 cohorts classified as low-risk for BRD and seven cohorts classified as high-risk for BRD. Ten cohorts were heifers, 18 cohorts were steers, and two cohorts contained both sexes. The number of days a cohort was in the feedlot ranged from 148 to 285 days (mean: 192; SD: 31.6) and the mean daily weight gain was 1.31 kg (SD: 0.15). The mean cumulative incidence of crude morbidity and crude mortality across all cohorts was 13.7% (SD: 0.18) and 3.1%, (SD: 0.05), respectively. Only crude (not cause-specific) morbidity and mortality were analyzed; no diagnoses of salmonellosis were made by the participating feedlot's personnel.

Sampling occurred during the weeks of October 8, October 29, November 5, November 12, December 3, December 10 (all 2006), and January 14, 2007 for arrival sampling. Weeks for preharvest fecal sampling were February 25, March 11, April 1, April 29, May 6, June 3, June 10, June 24, and August 5, 2007; one cohort was not re-sampled at preharvest. Continuous independent variables that were categorized included morbidity (≤ 10%, > 10%), mortality (≤ 1.0%, > 1.0%), days on feed (≤ 200, > 200), mean daily weight gain (≤ 1.36 kg, > 1.36 kg), and mean entry weight (≤ 273 kg, > 273 kg). Evaluations of associations were conducted using multiple bivariable models (rather than multivariable models) due to strong correlations among the independent variables.

Mean fecal prevalence of *Salmonella* at feedlot arrival across all cohorts was 64.7% (SD: 7.9) and ranged from 16.7 to 100% (Figure 3.1). Respiratory disease risk category ($p = 0.93$), gender ($p = 0.10$), month of enrollment ($p = 0.25$), morbidity ($p = 0.55$), and mortality ($p = 0.26$) were not associated with *Salmonella* prevalence at entry. Cohort mean entry weight was negatively associated with the prevalence of *Salmonella* ($p = 0.02$); the mean prevalence estimates of *Salmonella* in cohorts with low and high mean entry weights were 82.7% (range 76.6 to 88.8%) and 61.1% (range 57.6 to 64.6%), respectively.

Mean preharvest fecal prevalence of *Salmonella* was 72.6% (SD: 8.3). Across cohorts, the prevalence of *Salmonella* preharvest ranged from 0 to 100% (Figure 3.1). Prevalence was higher ($p = 0.04$) in cattle categorized as high risk for BRD (mean = 83.9%) than in low-risk cattle (mean = 69.7%). Preharvest prevalence was higher ($p = 0.02$) in cohorts of cattle that were

in the feedlot longer than 200 days (mean = 79.5%) than in cohorts that were fed up to 200 days (mean = 60.1%). Morbidity ($p = 0.01$) and mortality ($p = 0.03$) were positively associated with preharvest *Salmonella* prevalence; prevalence estimates for high and low morbidity cohorts were 90.6% and 60.0%, respectively, and for high and low mortality cohorts they were 85.5% and 54.4%, respectively. Daily weight gain was not associated with preharvest *Salmonella* prevalence. The cohort prevalence of *Salmonella* at feedlot entry was not correlated with the prevalence immediately prior to harvest (n = 29, $p = 0.64$, Spearman's rho = -0.09).

Of the *Salmonella* isolates characterized (Table 3.1), most were serogroup E (567), C1 (398), or C3 (123). Predominant serotypes recovered were Anatum (347), Montevideo (261), Orion (206), Kentucky (123), Mbandaka (110), and Newport (38) (Table 3.1). *Salmonella* isolates of the same serotype were found at both feedlot arrival and preharvest within 23 of 29 cohorts sampled. In all, there were 518 *Salmonella* isolates that had matching serotypes within a cohort at both sampling times; thus, 518 were further characterized by PFGE. There were a total of 26 distinguishable PFGE types and 49 subtypes. Approximately half (48.3%) of these isolates belong to one of two subtypes. Eleven of 26 (42.3%) types and 10 of 49 (20.4%) subtypes were represented by at least ten isolates; whereas 14 types (53.8%) and 21 subtypes (42.9%) were detected only once. The number of *Salmonella* isolates by PFGE subtype and date of sampling for each cohort where *Salmonella* was recovered within a cohort at feedlot arrival and preharvest are shown in Tables 3.2 (serogroup C isolates) and 3.3 (serogroup E isolates).

Eligible cohorts (n = 23) had similar types (21, 91.3%) and subtypes (14, 60.9%) of *Salmonella* recovered both at feedlot entry and preharvest. Among the 518 isolates that were of the same serotype within a cohort, 261 (50.4%) were of PFGE subtypes recovered at both sampling times. Proportions of isolates with matching subtypes for each *Salmonella* serotype are shown in Table 3.4. Some PFGE subtypes, for examples subtype *a* of serotype Anatum (Table 3.3) and subtype *ff* of serotype Montevideo (Table 3.2), persisted within several cattle cohorts (9 and 6, respectively). Specific subtypes persisted up to 285 days, which was the longest feeding period in the study. The probability of a cohort having at least one *Salmonella* subtype that persisted through the feeding period was not associated with mean entry weight ($p = 0.39$), respiratory disease risk category ($p = 0.78$), gender ($p = 0.67$), month of enrollment ($p = 0.47$), days on feed ($p = 0.88$), morbidity ($p = 0.32$), mortality ($p = 0.64$), or differences in *Salmonella* prevalence among sampling times ($p = 0.28$). For cohorts in which a PFGE subtype of

Salmonella was found to persist, the probability of detecting the subtype at preharvest sampling was not associated with respiratory disease risk category ($p = 0.69$), gender ($p = 0.86$), month of enrollment ($p = 0.69$), days on feed ($p = 0.83$), morbidity ($p = 0.27$), mortality ($p = 0.86$), or change in cohort prevalence of *Salmonella* ($p = 0.46$).

Discussion

Our study indicates that the prevalence of *Salmonella* in cohorts of cattle at arrival at the feedlot may not be associated with the prevalence of *Salmonella* immediately prior to harvest. However, specific subtypes of *Salmonella* appear to persist within cohorts throughout the feeding period (148 to 285 days in our study). Our study was based in only one feedlot, which had a high overall prevalence of *Salmonella* within the study population. Our data indicate that cohorts of cattle entering the feedlot at lighter mean body weights, fed for a longer period of time, and having higher cumulative incidence of morbidity and mortality may be more likely to shed *Salmonella* in their feces. However, a more in-depth assessment of these correlated cohort-level risk factors is necessary, since a multivariable analysis separating the effects of these individual factors was not appropriate for our data.

The fecal prevalence of *Salmonella* that we observed was relatively high compared to other studies that have determined the *Salmonella* prevalence in feedlot cattle (Fedorka-Cray *et al.*, 1998; Dargatz *et al.*, 2003; Loneragan *et al.*, 2005; Kunze *et al.*, 2008). Although some of these differences may be attributed to variation in diagnostic protocols, a relatively high fecal prevalence of *Salmonella* also may be expected for this geographical region (Kunze *et al.*, 2008; Alam *et al.*, 2009). As seen in Figure 3.1, there was considerable variability in prevalence estimates across cohorts at each sampling time in this study. We have observed this variability in the fecal prevalence of *Salmonella* in feedlot cattle populations in previous studies (Alam *et al.*, 2009; Dodd *et al.*, 2010).

There have been few published longitudinal studies of *Salmonella* prevalence within cohorts of commercial feedlot cattle. One study reported a decrease in fecal shedding of *Salmonella* in 120 commercial feedlot steers from feedlot arrival (40%) to harvest (0%) (Galland *et al.*, 2000); however, a direct comparison with our results cannot be made as there were several demographic differences between the study populations, and their study used different culture methods to estimate prevalence. Another longitudinal study followed shedding of *Salmonella* in

144 research feedlot steers in North Dakota; prevalence increased from 0.7 to 64.0% during the feeding period (Khaitsa *et al.*, 2007). In a longitudinal study of five commercial feedlots that used manila-hemp rope sampling devices to monitor *Salmonella*, researchers demonstrated differences in the proportion of *Salmonella*-positive pens by week within season and feedlot (Smith *et al.*, 2005). However, the probability of detecting *Salmonella* during the week of harvest was not significantly different than for other weeks during the feeding period (Smith *et al.*, 2005). Previous longitudinal data from a vaccine field trial at a commercial Kansas feedlot revealed that fecal shedding of *Salmonella* throughout the feeding period was highly variable by time and among cohorts in both vaccine and control groups, with a decrease in prevalence immediately prior to summer harvest (Dodd *et al.*, 2010). In contrast, a study of 100 US feedlots showed a higher prevalence of *Salmonella* in cohorts of commercial feedlot cattle that were close to harvest (7.4%) than cohorts of cattle that had recently entered feedlots (3.5%) (Fedorka-Cray *et al.*, 1998). In our current study, the within-cohort fecal prevalence of *Salmonella* immediately prior to harvest was highly variable and was not correlated with the prevalence at feedlot entry. This finding may reflect the transient nature or seasonality of fecal shedding of *Salmonella* in feedlot cattle that could be due to environmental conditions; however, unexplained variability in shedding remains (Barkocy-Gallagher *et al.*, 2003; Smith *et al.*, 2005; Rhoades *et al.*, 2009).

Of the potential risk factors evaluated in our study, only mean entry weight was associated with the *Salmonella* prevalence at feedlot arrival. We had a limited number of cohorts and categorized several continuous variables due to the lack of linear relationship with response variables, which may have decreased the power to detect effects of these factors. Mean entry weight was not associated with the preharvest prevalence of *Salmonella*; however, cohorts of lighter weight cattle usually incur a longer feeding period and have higher cumulative incidence of crude morbidity and mortality (Kelly *et al.*, 1986). Higher days on feed, morbidity, and mortality have been associated with a higher risk for fecal shedding of *Salmonella* in feedlot cattle (Losinger *et al.*, 1997; Fedorka-Cray *et al.*, 1998; Smith, 2002). In our study, preharvest prevalence was positively associated with high BRD risk, longer days on feed, high morbidity, and high mortality. Thus, risk factors we found to be associated with *Salmonella* prevalence estimates at feedlot arrival and preharvest are likely related, since we are describing similar cattle populations at different sampling times. Several of the variables in our data were highly correlated with each other (data not shown); hence, it was not possible to evaluate their

individual effects in a multivariable model. Therefore, interpretation of these unconditional associations should be made with caution. These associations indicate that an early predictor of preharvest *Salmonella* prevalence may be the perceived risk for respiratory disease; however, this assessment of risk is fairly subjective. The BRD risk category that is assigned to a cohort of calves at feedlot entry may be a proxy for several factors including transport distance, cattle source, and animal size/age (Sanderson *et al.*, 2008), and also would be inherently related to antimicrobial use. Days on feed, morbidity, and mortality are more objective predictors of the cohort prevalence of *Salmonella* than BRD risk category. Therefore, further assessments of these effects are needed to better understand the ecology of *Salmonella* in commercial feedlots.

Our finding of few predominant *Salmonella* serotypes is consistent with other feedlot studies (Fedorka-Cray *et al.*, 1998; Dargatz *et al.*, 2003; Kunze *et al.*, 2008). The five most frequent serotypes that we recovered are different than the most common serotypes isolated from clinically ill humans (Callaway *et al.*, 2008; CDC, 2010). Two serotypes, Anatum and Montevideo, represented 50.1% of *Salmonella* isolates recovered in our study. Loneragan *et al* reported that 48% of their *Salmonella* isolates were serotype Anatum (Loneragan *et al.*, 2005), and another cross-sectional study of cattle in abattoirs found 32.5% of fecal *Salmonella* isolates were Anatum (Kunze *et al.*, 2008). In a prospective cohort study to assess factors associated with fecal shedding of *Salmonella*, serotype Orion represented 46.5% of isolates and Anatum represented 19.8% of isolates (Alam *et al.*, 2009). Reasons for observing predominant *Salmonella* serotypes within a population of cattle are unknown, but may be due to the preferential ability of specific serotypes or strains to replicate inside or outside the host under the given conditions. Table 3.1 data indicate that the frequency of some serotypes may have increased throughout the feeding period (e.g., Anatum or Kentucky) while others may have decreased (e.g., Orion or Newport). Shifts in the relative proportion of *Salmonella* strains within a population requires further study as they may have important public health or animal health implications if the serotypes or strains differ in infectivity or virulence.

Salmonella can disseminate within and among cattle cohorts via fecal-oral transmission and will survive and persist outside hosts in agricultural environments. We found that serotypes and specific PFGE subtypes of *Salmonella* appear to persist within a cohort throughout the feeding period, and also can be found among multiple cohorts within the feedlot (Tables 3.2-3.3). Although our study was not designed to directly assess dissemination of *Salmonella* strains, most

PFGE subtypes that persisted were found more frequently immediately prior to harvest than they were found at feedlot arrival. As seen in Table 3.4, there was less diversity among the PFGE-characterized isolates that were recovered preharvest than those recovered at feedlot entry.

Studies of *Salmonella* subtype persistence among feedlot cattle cohorts have not been reported previously, although propagation of *Salmonella* has been studied in multiple livestock environments. *Salmonella* survived over 150 days in experimentally inoculated cattle fecal pats, and under some conditions had an initial 1.5 order of magnitude increase following pasture deposition (Sinton *et al.*, 2007). *Salmonella* Typhimurium survived over 133 days in cattle feces (Franz *et al.*, 2005), and a distinct PFGE type of *Salmonella* Typhimurium was found over a two-year period in swine feces and soil fertilized with swine manure (Baloda *et al.*, 2001). On a depopulated free-range poultry farm, *Salmonella* Enteritidis Phage Type 4 was recovered from litter and dried feces for up to 26 months (Davies *et al.*, 2003). Wildlife and flies also may be important vectors for *Salmonella*, as well as mechanisms for persistence, in livestock environments (Olsen *et al.*, 2000; Kirk *et al.*, 2002; Renter *et al.*, 2006). Several mechanisms for *Salmonella* transmission within a cohort may have existed within our study population.

Even though the cohort-level fecal prevalence of *Salmonella* at feedlot entry was not associated with the preharvest prevalence, it is interesting that some subtypes persisted within cohorts. The lack of a significant correlation among prevalence estimates may be due to the temporal variability in fecal shedding of *Salmonella* within a cohort. Feedlot pen environments were not sampled before the study commenced, so we cannot determine whether the *Salmonella* strains were also present in the feedlot environment. Hence, further studies that include environmental sampling are needed to determine how long *Salmonella* strains persist within commercial feedlots across feeding periods. Pulsed-field gel electrophoresis provides reasonable molecular differentiation of *Salmonella* and has been used extensively to determine genetic similarity among isolates (Fakhr *et al.*, 2005; Ribot *et al.*, 2006; Soyer *et al.*, 2010). Because of the discriminatory ability of PFGE analysis, identical subtypes found both at feedlot entry and immediately prior to harvest in the study cohorts were assumed to be isolates that persisted during the feeding period. Since sampling was limited and only one isolate per positive fecal sample was characterized, it is probable that our study underestimated the amount of strain persistence. Knowing that specific subtypes of *Salmonella* persist within a feedlot cohort, even when the cohort fecal prevalence may fluctuate, is important in understanding the ecology of

Salmonella in commercial feedlot environments. Although fecal shedding within a cohort may be transient, it appears that some of the *Salmonella* serotypes or PFGE subtypes persist. A study of *Salmonella* isolates from dairy cattle with salmonellosis indicated that some strains persist over time, and some PFGE types can be found in multiple farms (Fakhr *et al.*, 2005). Further studies are needed to determine whether *Salmonella* strains that persist in feedlot cattle during the feeding period are more likely to be present in higher concentrations in feces at harvest and lead to subsequent beef contamination.

Our repeated cross-sectional study provides further insight on fecal shedding of *Salmonella* by feedlot cattle; however, additional longitudinal data are needed to provide a better understanding of the ecology of *Salmonella* in commercial feedlots. The cohort-level risk factors that we found to be associated with *Salmonella* prevalence at arrival and preharvest need further evaluation, but our data provide preliminary evidence that specific subsets of the feedlot cattle population may have a higher risk for shedding *Salmonella*. Preharvest interventions for beef cattle need to affect the prevalence of *Salmonella* at harvest to potentially improve beef safety, but the persistence of specific subtypes of *Salmonella* throughout the feeding period may indicate that a more comprehensive approach to controlling *Salmonella* is needed. Future longitudinal studies involving multiple cattle sources, animal cohorts and feedlots are needed to evaluate the prevalence and persistence of *Salmonella* strains during the feeding period.

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Figure 3.1 Fecal prevalence of *Salmonella* at feedlot entry (arrival) and immediately prior to harvest (preharvest) for each cattle cohort (n=30) in the order that they were enrolled in the study. Error bars represent 95% exact confidence intervals for proportions. The cohort prevalence of *Salmonella* at feedlot entry was not correlated with the prevalence immediately prior to harvest (n = 29, *p* = 0.64, Spearman's rho = -0.09).

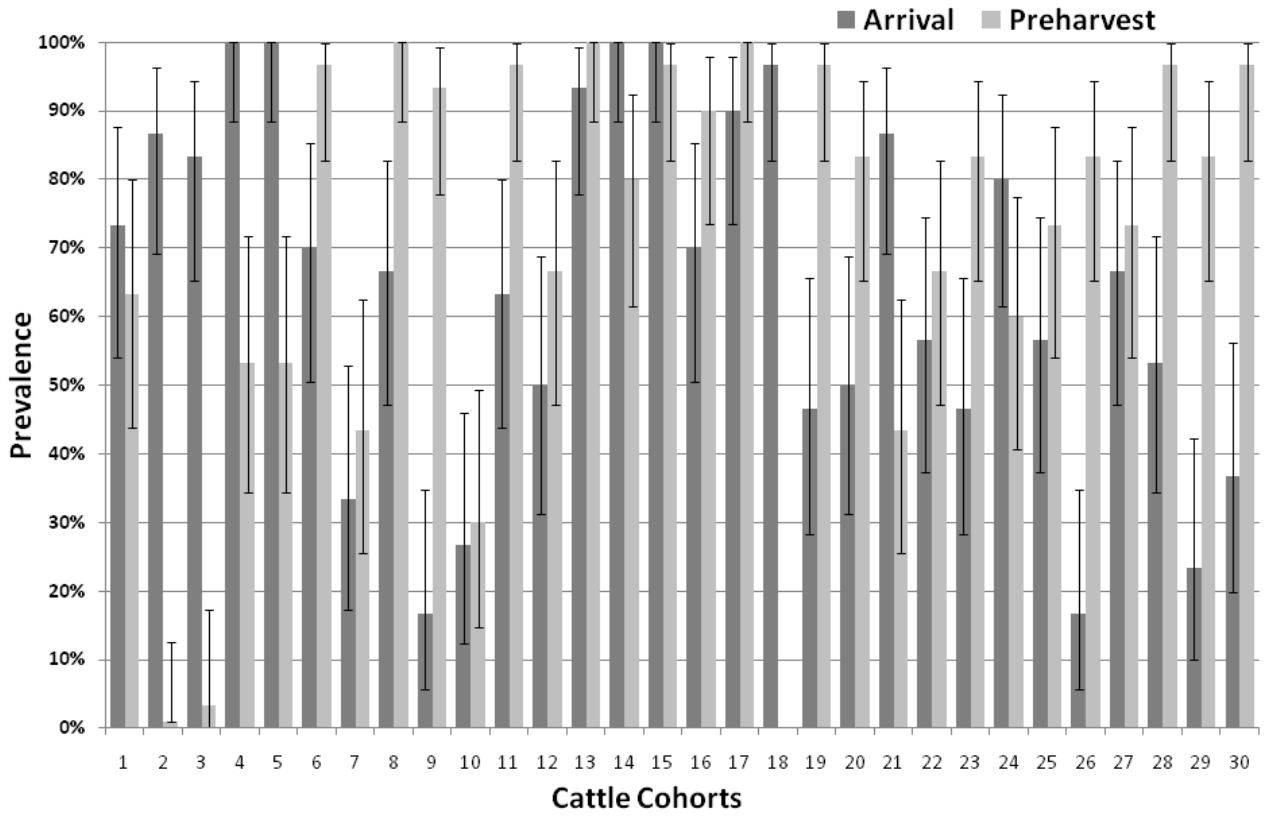

Table 3.1 *Salmonella* serotypes isolated from cattle fecal samples at feedlot arrival and prior to harvest (preharvest). Only one isolate was characterized from each positive fecal sample.

		Number of isolates (cattle cohorts)		
Serotype	*Serogroup*	*At arrival*	*Preharvest*	*Total*
Anatum[1]	E	105 (20)	242 (26)	347
Montevideo	C_1	104 (23)	157 (19)	261
Orion[2]	E	175 (24)	31 (3)	206
Kentucky	C_3	41 (15)	82 (14)	123
Mbandaka	C_1	42 (17)	68 (10)	110
Newport	C_2	37 (4)	1 (1)	38
Lille	C_1	0	21 (2)	21
Muenchen	C_2	16 (6)	0	16
Uganda[3]	E	7 (2)	0	7
Cerro	K	2 (2)	4 (2)	6
Muenster[4]	E_1	4 (3)	1 (1)	5
Meleagridis	E	3 (1)	0	3
Oranienburg	C_1	3 (2)	0	3
Senftenberg	E	1 (1)	2 (2)	3
Norwich	C_1	1 (1)	1 (1)	2
Reading	B	2 (1)	0	2
Agona	B	1 (1)	0	1
Bredeney	B	1 (1)	0	1
Cubana	G_2	1 (1)	0	1
Give	E	1 (1)	0	1
Schwarzengrund	B	0	1 (1)	1
Thomson	C_1	1 (1)	0	1
Nontypeable		34 (16)	21 (9)	55
Total:		582	632	1214

[1] Also includes Anatum var 15+ (n = 9) and Anatum var 15+34+ (n = 32)
[2] Includes Orion v15+ (n = 18) and Orion v15+34+ (n = 188)
[3] Also includes Uganda var 15+ (n = 1)
[4] Also includes Muenster var 15+34+ (n = 1)

Table 3.2 Counts of serogroup C *Salmonella* isolates by serotype, pulsed-field gel electrophoresis (PFGE) subtype[1], and sampling time for each cattle cohort that had the same serotype recovered at both feedlot arrival and prior to harvest (preharvest)[2]

[Next Page]

[1] Subtypes represent unique PFGE banding patterns following *Xba*1 digestion.
[2] Data collected immediately prior to harvest are shaded in gray.

		Count of serogroup C Salmonella *serotypes and PFGE subtypes*																						
		Kentucky							*Montevideo*															*Newport*
Cohort ID	*Date*	*q*	*r*	*s*	*t*	*u*	*v*	*w*	*ff*	*gg*	*hh*	*ii*	*jj*	*kk*	*ll*	*mm*	*nn*	*oo*	*pp*	*qq*	*rr*	*ss*	*tt*
1	10/11/06	-	-	-	-	-	-	-	-	-	-	-	-	-	4	4	-	-	-	-	-	-	-
	3/12/07	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	10/11/06	-	-	-	-	-	-	-	3	1	1	-	-	-	-	-	-	3	1	-	-	-	-
	4/2/07	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	10/11/06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3/12/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
5	10/11/06	-	-	-	1	-	-	-	1	-	-	-	-	7	-	-	-	-	-	-	-	-	14
	2/28/07	-	2	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	1
6	10/31/06	-	-	-	-	-	-	-	-	-	-	-	2	2	-	2	-	-	-	-	-	-	-
	5/7/07	-	-	-	-	-	-	-	1	-	-	2	-	-	-	-	-	-	-	-	-	-	-
7	11/7/06	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5/7/07	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	11/14/06	-	-	-	-	-	1	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
	4/30/07	-	-	7	3	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	11/14/06	-	-	1	1	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4/2/07	-	-	-	-	1	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	11/14/06	-	-	1	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6/11/07	-	-	-	-	3	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-
13	12/11/06	-	-	-	-	1	-	4	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	8/7/07	1	-	-	-	6	-	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	12/11/06	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	6/5/07	-	-	-	-	1	-	-	14	-	-	1	-	-	-	-	-	-	-	-	-	-	-
17	12/11/06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-
	5/7/07	-	-	-	-	-	-	-	1	-	-	3	-	-	-	-	-	-	-	-	-	-	-
18	1/17/07	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6/5/07	-	-	-	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	1/17/07	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	1	-	-	6	-	-	-
	6/11/07	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	1	-
23	1/17/07	-	-	-	-	-	-	-	1	-	-	-	-	2	-	-	-	-	-	-	-	-	-
	6/5/07	-	-	-	-	-	-	-	16	-	1	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.3 Counts of serogroup E *Salmonella* isolates by serotype, pulsed-field gel electrophoresis (PFGE) subtype[1], and sampling time for each cattle cohort that had the same serotype recovered at both feedlot arrival and prior to harvest (preharvest)[2]

[Next Page]

[1] Subtypes represent unique PFGE banding patterns following *Xba*1 digestion.
[2] Data collected immediately prior to harvest are shaded in gray.

		Count of serogroup E Salmonella *serotypes and PFGE subtypes*																									
		Anatum															*Mbandaka*								*Orion var 15+ 34+*		
Cohort ID	*Date*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*	*m*	*n*	*o*	*p*	*x*	*y*	*z*	*aa*	*bb*	*cc*	*dd*	*ee*	*uu*	*vv*	*ww*
1	10/11/06	-	1	-	-	-	1	-	-	-	3	-	-	5	1	-	-	-	-	-	-	-	-	3	-	-	-	-
	3/12/07	2	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
2	10/11/06	1	-	4	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	4/2/07	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	10/11/06	2	-	-	1	-	-	-	-	-	-	4	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3/12/07	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	10/11/06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	2	-	-	-	-
	3/12/07	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
5	10/11/06	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2/28/07	6	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	10/31/06	1	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	5/7/07	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	11/7/06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	1	-	-	-
	4/2/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
10	11/14/06	3	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-
	4/2/07	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
11	11/14/06	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	6/25/07	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-
12	11/14/06	1	-	-	-	2	-	1	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
	6/11/07	10	-	-	-	-	-	-	2	3	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
13	12/11/06	-	-	-	-	-	-	-	-	-	-	12	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	8/7/07	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
15	12/11/06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	5/7/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3	-
16	12/11/06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
	4/30/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
20	1/17/07	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6/5/07	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	1/17/07	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6/11/07	3	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	1/17/07	1	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-
	6/25/07	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19	-	-	-	-	-	-	-
23	1/17/07	4	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6/5/07	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.3 Summary of pulsed-field gel electrophoresis (PFGE) results for *Salmonella* serotypes present within cattle cohorts at both feedlot entry and prior to harvest (preharvest). Only one isolate was characterized from each positive fecal sample.

	Number of isolates characterized by PFGE		*Number of isolates with matching PFGE subtypes*[1]		*Percent of isolates with matching PFGE subtypes*	
Serotype	*Entry*	*Preharvest*	*Entry*	*Preharvest*	*Entry*	*Preharvest*
Anatum	79	144	22	111	27.8%	77.1%
Montevideo	58	101	11	56	19.0%	55.4%
Mbandaka	18	45	6	17	33.3%	37.8%
Kentucky	15	36	4	19	26.7%	52.8%
Newport	14	1	14	1	100.0%	100.0%
Orion v15+34+	1	6	0	0	0.0%	0.0%
Total:	185	333	57	204	30.8%	61.3%

[1] Isolates with a PFGE profile identical to another isolate that was recovered at a different sampling time within the same cohort.

CHAPTER 4 – Genetic Relatedness of *Escherichia coli* O157 Isolates from Cattle Feces and Pre-intervention Beef Carcasses

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Abstract

Our objective was to define and compare pulsed-field gel electrophoresis (PFGE) profiles of *E. coli* O157 isolates from cattle feces and carcass samples to evaluate relationships between beef carcass contamination and fecal shedding of *E. coli* O157 at harvest. We used PFGE separation of *Xba*1-digested DNA to characterize *E. coli* O157 isolates (n=174) from pre-evisceration carcasses (n=39) and feces (n=135) that were recovered from 37 *E. coli* O157-positive truckloads sampled at a commercial abattoir. Semi-quantitative fecal culture techniques differentiated high-shedding, low-shedding and negative cattle. Among all isolates, there were 17 PFGE types (95% homology) and 37 subtypes (100% homology). Specific subtypes were detected on multiple occasions and from different sample types within loads, among loads, and among days. Seventeen subtypes were recovered from carcasses; most were also recovered from feces of high-shedding cattle (13 subtypes) and low-shedding cattle (14 subtypes). Within truckload, the percentages of carcass isolates that were identical to high-shedder or low-shedder fecal isolates, as determined by PFGE, were 69.2% and 46.0%, respectively. Whereas among different truckloads within the same study day, the percentages of carcass isolates that were the same subtype as high-shedder or low-shedder fecal isolates were 35.3% and 58.8%, respectively. Our results suggest that cattle feces from both low- and high-shedders pose a potential risk for *E. coli* O157 contamination of carcasses. Truckload may be an important factor in the potential transmission of *E. coli* O157, but isolates from carcasses also may be similar to those from feces of cattle on different truckloads and harvest days.

Introduction

Escherichia coli O157 is a significant cause of foodborne illness in the United States. From 1982 to 2002, there were 350 reported outbreaks of *E. coli* O157 including 8,598 human illnesses, 1,493 hospitalizations, and 40 deaths (Rangel *et al.*, 2005). Although there are more cases of foodborne illness due to other pathogens each year, *E. coli* O157 outbreaks and related product recalls have generated substantial consumer risk awareness, impacting consumer demand for beef (Marsh *et al*., 2004; CDC, 2008). Properly cooking beef will kill the organisms, yet pre- and post-harvest interventions are still needed to lower *E. coli* O157 risk since cross-contamination and under-cooking still occur (Caprioli *et al.*, 2006; Verbeke *et al.*, 2007).

A primary pathway of *E. coli* O157 contamination of beef involves colonized cattle that shed organisms in feces, which subsequently contaminate hides (Loneragan *et al.*, 2005; Baker *et al.*, 2007). Most carcass contamination is believed to occur during the hide-removal and evisceration process (Barkocy-Gallagher *et al.*, 2003); *E. coli* O157 in cattle feces or on hides is associated with detectable levels of *E. coli* O157 on carcasses (Elder *et al.*, 2000; McEvoy *et al.*, 2003; Woerner *et al.*, 2006). Some cattle with *E. coli* O157 shed greater levels, more than 10^3 or 10^4 colony forming units (CFU) per g of feces, than other cattle within a given population (Omisakin *et al.*, 2003). High-shedders may pose a higher risk of carcass or hide contamination than cattle shedding lower concentrations, and may lead to situations where thresholds for effective intervention strategies are exceeded (Matthews *et al.*, 2006; Chase-Topping *et al.*, 2008; Fox *et al.*, 2008).

Assessing relatedness among *E. coli* O157 isolates recovered from points throughout beef production systems helps to better define the ecology and epidemiology of this pathogen during pre-harvest and harvest processes. Previously, Fox *et al.* (2008) described associations at harvest between fecal shedding (both low- and high-shedding levels) and pre-evisceration carcass contamination within truckloads of finished cattle. Inference was limited by factors inherent to the study design; however, genetic analysis of the recovered isolates could further define the potential transmission routes for carcass contamination. Our objective was to define and compare, within and among cattle cohorts, the PFGE profiles of *E. coli* O157 previously isolated from fecal and carcass samples to further define relationships between beef carcass contamination and fecal shedding of *E. coli* O157 at harvest.

Materials and Methods

Source of isolates

As previously described (Fox *et al.*, 2008), fecal and carcass samples were obtained from 1,503 cattle that arrived in 50 truckloads to a commercial abattoir in the Midwest U.S. during a 5-week period. Data on cattle origin were not available. Up to 32 cattle per truckload were sampled. Pre-evisceration carcass sponge samples, collected post-hide removal, were matched within animal to post-evisceration fecal samples collected from intact rectums. Gloves were changed between samples and measures were taken to prevent cross-contamination among samples (Fox *et al.*, 2008). All samples were transported in coolers with ice packs to the Kansas State University, College of Veterinary Medicine, Preharvest Food Safety Laboratory and stored under refrigeration (4°C) for processing within 48 h of collection.

Isolation of E. coli *O157*

Isolation and identification of *E. coli* O157 from carcass and fecal samples were previously described (Fox *et al.*, 2008). Briefly, carcass sponge samples were enriched in 2% brilliant green bile broth (Difco, Becton Dickinson, Sparks, MD) and fecal samples were enriched in GN broth (BD, Franklin Lakes, NJ) containing cefixime (Sigma-Aldrich; 50 ng/ml), cefsulodin (Sigma-Aldrich; 10 µg/ml), and vancomycin (Sigma-Aldrich; 8 µg/ml; GNccv). To detect high-shedding cattle (Sanderson *et al.*, 2007), a pre-enrichment fecal suspension was directly streaked onto sorbitol MacConkey agar (BD) containing cefixime (50 ng/ml) and potassium tellurite (2.5 µg/ml; CT-SMAC). Enriched samples were subjected to immunomagnetic separation (IMS) and plated onto CT-SMAC. Following 16 to 18 h incubation at 37°C, up to six non-sorbitol-fermenting colonies were transferred onto blood agar (Remel, Lenexa, KS) and incubated overnight at 37°C. Colonies were tested for indole production and latex agglutination of the O157 antigen (Oxoid Limited, Basingstoke, Hampshire, England); positives were tested by polymerase chain reaction (PCR) for *eae, stx*$_1$, *stx*$_2$ and *hyl*A genes (Fagan *et al.*, 1999). Isolates were considered *E. coli* O157 if they had *eae* and *hyl*A genes and at least one *stx* gene. Fecal samples were also classified based on the relative concentration of *E. coli* O157: cattle with fecal samples positive only on the IMS procedure were classified as low-shedder, while those with feces positive by the direct plate technique were classified as high-

shedder (Fox *et al.*, 2007; Sanderson *et al.*, 2007; Fox *et al.*, 2008). Isolates were stored on protect beads at -80°C for future characterization.

Pulsed-field gel electrophoresis

Isolates were analyzed by PFGE separation of *Xba*1-digested genomic DNA in accordance with the PulseNet U.S.A. Standardized Laboratory Protocol (Ribot *et al.*, 2006). Stored isolates were transferred onto blood agar and incubated at 37°C for 18 hours. Colonies were added to cell suspension buffer at room temperature and concentration was adjusted to an optical density of 1.3 to 1.4 at 610 nm. Cell suspensions were incorporated into gel plugs consisting of 1% SeaKem Gold® agarose, 1% SDS (BioWhittaker Molecular Applications, Rockland, Maine), and Proteinase K (Fisher Scientific, Fair Lawn, New Jersey). Cast gel plugs were then subjected to cell lysis in TE buffer (50mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosyl). Following washing in reagent grade, type 1 water, extracted DNA samples underwent restriction digestion with the *Xba*1 enzyme (Promega Corporation, Madison, Wisconsin). Along with *Salmonella* ser. Braenderup H9812 standards, each sample was cast into agarose plugs and loaded into wells. Restricted plug slices were subjected to electrophoresis, stained with an ethidium bromide solution, and viewed under UV light. Gel imaging software (Quantity One®, Bio Rad, Hercules, CA and BioNumerics®, Applied Maths, Austin, TX) was used to digitize, normalize, and assign bands for each isolate image. Band-sharing similarity coefficients were generated from the DNA fragments in the 10- to 550-kb range. Dendograms were constructed to provide a visual representation of the relationship among *E. coli* O157 isolates. Isolates were grouped based on banding pattern similarities where types and subtypes were defined as isolates having PFGE patterns of >95% or 100% Dice similarity, respectively (Sargeant *et al.*, 2006).

Data analysis

Truckload, animal, sample type, and all laboratory results were recorded and managed in spreadsheet format (Microsoft® Office Excel 2007, Microsoft, Redmond, WA). The PFGE results for the isolates were assessed in conjunction with epidemiologic data on the source of the isolates, including sample type, truckload, and day. Descriptive analyses of the frequency distribution of types and subtypes within each sample type, truckload, and sampling day were evaluated, and exact 95% binomial confidence intervals (CI) were calculated for proportions using the BETAINV function of Microsoft® Office Excel. Exact CI are reported in parentheses

for frequency measures. Comparisons of the 95% CI were used to assess the potential for proportions to differ ($P \leq 0.05$) while recognizing the strengths and weaknesses of this approach (Schenker *et al.*, 2001). In addition, we assessed and described the presence or absence of genetically similar *E. coli* O157 isolates longitudinally, including whether strains were persistently recovered from carcasses sampled on different days within the studied abattoir.

Results

Prevalence and sources of isolates

Escherichia coli O157 was isolated from fecal or carcass samples from 157 of 1,503 (10.4%) cattle originating from 37 of 50 (73.5%) truckloads (Fox *et al.*, 2008). Of the 174 recovered *E. coli* O157 isolates, 135 (77.6%) were from fecal samples and 39 (22.4%) were from carcass samples. Thirty-nine of 1,503 carcass samples (2.6%) representing 15 truckloads and 127 of 1,495 fecal samples (8.5%) representing 37 truckloads were positive. There were 55 fecal-positive cattle that were positive on direct plating (high-shedders) and 80 cattle that were only positive based on enrichment/IMS of fecal samples (low-shedders) (Table 4.1). Of 39 carcass-positive cattle, nine also had positive fecal samples (five high-shedders and four low-shedders). There were 120 fecal-positive cattle that were carcass-negative. The percentages of truckloads that had a least one high-shedder or low-shedder were 52.0% (37.4 to 66.3%) and 62% (47.1 to 75.3%), respectively (Fox *et al.*, 2008). All isolates had the *stx*2 gene and 90 (51.7%) isolates had the *stx*1 gene.

Pulsed-field gel electrophoresis

Among 174 isolates, there were a total of 17 distinguishable PFGE types (95% Dice similarity) and 37 subtypes (100% Dice similarity; no band differences). Among all isolates, two PFGE types were detected at least 30 times and four subtypes were detected at least 10 times (Table 4.2). Two of 17 types (11.8%), and 12 of 37 subtypes (32.4%) were represented only once. Fifty percent of all isolates were one of five subtypes, and 48.9% of all isolates were one of three types.

Overall, there were 17 *E. coli* O157 subtypes recovered from carcasses and 36 subtypes recovered from fecal samples. Twenty-four of the fecal isolate subtypes were recovered from high-shedding cattle, and 28 were recovered from low-shedding cattle (Table 4.1). Sixteen

subtypes were found in the feces of both high-shedding and low-shedding cattle. Thirty high-shedder (54.6%; 40.6 to 68.0%) and 37 low-shedder (46.3%; 35.0 to 57.8%) fecal isolates had identical PFGE subtypes as carcass isolates on the same load (Table 4.1).

Eleven of 37 (29.7%; 15.9 to 47.0%) subtypes were recovered from fecal or carcass samples from multiple truckloads within the same day (Table 4.2). Ten out of 37 (27.1%; 13.8 to 44.1%) subtypes were found on multiple truckloads on different days (Table 4.2), while four out of 17 (25.5%; 6.8 to 49.9%) subtypes from carcasses were found on carcasses from multiple loads on different days (Table 4.1). Only three (8.1%; 1.7 to 21.9%) subtypes from any sample type were found on multiple loads on consecutive days.

Thirteen of the 37 total subtypes (35.1%; 20.2 to 52.5%) were recovered from both carcasses and high-shedding cattle feces, whereas 14 (37.8%; 22.5 to 55.2%) were recovered from both carcass and low-shedding cattle feces. Eleven subtypes (29.7%) were recovered from all three sample types. All but one of the subtypes recovered from a carcass were also found in a fecal sample, and the subtype not found in feces was recovered from only one carcass.

Several *E. coli* O157 subtypes were recovered from multiple carcass or fecal samples within truckloads (Tables 4.1 and 4.2). Eight of 37 subtypes (21.6%; 9.8 to 38.2%) were found on more than one carcass within a load, and 18 (48.7%; 31.9 to 65.6%) were found in more than one fecal sample within truckload. One subtype was detected 10 times within a truckload and another was detected 15 times within a truckload (Table 4.2). There was a mean of 1.9 (range: 1-6) subtypes per positive truckload. Eleven subtypes (29.7%) were recovered from both carcass and fecal samples within a truckload (Table 4.3). The percentages of subtypes from carcasses that also were detected from a high-shedder, low-shedder, or any feces from within the same truckload were 70.6% (44.0 to 89.7%), 47.12% (23.0 to 72.2%), and 70.6% (44.0 to 89.7%), respectively. Within truckload, the percentages of carcass isolates that were identical to high-shedder, low-shedder, or any fecal isolate, as determined by PFGE, were 69.2% (52.4 to 83.0%), 46.0% (30.1 to 62.8%), and 69.2% (52.4 to 83.0%), respectively (Figure 4.1). Nine of 15 truckloads with a positive carcass had carcass subtypes that matched at least one high-shedder fecal isolate, 11 of 15 carcass-positive truckloads had a carcass subtype that matched a low-shedder subtype, while eight out of 15 carcass-positive truckloads had a carcass subtype that matched both low- and high-shedder subtypes in the same truckload (Table 4.3).

Four of 17 carcass subtypes (23.5%; 6.8 to 49.9%) were detected on carcasses from multiple truckloads, and three of these were on the same day. There were 11 fecal subtypes recovered from multiple fecal samples from different truckloads. Seven fecal subtypes were recovered from high-shedders from different truckloads, and four were found on truckloads harvested on the same day. Ten fecal subtypes were detected in feces from low-shedders from different truckloads with six of these on the same day. The percentages of carcass subtypes that also were detected from a high-shedder, low-shedder, or any feces among different truckloads within the same day were 35.3% (14.2 to 61.7%), 58.8% (32.9 to 81.6%), and 58.8% (32.9 to 81.6%), respectively. Among different truckloads within the same day, the percentages of carcass isolates that were identical to high-shedder, low-shedder, or any fecal isolate, as determined by PFGE, were 20.5% (9.3 to 36.5%), 48.7% (32.4 to 65.2%), and 51.3% (34.8 to 67.6%), respectively (Figure 4.1).

Discussion

We found that most of the *E. coli* O157 isolates recovered from pre-evisceration carcasses were the same PFGE subtype as isolates recovered from post-evisceration fecal samples from cattle within the same truckload. The percentages of carcass isolates that had identical PFGE patterns to that of fecal isolates from high- (69.2%) and low- (46.0%) shedding cattle within a truckload were fairly similar. However, comparing those numbers to corresponding percentages (35.3% and 58.8% respectively) among different truckloads within the same day revealed that the importance of high- versus low-shedding cattle may depend on whether the consideration of *E. coli* O157 dynamics is being made within or between truckloads (Figure 4.1). The transport cohort appears to be an important factor in the transmission of *E. coli* O157 at harvest, yet 32% of the subtypes were recovered from multiple truckloads and sampling days. Approximately half of the fecal isolates from high-shedders and low-shedders were the same PFGE subtype as carcass isolates on the same truckload. Although some have suggested that detecting high-shedders may be more efficient than detecting low-shedders and prioritizing the detection and mitigation of high-shedders within cattle cohorts might reduce risk of carcass contamination (Matthews *et al.*, 2006; Fox *et al.*, 2007; Sanderson *et al.*, 2007; Chase-Topping *et al.*, 2008), our findings suggest that pre-harvest intervention strategies need to mitigate the effects of both high- and low-shedding cattle within and among transport cohorts.

Defining associations between fecal shedding and carcass contamination may enhance the development of monitoring and/or intervention strategies to reduce *E. coli* O157 contamination of beef products. Molecular subtyping by pulsed-field gel electrophoresis (PFGE) has been used to study the genetic relatedness of *E. coli* O157 strains in foodborne disease outbreaks as well as in epidemiologic research (Faith *et al.*, 1996; Swaminathan *et al.*, 2001; Rangel *et al.*, 2005). Although PFGE protocols with different restriction enzyme combinations have been employed, the comparison of banding patterns produced by the same restriction enzyme(s) appears to provide useful estimates of relatedness among *E. coli* O157 isolates (Davis *et al.*, 2003). In this study, the total number of PFGE subtypes among isolates from carcasses and feces reflect the recognized genetic diversity of *E. coli* O157 within a population at an abattoir. The results of our PFGE analysis are consistent with observed frequencies among live cattle and beef carcasses (Rice, 1999; Barkocy-Gallagher *et al.*, 2001; Renter *et al.*, 2003). These data may not completely reflect genetic relationships among isolates, yet within the context of accompanying epidemiologic data, they allow us to infer reasonable estimates of relatedness (Davis *et al.*, 2003; Singer *et al.*, 2004). We chose a standard PFGE method using one restriction enzyme, which limited our visualization of restriction fragment patterns to approximately 20 bands (Singer *et al.*, 2004). These classification limitations may have affected our ability to differentiate some *E. coli* O157 isolates that we considered indistinguishable based on PFGE.

Some *E. coli* O157 PFGE subtypes were represented much more frequently than others, which is similar to other *Xba*1-PFGE comparison studies in cattle and beef carcasses (Rice, 1999; Barkocy-Gallagher *et al.*, 2001; Renter *et al.*, 2003). In this study, only one isolate from each sample underwent PFGE analysis, so the existence of other subtypes within a positive sample was unknown. Other studies have shown considerable heterogeneity of subtypes among individual samples (Faith *et al.*, 1996; Renter *et al.*, 2003), so it is possible that our study underestimated the existence of related *E. coli* O157 isolates among cattle, as well as the diversity of isolates within this population. Despite this limitation of the study, the genetic relatedness of detected isolates support associations of *E. coli* O157 among cattle and the potential transmission of specific subtypes from feces to carcass.

Our *E. coli* O157 isolates came from a study that used two culture methods to differentiate relatively low- versus high-shedding levels of *E. coli* O157 in fecal-positive cattle

(Fox *et al*., 2008). They found that the presence of a high-shedder within a truckload of cattle was the strongest predictor of carcass contamination. High *E. coli* O157 concentrations in cattle feces usually can be detected with the relatively rapid, less expensive, direct-plating technique, which has an estimated diagnostic sensitivity and specificity of 82.6% and 92.3%, respectively, for a breakpoint of 5×10^4 CFU/g feces (Sanderson *et al*., 2007). Concentrations as low as 10^2 CFU/g can be detected with approximately 90% sensitivity by culture methods incorporating IMS (Fox *et al*., 2007). A previous study demonstrated that a majority of cattle with low-level *E. coli* O157 carriage were fecal-positive only by using an IMS protocol and suggested a breakpoint fecal concentration of *E. coli* O157 of 10^3 CFU/g for differentiating high-shedders (Low et al., 2005). Although some previous studies have used experimentally inoculated cattle instead of naturally colonized cattle to estimate concentrations and test parameters, any misclassification in our study likely would have been non-differential with regards to classifying fecal-positive cattle into two groups based on relative concentration of fecal shedding. Although we did not make a direct attempt to enumerate *E. coli* O157 organisms within positive samples in this study, this classification scheme was still useful in examining associations while considering the heterogeneity of *E. coli* O157 fecal shedding in cattle populations.

High-shedders can contribute to the hide contamination of cohorts during transport to harvest and may be directly associated with carcass contamination (Bach *et al.*, 2004; Fox *et al.*, 2008). Overall prevalence of fecal shedding within cattle populations is also associated with hide and carcass contamination (Elder *et al.*, 2000), yet overall fecal prevalence may be affected by the presence or frequency of high-shedders (Cobbold *et al.*, 2007). Therefore, both fecal shedding concentration and overall prevalence of *E. coli* O157 within a pen or truckload may contribute to hide and eventual carcass contamination. In this study, one in three cattle testing positive for *E. coli* O157, and one in 27 (3.7%) of the cattle sampled, were identified as high-shedders (Fox *et al.*, 2008). Another study, which enumerated fecal isolates in harvested cattle, determined that only one in 11 (9.0%) cattle testing positive for *E. coli* O157 at harvest were high-shedders, but estimated that over 96% of the shed organisms originated from these high-shedders (Omisakin *et al.*, 2003). Some suggest that testing methods which primarily detect high-shedders, such as direct plating, may be a valid and feasible approach to strategically monitoring the risk of *E. coli* O157 contamination at harvest (Fox *et al.*, 2007; Sanderson *et al.*, 2007).

Pre-evisceration carcass swab samples and post-evisceration fecal samples were chosen in order to examine potential pre- and post-harvest associations of *E. coli* O157. Associations among subtypes from fecal and carcass samples may indicate that *E. coli* O157 isolates were transmitted among cattle, particularly since only 20.5% of carcass positive cattle were also positive in their feces. Approximately the same number of *E. coli* O157 subtypes from high-shedder and low-shedder cattle feces were also recovered from carcasses. This suggests that the risk of subsequent carcass contamination may be similar for both high- and low-shedding cattle; however, our data suggest the cohort effect may modify this relationship. Only one subtype from a carcass was not found in any fecal sample, which suggests a majority of *E. coli* O157 on carcasses at harvest may be detected in feces. However, previous literature suggests that *E. coli* O157 isolates detected on beef carcasses also can be detected on cattle hides (Barkocy-Gallagher *et al.*, 2001; Tutenel *et al.*, 2003).

Within the truckload, several subtypes were found on more than one carcass, in more than one fecal sample, or in both carcass and fecal samples. Some subtypes appeared to cluster among loads and on consecutive sampling days (Table 4.2). Although these data do not reveal extensive temporal associations, they support the hypothesis of transmission of *E. coli* O157 in feces between cattle at the feedlot or during transportation, lairage, or harvest (Akiba *et al.*, 2000; Minihan *et al.*, 2003). Data from pre-transport, post-transport, and lairage fecal and hide samples could have revealed further insight into the movement of *E. coli* O157 isolates among finished cattle within our study population.

The frequency of high-shedder subtypes matching carcass subtypes within truckload was not higher than the frequency of low-shedder subtypes matching carcass subtypes. Similarities among proportions may have been due to low statistical power in this study as there were only 17 subtypes isolated from carcasses. However, our determination of genetic relatedness suggests that more than half of *E. coli* O157 subtypes (58.8%) from carcasses were also present in cattle feces within the same load. The plausibility that the number of high-shedding cattle may pose a greater risk of carcass contamination than the overall number of cattle shedding *E. coli* O157 has been suggested in other studies (Omisakin *et al.*, 2003; Fegan *et al.*, 2004; Matthews *et al.*, 2006). Fox *et al* studied the same cattle population as we did, and found that high-shedder cattle, low-shedder cattle, and combined fecal prevalence were all significantly associated with carcass-

positive cattle within a truckload. However, the odds of carcass contamination were highest when a high-shedder was present within the truckload (Fox *et al.*, 2008).

Percentages of carcass isolates that matched high-shedder or low-shedder fecal isolates were similar both within truckloads and among truckloads within the same day (Figure 4.1). However, comparing the distributional trends of these data, within versus among truckloads, may indicate that high-shedders could play a more important role in transmission of *E. coli* O157 within truckloads than among truckloads. These data suggest that the truckload remains an important transmission factor in the ecology of *E. coli* O157 immediately prior to harvest, and perhaps the relative contribution of high versus low shedding cattle on carcass contamination risk depends on whether the assessment is made within or among truckloads.

Several identical subtypes were recovered from fecal samples taken from different truckloads, and several carcass subtypes also were detected from fecal samples among loads within the same day. This apparent transmission or persistence of *E. coli* O157 isolates among cattle in different truckloads, or their environments may be related to the pen or feedlot origin of cattle, the comingling of cattle during lairage, or dispersion of individual *E. coli* O157 isolates during the harvest process.

We described the relatedness of *E. coli* O157 isolates from feces and pre-evisceration carcasses within and among truckloads of finished beef cattle at harvest. We demonstrated that specific PFGE subtypes may be detected on multiple occasions and from different sample types within loads, among loads, and among days. Our results support previously suggested associations between overall *E. coli* O157 prevalence, fecal shedding and carcass contamination within truckload. Hence, this study provides additional evidence that pre-evisceration carcass contamination might be decreased by mitigating the effect of high- and low-shedders within a truckload. However, our results also suggest that the frequency distribution of carcass isolates that have identical PFGE patterns to that of fecal isolates from high- and low-shedding cattle within a truckload may differ from the corresponding distribution among different truckloads on the same day. Further investigations are needed to assess the relative importance of mitigating high-shedders, low-shedders, or any fecal shedder within and among transport cohorts in order to decrease the risk of carcass contamination.

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Figure 4.1 Percentages of *Escherichia coli* O157 carcass isolates that were the same PFGE banding pattern (following Xba1 digestion) as fecal isolates recovered from high- or low-shedding cattle within the same truckload or different truckloads on the same sampling day.

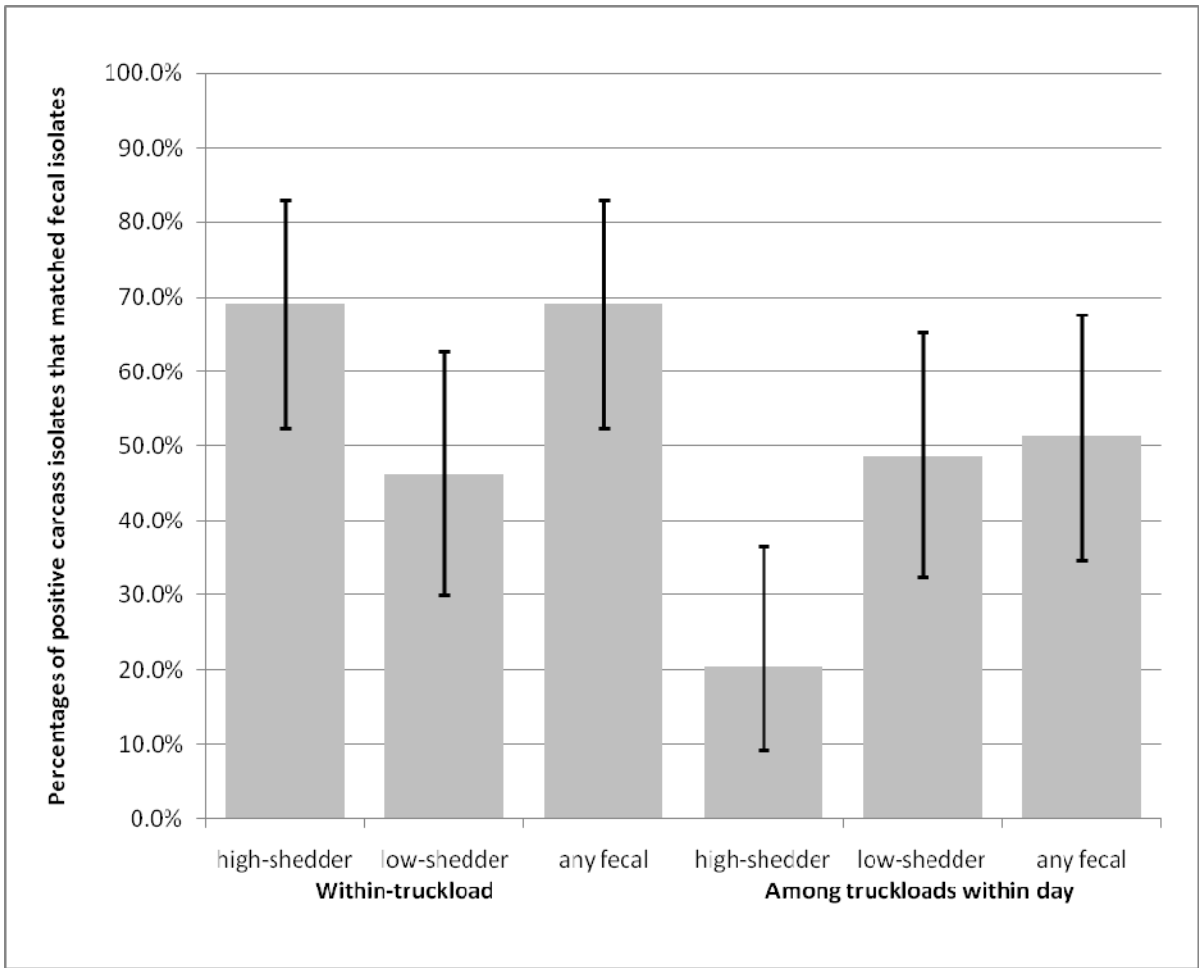

Table 4.1 Counts of *Escherichia coli* O157 isolates[1] and corresponding pulsed-field gel electrophoresis (PFGE) subtypes[2] by truckload, sampling date, and sample type.

Truck Load	Sampling Date	Number of isolates (PFGE subtypes)			Number of fecal isolates with a carcass-matching PFGE subtype	
		Carcass	Fecal High-shedder [3]	Fecal Low-shedder [4]	High-shedder	Low-shedder
A1	5/14/07	0	0	0	-	-
A2		0	0	0	-	-
A3		3(1)	2(1)	5(2)	2	4
B1	5/15/07	0	1(1)	1(1)	-	-
B2		0	0	0	-	-
B3		0	0	1(1)	-	-
B4		1(1)	1(1)	3(1)	0	0
B5		0	0	3(2)	-	-
B6		0	1(1)	3(1)	-	-
B7		0	0	1(1)	-	-
C1	5/21/07	0	1(1)	0	-	-
C2		1(1)	1(1)	0	1	-
C3		0	0	1(1)	-	-
C4		0	0	0	-	-
C5		1(1)	3(3)	3(2)	1	2
C6		0	0	1(1)	-	-
D1	5/22/07	0	0	0	-	-
D2		0	1(1)	0	-	-
E1	6/4/07	1(1)	6(2)	14(5)	4	10
E2		6(4)	4(4)	3(2)	3	3
E3		3(2)	7(2)	1(1)	6	0
F1	6/6/07	0	5(1)	1(1)	-	-
F2		0	1(1)	4(2)	-	-
F3		0	0	1(1)	-	-
F4		0	0	0	-	-
F5		0	1(1)	2(2)	-	-
G1	6/11/07	1(1)	1(1)	1(1)	1	0
G2		2(2)	1(1)	0	1	-
G3		0	0	0	-	-
G4		0	0	1(1)	-	-
G5		2(2)	2(1)	5(2)	2	3
G6		0	1(1)	1(1)	-	-
G7		0	3(2)	1(1)	-	-
H1	6/13/07	0	1(1)	0	-	-
H2		0	0	1(1)	-	-
H3		0	0	1(1)	-	-
H4		0	0	0	-	-
H5		3(3)	2(1)	5(2)	2	5
H6		8(2)	3(1)	3(2)	3	2
I1	6/18/07	4(1)	2(2)	1(1)	1	0
I2		0	0	2(2)	-	-
I3		2(1)	2(1)	5(1)	2	5
J1	6/19/07	1(1)	1(1)	3(1)	1	3
J2		0	0	0	-	-
J3		0	0	2(2)	-	-
K1	6/20/07	0	0	0	-	-
K2		0	0	0	-	-
K3		0	0	0	-	-
K4		0	1(1)	0	-	-
Total		39(17)	55(24)	80(28)	30	37

[1] There was only one isolate characterized per sample.
[2] Subtypes represent unique PFGE banding patterns following *Xba*1 digestion
[3] Cattle with a fecal sample that was culture positive by a direct plate technique were classified as high-shedders.
[4] Cattle with a fecal sample that was culture positive only on an IMS procedure were classified as low-shedders.

Table 4.2 Counts of *Escherichia coli* O157 isolates by pulsed-field gel electrophoresis (PFGE) subtype and date of sampling for each truckload where *E. coli* O157 was recovered from carcass or fecal samples.

		PFGE Subtypes from all sample types																																		
Load	*Date*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*	*m*	*n*	*o*	*p*	*q*	*r*	*s*	*t*	*u*	*v*	*w*	*x*	*y*	*z*	*aa*	*bb*	*cc*	*dd*	*ee*	*ff*	*gg*	*hh*	*ii*	*jj*	*kk*
A3	5/14/07	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-
B1	5/15/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B3		-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B4		-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
B5		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B6		-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B7		-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C1	5/21/07	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C2		-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C3		-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C5		-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C6		-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D2	5/22/07	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E1	6/4/07	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	15	-	-	1	2	-
E2		-	-	-	-	-	-	-	-	1	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	4	3	-
E3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	1	1	-	1	-	-
F1	6/6/07	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F2		-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
F3		-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F5		-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-
G1	6/11/07	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G2		-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G4		-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G5		-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G6		-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
G7		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
H1	6/13/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H2		-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H5		-	5	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H6		-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	1	-	-	-	-	-	-	-	-	-	-	-	-	-
I1	6/18/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	5	-	-	-	-	-	-	1	-	-	-	-
I2		1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
I3		-	-	-	-	-	-	-	-	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
J1	6/19/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-
J3		-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
K4	6/20/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4.3 Distribution of *Escherichia coli* O157pulsed-field gel electrophoresis (PFGE) subtypes[1] recovered by carcass sampled, as well as negative (-) pre-evisceration carcasses within cattle truckload by order of slaughter.

		Carcass order within load																														
Truck Load	Sample Date	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
A3	5/14/07	-	-	-	b[abc]	-	-	-	-	-	-	-	-	-	-	-	-	-	b[abc]	-	-	-	-	-	-	-	-	-	-	b[abc]	-	-	-
B4	5/15/07	-	-	-	-	-	-	-	-	-	-	y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.
C2	5/21/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	m[a]	-	-	-	-	-
C5		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	w[ab]	-	-	-	-	-	-	-	-
E1	6/4/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ff[ab]	-	-	-	-	-	-	-	-	-
E2		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ii[ab]	-	-	k[c]	k[c]	jj[ab]	-	hh[ac]	-	hh[ac]	-	-	-
E3		-	-	-	-	-	-	-	-	-	-	-	-	ff	aa[ac]	aa[ac]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.
G1	6/11/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	g[a]	-	-	-
G2		-	-	-	-	-	t	-	-	-	-	m[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G5		-	-	-	-	-	-	-	-	-	s[ab]	-	-	-	-	-	-	-	-	-	v	-	-	-	-	-	-	-	-	-	-	-	-
H5	6/13/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	w[b]	e	-	-	-	-	-	-	b[ab]	-	-
H6		-	b[c]	-	-	-	-	-	w[abc]	-	-	w[abc]	-	-	-	-	-	w[abc]	b[c]	-	w[abc]	-	w[abc]	b[c]	-	-	-	-	-	-	-	-	-
I1	6/18/07	-	-	-	-	-	-	-	-	-	-	-	z[ac]	-	-	-	-	-	-	z[ac]	-	z[ac]	-	-	-	z[ac]	-	-	-	-	-	-	.
I3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	j[abc]	-	-	-	-	j[abc]	-	-	-	-	-	-	-	-	-	-	-	-
J1	6/19/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ff[ab]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] Subtypes represent unique PFGE banding patterns following *Xba*1 digestion (designations correspond to those of Table 4.2). Superscripts (a-c) indicate where carcass isolates were the same subtype as other isolates recovered within the load.
[a] This subtype was also present in a fecal isolate from a high-shedder within the same load.
[b] This subtype was also present in a fecal isolate from a low-shedder within the same load.
[c] This subtype was also present in a carcass isolate within the same load.

CHAPTER 5 – Modeling Preharvest Interventions for *Escherichia coli* O157 Contamination of Beef Cattle Carcasses

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Abstract

Field studies evaluating the effects of multiple concurrent preharvest interventions for *Escherichia coli* O157 are not often feasible; however, modeling techniques may provide useful information on these effects, while also identifying crucial information gaps that can guide future research. We constructed a risk assessment model with data obtained from a systematic review of scientific literature. Parameter distributions were incorporated into a stochastic Monte-Carlo modeling framework to examine the impacts of different combinations of preharvest and harvest interventions for *E. coli* O157 on the risk of beef carcass contamination. We estimated the risk of *E. coli* O157 carcass contamination conditional on preharvest fecal prevalence estimates, inclusion of feed additive(s) in diet, vaccination for *E. coli* O157, transport and lairage effects, hide intervention(s), and carcass intervention(s). Prevalence parameters for *E. coli* O157 were assumed to encompass potential effects of concentration; therefore, concentration effects were not specifically evaluated. Sensitivity analyses revealed that fecal prevalence, fecal to hide transfer, hide to carcass transfer, and carcass intervention efficacy significantly impacted the risk of carcass contamination (correlation coefficients = 0.37, 0.56, 0.58, and -0.29, respectively). Results indicated that combinations of preharvest interventions may be particularly important for

supplementing harvest interventions during periods of higher variability in fecal shedding prevalence (i.e., summer). Further assessments of the relationships among fecal prevalence and concentration, hide contamination and subsequent carcass contamination are needed to further define risks and intervention impacts for *E. coli* O157 contamination of beef.

Introduction

Escherichia coli O157 has been found in several food production systems and remains an important cause of human illness (Rangel *et al.*, 2005). Preharvest fecal shedding of *E. coli* O157 in cattle and subsequent hide and carcass contamination within groups of cattle may increase the risk of human foodborne illness following consumption of undercooked beef products (Woerner *et al.*, 2006). As previously reviewed, several preharvest interventions for *E. coli* O157 in cattle have been proposed that may decrease carcass contamination and reduce the risk of human foodborne illness (Loneragan *et al.*, 2005; Woerner *et al.*, 2006); however, little is known about the overall impacts of multiple concurrent interventions upon the risk for carcass contamination at harvest. The feasibility and parsimony of preharvest control strategies for *E. coli* O157, comprised of single or multiple interventions, may depend on their ability to reduce the colonization of cattle so that the effective thresholds of harvest interventions are not exceeded. The impact of preharvest interventions on risk of contamination of carcasses in conjunction with existing harvest interventions is not well understood.

Individual cattle shed *E. coli* O157 in feces intermittently (Besser *et al.*, 1997). Likewise, the fecal prevalence of *E. coli* O157 immediately prior to harvest (preharvest) within cohorts of cattle also has been shown to vary significantly (Hancock *et al.*, 1997; Barkocy-Gallagher *et al.*, 2003; Arthur *et al.*, 2009). Prevalence peaks within a cohort may be seasonal and occur more often in the summer, as evidenced by cross-sectional studies that sampled cattle feces immediately prior to or during the harvest process at different seasons (Barkocy-Gallagher *et al.*, 2003; Renter *et al.*, 2008). Some studies have used manila ropes to sample cohorts of feedlot cattle and also have found increased prevalence of *E. coli* O157 during summer months (Smith *et al.*, 2005; Stephens *et al.*, 2009). Conversely, some cross-sectional studies in feedlot cattle have

not indicated a seasonal tendency in fecal prevalence immediately prior to harvest (Khaitsa *et al.*, 2006; Arthur *et al.*, 2009; Stephens *et al.*, 2009). Regardless of when the prevalence of *E. coli* O157 is relatively high within cohort of cattle, prevalence peaks may increase the importance of interventions during the cattle feeding period and at harvest. However, these complex relationships are often difficult to investigate within a single observational study or randomized controlled trial.

A systematic review by Sargeant *et al*. (2007) indicated that several preharvest interventions available during the feeding period may reduce fecal shedding of *E. coli* O157 in feedlot cattle. Control strategies include vaccination for *E. coli* O157 or dietary modifications, such as direct-fed microbials (DFM), sodium chlorate, bacteriophages, antibiotics, and other feed additives (Sargeant *et al.*, 2007). Little is known about the impact of combinations of these interventions on the eventual risk of contamination of cattle carcasses at harvest (Sargeant *et al.*, 2007; Cernicchiaro *et al.*, 2010). Study designs that allow assessment of multiple concurrent preharvest interventions may not be feasible, and thus may not be reasonable approaches to elucidate multiple-hurdle control strategies.

Mathematical simulation models allow researchers to assess the impacts of multiple interventions within a complex system and can be used to estimate the effect and value of multiple interventions for *E. coli* O157. An epidemiologic modeling approach can be justified by its relative feasibility over multivariable randomized controlled trials or observational studies, and can clarify assumptions about empirical data (Hethcote, 2009). Our objective was to demonstrate the range of potential effects within the framework of preharvest and harvest interventions, conditional upon expected variations in fecal shedding of *E. coli* O157 in feedlot cattle and subsequent pathogen transfer at harvest. To meet this objective, we utilized available data from the scientific literature and constructed a Monte-Carlo model to stochastically simulate relationships among fecal and hide prevalence of *E. coli* O157 in commercial feedlot cattle, combinations of interventions, and associated risks of carcass contamination.

Materials and Methods

Model parameter development

We conducted a systematic search of the scientific literature to identify preharvest- and harvest-level data regarding the epidemiology and ecology of *E. coli* O157 in commercial feedlot cattle. PubMed and Agricola databases were searched to locate published abstracts from 1990 to 2010. The overall search term was (O157 OR O157:H7 OR E. coli O157 OR E. coli O157:H7 OR Escherichia coli O157) AND (cattle OR calf OR calves OR steer) AND (feedlot OR feedyard OR truckload) AND (feces OR hide OR carcass OR transport OR lairage OR harvest OR slaughter OR wash OR DFM OR probiotic OR season OR vaccine); 126 potential manuscripts were identified using these search criteria. Modified search terms targeting cattle hide and carcass interventions were employed to retrieve 39 additional potential manuscripts. Abstracts were screened for study type, design, and general content before selection for full manuscript review. Manuscripts were then critically reviewed according to established standards to assess the internal and external validity, potential bias, and relevancy of the data (Dohoo *et al.*, 2009; Sargeant *et al.*, 2009; O'Connor *et al.*, 2010).

We constructed a conceptual risk model (Figure 5.1) to represent groups of factors that influence risk of carcass contamination. Model foci were selected based upon current and potential use of interventions within the beef industry. Commercially available interventions were grouped into categories in accordance with the conceptual risk model. Preharvest interventions included vaccines and direct-fed microbials. To derive parameter estimates for the model, appropriate data were compiled to build parameter distributions. Parameter estimates derived from multiple studies were assigned weights according to within study variance. If variance estimates were unknown, the number of studied cohorts (groups of cattle in feedlot pens or truckloads) was used as a weight for the estimate (Dohoo *et al.*, 2009). Because our intent was to model the North American feedlot system, study populations outside of the North American feedlot cattle industry were excluded. Additional exclusions were fecal prevalence data of unknown season, or data derived from less sensitive detection methods (e.g., direct

plating instead of an immunomagnetic separation protocol), and intervention efficacy data derived from artificial challenge/inoculation studies or non-commercial feedlot cattle settings. Fecal prevalence data from harvest (e.g., samples from the terminal rectum during the slaughter process) also were excluded because the model framework required fecal prevalence estimates that were measured prior to transport and lairage effects.

Model development

Parameters were incorporated into a stochastic Monte-Carlo modeling framework using @Risk for Excel (v. 5.5; Palisade, Corp; Ithaca, NY) to examine the impact of combinations of feedlot and harvest interventions for *E. coli* O157 on risk of carcass contamination. Model parameter definitions, distributions, and primary references are shown in Table 5.1. Within each iteration, stochastic model parameters, including baseline pre-intervention fecal prevalence of *E. coli* O157 within a cohort of feedlot cattle, were randomly chosen from their respective parameter distributions. In this manuscript, we have defined feedlot or feeding as the period in which cattle are raised in a feedlot (approximately 4 – 7 month period), preharvest as the time immediately prior to harvest (day of harvest, when cattle leave the feedlot and are transported to a slaughter facility), and harvest as the slaughter process.

Efficacy of feedlot interventions for *E. coli* O157 were modeled as proportional reductions in baseline fecal prevalence. Combined feedlot interventions (vaccine and DFM) were modeled as successive proportional reductions in fecal prevalence. A combined transport and lairage effect was modeled as a proportional change in fecal prevalence of *E. coli* O157 (after effects of any feedlot interventions were incorporated). Fecal to hide transfer of *E. coli* O157 was then modeled as a stochastic ratio of the subsequent fecal prevalence to hide prevalence within a cohort; post-transport and lairage fecal prevalence was multiplied by the cohort-level fecal to hide prevalence transfer ratio to obtain the prevalence of contaminated hides. This risk of hide contamination as a preliminary model output was truncated from 0 to 1.0 for all scenarios. A proportional reduction in the prevalence of *E. coli* O157 on hides within a cohort was applied by using a randomly selected hide intervention efficacy parameter from the

respective distribution. The hide prevalence, post-hide intervention, was multiplied by the cohort-level hide to carcass prevalence transfer ratio in order to obtain the prevalence of contaminated carcasses. Carcass intervention efficacy was then applied to the prevalence of pre-intervention carcasses contaminated with *E. coli* O157 as a proportional decrease in the prevalence of carcasses contaminated with *E. coli* O157.

Model simulation

We used 50,000 iterations for each model scenario simulation in order to ensure consistent model convergence for the mean of each outcome (95% CI +/- 3%). A fixed simulation seed (seed = 1) was used to ensure reproducibility in model outcomes. Scenarios included multivariable combinations of baseline seasonal prevalence, vaccine intervention, feed additive intervention, transport/lairage effect, fecal to hide transfer, hide intervention efficacy, hide to carcass transfer, and carcass intervention efficacy. Combinations of feedlot interventions, including no feedlot intervention (NFI), vaccination only (VAC), feed additive only (FA), and both vaccination and feed additive (VAC-FA), were modeled as separate scenarios. Stochastic parameters for hide and carcass intervention efficacy were included in all scenarios.

Sensitivity analysis and validation

Sensitivity analyses were performed to evaluate the effect of parameters on the risk of carcass contamination by examining tornado graphs, Spearman rank correlation coefficients, and by fixing input parameters at the 5^{th}, 50^{th} and 95^{th} percentile and comparing risk of carcass contamination (Vose, 2008). A simulation table for the impact of combinations of the 5^{th}, 50^{th}, and 95^{th} percentiles of baseline fecal prevalence, feedlot intervention, and hide intervention upon the prevalence of *E. coli* O157 on post-interventions carcasses was constructed.

Model outputs for fecal, hide, and carcass prevalence data were compared to data from studies that were not used to build model parameters. Model diagnostics were performed to detect abnormal intermediate model output (e.g., prevalence above 100%). Although a full model validation was not possible, outputs at various stages of the model were evaluated for

consistency with other empirical data from manuscripts not used to build model parameters (Barkocy-Gallagher *et al.*, 2003; Brichta-Harhay *et al.*, 2008; Arthur *et al.*, 2009).

Results

Impact of baseline fecal prevalence and feedlot interventions

The distributions for risk of carcass contamination with *E. coli* O157 for model scenarios using combinations of seasonal fecal prevalence and feedlot interventions, including no feedlot intervention (NFI), vaccination only (VAC), feed additive only (FA), and both vaccination and feed additive (VAC-FA) are shown in Figure 5.2. For each feedlot intervention scenario, the median risk of carcass contamination was approximately two times higher with summer fecal prevalence than winter fecal prevalence, as shown in Table 5.2. In summer and winter scenarios, feedlot interventions reduced the median risk of carcass contamination by approximately three times. The mean risk of post-intervention carcass contamination was below 1.0% for all scenarios except summer fecal prevalence with no feedlot intervention.

Sensitivity analysis

Sensitivity analysis revealed that hide to carcass transfer, fecal to hide transfer, baseline fecal prevalence, and carcass intervention efficacy highly impacted the risk of carcass contamination in each model scenario. Spearman rank correlation coefficients for model input parameters with the risk of carcass contamination are shown in Figure 5.3 for the summer VAC-FA model scenario; this tornado graph is representative of the other model scenarios for combinations of seasonal fecal prevalence and feedlot interventions, as shown in Table 5.3.

The median percentages of carcasses contaminated with *E. coli* O157 for 48 model scenarios that used the 5^{th}, 50^{th} and 95^{th} percentiles one by one as deterministic values for each of the input parameters in separate simulations while other parameters were allowed to vary are shown in Table 5.4. The median percentage of contaminated carcasses was highest within any seasonal fecal prevalence and within any feedlot intervention scenario when hide to carcass or fecal to hide transfer ratios were set deterministically at their 95^{th} percentile. Likewise, the

median percentage of carcasses contaminated with *E. coli* O157 was lowest within any seasonal fecal prevalence and within any feedlot intervention scenario when hide to carcass or fecal to hide transfer ratios were set deterministically at their 5th percentile. In this sensitivity analysis, the percentage of contaminated carcasses was highest when no feedlot interventions were employed during the summer season and the hide to carcass prevalence transfer was deterministically set at the 95th percentile.

The change in the percentage of contaminated carcasses in model simulations comparing the 5th and 95th percentile deterministic settings for input parameters is shown in Table 5.5. For all parameters, the absolute value of differences increased in the order of VAC-FA to FA to VAC to NFI scenarios. The largest change in carcass contamination risk occurred when hide to carcass transfer was changed from its 5th to the 95th percentile, which also suggests the model is sensitive to this parameter (Table 5.5). The next largest change in carcass contamination risk occurred when fecal to hide transfer was changed from its 5th to 95th percentile. In this analysis, the FA scenarios mitigated more carcass contamination than the VAC scenarios, and VAC-FA scenarios mitigated more carcass contamination risk than the VAC and FA scenarios (Table 5.5).

Model performance assessment

Convergence of model output was obtained in each simulation. The NFI, VAC, FA, and VAC-FA model scenario outputs for risk of carcass contamination were consistent with relevant data derived from three observational studies (Barkocy-Gallagher *et al.*, 2003; Brichta-Harhay *et al.*, 2008; Arthur *et al.*, 2009) that were not used to build input parameters for our models; these data are summarized in Table 5.6. The model output prediction intervals were consistent with empirical estimates of mean prevalence of summer and winter hide and pre-intervention carcass contamination from Brichta-Harhay et al. (Brichta-Harhay *et al.*, 2008). Model outputs also were consistent with *E. coli* O157 prevalence on hides at harvest, pre-intervention carcasses, and post-intervention carcasses at three fed-beef harvest facilities (Barkocy-Gallagher *et al.*, 2003). Model outputs were consistent with data from Arthur et al. (Arthur *et al.*, 2009); when we restricted the summer fecal prevalence of *E. coli* O157 in our model to greater than 20%, hide

prevalence was at least 80% approximately 50.6% of the time. When we restricted the summer fecal prevalence of *E. coli* O157 in our model to less than 20%, 86.4% of the time, hide prevalence was less than 80%. As shown in Table 5.6, model output prediction intervals for hide prevalence were consistent with the observations of Arthur et al.

Discussion

We used a Monte-Carlo model to estimate the risk of carcass contamination in a cohort of feedlot cattle conditional on baseline preharvest fecal prevalence of *E. coli* O157, feed additive inclusion in diet, vaccination for *E. coli* O157, transport and lairage effects, hide intervention, and carcass intervention. Feedlot interventions to control fecal and hide prevalence of *E. coli* O157 may be important for supplementing harvest interventions during periods of higher variability in fecal shedding prevalence. In our model, fecal to hide transfer, hide to carcass transfer, fecal prevalence, and carcass intervention efficacy were the most influential input parameters on the risk of carcass contamination at harvest. Because of the relative importance, yet sparse amount of data defining fecal to hide and hide to carcass transfer parameters, further targeted field studies are warranted to better define these relationships.

Models can aid the analysis and assessment of factors in complex infectious disease systems and are now a component of public health decision making policies in several countries (Hethcote, 2009). Models may be useful to investigate potential areas of mitigation and provide a representation of the effects of feedlot interventions on contamination of cattle carcasses with *E. coli* O157, especially when observational studies and randomized clinical trials are not feasible. Our model estimated the risk of carcass contamination with *E. coli* O157 with combinations of interventions and highlighted the need for more data to better define the relationships between fecal and hide prevalence and hide and carcass prevalence. In addition, our model emphasized the need for multiple concurrent interventions during periods of high variability in fecal prevalence.

Preharvest and harvest models of *E. coli* O157 interventions in beef production systems have been published previously (Wood *et al.*, 2007; Signorini *et al.*, 2009; Signorini *et al.*, 2010).

One study used a spatial simulation model to assess the potential impact of herd size, water trough and pen hygiene, and vaccination on the fecal prevalence of *E. coli* O157 in cattle within an intensively-managed grazing cattle system (Wood *et al.*, 2007). A metapopulation model examined the population dynamics of *E. coli* O157 within feedlot cattle pens based upon *E. coli* O157 habitats in cattle, water, feed, and the remaining feedlot pen environment (Ayscue *et al.*, 2009). Another recent study used mathematical modeling to assess the risk of *E. coli* O157 foodborne illness in humans following feedlot vaccination of cattle for *E. coli* O157. This study included an economic analysis and determined break-even costs in accordance with variation in vaccine efficacy (Withee *et al.*, 2009). A recent study in Argentina utilized Monte-Carlo risk analysis techniques to assess the risk of human illness from verocytotoxigenic *E. coli* based on postharvest interventions (Signorini *et al.*, 2009). Although models of these complex biological systems can provide important information, their usefulness can be limited by model framework and assumptions.

Our model suggests that peaks in preharvest fecal prevalence of *E. coli* O157 may substantially increase the risk of carcass contamination at harvest within a feedlot cattle cohort, even when hide and carcass interventions are employed. Data from the scientific literature reveal that fecal prevalence of *E. coli* O157 within a cohort of commercial feedlot cattle may range from 0 to over 93% (Sargeant *et al.*, 2003; Khaitsa *et al.*, 2006), and this preharvest prevalence has been associated with risk of carcass contamination within the cohort (Elder *et al.*, 2000; Woerner *et al.*, 2006). Previous simulation data also have indicated that feedlot interventions which reduce the preharvest fecal prevalence of *E. coli* O157 may have significant impact on the risk of carcass contamination at harvest (Jordan *et al.*, 1999). Our model output was consistent with this concept; individual and combined feedlot interventions appeared to reduce the risk of carcass contamination given any fecal prevalence of *E. coli* O157, but were relatively more important in cohorts with higher levels of fecal prevalence. Cohorts with high fecal prevalence were at increased risk for carcass contamination and this high prevalence was more likely to occur in summer.

As shown in the sensitivity analysis, extreme values of the fecal to hide and hide to

carcass transfer parameters were very influential in this model. Seasonal fecal prevalence and carcass intervention efficacy were also influential. This suggests that the distributions of these parameters should be closely scrutinized and, if possible, more clearly defined in future studies to guide optimal intervention efforts. Cohort-level data demonstrating the relationships of fecal, hide, and carcass prevalence in commercial feedlot cattle were available from only one study (Jacob *et al.*, 2010). Therefore, these transfer parameter distributions have considerable uncertainty along with their inherent variability. Although other studies reported some of these relationships as study means, we did not construct transfer ratio parameter distributions from overall mean prevalence estimates (e.g., overall mean hide prevalence to overall mean carcass prevalence) if we could not determine the relationship of fecal to hide to carcass prevalence at the cohort level (Woerner *et al.*, 2006; Brichta-Harhay *et al.*, 2008; Fegan *et al.*, 2009). Since fecal prevalence parameters were constructed with more data from several studies, the fecal prevalence distribution primarily represents variability rather than uncertainty. More data are needed to decrease the uncertainty in fecal to hide and hide to carcass parameter distributions.

Although the comparison of model output with empirical data was not a formal validation, this comparison did allow assessment of model simulation performance. Three data sets were used for comparison to the model output because they did not meet inclusion criteria for the model parameters and hence, were not used to build the model. Some studies offered comparative values for hide or carcass contamination even if the fecal prevalence was not measured in the study. Empirical data from individual studies on the cohort-level relationship of fecal, hide, and carcass prevalence (within a single study) were not available to formally validate the model. Examination of data from future observational studies may allow a more comprehensive validation of this model. Regardless, our model is consistent with available data estimating hide and carcass contamination. In the Arthur et al. (2009) longitudinal study of feedlot cattle, observed hide prevalence of *E. coli* O157 was greater than 80% whenever fecal prevalence was greater than 20% within a cohort during the feeding period. We were able to produce results consistent with these observations when fecal prevalence in our model was limited to greater than 20%. Further, when fecal prevalence in our model was limited to less

than 20%, hide prevalence ≥ 80% was a rare occurrence.

We chose to model combined feedlot interventions as successive reductions in baseline fecal prevalence of *E. coli* O157, but this may have been an oversimplification of the impact of combined interventions. This assumption that two interventions have an additive effect may overestimate their true combined impact, or it could underestimate the impact if the interventions have a synergistic relationship in lowering prevalence or concentration of *E. coli* O157 and subsequently transmission from calf to calf. Because data were not consistently available, we did not model fecal concentration of *E. coli* O157. However, feedlot interventions may impact the concentration of *E. coli* O157 in feces shed by individual cattle, and/or impact fecal prevalence of *E. coli* O157 within a cohort (Thornton *et al.*, 2009). In addition to fecal prevalence, higher concentrations of *E. coli* O157 shed in feces may be an important contributor to hide and carcass contamination. In a review of the impact of cattle shedding high levels of *E. coli* O157 in feces (>10^4 CFU/g feces) upon carcass contamination, Arthur *et al*. (2010) hypothesized that interventions at harvest may have critical thresholds for the bacterial loads of individual cattle within a cohort. The cumulative capacity of hide and carcass interventions may become overwhelmed if the pathogen concentrations on a hide or carcass exceed these thresholds (Arthur *et al.*, 2010). The relationship between fecal prevalence and fecal concentration of *E. coli* O157 in feedlot cattle may be complex and creates challenges in determining the impact of combinations of preharvest and harvest interventions. Observational studies and randomized controlled trials employing multiple interventions may be needed in order to better understand the impact of combinations of feedlot- and harvest-level interventions for *E. coli* O157 on prevalence and concentration in feces and on hides and carcasses.

The cohort-level fecal prevalence of *E. coli* O157 was chosen as the initial measurement in our conceptual model to investigate the risk of eventual carcass contamination. Since the fecal prevalence of *E. coli* O157 in feedlot cattle immediately prior to harvest may be higher in the summer than in the winter season, we selected preharvest fecal prevalence data from studies that provided estimates specific to the summer or winter season. Because we excluded prevalence estimates derived from data from feedlot cattle at harvest, we found fewer preharvest data for

winter than summer season. We also chose to exclude data from studies that used less sensitive detection methods such as direct plating or culture without immunomagnetic separation, other forms of preharvest sampling methods (e.g. manila ropes), or sample pooling. Although we acknowledge the importance and practicality of various detection methods, we could not reliably convert data from alternative detection methods to the standard prevalence data reported in a majority of studies.

Fecal shedding of *E. coli* O157 in cattle is both transient and heterogeneous; individual cattle are likely to shed *E. coli* O157 for variable times and at different concentrations in feces (Hancock *et al.*, 1997; Sanderson *et al.*, 1999; Sargeant *et al.*, 2000; Omisakin *et al.*, 2003). Limited data address the relationship between fecal prevalence, fecal concentration, hide prevalence and carcass contamination risk (Omisakin *et al.*, 2003; Fox *et al.*, 2008; Arthur *et al.*, 2009; Jacob *et al.*, 2010). Some researchers have suggested that control methods for *E. coli* O157 in cattle should target cattle that are shedding higher concentrations in feces. Omisakin *et al*. estimated the prevalence and concentration of *E. coli* O157 in cattle feces at harvest and asserted that four out of 44 positive animals were responsible for 96% of the total *E. coli* O157 organisms produced (Omisakin *et al.*, 2003). A published modeling study suggested that approximately 20% of cattle shedding *E. coli* O157 were responsible for 80% of the transmission within cattle herds in Scotland (Matthews *et al.*, 2006). These studies highlight the importance of determining the fecal concentration of *E. coli* O157 in cattle, but little is known about the longitudinal relationships between herd or pen prevalence and individual fecal concentrations. A recent study was used to demonstrate the association between fecal concentrations of *E. coli* O157 and hide contamination. Cohort-level fecal prevalence estimates over 20% were positively associated with hide prevalence estimates of over 80%; however no samples were collected at slaughter (Arthur *et al.*, 2009). This longitudinal study indicated that both fecal prevalence and fecal concentration were important factors in predicting hide contamination with *E. coli* O157, yet the relative importance and interrelationship of fecal prevalence and concentration is unclear. Previous studies have shown that the proportion of high-shedding animals is positively correlated with the overall number of animals shedding within a cohort (Cobbold *et al.*, 2007; Fox *et al.*,

2008; Jacob *et al.*, 2010). Yet, fecal concentration of *E. coli* O157 may be a critical component of models, particularly if the proportion of high-shedding animals is not consistently and positively correlated with the overall number of animals shedding within a cohort. Also, if preharvest intervention efficacy varies according to fecal prevalence and/or concentration, our model may not have fully captured these intervention effects. For this model we assumed that data from the scientific literature that depicted relationships between fecal and hide prevalence or fecal and carcass prevalence inherently accounted for the presence of high-shedding animals. Future models that account for fecal concentration may provide more accurate assessments of carcass contamination risk.

Although we used model scenarios based on either a summer (higher) or winter (lower) preharvest fecal prevalence, our analysis of data from studies measuring preharvest fecal prevalence provided surprising similar means for fecal prevalence between these seasons. Mean cohort-level summer preharvest fecal prevalence was 12.1% while winter prevalence was 10.8%; however, distributions for these parameters allowed a much wider range of values for summer (up to 93% cohort-level prevalence) than for winter (up to 21%). These differences in the distribution for the baseline fecal prevalence parameter resulted in notable differences in the eventual risk of carcass contamination. Since the means for fecal prevalence were similar between seasons, our model may suggest that prevalence variability, rather than the mean prevalence, may be a more important driver of carcass contamination risk. In addition, preharvest interventions may be most important during peaks in *E. coli* O157 prevalence, regardless of season. Currently, season may be the best indicator of high fecal prevalence risk in cohorts of feedlot cattle; methods to indentify high prevalence pens for additional interventions might be useful.

The two categories of feedlot interventions for *E. coli* O157 in this model were commercially available vaccines and feed additives. Current data support that these interventions may significantly impact the fecal shedding of *E. coli* O157 in commercial feedlot cattle. Several feed additive studies investigated the use of direct-fed microbials (Brashears *et al.*, 2003; Elam *et al.*, 2003; Younts-Dahl *et al.*, 2004; Younts-Dahl *et al.*, 2005; Stephens *et al.*,

2007; Stephens *et al.*, 2010). Direct-fed microbials are cattle feed additives that contain viable microorganisms, commonly *Lactobacillus* spp. strains, and have been considered to improve animal growth and reduce fecal shedding of *E. coli* O157 in commercial feedlot cattle (Brashears *et al.*, 2003; Elam *et al.*, 2003; Younts-Dahl *et al.*, 2004; Younts-Dahl *et al.*, 2005; Stephens *et al.*, 2007; Stephens *et al.*, 2010). Although other potential feed additives exist, such as bacteriophage and sodium chlorate, our inclusion criteria for research data was restricted to randomized controlled trials of commercially available interventions; this restricted our feed additive parameter distribution to studies involving DFMs.

Included studies for *E. coli* O157 vaccination of cattle were on vaccine technologies that targeted either the type III secreted proteins or siderophore receptor and porin protein mechanisms of *E. coli* O157 bacteria. The type III secreted proteins vaccine works on the principle that these secreted proteins are required for cattle colonization of the terminal rectum with *E. coli* O157. Vaccinated cattle are less likely to shed *E. coli* O157 in the feces and less likely to be colonized in the terminal rectum (Peterson *et al.*, 2007; Moxley *et al.*, 2009; Smith *et al.*, 2009; Smith *et al.*, 2009). The *E. coli* O157 SRP vaccine targets the siderophore receptor and porin proteins of the bacteria. This vaccine tends to reduce fecal prevalence and concentration of *E. coli* O157 in inoculated calves (Thornton *et al.*, 2009), but has not been evaluated extensively in commercial feedlot settings (Fox *et al.*, 2009; Thomson *et al.*, 2009). Although other feedlot-level interventions exist, vaccination and feed additive intervention categories in our model framework provided a multi-intervention yet parsimonious approach in modeling the impact of feedlot interventions on the eventual risk of carcass contamination with *E. coli* O157. In our model, these interventions could conceptually represent any feedlot-level intervention affecting fecal prevalence of *E. coli* O157.

Fecal to hide transfer of *E. coli* O157 was modeled as a ratio of fecal prevalence to hide prevalence within a cohort following an adjustment of fecal prevalence by a transport and lairage effect, because we had relatively more cohort-level data to describe the ratio of fecal to hide prevalence at this time (Jacob *et al.*, 2010). As discussed by Jordan et al. (1999), a model of the transfer of *E .coli* O157 from cattle feces to hides should include cattle hygiene measurements

(e.g., tag scores) and pathogen concentration (Jordon *et al*., 1999), for which we had insufficient data.

Although some enumeration data exist to describe the concentration of *E. coli* O157 organisms on contaminated cattle hides and carcasses, the relationships between hide and carcass prevalence and hide and carcass concentration is unknown at the cohort level (Brichta-Harhay *et al.*, 2008; Kalchayanand *et al.*, 2009); the lack of data prevented us from constructing reliable distributions for these parameters. This limitation may have also decreased the accuracy of our hide and carcass intervention parameters, since many studies assessing hide and carcass intervention efficacy report reductions in pathogen concentration rather than reductions in the prevalence of contamination on hides or carcasses. Because of sparse data, we also assumed that the hide to carcass transfer parameter in our model encompassed the effects of non-hide contamination sources at this processing step. Furthermore, substantial data on the efficacy of hide and carcass interventions may not be reported in the scientific literature, but instead may be generated and retained within operators in the beef processing industry. With more empirical data, future models may more reliably account for hide and carcass pathogen loads in estimating the risk of carcass contamination as well as subsequent product contamination and human disease risk.

Since fecal to hide transfer, carcass intervention efficacy, and hide to carcass transfer parameters exerted significant influence on the risk of carcass contamination with *E. coli* O157 in our model, further defining of the distribution of these parameters is warranted. Future studies of these factors may reduce uncertainty and indentify controllable variability, which may lead to improved interventions at these contamination pathway steps. Fecal to hide and hide to carcass transfer may be impacted by cattle hygiene, pathogen load, and processing plant methods (Brichta-Harhay *et al.*, 2008); yet few data are available to define these effects. For the transfer parameters, we used pen-level data from Jacob et al. (2010) as it was important to know the range of individual cohort-level transfer rather than an overall mean transfer across cohorts. Our model suggests that relatively small changes in these parameters may have substantial impact on the eventual risk of carcass contamination with *E. coli* O157.

Our stochastic model estimated the impact of specific combinations of preharvest and harvest interventions on the risk of contamination of cattle carcasses with *E. coli* O157. Combinations of preharvest interventions may be particularly valuable for food safety during periods of high fecal prevalence. The sensitivity of our model to specific input parameters suggests potential prime opportunities for intervention development and data needs that could be addressed in future clinical trials or observational studies. Further, longitudinal observational studies investigating the relationships among fecal prevalence and concentration, hide contamination and concentration, and subsequent carcass contamination at the cohort level are needed to further define modifiable determinants of risk of *E. coli* O157 contamination in beef originating from feedlot cattle.

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Figure 5.1 Conceptual model for the pathway of *Escherichia coli* O157 contamination in cohorts of feedlot cattle from feedlot to harvest.

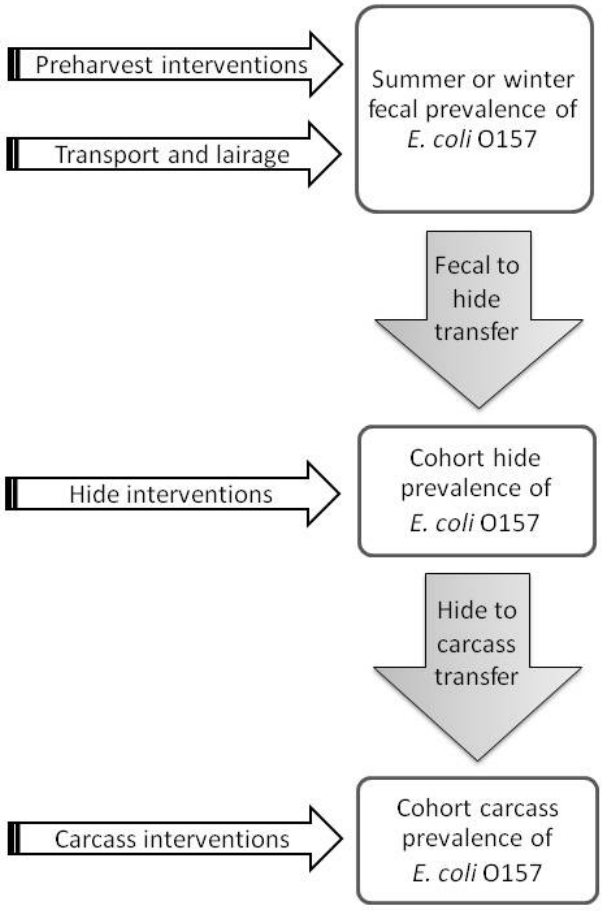

Figure 5.2 Box plot depicting the median risk of cattle carcass contamination with *Escherichia coli* O157 for model scenarios using combinations of summer (S) and winter (W) fecal prevalence and feedlot interventions, including no feedlot intervention (NFI), vaccination only (VAC), feed additive only (FA), and both vaccination and feed additive (VAC-FA).

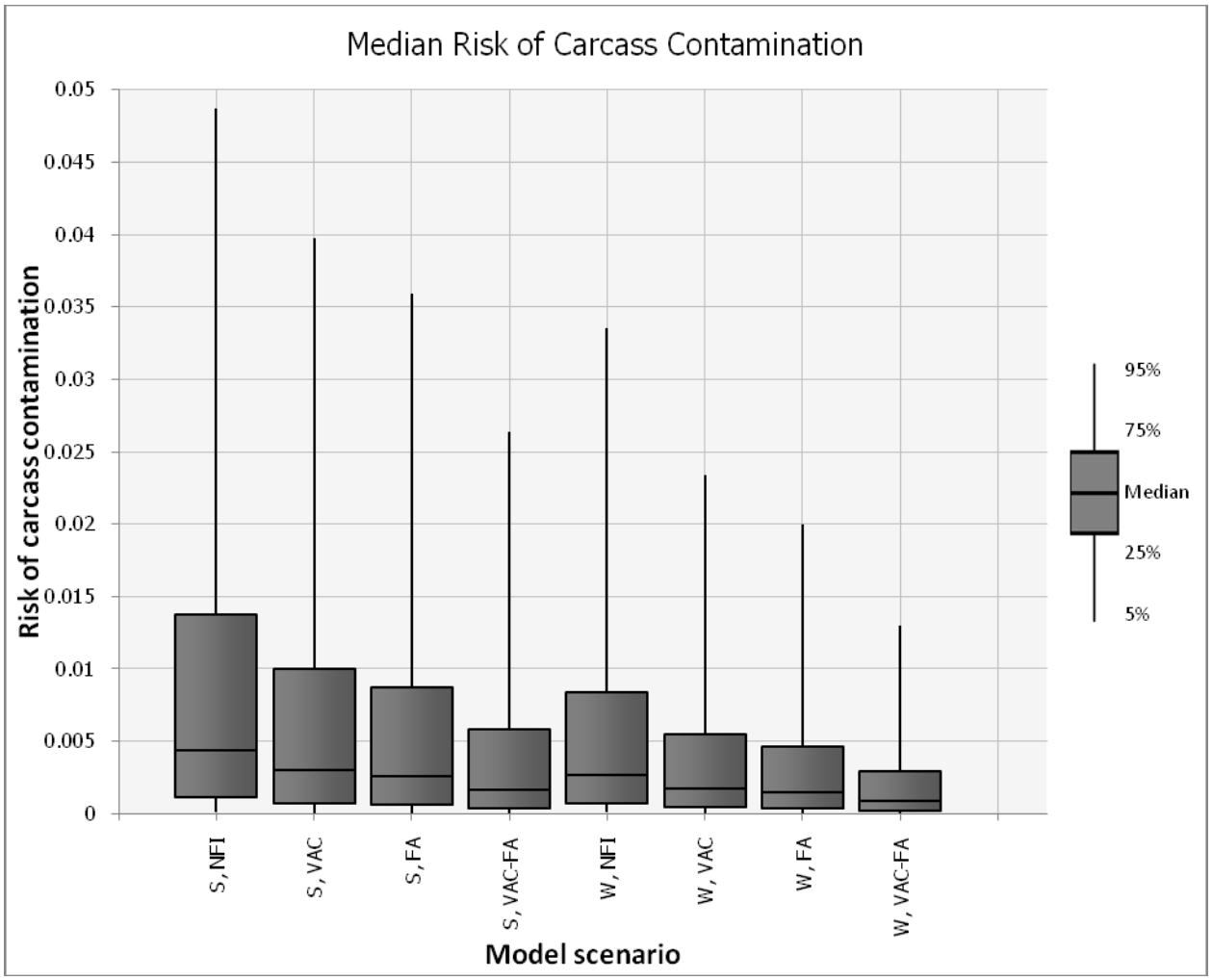

Figure 5.3 Sensitivity analysis showing Spearman rank correlation coefficients between model input parameters and the risk of cattle carcass contamination with *Escherichia coli* O157 for model simulations using summer (S) fecal prevalence and both vaccination and feed additive (VAC-FA).

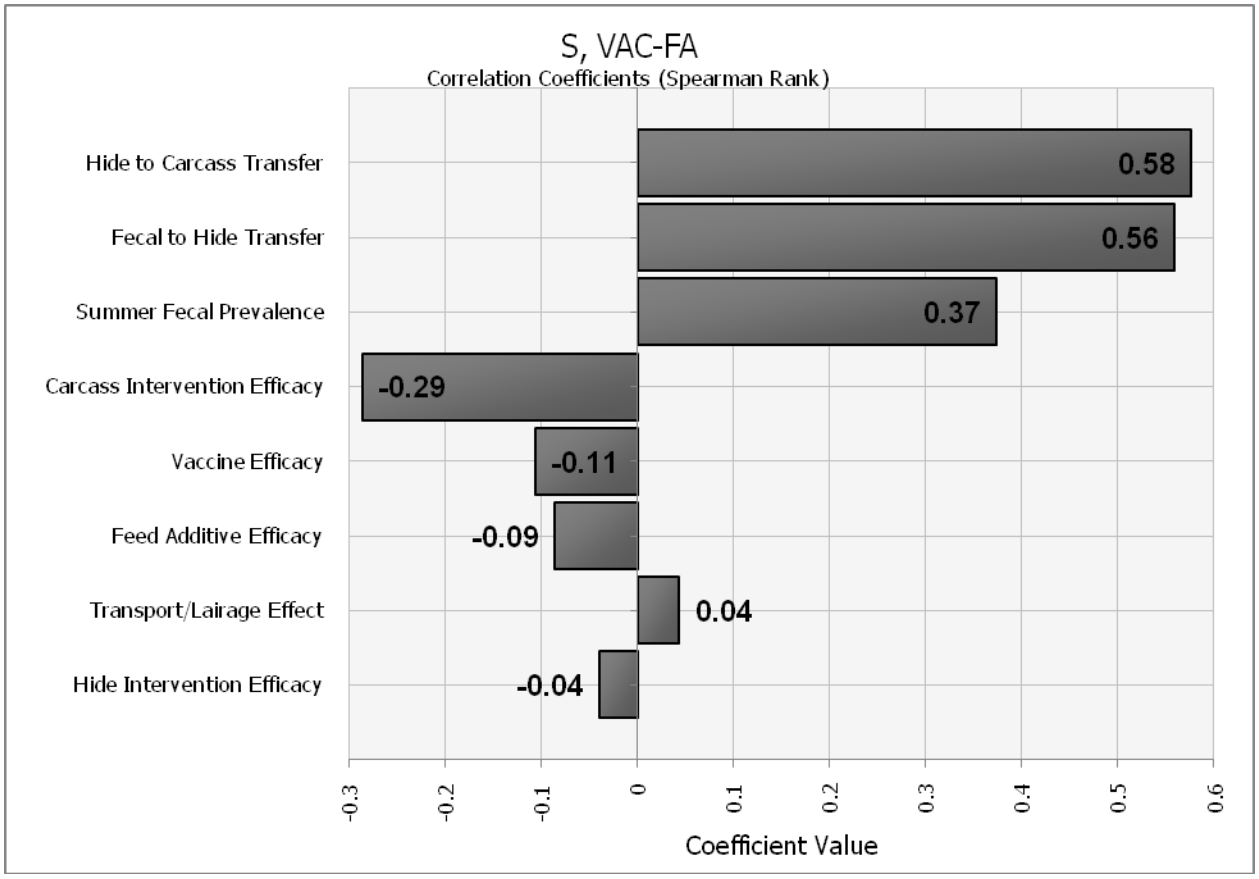

Table 5.1 Input parameters, distributions, and references for modeling the risk of carcass contamination with *Escherichia coli* O157 in cohorts of commercial feedlot cattle.

Parameter	Minimum	Most Likely	Maximum	Unit	Distribution	Reference
Summer fecal prevalence	0.07	0.12	0.93	%	Pert	Arthur *et al*., 2007; Callaway *et al*., 2006; Cobbold *et al*., 2007; Dewell *et al*., 2005; Dewell *et al*., 2008; Loneragan *et al*., 2005; Niu *et al*.,; Renter *et al*., 2008; Sanderson *et al*., 2006; Sargeant *et al*., 2003; Smith *et al*., 2001; Stephens *et al*., 2009
Winter fecal prevalence	0.05	0.11	0.21	%	Pert	Khaitsa *et al*., 2006; Renter *et al*., 2008; Stephens *et al*., 2009
Vaccine efficacy	0.08	0.29	0.85	%	Pert	Moxley *et al*., 2009; Peterson *et al*., 2007; Potter *et al*., 2004, Smith *et al*., 2009 ; Thomson *et al*., 2009; Woerner *et al*., 2006; Van Donkersgoed *et al.,* 2005
Feed additive efficacy	0.21	0.45	0.74	%	Pert	Arthur *et al*., 2010; Brashears *et al*., 2003; Cernicchiaro *et al*., 2010; Elam *et al*., 2003; Peterson *et al.,* 2007; Stephens *et al*., 2007a&b; Tabe *et al*., 2008; Woerner *et al*., 2006; Younts-Dahl *et al*., 2004; Younts-Dahl *et al*., 2005
Transport and lairage effect	-0.12	0.23	0.42	%	Pert	Childs *et al*., 2006; Dewell *et al*., 2008; Fegan *et al*., 2009; Woerner *et al*., 2006
Fecal to hide prevalence transfer	0.00	3.00	15.50	Ratio	Exponential[a]	Jacob *et al*., 2010
Hide intervention efficacy	0.39	0.57	0.62	%	Pert	Arthur *et al*., 2007; Bosilevac *et al*., 2004, Bosilevac *et al*., 2005
Hide to carcass prevalence transfer	0.00	0.26	1.25	Ratio	Exponential[a]	Jacob *et al*., 2010
Carcass intervention efficacy	0.35	0.86	0.98	%	Pert	Arthur *et al*., 2004; Bosilevac *et al*., 2006; Elder *et al*., 2000

[a] The most likely value was used for the β parameter in exponential distributions; these distributions were truncated at minimum and maximum values.

Table 5.2 Model outpu for the 2.5th, 50th, and 97.5th percentile probability of carcass contamination with *Escherichia coli* O157 for combinations of summer (S) and winter (W) fecal prevalence and no feedlot intervention (NFI), vaccine (VAC), feed additive (FA), and vaccination and feed additive (VAC-FA) models.

Scenario	2.5%	50%	97.50%
S, VAC-FA	0.00001	0.00165	0.04053
S, VAC	0.00002	0.00299	0.05713
S, FA	0.00002	0.00256	0.05209
S, NFI	0.00004	0.00440	0.06828
W, VAC-FA	0.00001	0.00091	0.01938
W, VAC	0.00002	0.00171	0.03446
W, FA	0.00001	0.00143	0.02917
W, NFI	0.00003	0.00268	0.04809

Table 5.3 Spearman rank correlation coefficients between model parameters and the risk of carcass contamination with Escherichia coli O157 for combinations of summer and winter fecal prevalence and no feedlot intervention (NFI), vaccine (VAC), feed additive (FA), and vaccination and feed additive (VAC-FA) models.

	Spearman rank correlation coefficient							
	Summer				Winter			
Model parameter	NFI	VAC	FA	VAC-FA	NFI	VAC	FA	VAC-FA
Fecal prevalence	0.33	0.36	0.37	0.37	0.22	0.22	0.22	0.22
Feed additive efficacy	n/a	n/a	-0.09	-0.09	n/a	n/a	-0.10	-0.10
Vaccine efficacy	n/a	-0.09	n/a	-0.11	n/a	-0.11	n/a	-0.11
Transport and lairage effect	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.04
Fecal to hide transfer	0.50	0.53	0.55	0.56	0.60	0.61	0.61	0.61
Hide intervention efficacy	-0.05	-0.05	-0.05	-0.04	-0.05	-0.05	-0.05	-0.05
Hide to carcass transfer	0.63	0.60	0.59	0.58	0.62	0.61	0.61	0.61
Carcass intervention efficacy	-0.33	-0.31	-0.31	-0.29	-0.33	-0.32	-0.32	-0.32

Table 5.4 Sensitivity analysis showing the impact of 5th, 50th, and 95th percentile deterministic settings for input parameters on the percentage of carcasses within a cohort contaminated with *Escherichia coli* O157 for no feedlot intervention (NFI), vaccine (VAC), feed additive (FA), and vaccination and feed additive (VAC-FA) models

	Parameter distribution	Deterministic		Median percentage of contaminated carcasses			
Input parameter	percentile	value	Season	NFI	VAC	FA	VAC-FA
Summer fecal prevalence	5th	0.035	summer	0.10	0.06	0.05	0.03
	50th	0.210	summer	0.53	0.36	0.31	0.19
	95th	0.529	summer	0.90	0.72	0.65	0.46
Winter fecal prevalence	5th	0.041	winter	0.11	0.07	0.06	0.04
	50th	0.107	winter	0.29	0.19	0.16	0.10
	95th	0.172	winter	0.45	0.30	0.25	0.16
Vaccine efficacy	5th	0.147	summer	N/A	0.39	N/A	0.22
			winter	N/A	0.23	N/A	0.12
	50th	0.338	summer	N/A	0.31	N/A	0.17
			winter	N/A	0.18	N/A	0.09
	95th	0.601	summer	N/A	0.19	N/A	0.10
			winter	N/A	0.11	N/A	0.06
Feed additive efficacy	5th	0.297	summer	N/A	N/A	0.33	0.21
			winter	N/A	N/A	0.19	0.12
	50th	0.456	summer	N/A	N/A	0.26	0.17
			winter	N/A	N/A	0.15	0.09
	95th	0.626	summer	N/A	N/A	0.18	0.12
			winter	N/A	N/A	0.10	0.06
Transport /lairage fecal prevalence increase	5th	0.032	summer	0.39	0.26	0.22	0.14
			winter	0.23	0.14	0.12	0.08
	50th	0.211	summer	0.44	0.30	0.26	0.17
			winter	0.27	0.17	0.14	0.09
	95th	0.356	summer	0.48	0.33	0.29	0.19
			winter	0.30	0.19	0.16	0.10
Fecal to hide prevalence transfer	5th	0.150	summer	0.04	0.03	0.02	0.01
			winter	0.02	0.01	0.01	0.01
	50th	2.062	summer	0.58	0.38	0.32	0.20
			winter	0.32	0.20	0.17	0.11
	95th	8.680	summer	1.18	1.04	0.97	0.75
			winter	1.13	0.83	0.71	0.45
Hide intervention efficacy	5th	0.472	summer	0.51	0.35	0.30	0.19
			winter	0.31	0.20	0.17	0.10
	50th	0.552	summer	0.43	0.30	0.25	0.16
			winter	0.26	0.17	0.14	0.09
	95th	0.606	summer	0.38	0.26	0.22	0.14
			winter	0.23	0.15	0.12	0.08
Hide to carcass prevalence transfer	5th	0.013	summer	0.04	0.03	0.02	0.01
			winter	0.02	0.01	0.01	0.01
	50th	0.178	summer	0.56	0.37	0.31	0.20
			winter	0.32	0.20	0.17	0.11
	95th	0.741	summer	2.32	1.55	1.30	0.83
			winter	1.34	0.85	0.72	0.45
Carcass intervention efficacy	5th	0.594	summer	1.05	0.71	0.61	0.39
			winter	0.64	0.40	0.34	0.21
	50th	0.811	summer	0.49	0.33	0.28	0.18
			winter	0.30	0.19	0.16	0.10
	95th	0.945	summer	0.14	0.10	0.08	0.05
			winter	0.09	0.05	0.05	0.03

Table 5.5 Change in the percentage of carcasses contaminated with *Escherichia coli* O157 in model simulations using the 5th and 95th percentile settings for input parameters for no feedlot intervention (NFI), vaccine (VAC), feed additive (FA), and vaccination and feed additive (VAC-FA) models.

Input Distribution	Season	NFI	VAC	FA	VAC-FA
Summer fecal prevalence	Summer	0.81	0.66	0.60	0.43
Winter fecal prevalence	Winter	0.34	0.22	0.19	0.12
Vaccine efficacy	Summer	N/A	-0.19	N/A	-0.12
	Winter	N/A	-0.28	N/A	-0.06
Feed additive efficacy	Summer	N/A	N/A	-0.15	-0.10
	Winter	N/A	N/A	-0.09	-0.06
Transport /lairage fecal prevalence increase	Summer	0.09	0.07	0.06	0.04
	Winter	0.07	0.04	0.04	0.02
Fecal to hide prevalence transfer	Summer	1.14	1.01	0.95	0.73
	Winter	1.11	0.81	0.70	0.45
Hide intervention efficacy	Summer	-0.13	-0.09	-0.08	-0.05
	Winter	-0.08	-0.05	-0.04	-0.03
Hide to carcass prevalence transfer	Summer	2.28	1.52	1.28	0.81
	Winter	1.32	0.83	0.71	0.44
Carcass intervention efficacy	Summer	-0.91	-0.62	-0.53	-0.34
	Winter	-0.55	-0.35	-0.29	-0.18

Table 5.6 Empirical data from previous studies that were used to assess model simulation accuracy and outputs from the model of carcass contamination with *Escherichia coli* O157 that we reported herein.

Study	Sample size	Season	Prevalence (95% Prediction Interval)		
			Hide	Pre-intervention carcass	Post-intervention carcass
Brichta-Harhay et al., 2008	180	summer	55.7%	18.3%	N/A
		winter	50.4%	19.5%	N/A
Barkocy-Gallagher et al., 2003	300	summer	73.5%	40.8%	1.0%
		winter	29.4%	1.2%	0.0%
Arthur et al., 2009	319[a]	summer	37.2%	N/A	N/A
		winter	63.4%	N/A	N/A
Model output (NFI)	N/A	summer	51.1% (0.9-100.0%)	5.8% (0.0-29.4%)	1.2% (0.0-6.9%)
		winter	34.0% (0.8-100.0%)	3.8% (0.0-21.4%)	0.8% (0.0-4.9%)

[a] Although 319 animals were in the study, results were analyzed at the cohort level (n = 10)

CHAPTER 6 - Conclusions

Several conclusions on the epidemiology of *Salmonella* and *Escherichia coli* O157 in the preharvest phase of beef cattle production can be reached based on the review of scientific literature and the subsequent research described within this dissertation. Previous research has focused more on the epidemiology of *E. coli* O157 than on *Salmonella* in beef cattle production systems, yet both pose a serious risk to cattle carcass contamination and the eventual risk of foodborne illness in humans. Insufficient information is available to effectively identify and validate several preharvest and harvest interventions in varied commercial beef production systems. Understanding the transfer of *E. coli* O157 and *Salmonella* from cattle feces to hides to carcasses likely depends on both pathogen prevalence and concentration at each step in the contamination pathway. Previous research suggests that preharvest interventions for *Salmonella* or *E. coli* O157 are important in reducing the risk of carcass contamination at harvest, yet data are limited on the impact of preharvest interventions on the prevalence and concentration of these organisms in feces, hides and carcasses. Furthermore, data regarding the transmission and persistence of *E. coli* O157 and *Salmonella* within and among cohorts of cattle are sparse. Review of the literature indicates that cohort-level studies are needed to develop a better understanding of the epidemiology of *Salmonella* and *E. coli* O157 and evaluate the impact of combinations of preharvest interventions in commercial feedlot cattle.

We used diverse study designs to further evaluate the epidemiology of *Salmonella* and *E. coli* O157 in beef cattle production systems. Our first study was a randomized controlled trial that indicated a commercially available vaccine (*Salmonella* Newport SRP®) did not significantly affect health and performance of feedlot cattle, nor did it appear to affect the fecal prevalence of *Salmonella* in vaccinated cattle. In this study, the low fecal prevalence of *Salmonella* combined with the longitudinal variability in fecal prevalence among replicates may have inhibited our ability to detect a significant vaccine effect. However, the study did provide further useful information. Fecal prevalence of *Salmonella* varied by arrival cohort throughout the study period (> 200 days in the feedlot), suggesting that cattle source may be an important risk factor for fecal shedding of *Salmonella*. This was the first peer-reviewed study to evaluate the effects of the *Salmonella* Newport SRP® vaccine in cattle within a commercial feedlot production setting; further trials of this vaccine administration in different feeder cattle

production settings may allow further assessment of this vaccine. Future study designs may need to include long-term herd vaccination strategies and/or regional vaccine coverage of several adjacent cohorts within a feedlot and within feedlot replicates. Because the control of *Salmonella* in commercial feedlot production systems may enhance food safety, and potentially improve cattle health and performance, further studies are necessary to validate preharvest interventions such as the *Salmonella* Newport SRP® vaccine.

Several risk factors may be associated with the fecal prevalence of *Salmonella* in feedlot cattle, such as the source of cattle as mentioned above. We used a repeated cross-sectional study to identify factors associated with the fecal prevalence of *Salmonella* in cattle at feedlot entry and immediately prior to harvest. We also determined if specific *Salmonella* strains (as differentiated by pulsed-field gel electrophoresis (PFGE)), which were isolated from feces of newly arrived cattle in the feedlot, were similar to strains isolated from those same cattle prior to being shipped for harvest. Surprisingly, the fecal prevalence of *Salmonella* within cohorts of cattle at feedlot arrival was not associated with the prevalence immediately prior to harvest. However, we found that specific serotypes, and also specific (PFGE) subtypes of *Salmonella*, appear to persist within a cohort throughout the feeding period, and can also be found among multiple cohorts within a feedlot. Furthermore, we observed that cohorts of cattle entering the feedlot at lighter mean body weights, fed for a longer period of time, and having higher cumulative incidence of morbidity and mortality may be more likely to shed *Salmonella* in their feces. However, we could not conduct a multivariable analysis of our data (and thus evaluate the individual effects of the aforementioned risk factors), since the sample size was limited and these cohort-level variables were correlated with each other. A more in-depth assessment of these risk factors may be necessary. Our data provided preliminary evidence that specific subsets of the feedlot cattle population may have a higher risk for shedding *Salmonella*. Factors like mean entry weight may be useful predictors of risk for shedding *Salmonella* in feces, but may not provide a direct method to mitigate this risk. Preharvest interventions for beef cattle need to affect the prevalence of *Salmonella* at the time of harvest to potentially improve beef safety, but the persistence of specific PFGE subtypes of *Salmonella* within and among cohorts throughout the feeding period may indicate that an approach to controlling *Salmonella* that impacts upstream factors in the production system is needed.

In another study, we defined and compared PFGE subtypes of *E. coli* O157 isolates from cattle feces and carcass samples at harvest to determine relationships between fecal shedding and carcass contamination. Truckload appeared to be an important factor, and feces from cattle shedding both high- and low-concentrations of *E. coli* O157 posed a risk for carcass contamination. We did not know the original feedlot cohort sources to make feedlot-level inferences. Interestingly, we found that most of the *E. coli* O157 isolates recovered from pre-evisceration carcasses were the same PFGE subtype as the isolates recovered from post-evisceration fecal samples from cattle within the same truckload. Generally, our findings suggested that pre-harvest intervention strategies for *E. coli* O157 need to mitigate the effects of both high- and low-shedding cattle, but the risk of carcass contamination is highly impacted by transmission of *E. coli* O157 within transport and harvest cohorts.

Mathematical simulation modeling is one method to assess the impacts of multiple interventions within a complex system; more specifically to this case, models can be used to estimate the effect and value of preharvest and harvest interventions for foodborne pathogens in beef production systems. We used a Monte-Carlo model to estimate the risk of carcass contamination in cohorts of cattle conditional on seasonal preharvest fecal prevalence of *E. coli* O157, vaccination for *E. coli* O157, feed additive inclusion in diet, transport and lairage effects, hide intervention(s), and carcass intervention(s). In our model, fecal to hide transfer, hide to carcass transfer, fecal prevalence, and carcass intervention efficacy were the most influential input parameters on the risk of carcass contamination at harvest. Parameters that were less influential included vaccine efficacy, feed additive efficacy, transport and lairage effect, and hide intervention efficacy. Because of the relative importance, yet sparse amount of data defining transfer parameters, further targeted field studies are warranted to better define these relationships. Our model suggested that relatively small changes in the influential parameters may have substantial impact on the eventual risk of carcass contamination with *E. coli* O157. Hence, reductions in fecal prevalence or improvements in carcass interventions may reap significant benefits in lowering the risk of carcass contamination. Our study also suggested that combining multiple preharvest interventions may be most important for public health during periods of high fecal prevalence. The sensitivity of our model to specific input parameters suggested an emphasis for future clinical trials, observational studies, and intervention development. Further longitudinal observational studies investigating the relationship of fecal

prevalence and concentration with hide contamination and subsequent carcass contamination at the cohort level are needed to further define risk of *E. coli* O157 contamination in beef originating from feedlot cattle.

Overall, the research described in this dissertation demonstrates the complex interrelationships among cattle management factors (including cattle source and transport groups), targeted interventions and microbial persistence that must be considered in order to mitigate the risks associated with *Salmonella* and *E. coli* O157 in beef production systems. To lower the risk of foodborne illness, we need to continue to develop a better understanding of the relationships among prevalence and concentration of *E. coli* O157 and *Salmonella* at each step in the contamination pathway within the beef production system. Concurrent evaluation of multiple preharvest and harvest interventions along this contamination pathway are often difficult and expensive to conduct, yet necessary to understand the complex relationships within a biological system and validate information gleaned from mathematical models. Future researchers need to strategically assess the impact of interventions for *Salmonella* and *E. coli* O157 at the cohort level in a variety of commercial cattle environments. Research described herein has furthered knowledge of the complex epidemiology of bacterial foodborne pathogens in beef production systems and demonstrated that comprehensive approaches will be necessary to improve public health and lower the incidence of foodborne illness attributed to *Salmonella* and *E. coli* O157 in beef products.