DISSERTATION

PERI-SLAUGHTER ECOLOGY OF *ESCHERICHIA COLI* O157 AND *SALMONELLA ENTERICA* IN FEEDLOT BEEF CATTLE

Submitted by

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In partial fulfillment of the requirements

For the degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2007

COLORADO STATE UNIVERSITY

April 11, 2008

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY GRANT ALAN DEWELL ENTITLED PERI-SLAUGHTER ECOLOGY OF *ESCHERICHIA COLI* O157 IN FEEDLOT BEEF CATTLE BE ACCEPTED AS FULFILLING IN PART THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

PERI-SLAUGHTER ECOLOGY OF
ESCHERICHIA COLI O157 AND *SALMONELLA ENTERICA*
IN FEEDLOT BEEF CATTLE

Escherichia coli O157 and *Salmonella enterica* are significant causes of foodborne illness throughout the world. The prevalence of *E. coli* O157 and *S. enterica* immediately prior to shipment and harvest is an important facet of the ecology of these organisms. The potential for disease outbreaks has led to increased research and development of control strategies within feedlots and slaughter plants to reduce potential for contamination of carcasses during the slaughter process. Despite years of research and implementation of mitigation strategies by the beef industry, *E. coli* O157 and *S. enterica* continue to be implicated in food-borne disease outbreaks and are responsible for recalls of millions of pounds of beef each year.

Risk factors for prevalence of *E. coli* O157 immediately prior to slaughter and the genotypic relationship between feedlot and slaughter isolates was investigated. The odds of *E. coli* O157 positive fecal samples from cattle fed brewers grains were 6 times that for cattle not fed brewers grains. The odds of *E. coli* O157 positive fecal samples from pens of cattle from Central Nebraska was 9 times that for pens of cattle from Eastern Colorado. These findings demonstrate that the presence of *E. coli* O157 in fecal samples from finished feedlot cattle is associated with feeding of brewers grain and geographic location. The genetic relatedness of *E. coli* O157 isolates from feedlot and slaughter plant beef can be used to investigate sources of contamination. Pulsed-field gel

electrophoresis was used to determine the genotypic relatedness of *E. coli* O157 isolates from fecal hide and carcass samples from 15 pens of cattle. From the 110 feedlot fecal sample isolates, 65 different genetic types were identified; 7 of which had multiple related isolates. At the pen level for all the different sample types (fecal, hide, and carcass) there were 0 to 14 different genetic types identified per pen with up to 4 types having multiple isolates. Within the sampled pens, 64% of the hide samples at the abattoir corresponded to a feedlot isolate. For carcass samples, 59% of isolates had a corresponding feedlot isolate. These findings indicate that although the majority of *E. coli* O157 isolates at the slaughter plant correspond to the feedlot pen of origin up to 40% of isolates may have been introduced after cattle left the feedlot. Therefore other sources of contamination should be considered.

Transportation of cattle from the feedlot to the slaughter plant could influence hide contamination of *Escherichia coli* O157 or *Salmonella enterica.* A cross-sectional study was initiated to evaluate the risk factors for hide contamination at slaughter. A multilevel Poisson model was used to evaluate if transportation and lairage were associated with changes in prevalence of fecal shedding or hide contamination of *E. coli* O157 or *Salmonella enterica* in finished beef cattle. Cattle held in *E. coli* O157 positive lairage pens had eight times greater risk of having *E. coli* O157 positive slaughter hide samples compared to cattle held in culture-negative pens (RR, 8.0; 95% CI, 1.6-38.8). Cattle that were held in lairage pens contaminated with feces had three times greater risk for *E. coli* O157 positive slaughter hide samples compared to cattle held in clean pens (RR, 3.1; 95% CI, 1.2-7.9). Cattle that were transported for long distances (> 160.9 km) had twice

the risk of having *E. coli* O157 positive hide samples at slaughter as to cattle transported a shorter distance (RR, 2.4; 95% CI, 1.1-5.1). Cattle with positive *Salmonella enterica* hide samples at the feedlot had almost twice the risk of having *S. enterica* positive slaughter hide samples compared to cattle without *S. enterica* positive feedlot hide samples (RR, 1.9: 95% CI, 1.2-3.0). Cattle transported in trailers with positive *S. enterica* samples had over twice the risk of having *S. enterica* positive slaughter hide samples compared to cattle transported in culture negative trailers (RR, 2.3: 95% CI, 1.4-3.6). Cattle transported for long distances (> 160.9 km) had over twice the risk of having *S. enterica* positive hide samples at slaughter compared to cattle transported shorter distances (RR, 2.3; 95% CI, 1.4-3.7). Cattle held in lairage pens contaminated with feces had almost twice the risk for *S. enterica* positive slaughter hide samples compared to cattle held in clean pens (RR, 1.8; 95% CI, 1.1-3.1). Cattle held off feed longer than 18 hours before loading had a greater risk of having *S. enterica* positive slaughter hide samples compared to cattle held off feed for shorter times (RR, 1.7; 95% CI, 1.0-2.9). Cattle that were agitated during loading had a higher risk of having *S. enterica* positive slaughter hide samples compared to cattle that were calm (RR, 2.2; 95% CI, 1.3-3.6). These findings suggest that transportation and lairage should be considered in *E. coli* O157 and *Salmonella enterica* control strategies.

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ACKNOWLEDGMENTS

I am indebted to several people for investing their time and energy as I pursued this doctoral degree. Without their input, this opportunity would not have been nearly as rewarding or intellectually stimulation.

Dr. Mo Salman, my major advisor, has been instrumental as I progressed through this challenge. Encouraging me both in my work as well as my education, I have sought Dr. Salman's advice many times. Dr. Salman has the enviable gift of being able to see what things could or should be. In addition to being a gifted modeler and epidemiologist, Dr. Salman possesses a unique combination of traits such as perceptiveness, visionary thinking, and a positive and encouraging attitude. These make him a tremendous advisor and colleague and without his vision, advice, and encouragement, I would not have learned nearly as much.

Dr. Wagner provided valuable input and was especially helpful in my approach to statistical methods. He invested many hours into my learning which truly advanced my understanding of statistical analysis and study design. I appreciated his attention to detail, high standards for epidemiological design and analysis, ability to teach and always being available to help me.

Dr. Belk has always been open to discuss my ideas and provide guidance as I have progressed in my studies and professional career. He has helped me chart my future from his forward thinking ideas and his willingness to share his expertise.

Dr. Hyatt has helped me in understanding the organization and details necessary for developing and conducting research projects. She provided me the latitude to try new things and helped me maintain balance and humor to see me through the program.

Dr. Dargatz has always shown confidence in me and he has been instrumental to my success throughout my career. He is always willing to discuss things with me and values my ideas. He has added a unique and valuable perspective to my endeavors.

I also would like to acknowledge the financial support for these projects that was received from the USDA CSREES National Integrated Food Safety Initiative. Sincere thanks is also extended to scientists at the Rocky Mountain Regional Animal Health Laboratory, Colorado Department of Agriculture for their services in laboratory analysis, the many microbiology students who spent many hours culturing and testing samples, as well as the graduate research assistants at the Center for Red Meat Safety, Colorado State University for their assistance in sample collection. Joni Triantis in the APHI lab also has been a tremendous asset for me to understand laboratory methods.

Lastly I would like to thank my wife, Reneé, for the extra burdens that she accepted, the encouragement she provided and confidence in me. Throughout all we have done together she has always helped me to accomplish my goals and this would not have been possible without her.

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INTRODUCTION

This chapter provides an overview of the structure and objectives of this dissertation. The focus of the dissertation is the ecology of *Escherichia coli* O157 and *Salmonella enterica* in U.S. market ready feedlot cattle.

Chapter 2 is a literature review of the human implications, and feedlot and slaughter plant ecology for *E. coli* O157 and *Salmonella enterica.*

Chapter 3 is the first of three papers investigating the ecology of *E. coli* O157 in peri-slaughter beef cattle. This paper, which has been published in *Foodborne Pathogens and Disease* (Dewell et al., 2005), presents a factor analysis of the prevalence of *E. coli* O157 in market ready beef cattle.

Chapter 4 expounds on the genotypic relatedness of the *E. coli* O157 isolates from the study presented in Chapter 3. This chapter, which will be submitted to the *Journal of Food Protection* (Dewell et al., 2008c), describes the genotypic relatedness of the *E. coli* O157 isolates obtained from live cattle and beef carcasses.

Chapter 5 presents the results of a study investigating the effect of transport and lairage on *E. coli* O157 in finished beef cattle. This chapter, which has been accepted by the *Journal of Food Protection* (Dewell et al., 2008a), describes risk factors for increased in hide contamination with *E. coli* O157 following transport and lairage.

Chapter 6 presents the results of a study investigating the effect of transport and lairage on *Salmonella enterica* in finished beef cattle. This chapter, which has been submitted to the *Journal of Food Protection* (Dewell et al., 2008b), describes risk factors for increased in hide contamination with *Salmonella enterica* following transport and lairage.

Chapter 7 provides a summary of the conclusions from Chapters 3 through 6.

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CHAPTER 2

REVIEW OF *ESCHERICHIA COLI* O157 AND *SALMONELLA ENTERICA* IN U.S. FEEDLOT CATTLE

Summary

Escherichia coli O157 and *Salmonella enterica* are important causes of foodborne illness throughout the world. The prevalence of *E. coli* O157 and *S. enterica* immediately prior to shipment and harvest is an important facet of the ecology of these organisms. The potential for disease outbreaks has led to increased research and development of control strategies within feedlots and slaughter plants to reduce potential for contamination of carcasses during the slaughter process. Despite years of research and implementation of mitigation strategies by the beef industry, *E. coli* O157 and *S. enterica* continue to be implicated in beef-related food-borne disease outbreaks. Several epidemiological studies have been implemented related to the food safety issues of *E. coli* and *S. enterica* (Hancock et al., 1997; Van Donkersgoed et al., 1999; Elder et al., 2000; Dargatz et al., 1997; Dargatz et al., 2003; Sargeant et al., 2003; Smith et al., 2005a; Smith et al., 2005b). Although these studies have led to major understanding of the ecology and the behavior of these two pathogenic bacteria in terms of their spread and pathogenesis, incorporation of these findings into pre-harvest mitigation strategies by the industry has not been accomplished. The pre-harvest beef industry is willing to continue modifying their practices if significant benefits are clearly outlined.

Introduction

As outbreaks of food-borne disease continue to occur in populations of humans in the USA and worldwide, management practices used in livestock production systems have come under increased scrutiny. Increasingly, the burden to assess and mitigate the risk of zoonotic pathogens contaminating foods destined for human consumption is being placed on producers and slaughter plants. Zoonotic pathogens reported to be responsible for many of the recent outbreaks of food-borne disease include of *Escherichia coli* O157, *Salmonella enterica*, *Listeria monocytogenes*, and *Campylobacter* spp. (Mead et al., 1999). Foods commonly implicated in these outbreaks include milk, cheese, fruit juices, eggs, and meat (Mead et al., 1999). Because these pathogens also are present in livestock, control of contamination of food products should include control of the pathogens within livestock populations. In the USA, beef cattle are finished in commercial feedlots, and are shipped directly from these facilities to harvest. Therefore, efforts at mitigating the risk of zoonotic pathogens contaminating meat destined for human consumption can be effectively focused at the feedlot and slaughter plant levels.

Literature Review

E. coli O157 in humans

Pathogenic strains of *E. coli* O157 have been identified in outbreaks of food-borne illness in humans in the past two decades (Riley et al., 1983; Dorn, 1993; Griffin, 1995; Su & Brandt, 1995). From 1982 to 2002, there were 8,598 cases from 350 outbreaks that resulted in 40 deaths (Rangel et al., 2005). Some strains of *E. coli* O157 produce one or

two shiga-like toxins which are at least partly responsible for the most severe pathogenic effects seen in humans, such as bloody diarrhea and hemolytic uremic syndrome (Chapman et al., 1993; Griffin, 1995; Mahon et al., 1997). Virulence depends on toxin production and, potentially, on the presence of other characteristics such as the attaching and effacing genes that have been reported in some *E. coli* O157 strains (Griffin, 1995; Franck et al., 1998). The infectious dose for *E. coli* O157 for humans has been reported to be less than 100 colony forming units (Griffin, 1995). Although everybody is at risk for contracting *E. coli* O157, children, elderly and immune-compromised individuals are most susceptible (Lawson, 2004). A recent study reported that 1.1% of retail ground beef samples were positive for *E. coli* O157 (Samadpour et al., 2006) indicating potential risk for humans.

E. coli O157 in cattle in feedlots and slaughter plants

Epidemiologic studies and culturing of suspect foods have linked many outbreaks of food-borne *E. coli* O157 to contaminated beef products. Prevalence studies in beef cattle have supported this link (Chapman et al., 1993; Hancock et al., 1997; Elder et al., 2000; al-Saigh et al., 2004).

Many researchers have suggested that *E. coli* O157 should be considered a ubiquitous organism in cattle feedlots in North America (Hancock et al., 1997; Hancock et al., 2001; Van Donkersgoed et al., 1999). However, individual animals only transiently shed organisms (Sargeant et al., 2000; Rasmussen & Casey, 2001; Sanchez et al., 2002). Besser et al. (2001) reported that *E. coli* O157 can be transmitted between animals at low

doses. Keen and Elder (2002) and Stephens et al. (2007) reported that *E. coli* O157 can be cultured from the oral cavity and hides in addition to fecal samples. Recent studies have reported that *E. coli* O157 colonizes the bovine colon (Grauke et al., 2002; Laven et al., 2003; Naylor et al., 2003). A higher level of fecal prevalence for *E .coli* O157 has been detected in younger animals compared to mature beef cattle (Hancock et al, 1998; Van Donkersgoed et al., 1999; Gannon et al., 2002). One study hypothesized that horizontal transmission of *E. coli* O157 can occur between cattle during social grooming (McGee et al., 2004).

There are many reports of *E. coli* O157 organisms persisting in the farm environment for extended periods of time (Shere et al., 1998; LeJeune et al., 2004; Davis et al., 2005; Liebana et al., 2005; Williams et al., 2005). However it is unclear if the environment is the primary source for new infections in cattle or an indication of past contamination. LeJeune et al. (2004) reported that the same clonal type persisted over time despite high cattle turnover indicating that the feedlot environment was an important source for *E. coli* O157. In this study, 60% of the isolates were grouped into four closely related genotypes that were recovered from each sampled pen throughout the course of the study (LeJeune et al., 2004). However, Sanderson et al. (2006) reported that new genetic types were introduced to the feedlot by incoming cattle. After introduction, the new strain became the predominant genotype within the feedlot (Sanderson et al., 2006).

In 1997, the animal-level fecal prevalence of *E. coli* O157 was estimated to be 1.6% in feedlot cattle from 13 states, with a feedlot-level prevalence of 63% (Hancock et al.,

1997). This study was conducted during the winter months (October through December) and relied on traditional culturing methodologies to isolate *E. coli* O157. In contrast, Keen & Elder (2000) published a within feedlot animal-level prevalence range of 0% to 73% (mean of 28.2%) for feedlots in Nebraska and Kansas, with 78.6% of sampled pens having at least one positive animal. Additionally, a study by USDA National Animal Health Monitoring System reported an overall prevalence of 11% from October, 1999 through September, 2000 (USDA, 2001a). The Keen & Elder study was conducted in the summer months and incorporated an immunomagnetic bead separation step in the culturing methodology, which has been shown to improve detection of *E. coli* O157 (USDA, 2001a; Van Donkersgoed et al., 1999). Several studies have found a seasonal elevated prevalence of *E. coli* O157 in cattle feces during warmer months (Hancock et al., 1997; Van Donkersgoed et al., 1999; Hancock et al., 2001; Van Donkersgoed et al., 2001; USDA, 2001; Laven et al., 2003). In one study the prevalence ranged from 19.7% in the summer to 0.7% in the winter (Van Donkersgoed et al., 1999). However, Alam and Zurek, (2006) recently reported that there was not any seasonal effect on *E. coli* O157 in a Kansas feedyard. In this study, the prevalence was 18% in February, 12% in August and less than 9% in September, October, November, and January (Alam and Zurek, 2006). However, this study did not sample cattle in May, June or July.

At the slaughter plant, fecal prevalence of *E. coli* O157 peaks in the summer time while hide and pre-evisceration carcass prevalence remains high from Spring through the Fall (Barkocy-Gallagher et al., 2003). Woerner et al. (2006) reported that feedlot cattle with fecal prevalence greater than 20% were more apt to have hide and carcass contamination

with *E. coli* O157. Contamination of carcasses by *E. coli* O157 and other pathogens primarily has been attributed to transfer of pathogens from the hide onto the carcass (Barkocy-Gallagher et al., 2003; McEvoy et al., 2003; Arthur et al., 2004)

Risk factors associated with *E. coli* O157 in feedlot cattle

Several environmental or management factors have been reported to be associated with increased fecal shedding of *E. coli* O157. The condition (wet versus dry) of the pen floor has been associated with the prevalence or presence of *E. coli* O157 in cattle feces (Smith et al., 2001, Sargeant et al., 2004a, Smith et al., 2005). However, intervention strategies to manage pen floor condition have not resulted in decreased prevalence of *E. coli* O157 (Khaitsa et al., 2002). Sargeant et al., (2004a) also reported that wind velocity was positively associated with the presence of *E. coli* O157 in fecal samples and that the increasing height of the feed bunk was positively associated with presence of *E. coli* O157 in cattle feces. In the same study, Sargeant et al. (2004b) found 15% of feed samples were positive for *E. coli* O157 but did not report that wind velocity was associated with presence of *E. coli* O157 in feed.

Alam and Zurek (2004) reported that house flies can carry *E. coli* O157 in their fecal matter and may be a vector for transmission of the pathogen. Ahmad et al. (2007) reported that experimentally inoculated house flies could transmit *E. coli* O157 to calves. Sargeant et al. (2004a) reported that increased frequency of cats in the cattle pen areas was positively associated with *E. coli* O157.

The effect of various feed components has been investigated by several researchers. Cattle on barley based diets have been reported to have a higher prevalence of *E. coli* O157 than cattle on corn diets (Dargatz et al., 1997; Buchko et al., 2000; Berg et al., 2004). A recent study reported that cattle fed a diet consisting of dry rolled grains had lower fecal prevalence of *E. coli* O157 than cattle fed steam flaked grains (Fox et al., 2007). Additionally, feetlot cattle fed distiller's grain have been reported to have increased fecal shedding of *E. coli* O157 (Jacob et al., 2007). Dargatz et al. (1997) reported that cattle fed soy meal were less likely to be positive for *E. coli* O157. Van Baale et al. (2004) reported that cattle on forage diets shed *E. coli* O157 for longer duration and at higher levels than cattle on grain diet. Dargatz et al. (1997) reported no association with feeding of antimicrobial agents, probiotics and certain other supplements. Hancock et al. (1998) reported that feed changes are associated with shedding of *E. coli* O157. Reports differ on the effect of switching from a concentrate to a hay-based diet on prevalence of *E. coli* O157. Keen et al. (1999) found that cattle that were switched to hay had a lower prevalence for *E. coli* O157. However, Hovde et al. (1999) and Grauke et al. (2003) reported no effect or increased shedding of *E. coli* O157 with roughage diets.

Early studies indicated that feed and/or water may be important sources of dissemination of *E. coli* O157 in cattle feedlots (Hancock et al., 2001; LeJeune et al., 2001a; LeJeune et al., 2001b; Van Donkersgoed et al., 2001; Sargeant et al., 2003). To date intervention strategies targeting water sources have not been successful at controlling *E. coli* O157 (LeJeune et al., 2004).

There have been many management factors that have been reported to be associated with increased fecal shedding of *E. coli* O157. Dargatz et al. (1997) reported that lower body weights at entry to the feedlot, and type of ration were associated with the prevalence of *E. coli* O157. Dargatz et al. (1997) also reported no association with general health or density of animals in the pen. Sargeant et al. (2004a) reported that a history of an injectable mass medication of an antibiotic had a positive association with the presence of *E. coli* O157 in the pen while antibiotics in the feed or water had a negative association. Since it had been over 90 days since the cattle had been treated with injectable antibiotics, it is unclear if association is due to use of mass medication or possibly inherent to the type of cattle that need mass medication. Management factors are important as they might be controlled during the feeding period and because they could be incorporated into risk assessment models. However, due to the large amount of unexplained variability, changes in on-farm management of cattle alone are unlikely to prevent the presence of *E. coli* O157 in feedlot cattle (Sargeant et al., 2004).

Reports differ as to the association between prevalence of shedding and gender of cattle in the pen. Some researchers detected no difference in prevalence between steers and heifers (Keen & Elder, 2000; Van Donkersgoed et al., 1999), whereas Dargatz et al. (1997) reported a negative association between detection in pens and a higher proportion of heifers. Van Donkersgoed et al. (1999) reported an increased prevalence during the summer months. Evaluation of the role of these types of factors might allow for

differential management of groups of animals to compensate for risk factors that are not alterable.

Control of *E. coli* O157 in cattle

During the last 15 years there has been increased interest in food safety research. For beef cattle much of this research has focused on mitigation strategies within slaughter plants or understanding the ecology of *Escherichia coli* O157 within the feedlot environment. A number of novel ideas have been presented as potential strategies that could be used by the feedlot operators to further minimize the prevalence of *E. coli* O157 near harvest. Competitive inhibition with *Lactobacillus* species have been shown to be effective at reducing *E. coli* O157 shedding in feedlot cattle (Brashears et al. 2003; Younts-Dahl et al., 2004; Younts-Dahl et al., 2005; Peterson et al., 2007a; Stephens et al., 2007). Other strategies such as vaccination of cattle (Potter et al, 2004; Van Donkersgoed et al., 2005; Thornton et al., 2007; Peterson et al., 2007b), feeding of kelp products (Braden et al., 2004), use of sodium chlorate (Callaway et al., 2002), or neomycin for pre-slaughter cattle (Elder, 2002; Ransom, 2003) also have been investigated. Only neomycin has shown a consistent and dramatically lowered prevalence of *E. coli* O157 (Elder, 2002; Ransom, 2003), but FDA approval is very unlikely. Recently, bacteriophages against *E. coli* O157 have been investigated (Bach et al., 2003, Tanji et al., 2005; Sheng et al., 2006) but have not shown conclusively to be effective.

E. coli O157 during transportation and lairage

In addition to contamination of beef products via contact with bovine feces, *E. coli* O157 also may contaminate these products via contact with contaminated hide surfaces at slaughter (Elder et al., 2000; Barkocy-Gallagher et al., 2003). In one report, the prevalence of *E. coli* O157 on the hides of live animals was generally associated with the fecal prevalence (Keen & Elder, 2002). This finding suggests that strategies aimed at reducing prevalence of fecal shedding of *E. coli* O157 would likely impact the level of hide contamination as well. However, strategies aimed at mitigating risk of contamination of meat products via hide contact may differ from those mitigating risk of contamination via fecal contact at the slaughter plant.

A study from the United Kingdom of 73 different cattle consignments implied that the floor of the lairage pens or of the stunning box was the source of the prevalent clone (75% of isolates) cultured from hides of slaughtered cattle (Avery et al., 2002). Another UK study of lairage environments found that 27.2% of samples from selected sites along the unloading-to-slaughter routes were positive for *E. coli* O157. The sites that were most frequently contaminated were the holding pen floors, entrance gate to the stun box, and the stun box floor (Small et al., 2002). A study in Ireland did not demonstrate any difference in fecal prevalence for *E. coli* O157 due to transport or lairage (Minihan et al., 2003). Investigation of hide contamination at slaughter in Scottish cattle identified an increased risk of a carcass testing positive for *E. coli* O157 if an adjacent carcass had an *E. coli* O157 contaminated hide, if cattle were transported to the slaughter plant by a commercial hauler, or cattle were restrained in a squeeze chute during lairage (Mather et al., 2007). A recent study linked isolates from transportation trailers, and lairage pen

water troughs, with isolates obtained from cattle hides at slaughter via DNA fingerprinting (Childs et al., 2006)

There have been several recent publications evaluating effects of transportation of beef cattle on shedding of enteric pathogens. Bach et al. (2004) reported that non-preconditioned, weaned calves transported long distances had higher *E. coli* O157 fecal shedding prevalence than other calf groups. In contrast, Barham et al. (2002) did not demonstrate a significant difference in the fecal or hide prevalence of *E. coli* O157 following transportation, although they did find a significant increase in fecal and hide prevalence of *Salmonella* spp. after transportation of cattle to slaughter. Reicks et al. (2007) found a significant increase in shedding prevalence following transportation of cattle to slaughter for both *E. coli* O157 and *Salmonella* spp., although differences in *E. coli* O157 shedding were not biologically significant nor were any other risk factors for shedding identified. Arthur et al. (2007) reported that transport to and lairage at slaughter plants led to increased prevalence of *E. coli* O157 on cattle hides compared to feedlot prevalence. A limiting factor with these three studies was that all the cattle were from the same feedyard and slaughtered at the same facility, making it difficult to extrapolate the results beyond the study population.

Salmonella in humans

According to Centers for Disease Control and Prevention (CDC) statistics, there are approximately 40,000 reported cases of human salmonellosis in the United States each year (CDC, 2006a). Young children are most likely to contract salmonellosis (CDC,

2006a). The CDC estimates that there are 1.3 million food-borne human cases of salmonellosis among people every year because most cases go unreported (Mead et al., 1999). *Salmonella* Enteritidis and *S.* Typhimurium are responsible for half of the human cases of salmonellosis (CDC, 2005). In the spring of 2002, ground beef was implicated in an outbreak of multidrug-resistant *S.* Newport that was responsible for illness in 47 people in the northeastern US (CDC, 2002b). Another *Salmonella* outbreak linked to ground beef affected 31 people in nine states in 2004 (CDC, 2006b). *Salmonella* also has been found in retail packages of ground beef in several studies (White et al., 2001, Zhao et al., 2002; Sorensen et al., 2002; Samadpour et al., 2006). These studies confirm that *Salmonella enterica* is an important concern for the beef industry.

Salmonella in cattle in feedlots and slaughter plants

The USDA's National Animal Health Monitoring System (NAHMS) reports in its Feedlot '99 study, that 6.3% of fecal samples from feedlot cattle were positive for *Salmonella* regardless of duration of feeding (USDA, 2001b). Callaway et al. (2006) reported an 11.7% fecal prevalence of *Salmonella* in feedlot cattle in the Southern Plains region of the United States. Galland et al. (2000) reported a *Salmonella* prevalence of 40% in feeder steer fecal samples at entry into the feedlot, which was down to 0% at slaughter. The stress associated with marketing calves and their introduction to a feedyard environment has been implicated as the cause for the increased fecal prevalence during the early feeding period (Corrier et al., 1990). However, Khaitsa et al. (2007) reported that the prevalence of *Salmonella* in the feces of feedlot cattle increased from 1% on arrival to 62% at finish after 7 months on feed. Stephens et al. (2007) recently

reported higher prevalences of *Salmonella* could be cultured from hide surfaces (flank 74%, back 76%, neck 76%, ventrum 86%, perineum 88% and hock 94%), oral cavity (94%), recto-anal junction (64%) and feces (50%) in feedlot cattle.

In a Canadian study, Van Donkersgoed et al. (1999) reported a fecal prevalence of 0.08% in cattle at slaughter. Fegan et al. (2004) reported a fecal prevalence of 6.8% in cattle presented for slaughter in Australia. Bacon et al. (2002) reported a hide prevalence of 15.4% at slaughter with a carcass contamination prevalence of only 1.3% in U.S. slaughter cattle. Barckocy-Gallagher et al. (2003) reported that in Midwestern slaughter plants the prevalence of *Salmonella* was 4.4% in feces, 71.0% on hides and 12.7% on pre-evisceration carcass samples. In this study, prevalence of *Salmonella* peaked in the summer for cattle at slaughter. An increased seasonal prevalence in the summer also was reported by McEvoy et al. (2003). The large variation in Salmonella prevalence may be partially influenced by culture techniques. Van Donkersgoed et al. (1999) inoculated tetrathionate broth with feces and incubated for 48 hours, then subcultured to Rappaport Vassiliadis broth for 24 hours and then plated onto brilliant green sulfa agar for 24 hours. Barckocy-Gallagher et al. (2003) inoculated tryptic soy broth with feces and incubated for 18 hours, then subcultured to tetrathionate broth for 24 hours, immunomagnetic seperations then was used to select *Salmonella* organisms, the immunomagnetic beads then were added to Rappaport-Vassiliadis broth for 18 hours then plated to a Hektoen enteric agar with novobiocin for 24 hours. Rivera et al. (2004) found *Salmonella* on fabrication floor conveyer belts.

Risk factors for *Salmonella enterica* in feedlot cattle

There has been scarcity of studies investigating risk factors for *Salmonella enterica* in feedlot cattle. Davis et al. (2003b) reported that *Salmonella* positive feed could play a role in the transmission of *S. enterica* in feedlot cattle. Dargatz et al. (2005) reported that 5.3% of feed commodities were positive for *S. enterica.* Losinger et al. (1997) reported that feeding tallow or cottonseed was positively associcated with an increased prevalence of *Salmonella* in a pen. Smith et al. (2005) reported that pen condition and recovery of *Salmonella* from water tank was positively associated with the pen being positive for *Salmonella.*

Salmonella during transportation and lairage

Shedding of *Salmonella* spp. in beef cattle is reported to increase in association with stress such as transportation (Corrier et al., 1990; Barham et al., 2002; Beach et al., 2002a). This phenomenon also has been documented in swine during transportation and lairage (Berends et al., 1996; Isaacson et al., 1999; McKean et al., 2001; Hurd et al., 2002). However, when pigs were experimentally lairaged for 18 hours in clean facilities prior to transporting them to the slaughterhouse, the isolation rate of *Salmonella* decreased (Hurd et al., 2001) indicating that the environment can affect *Salmonella* recovery rates. Additional studies have shown that *Salmonella* is present in lairage pens in both cattle and swine abattoirs (Swanenburg et al., 2001; Small et al., 2002). However, none of these studies have examined the risk of carcass contamination by *Salmonella* organisms during transportation and lairage of cattle.

Rivera-Betancourt et al. (2004) observed that 25 to 52% of lairage pen samples were positive for *Salmonella* in two slaughter plants in the U.S. A UK study of lairage environments found that 6.1% of samples from selected sites along the unloading-to-slaughter routes were culture-positive for *Salmonella* (Small et al., 2002). In that study, the sites at the slaughter plant that were most frequently contaminated were the holding pen floors, entrance gate to the stun box, and the stun box floor (Small et al., 2002 Also, *Salmonella* can persist in the lairage environment from one day to next even after cleaning (Small et al., 2006) These studies (Small et al., 2002; Rivera-Betancourt et al., 2004; Small et al., 2006) identified the presence of environmental contamination at slaughter. However, cattle were not sampled at the farm of origin to determine if contamination was present prior to transportation and lairage. Additionally, fecal prevalence of *Salmonella enterica* in cattle can increase following stress of transportation (Corrier et al., 1990; Galland et al., 2000).

Several recent studies evaluated effects of transportation of beef cattle to slaughter on shedding of enteric pathogens. Beach et al. (2002) observed a significant increase in *Salmonella* contamination of hides following transportation from the feedlot to the slaughter plant but did not identify any risk factors. Barham et al. (2002) found a significant increase in *Salmonella* spp. prevalence in feces and on hides after transportation, although they did not demonstrate a significant difference in the fecal or hide prevalence of *E. coli* O157 following transportation. Reicks et al. (2007) found a significant increase in shedding prevalence of both *Salmonella* and *E. coli* O157 following transportation.

Stress in finished beef cattle

Stress as a result of transportation and/or lairage has been implicated to influence the prevalence of *E. coli* O157 or *Salmonella* spp. in cattle (Corrier et al., 1990; Barham et al., 2002). In swine, cortisol levels have been unrewarding as a measure of stress in transported and lairaged pigs (Brown et al., 1999; Perez et al., 2002). Much of this variability is attributed to differences in psychological stress that an individual or group of animals exhibit (Grandin, 1997). Grandin (1998, 2001, 2002) has developed objective measures of stress and welfare, which are used by feedlots and slaughter plants to score the handling of cattle. These objective systems measure the behavior of animals by scoring prod usage, running, slipping, falling, or banging into structures, and vocalization (Grandin, 1998, 2001, 2002). The role that stress may have on the prevalence of *E. coli* O157 or *Salmonella* spp. in finished feedlot cattle has not been adequately examined using an objective scoring system.

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) has been used to investigate outbreaks of human disease (Barrett et al., 1994; Gerner-Smidt et al., 2005) and by researchers to compare isolates among and between groups of cattle (Hancock et al., 1998; Rice et al., 1999; Renter et al., 2003; LeJune et al., 2004; Childs et al., 2006). Additionally, molecular subtyping is being used to compare isolates from cattle before and after slaughter (Barkocy-Gallagher et al., 2001; Tutenel et al., 2003; Childs et al., 2006; Arthur et al.,

2007). Genotyping can be used to help identify potential sources of *E. coli* O157 on beef carcasses.

Pulsed-field gel electrophoresis (PFGE) utilizes restriction endonuclease enzymes to cut the entire bacterial genome to produce 10 to 20 DNA fragments between 10 and 800 kb (Pagotto et al. 2005). Electrophoresis then is utilized to separate the fragments by size so restriction patterns can be compared between isolates. Periodic inversions of the electric field allow for the reorientation of DNA strand trapped in the agarose gel. PFGE banding patterns can then be evaluated for similarity with the Dice similarity coefficient. The Dice coefficient is calculated by dividing 2 times the number of matching bands by the total number of bands being compared (Dice, 1945). The Dice coefficient assumes bands of identical size are genetically homologous. Theoretically, two nonhomologous genomes could be cleaved to yield fragments of similar sizes (Davis, et al., 2003a).

Historically, the single restriction enzyme XbaI has been used for disease surveillance and outbreak investigations by PulseNet (Ribot et al., 2006). This one day method was developed for rapid turnaround and reproducibility between PulseNet labs (Ribot et al., 2001; Ribot et al., 2006). However, for genome sizing and mapping other PFGE methods are used to produce higher band resolution (Chang and Taylor, 1990). To make relationship decisions among isolates with a single restriction enzyme requires spatial, temporal and/or other epidemiological data (Pearl et al., 2007). PFGE has not been stringently evaluated to measure genetic relatedness for unrelated isolates (Davis et al., 2003a). Different restriction enzymes can assign different relatedness patterns (Harsono

et al., 1993; Rice et al., 1999). Single enzyme PFGE may not correctly resolve genetic relatedness because matching bands may represent homologous genetic material or bands of similar size may resolve into individual bands (Davis et al., 2003a). Without appropriate epidemiological data six or more restriction enzymes are needed to indicate genetic relatedness.

PFGE is extensively used because it is simple and inexpensive. However, results are not easily compared between labs and banding patterns are subjectively decided (Noller et al., 2003). Other methods have been investigated but PFGE remains the gold standard because it has higher discriminative power (Heir et al., 2000; Foley et al., 2004). The advantage of sequence based methods is they are easily standardized and automated. Methods that have been evaluated include fluorescent amplified-fragment-length polymorphism (Heir et al., 2000), multilocus sequence typing (Noller et al., 2003a; Foley et al., 2004), and repetitive-element PCR (Foley et al., 2004). Multilocus variable-number tandem repeat analysis has been shown to correctly identify outbreak isolates from sporadic isolates and to discriminate PFGE strains within clusters (Noller et al., 2003b).

Several studies comparing non-epidemiological related isolates have identified indistinguishable strains from different geographical locations (Faith et al., 1996; Rice et al., 1999; Van Donkersgoed et al., 2001; Davis et al., 2003c; Arthur et al., 2007). Arthur et al. (2007) had indistinguishable strains from geographical regions hundreds of miles apart when utilizing 3 enzymes. Therefore, source tracking of isolates without clear

epidemiological connections should be avoided. Additionally, genetic diversity of *E. coli* O157 isolates within herds has been demonstrated (Faith et al., 1996; Rice et al., 1999; Renter et al., 2003; Vali et al., 2004). Vali et al. (2007) demonstrated that in order to account for *E. coli* O157 diversity in a sample at least five colonies per sample should be analyzed to identify different PFGE subtypes.

Conclusion

In summary, although there has been extensive research on *E. coli* O157 and to a lesser degree *Salmonella enterica* in feedlot and slaughter ready cattle, there remains critical gaps in the knowledge and understanding of the ecology and subsequent control of these organisms. These bacteria have shown the ability to persist in cattle populations over time and are easily transmitted between individual cattle. Due to the biological ecosystem and interactions between environment, mammalian host and the bacterial organism, repeatability of risk factors has been difficult between studies. Further work based on hypothesis driven research is needed to understand and ultimately control human pathogens in beef cattle production settings.

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CHAPTER 3

PREVALENCE OF AND RISK FACTORS FOR *ESCHERICHIA COLI* O157 IN MARKET-READY BEEF CATTLE FROM 12 U.S. FEEDLOTS

Summary

Determination of *E. coli* O157 prevalence immediately prior to shipment and harvest is an important facet of the ecology of this organism that requires further elucidation. As part of a larger study to measure the effects of within-pen prevalence of *E. coli* O157 on subsequent carcass contamination, fecal samples from 15 pens of cattle in each of 12 different feedlots in 3 states (Colorado, Nebraska and Montana) were collected from June through September 2002. Thirty fresh fecal samples were collected from each pen floor within 36 hours of shipment to slaughter. Fecal samples underwent standard enrichment, immunomagnetic separation and isolation procedures for *E. coli* O157. Multivariable logistic regression was used to determine which factors best predicted pen-level positive culture results, and to estimate the magnitude of association between each factor and the outcome, while adjusting for other factors in the model. Thirteen (86.7%) of the 15 pens had at least one positive sample and the within-pen prevalence of *E. coli* O157 in positive pens ranged from 3.3% to 77.8%. The odds of *E. coli* O157 positive fecal samples from cattle fed brewers grains were 6 times that for cattle not fed brewers grains. The odds of *E. coli* O157 positive fecal samples from pens of cattle from Central Nebraska was 9 times that for pens of cattle from Eastern Colorado. These data demonstrate that the presence of *E. coli* O157 in fecal samples from finished feedlot cattle is associated with feeding of brewers grain and geographic location. Additional

studies to further characterize geographic distribution of *E. coli* O157 and to investigate pen-level intervention strategies should be conducted.

Introduction

Epidemiologic studies and culturing of suspect foods have linked outbreaks of foodborne *Escherichia coli* O157 infections to contaminated beef products, and prevalence studies in beef cattle have supported this link (Chapman et al., 1993; Hancock et al., 1997). Recent prevalence estimates for prevalence of *E. coli* O157 among feedlot cattle range from 10% to 28% (Elder et al., 2000; USDA, 2001; Sargeant et al., 2003). However, the prevalence among feedlots has been reported to be as high as 96% (Sargeant et al., 2003) and prevalence within pens ranged from 52% to 72% (Elder et al., 2000; USDA, 2001; Sargeant et al., 2003). The sample type, sample weight, culture method, and season of sampling varied among studies and might have accounted for some of the variation in prevalence estimates.

Despite extensive research and the incorporation of various mitigation strategies during harvest, human outbreaks of *E. coli* O157 associated with beef products continue to occur. Recently, research to develop strategies for control of *E. coli* O157 has focused on beef cattle feedlots. Several novel ideas have been presented as potential strategies for use in the feedlot sector to further minimize the prevalence and shedding of *E. coli* O157. Among these proposed strategies are competitive inhibition by *Lactobacillus* species (Brashears et al. 2003; Younts-Dahl et al., 2004), vaccination of cattle (Potter et al., 2003), feeding of kelp products (Barham et al., 2001), and oral administration of sodium

chlorate (Callaway et al., 2002) or neomycin sulfate pre-slaughter (Ransom, 2003). Many of these strategies show potential to reduce shedding of *E. coli* O157 in the feces of cattle. Although this may be a potential mitigation point, the tactics must result in reduced contamination of beef carcasses as well as reduced shedding by cattle. The fundamental assumption for these proposed strategies is that reduction in fecal shedding will reduce subsequent contamination in the slaughter plant. However, this assumption does not account for the level of these bacteria in the environment and on cattle hides, both plausible sources of carcass contamination (Elder et al., 2000; Keen & Elder, 2002).

Other studies have not been designed to target populations of feedlot cattle immediately prior to harvest. Elder et al. (2000) used feces collected at the slaughter facility to estimate prevalence. Sampling results at this time point may be influenced by factors associated with transportation from the feedlot to the slaughter plant and may not accurately reflect feedlot prevalence. The characterization of *E. coli* O157 prevalence just before shipment and subsequent harvest is an important facet of ecology of the organism that needs to be investigated. A better understanding of *E. coli* O157 at this time can lead to targeted intervention strategies and the development of comprehensive risk analyses. The objective of this study was to determine the within-pen prevalence of *E. coli* O157 in feedlot cattle immediately prior to slaughter and to determine the associated risk factors. The feedlot prevalence study was a part of a cohort study to estimate the prevalence and possible risk factors for *E. coli* O157 on the hide, in the colon and on the carcass.

Materials and Methods

This cross-sectional study was designed to estimate within-pen prevalence of *E. coli* O157 shedding by market-ready beef cattle. Sampling occurred between June and September, which has previously been identified as a high prevalence period (Van Donkersgoed et al., 1999; Elder et al., 2000).

Study population – Participating feedlots were open-air, dirt-floored pens typical of Western USA commercial facilities located in Colorado, Nebraska and Montana. Fifteen pens of cattle were selected for sampling based on a targeted sampling time at participating slaughter plants. Thirty fecal samples were collected from fresh fecal pats on the pen floor from each selected pens within 36 hours before the animals were sent to slaughter.

Data collection – A questionnaire, designed to capture information on the pen of cattle being sampled, was administered to the feedlot manager. Data collected included the mean age, mean weight, days on feed, head count, gender and diet of cattle. Data also were collected regarding the temperature, humidity, pen condition, and pen dimension.

Sampling protocol – Approximately 10 grams of feces were collected from 30 fresh fecal pats off the pen floor. In order to obtain a representative sample, the collector walked a Z shaped pattern starting at the front of the pen, walking parallel to the feed-bunk, and then angling across the pen and along the back fence. Samples were collected with sterile wooden applicators and placed in sterile plastic bags. Samples were then transported in

Styrofoam coolers with icepacks to the Rocky Mountain Regional Animal Health Laboratory for culture.

Culture Protocol – Samples were processed on the day of collection. Briefly, 10 g of feces were suspended in Gram Negative broth (GN; Fisher Scientific, Pittsburgh, PA) containing vancomycin (8mg/L; Sigma, St. Louis, MO), cefixime (0.25 mg/L; Dynal Laboratories, Lake Success, NY) and cefsuludin (10mg/L; Sigma). Samples were then incubated for 2 hours at 25°C and then 6 hours at 42°C.

Enrichment was followed by immunomagnetic bead separation, which consisted of a 30 minute incubation of 1 ml aliquots of the inoculated GN broth with 20 µl of anti-O157 immunomagnetic beads (Dynal Laboratories, Lake Success, NY) on a rocker at room temperature (Elder et al., 2000). The bead suspension was washed 3 times in 1 ml of phosphate buffered saline/0.05%Tween 20 on a magnetic separation rack and then resuspended in 100 µl of PBS/0.05%Tween 20. Fifty µL of the bead suspension were then plated on a sorbitol MacConkey plate containing cefixime (0.5 mg/l) and potassium tellurite (2.5 mg/l), and a Rainbow agar (Biolog, Inc., Hayward, Calif.) supplemented with 20 mg/l of novobiocin (Sigma) and 0.8 mg/l of potassium tellurite (Sigma). Plates were incubated 18 hours at 37°C.

Identification of isolates as *E. coli* O157 – After incubation, up to 3 sorbitol-negative colonies exhibiting colony morphology typical of *E. coli* O157 were picked from either plate as suspect *E. coli* O157, then these colonies were used to inoculate MacConkey

broth, which were incubated at 37°C overnight. Isolates were then screened with an *E. coli* O157 indirect ELISA using monoclonal antibodies specific for O157 lipopolysaccharide. Positive isolates were biochemically confirmed as *E. coli* with API 20E strips (Biomerieux, Hazlewood, MO) and further confirmed as *E. coli* O157 by *rfb* O157 PCR

Statistical analyses – Within-pen prevalence was determined by dividing the number of positive fecal samples confirmed as *E.coli* O157 by the number of tested fecal samples within a pen. The unit of the analysis was the pen. The outcome variable was the number of pats within a pen of cattle with *E. coli* O157-positive culture results. Multivariable logistic regression was used[1] to determine which factors best predicted pen-level positive culture results, and to estimate the magnitude of association between each factor and the outcome, while adjusting for other factors in the model. Fixed effects factors eligible for inclusion in the model were gender, temperature, humidity, pen condition, days since water tank cleaned, days since pen scraped, density of cattle in pen, days on feed, geographic location by state and dietary components (corn processing, barley, brewers grain, percent concentrate, corn silage, alfalfa hay, tallow, ionophores, probiotics, and antibiotics). Diet variables were for the current diet and had been fed for at least 30 days. Continuous variables that didn't meet the assumption of linearity in the log odds were converted to categorical variables based on quartiles. Pen was included in the model as a random effect term to account for clustering of fecal samples with pen. Univariable logistic regression was used to screen variables for inclusion in the

[1] Egret for Windows 2.0, Cytel Software Corporation, Cambridge, MA

multivariable model. Variables with $p < 0.20$ on likelihood ratio testing were eligible for inclusion in multivariable modeling. The best-fitting univariable model was used as the foundation for the multivariable model. Additional variables were added singly in descending order of likelihood ratio p-value, and were retained if they significantly improved model fit (likelihood ratio $p<0.05$). Odds ratios (OR) and corresponding 95% confidence intervals (CI) were used as a quantitative measurement of association.

Results

For the 15 pens of cattle from 12 feedlots, pen size ranged from 170 to 475 head, with a mean of 283 head per pen. Cattle were fed a concentrated ration in the feedlot for 100 to 190 days, with a mean of 154 days at the time of sampling. Overall, 24.7% (111/450) of fecal samples were positive for *E. coli* O157. Thirteen (86.7%) of the 15 pens had at least one positive sample (86.7 percent). Individual positive pens ranged from 3.3% to 76.7% *E. coli* O157-positive samples. Overall, there was a median of 6 positive samples per pen (range, 0 to 23 positive samples; Table 3.1).

Univariable analysis was used to identify potential explanatory variables (Table 3.2). Significant variables in univariable analysis were geographic location, humidity, probiotics, brewers grain, corn silage, gender, barley, pen condition, antibiotics, alfalfa hay, tallow, pen density, and temperature. The model would not converge with the random effect term (pen) to account for clustering due to pen effects. The best-fitting variable (based on model deviance) was geographic location.

Table 3.1 *E. coli* O157 pen-level prevalence with descriptive data

Geographic Location	Number of cattle	Days on feed	Brewers Grain	Animal Density (quartile)	Prevalence (%)
E. Colorado	475	131	No	2	0.0
E. Colorado	192	150	No	1	3.3
E. Colorado	220	190	Yes	2	6.7
E. Colorado	376	144	No	4	10.0
E. Colorado	219	166	No	4	16.7
E. Colorado	341	154	No	4	26.7
E. Colorado	322	162	No	3	30.0
E. Colorado	286	170	Yes	1	43.3
E. Colorado	218	170	No	3	50.0
C. Nebraska	170	156	No	2	20.0
C. Nebraska	184	100	No	1	40.0
C. Nebraska	398	155	No	1	43.3
C. Nebraska	287	150	Yes	1	76.7
Other	261	170	No	1	0.0
Other	292	140	No	3	3.3

Geographic location, pen density and brewers grain were included in the final multivariable model which had a deviance of 10.7 and 8 degrees of freedom. In the multivariable model, fecal samples from cattle fed brewers grain were 6 times more likely to culture positive for *E. coli* O157 when compared to fecal samples from cattle not fed brewers grain. Fecal samples from cattle with different density of cattle were 0.2 to 8.8 times as likely to culture positive for *E. coli* O157 when compared to fecal samples from cattle in the first pen density quartile. Fecal samples from cattle located in Central Nebraska were nine times more likely to have positive fecal samples when compared to cattle located in Eastern Colorado. Cattle from other locations were 22 times less likely to have positive fecal samples when compared to cattle located in Eastern Colorado (Table 3.3).

Table 3.2. Univariable analyses of risk factors for *E. coli* O157-positive feedlot pen floor samples as assessed by fecal culture

Factor	p-value	Odds Ratio	95% Confidence Interval
Gender (steer, heifer)	<0.001	0.42	0.27 – 0.65
Temperature (°C)	0.030	0.95	0.91 – 1.00
Humidity (quartile)	<0.001	1.03	1.02 – 1.04
Pen Condition (dry, wet)	0.001	2.15	1.36 – 3.40
Water tank cleaned (days)	0.042	0.98	0.96 – 1.00
Pen scraped (days)	0.662	1.00	1.00 – 1.00
Density (quartile)	0.180	0.86	0.76 – 0.97
Days on Feed (days)	0.601	1.00	0.99 – 1.01
Geographic location (E. CO, C. NE, other)	<0.001	3.1	1.96 – 4.98
Corn Processing (dry rolled, steam flaked, cracked)	0.334	1.19	0.84 – 1.68
Barley (yes, no)	0.023	0.10	0.01 – 0.72
Brewers grain (yes, no)	<0.001	2.87	1.76 – 4.69
Percent concentrate (%)	0.001	1.05	1.02 – 1.08
Corn Silage (yes, no)	<0.000	0.42	0.27 – 0.65
Alfalfa Hay (yes, no)	0.008	1.8	1.17 – 2.78
Tallow (yes, no)	0.008	0.56	0.36 – 0.86
Ionophores (yes, no)	0.305	0.73	0.40 – 1.33
Probiotics (yes, no)	<0.000	2.69	1.72 – 4.20
Antibiotics (yes, no)	0.002	0.50	0.33 – 0.78

Discussion

Within-pen prevalence estimates in feedlot cattle in this study were comparable to previous reports (Elder et al., 2000; Sargeant et al., 2003). However, this is the first study that has targeted feedlot cattle immediately prior to slaughter. Non-independence of culture results within a pen could not be accounted for in the model because the model failed to converge when a random effect term was added for pen. The lack of convergence may have resulted from the outcome variable and the random effect term being at the pen level.

Table 3.3. Best-fitting multivariable model of risk factors associated with *E. coli* O157-positive feedlot pen floor samples as assessed by fecal culture

Factor	Number Pens	Mean prevalence (%)	Odds Ratio	95% Confidence Interval
Geographic Location				
Eastern Colorado	9	20.7	1.0	
Central Nebraska	4	45.0	9.3	3.92 – 22.26
Other	2	1.7	0.05	0.01 – 0.35
Pen Density (animals/m^2)				
1 (0.02 – 0.04)	6	34.4	1.0	
2 (0.041 -0.05)	3	8.9	0.2	0.10 – 0.54
3 (0.051 – 0.06)	3	27.8	8.8	3.11 – 25.01
4 (0.061 – 0.09)	3	17.8	2.8	0.98 – 8.20
Brewers Grain				
No	12	20.3	1.0	
Yes	3	42.2	6.6	2.87 – 15.31

The authors are not aware of another published study that has shown that the prevalence of *E. coli* O157 in feedlot cattle is associated with geographic location of the feedlot. A USDA study (2001) reported that there was not a regional difference in prevalence. These regions contained three or more states and may not have been able to detect a difference across such a broad area. The specific regions combined in this study (Eastern Colorado and Central Nebraska) possess unique environmental differences that may not have been accounted for when these geographical areas were pooled. Increased precipitation and humidity in Central Nebraska (CN) when compared to Eastern Colorado (EC) as well as differences in soil composition may affect survival and/or proliferation of the organism in the environment. Management practices related to geographic location also may have been difficult to account for when the regions were combined. These potential differences include, but are not limited to, the probable differences in sources of cattle between areas, and that sampling occurred during a severe drought in which environmental conditions may have altered the shedding patterns.

Another factor that should be considered is that the increased distance from the CN feedlots to the laboratory generated a "pre-enrichment" time that increased the probability of detecting *E. coli* O157 in those samples, although transportation of the samples with chilling should have minimized this effect. Ideally, another study designed to further explore the association of geographical differences with distribution of *E. coli* O157 across many more regions over time would be provide valuable clarification.

Other dietary factors such as barley or roughage have been hypothesized to affect the shedding of *E. coli* O157. Brewers grain has not been reported to be associated with increased shedding of *E. coli* O157. This association may be related to components within the brewers grain or to management factors associated with its usage. The observed association of *E. coli* O157 shedding related to animal density within the pen fluctuates up and down between quartiles. This fluctuation may be due to the small number of pens being divided into quartiles because of non-linearity.

Temperature and humidity were measured on the day of sampling. However, since it is unlikely that prevalence would change significantly on a daily basis, average temperature and humidity the previous week may have been more meaningful. The association between location, temperature, and humidity may explain why temperature and humidity, both significant variables in univariable analysis, did not enter a model that already included geographic location. In the multivariable model, there was no evidence that gender (Dargatz et al., 1997) or pen condition (Smith et al., 2001) were factors related to pen-level prevalence as previously reported. Potentially, culture techniques based on

quantitation of *E. coli* O157 within the sample instead of enrichment culture for prevalence based on presence or absence of the pathogen may provide different risk factors or allow more precise estimation of odds ratios.

Conclusions

This study demonstrated that feedlot pen-level prevalence of *E. coli* O157 is associated with geographic location, brewers grain and animal density within the pen. Additional investigations to further characterize geographic distribution of *E. coli* O157and associated factors should be conducted. This study was an integral component of a larger project designed to determine pre-harvest risk factors associated with contamination of beef carcasses and then develop subsequent mitigation factors to reduce contamination at the feedlot.

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CHAPTER 4

GENOTYPIC RELATEDNESS OF *ESCHERICHIA COLI* O157 ISOLATES FROM LIVE CATTLE AND BEEF CARCASSES

Summary

The genetic relatedness of *E. coli* O157 isolates from feedlot and slaughter plant beef were determined. At the feedlot, 15 pens of cattle were selected for sampling based on a targeted sampling time at participating slaughter plants. Thirty fecal samples were collected from fresh fecal pats on the pen floor from each selected pen within 36 hours of when the animals were sent to slaughter. At the slaughter plant, 10 hide and 10 colon samples were obtained during processing. Twenty carcasses also were sampled during processing; ten before evisceration and ten after evisceration. These carcasses were re-sampled after final intervention strategies were completed. The DNA fingerprints of the *E. coli* isolates were determined with pulsed field gel electrophoresis and genotypic relatedness was determined. From the 110 feedlot fecal sample isolates, 65 different genetic types were identified; 7 of which had multiple related isolates. At the pen level for all the different sample types (fecal, hide, and carcass) there were 0 to 14 different genetic types identified per pen with up to 4 types having multiple isolates. Within the sampled pens, 64% of the hide samples at the slaughter plant corresponded to an isolate from the feedlot from which the cattle originated. For carcass samples, 59% of isolates had a corresponding feedlot isolate. These findings indicate that although the majority of *E. coli* O157 isolates at the slaughter plant correspond to the feedlot pen of origin up to

40% of isolates may have been introduced after cattle left the feedlot. Therefore other sources of contamination should be considered.

Introduction

Escherichia coli O157 is a ubiquitous organism in cattle populations (Hancock et al., 1997; Hancock et al., 2001; Van Donkersgoed et al., 1999) and can cause severe gastroenteritis and death in humans (Mahon et al., 1997; Lawson, 2004). Beef carcasses are contaminated with *E. coli* O157 during processing at slaughter. Potential sources for contamination include cross-contamination from knives and equipment, rupture of gastrointestinal tract and from hides of cattle (McEvoy et al. 2003, Fegan et al., 2005). Hides are the most likely source of the majority of beef carcass contamination with *E. coli* O157 (Elder et al., 2000; Woerner et al., 2006). Cattle hides may harbor *E. coli* O157 from fecal contamination at the feedlot of origin (Keen and Elder, 2002), during transportation to slaughter plants (Dewell et al., 2008; Cuesta Alonso et al., 2007) and in lairage pens (Small et al., 2002; Tutenel et al., 2003; Dewell et al., 2008). Determination of appropriate intervention strategies requires identification of routes of contamination and source of *E. coli* O157.

Pulsed-field gel electrophoresis (PFGE) has been used to investigate outbreaks of human disease (Gerner-Smidt et al., 2005) and by researchers to compare isolates among and between groups of cattle (Hancock et al., 1998; Rice et al., 1999; Renter et al., 2003; LeJune et al., 2004; Childs et al., 2006). However, use of molecular subtyping to compare isolates from live cattle to slaughter isolates are limited (Barkocy-Gallagher et

al., 2001; Tutenel et al., 2003; Childs et al., 2006; Arthur et al., 2007). The percent of slaughter plant isolates that can be genetically linked to pre-harvest isolates has been reported from 29 to 68% (Arthur et al., 2007; Barkocy-Gallagher et al., 2001). Genotyping can be used to help identify potential sources of *E. coli* O157 on beef carcasses. The objective of this study was to explore the potential use of PFGE technique to determine the source of contamination by comparing the genetic relatedness of *E. coli* O157 isolates from beef cattle feces, hides and carcasses.

Materials and Methods

A cross-sectional study was designed to estimate within-pen prevalence of *E. coli* O157 shedding by market-ready beef cattle and subsequent carcass contamination (Dewell et al., 2005; Woerner et al., 2006). Sampling occurred between June and September, 2002. Participating feedlots were open-air, dirt-floored pens typical of Western USA commercial facilities located in Colorado, Nebraska and Montana. Fifteen pens of cattle from 12 feedlots were selected for sampling based on a targeted sampling time at participating slaughter plants. Thirty fecal samples were collected from fresh fecal pats on the pen floor from each selected pens within 24 to 36 hours before the animals were sent to slaughter. At slaughter, samples were collected from hides and carcasses at multiple locations throughout the harvesting process. Ten hide samples were obtained by swabbing three 100-cm^2 areas (round, flank, and brisket) using a sterile template and sterile sponge. Carcass samples were obtained (using the same three-site sponge swabbing method) at three different time points during harvesting: (i) 10 pre-evisceration carcasses, (ii) 10 post-evisceration carcasses, and (iii) 20 post–final intervention (opposite

side of pre- or post-evisceration carcasses). Ten random fecal samples were obtained from the distal colon after evisceration.

Samples were processed on the day of collection. Briefly, 10 g of feces were suspended in Gram Negative broth containing vancomycin (8mg/L), cefixime (0.25 mg/L) and cefsulodin (10mg/L). Samples then were incubated for 2 hours at 25°C and then 6 hours at 42°C. Sponges (hide and carcass samples) were incubated with 90 ml of brilliant green bile 2% (40 g/L) for 6 hours at 37°C. Enrichment was followed by immunomagnetic bead separation, which consisted of a 30 minute incubation of 1 ml aliquots of the inoculated broth with 20 µl of anti-O157 immunomagnetic beads on a rocker at room temperature. The bead suspension was washed 3 times in 1 ml of phosphate buffered saline/0.05%Tween 20 on a magnetic separation rack and then resuspended in 100 µl of PBS/0.05%Tween 20. Fifty µL of the bead suspension was plated on a sorbitol MacConkey plate containing cefixime (0.5 mg/l) and potassium tellurite (2.5 mg/l). Plates were incubated 18 hours at 37°C.

After incubation, up to 3 sorbitol-negative colonies exhibiting colony morphology typical of *E. coli* O157 were picked as suspect *E. coli* O157, then these colonies were inoculated into MacConkey broth, which were incubated at 37°C overnight. Isolates were screened with an *E. coli* O157 indirect ELISA using monoclonal antibodies specific for O157 lipopolysaccharide. Positive isolates were biochemically confirmed as *E. coli* with API 20E strips (bioMerieux, Hazlewood, MO) and further confirmed as *E. coli* O157 by *rfb*

O157 PCR. Following confirmation, virulence factors were characterized using *stx*$_1$, *stx*$_2$, *eaeA*, and *hlyA* PCR.

All confirmed *E. coli* O157 isolates were subtyped by PFGE separation of XbaI-digested DNA using standardized methods (CDC, 1998). Banding patterns were analyzed by pen using Gene Directory gel comparison software by Syngene (Cambridge, UK), using Dice similarity coefficients calculated with the unweighted pair group methods arithmetic average algorithm for clustering. Genetic types were defined as isolates that grouped together and had approximately 95% or greater Dice similarity.

Results

For the 15 pens of cattle, pen size ranged from 170 to 475 head, with a mean of 283 head per pen. Cattle were fed a concentrated ration in the feedlot for 100 to 190 days, with a mean of 154 days at the time of sampling. Live weight of cattle ranged from 490 to 650 kg, with a mean of 579 kg. Overall, 191 of 1,328 samples (14.4%) were positive for *E. coli* O157 (Table 4.1). All 191 of the *E. coli* O157 isolates were positive for *eaeA* and *hlyA*. Ninety-eight of these isolates were positive for both *stx*$_1$ and *stx*$_2$. Ninety-two isolates were *stx*$_1$ negative and *stx*$_2$ positive. One isolate was *stx*$_1$ and *stx*$_2$ negative.

From the 111 feedlot fecal sample isolates, 65 different genetic types were identified; 7 of which had multiple related isolates. At the pen level, for all the different sample types (fecal, hide, and carcass) there were 0 to 14 different genetic types identified per pen with up to 4 types having multiple isolates (Table 4.2). Within the sampled pens, 64% of the

hide samples at the slaughter plant corresponded to a feedlot isolate. For carcass samples, 59% of isolates had a corresponding feedlot isolate.

Table 4.1. Prevelance of *E. coli* O157 by sample source

Source	Samples	Positive	Percent	Range
Pen Floor Fecal	450	111	24.7%	0 - 77%
Slaughter Hide	150	22	14.7%	0 - 50%
Slaughter Colon	145	40	27.6%	0 - 100%
Pre-Evisceration Carcass	149	15	10.1%	0 - 60%
Post-Evisceration Carcass	144	2	1.4%	0 - 20%
Final Intervention Carcass	290	1	0.3%	0 - 5%
Total	1328	191	14.4%	

Results of all 15 individual pen dendrograms are not included. Results from one pen with several unrelated isolates are included here. Pen 102 had 3 different clusters (Figure 4.1). Cluster A had 27 isolates that could be differentiated into 8 different genetic subtypes. All the isolates from cluster A were from pen floor fecal samples or hide samples at the slaughter plant. Cluster B and C were only 70 and 60% related to Cluster A respectively. Cluster B consisted of 3 isolates and Cluster C of 1 isolate. All of the isolates from Cluster B and C were from colon fecal samples obtained after evisceration.

Discussion

These findings indicate that the DNA fingerprints of *E. coli* O157 isolates from beef cattle are diverse and that not all slaughter plant isolates (hide and carcass) have a corresponding related isolate from the pen of origin. Barkocy-Gallagher et al., (2001) reported that 68.2% of carcass isolates matched pre-harvest isolates. However, all

sampling, including pre-harvest fecal and hide samples, were done at the slaughter plant. Therefore, hide contamination from transportation trailers or the lairage pen may have resulted in the slightly higher percent of linkage to "pre-harvest" samples compared to our study. Arthur et al. (2007) reported a lower percentage (29.2%) of post-harvest isolates (hide and carcass) that matched pre-transport PFGE profiles. The high hide prevalence post-harvest (94.4%) compared to 50.3% pre-harvest may contribute to some of the disparity in matching pre and post-harvest PFGE types.

Table 4.2. PFGE characterization by pen

Pen	Number *E. coli* O157 Isolates	Genetic Subtypes	Clonal types (number clonal isolates)	Clusters
101	11	9	1 (3)	3
102	31	11	3 (16, 5, 2)	3
103	6	5	1 (2)	2
104	1	1		1
105	5	4	1 (2)	2
106	2	2		2
107	23	18	2 (5,2)	2
108	7	5	1 (3)	2
109	26	12	3 (12, 3, 2)	4
110	9	9		3
111	25	16	4 (4, 3, 4, 2)	4
112	2	2		1
113	1	1		1
114	26	7	4 (6, 6, 8, 3)	4
115	16	13	1 (4)	3

The individual pen dendrogram for pen 102 was unique. This pen of cattle was lairaged overnight prior to slaughter. Identification of four isolates that were completely unrelated to feedlot samples that were only found in the colon at slaughter may indicate that cattle ingested new genetic subtypes of *E. coli* O157 possibly from lairage pen water tanks.

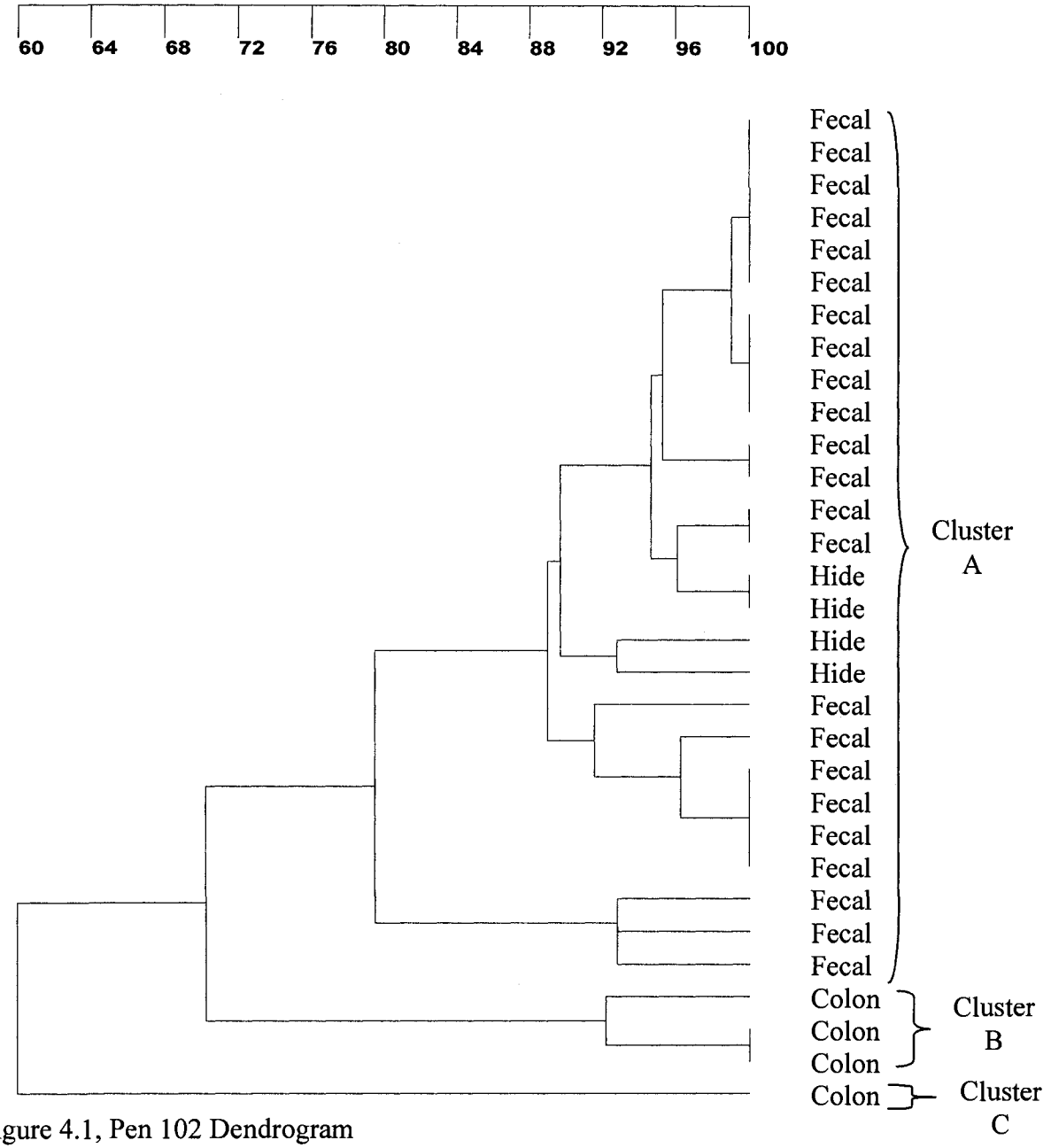

Figure 4.1, Pen 102 Dendrogram

The relatively high percentage of slaughter plant isolates that could not be linked to a feedlot isolate (41%) could be attributed to several different factors. Some of these discrepancies could be due to the introduction of new isolates after the cattle left the feedlot. Childs et al. (2006) identified genotypic matches between isolates obtained from

trailer side walls and post-harvest hide samples. Additionally, Arthur et al. (2007) matched 2% of the post-harvest samples to transportation trailers. Avery et al. (2002) reported in a study from the United Kingdom (UK) that the floors of the lairage pens or stunning boxes were the likely source of the most prevalent *E. coli* O157 clone (75% of isolates) cultured from hides of slaughtered cattle. Tutenel et al. (2003) reported that contact between animals after leaving the farm can affect the spread of *E. coli* O157 hide contamination. Another potential factor is that not all genetic types were identified with the feedlot sampling. Genetic diversity of *E. coli* O157 isolates within herds has been demonstrated (Faith et al., 1996; Rice et al., 1999; Renter et al., 2003; Vali et al., 2004). In our study only 30 samples per pen (170 to 475 head pens) were collected. The study was designed to correctly classify a pen as positive or negative and to estimate prevalence of *E .coli* O257 within the pen, not to obtain every unique genetic type. Potentially, rare genetic types were not sampled because the probability of collecting that particular type was low. Additionally, only one isolate per positive sample was retained for PFGE testing in our study. Vali et al. (2007) demonstrated that in order to account for *E. coli* O157 diversity in a sample at least five colonies per sample should be analyzed to identify different PFGE subtypes. Therefore, all genetic types from the feedlot or the slaughter plant may not be represented. Since this study only sampled cattle and carcasses no environmental isolates from transportation trailers, lairage pens, stun boxes or other lots of cattle were not available for comparison. Although some rare genetic types may have been missed in the feedlot fecal samples it is unlikely that these rare types account for 40% of carcass isolates.

Conclusion

In summary, this study indicates that although there is genetic variability in PFGE types among *E. coli* O157 isolates within pens of cattle there are also some sub-types with numerous identical isolates. Additionally, although the majority of carcass samples can be genetically linked to feedlot isolates over 40% of carcass isolates potentially could have been introduced after the cattle left the feedlot. Therefore, additional studies are needed to identify intervention strategies during transportation and lairage to reduce contamination. This may be especially important for cattle that are lairaged for extended periods at the slaughter plant. Further use of PFGE genetic typing between isolates obtained at slaughter and from potential contamination sources will aid in identification of sources of contamination of cattle with *E. coli* O157.

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CHAPTER 5

IMPACT OF TRANSPORATION AND LAIRAGE ON HIDE CONTAMINATION WITH *ESCHERICHIA COLI* O157 IN FINISHED BEEF CATTTLE

Summary

Transportation of cattle from the feedlot to the slaughter plant could influence hide contamination of *Escherichia coli* O157. A study was initiated to investigate the influence of transportation and lairage on shedding and hide contamination of *E. coli* O157. Fecal and hide samples were obtained from forty pens of harvest ready beef cattle at the feedlot prior to transport and again at the slaughter plant immediately after slaughter. Potential risk factors for hide contamination at the feedlot, during transport and at slaughter were evaluated. A multilevel Poisson regression model was used to evaluate if transportation and lairage were associated with hide contamination by *E. coli* O157 in finished beef cattle. Lots of cattle held in *E. coli* O157 positive lairage pens had eight times greater risk of having positive slaughter hide samples compared to cattle held in culture-negative pens (RR = 8.0; 95%CI, 1.6-38.8). Lots of cattle that were held in lairage pens contaminated with feces had three times greater risk for positive slaughter hide samples compared to cattle held in clean pens (RR = 3.1; 95%CI, 1.2-7.9). Lots of cattle that were transported for long distances (> 160.9 km) had twice the risk of having positive hide samples at slaughter compared to cattle transported a shorter distance (RR = 2.4; 95%CI, 1.1-5.1). These findings suggest that transportation and lairage should be considered in *E. coli* O157 control strategies.

Introduction

Escherichia coli O157 infections in humans have been associated with contaminated beef products in 41% of *E. coli* O157 foodborne outbreaks from 1982 to 2002 (*17*). The potential for disease outbreaks has led to increased research and development of mitigation strategies within slaughter plants to reduce contamination of carcasses during the slaughter process. Many novel ideas have been presented as potential strategies that could be used by the beef feedlot sector to further minimize the prevalence of *E. coli* O157 in cattle, particularly immediately prior to the time of harvest. These include competitive inhibition by *Lactobacillus* species (*24*), vaccination of cattle against *E. coli* O157 (*16*), feeding of kelp products (*6*), use of sodium chlorate as a feed additive (*7*), or neomycin treatment for pre-slaughter cattle (*10*). The fundamental assumption for these proposed strategies is that reduction in fecal shedding will reduce subsequent contamination of carcasses at the slaughter plant. However, this assumption does not necessarily account for the amount of residual contamination in the environment and on cattle hides which could ultimately serve as sources for re-contamination prior to slaughter (*9, 13*). To be successful, these interventions must not only result in reduced shedding by cattle but also reduced contamination of beef carcasses.

Pre-harvest food safety research in the pork industry has focused on *Salmonella* as a food-borne pathogen, with emphasis on the effects of transportation from the farm to the slaughter plant and to the time animals spend in lairage on *Salmonella* carcass contamination prevalence. Lairage is the period of time that animals are housed at the

slaughter plant before they are harvested. Time in lairage and contamination of the lairage pen with *Salmonella enterica* has been linked with increased prevalence of *S. enterica* in pork (*11, 12, 14, 19, 21*). There have been several recent publications evaluating effects of transportation of beef cattle on shedding of enteric pathogens. Bach et al. (*3*) reported that non-preconditioned, weaned calves transported long distances had higher *E. coli* O157 fecal prevalence than other calf groups. Barham et al. (*4*) did not demonstrate a significant difference in the fecal or hide prevalence of *E. coli* O157 following transportation, although they did find a significant increase in *Salmonella* spp. prevalence after transportation. Reicks et al. (*18*) found a significant increase in shedding prevalence following transportation for both *E. coli* O157 and *Salmonella* spp., although differences in *E. coli* O157 shedding were not practically significant nor were any other risk factors for shedding identified. Arthur et al. (*1*) reported that transport to and lairage at slaughter plants led to increased prevalence of *E. coli* O157 compared to feedlot prevalence.

Several characteristics of the transportation and lairage process have the potential to influence the presence of *E. coli* O157 on beef cattle hides. Cattle hides may become contaminated from pen-mates due to the concentration of animals in trucks during transportation and in small holding pens during lairage. *E. coli* O157 also may be transferred from contaminated trailers or holding pens to cattle hides. A study from the United Kingdom (UK) of 73 cattle consignments found that the floors of the lairage pens or stunning boxes were the likely source of the most prevalent *E. coli* O157 clone (75% of isolates) cultured from hides of slaughtered cattle (*2*). Another UK study of lairage

environments found that 27.2% of samples from selected sites along the unloading-to-slaughter routes were culture-positive for *E. coli* O157. In that study, the sites that were most frequently contaminated were the holding pen floors, entrance gate to the stun box, and the stun box floor (*20*). These two studies (*2, 20*) identified the potential of contamination at slaughter. However, cattle were not sampled at the farm of origin to determine if contamination was present prior to transportation and lairage.

The objective of this study was to evaluate the effects of transportation and lairage on *E. coli* O157 contamination of cattle hides at the time of slaughter. More specifically, this study examined effects of time and distance for transportation and lairage, cleanliness of transportation and lairage environments, recovery of *E. coli* O157 from these environments, and effects of behavior characteristics of the cattle on the likelihood of recovery at slaughter.

Materials and Methods

Study design - A cross-sectional design was used for this study of *E. coli* O157 hide contamination of market-ready beef cattle. Lots of cattle were followed from feedlot environments, through transportation and lairage, and into the slaughter plant. Enrollment and biological sampling occurred between June and September, which has previously been identified as a seasonal high prevalence period for *E. coli* O157 in beef cattle (*22, 9*).

Study population –Two slaughter plants agreed to participate in the study and pens of cattle were selected for enrollment based on dates and times of availability provided by the slaughter plants. Ultimately, 40 lots of cattle from 18 feedlots were enrolled. Cattle were housed in open-air, dirt-floored pens typical of commercial facilities located in Colorado and Nebraska. Twenty animals at the feedlot and at the slaughter plant were selected for sampling.

Data collection – Standardized questionnaires were administered to the feedlot managers in order to capture information about groups of cattle enrolled in the study(Questionnaire is available from M.D. Salman upon request). Data collected included the estimated mean age, mean weight, days on feed, head count, and gender. Data also were collected regarding the temperature, humidity, feedlot pen condition (dry, damp, wet or standing water) and feedlot water trough condition (clean or contaminated) at the time of biological sampling, and pen dimensions. Trailers used to transport cattle (clean, dry manure, wet manure, or slurry) and lairage pens (clean or contaminated) and water troughs (clean or contaminated) were scored for cleanliness prior to loading with cattle. Durations for the time cattle were held in handling facilities prior to loading, time required for loading onto trucks, transportation time from feedlots to slaughter plants, and duration of holding in lairage pens prior to slaughter were recorded, as were distances transported from feedlot to slaughter plant. Additionally, a number of handling and behavior indicators were recorded as a proxy to the overall stress that may occur during transportation and lairage. Indicators measured included objective measurement of electrical prod usage, falling and slipping, colliding with fences or other objects,

vocalization, and defecation, and subjective assessment of handling (gentle, moderate or rough), cattle behavior (calm, agitated or excited) were recorded during loading, unloading, and moving to knock box. Additionally, flight zone distance (none, moderate or large) of cattle was subjectively assessed during lairage. Flight zone refers the area around an animal or group of animals that will cause alarm and/or escape behavior if encroached upon. The size of the flight zone is determined by the disposition of the animals. Flight zone was determined by subjectively assessing the cattle's response to the observer approaching the lairage pen. If cattle did not react to the observer the score was none. If the cattle moved away from the observer the score was moderate. If the cattle moved as far away as the back fence of the pen would allow the score was large.

Sampling protocol – Twenty hide swab samples from each pen of cattle were collected from systematically-random selected individuals while the animals were being loaded for transport to the slaughter plant. At slaughter, another 20 individuals from each of the 40 lots were systematically selected for hide swab sampling at the slaughter plant prior to hide removal for sampling. Sponges moistened with buffered peptone water were used to swab a 100-cm^2 area along the dorsal surface of selected individuals and representative surfaces (floor and walls or rails) of the loading chute, transportation trailers, plus the lairage pens and water troughs. Approximately 10 grams of feces were collected from 20 fresh fecal pats off the feedlot pen floor after cattle had been removed from the pen. The collector walked a Z shaped pattern starting at the front of the pen, walking parallel to the feed-bunk, and then angling across the pen and along the back fence. Each fecal sample was collected with a sterile wooden applicator and placed in a sterile plastic bag. At the

slaughter plant, approximately 10 grams of feces were collected from 20 colons after evisceration. Samples then were transported in Styrofoam coolers with icepacks to the laboratory for culture.

Culture Protocol – Samples were processed on the day of collection following a previously published protocol (5). Briefly, either 10 g of feces or the hide sponges were suspended in 90 mL of tryptic soy broth and incubated for 2 hr at 25°C, then 6 hr at 42°C, and left overnight at 4°C. Overnight enrichment was followed by immunomagnetic separation (IMS), which consisted of 1mL of each sample being mixed with 1 mL of phosphate buffered saline/0.05% Tween 20 (PBST) and 20μL magnetic beads coated with anti-*E. coli* O157 antibodies (Dynal Biotech ASA, Oslo, Norway). Samples then were processed per manufacturer's instructions in an automated immunomagnetic separator (Dynal BeadRetriever, Thermo Electron Corporation, Finland). After processing, the beads were suspended in 100μL PBST and 50μL of the bead suspension was plated onto sorbitol MacConkey plates containing cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L).

Identification of *E. coli* O157 was completed after 18-hour incubation at 37°C. Briefly, up to five sorbitol-negative colonies exhibiting colony morphology typical of *E. coli* O157 were selected for further analysis. Each selected suspect colony was suspended in 200μL of sterile saline and tested for O157 antigens with latex agglutination (*E. coli*PRO™ O157 Latex Kit by Hardy Diagnostics). Colonies identified as positive were confirmed as *E. coli* O157 using rfb_{O157} PCR (*9, 15*).

Statistical analyses – All data were lot-level observations with no individual identification of animals. Data analysis was performed using MLwiN (Center of Multilevel Modeling, Bristol, UK) to account for clustering and random effects that were associated with the hierarchal structure of the data including slaughter plant and feedlot. Multilevel Poisson regression was used to evaluate the relationship between transportation, lairage and cattle behavior on hide culture results for *E. coli* O157 at slaughter. The outcome variable was frequency of positive hide cultures with an offset of the number of samples taken per pen at slaughter. Independent variables included pen-level exposure data previously described as well as the proportion of positive cultures obtained from feedlot, trailer and lairage pen. A univariable multilevel Poisson regression model was constructed as the first screening process to evaluate which variables should be further considered in multivariable modeling. Fixed effect factors eligible for inclusion in the model were gender, days on feed, temperature, humidity, pen condition, water trough condition, holding, loading, transport and lairage times, distance transported, lairage pen condition, behavioral and handling scoring during loading, unloading and slaughter, and flight zone in pen. Continuous variables that failed to meet the assumption of linearity in the log odds were converted to categorical variables based on quartiles. Univariable relative risks were calculated to determine the magnitude of association and its significance level, using $P \leq 0.1$ as the cutoff for factors to be included in the multivariable model. Forward selection was used to construct a multivariable model. Interaction terms were added to evaluate any effect modification between variables. Poisson regression modeling yields adjusted relative risk estimates and 95%

confidence intervals that relate to average risk of average individuals being positive from a given lot.

Results

The number of lots enrolled varied from 1 to 6 lots per feedlot. Cattle from 18 of the lots were harvested at one slaughter plant and 22 lots at the other plant. Numbers of cattle in lot groups ranged from 43 to 443 head, with a mean of 168 head per lot. Cattle were fed a concentrated corn-based ration while at the feedlots, and days on feed ranged between 110 and 187 days, with a mean of 176 days. At the time of sampling, cattle weighed between 500 and 644 kg, with a mean of 579 kg. Seven of 40 feedlot pens had standing water present and 33 were dry or damp. Cattle were transported between 8 and 333 km from feedlots to the slaughter plants, with a mean transportation distance of 125 km. Lairage times at the slaughter plant ranged from 0.2 to 11.8 hours with mean lairage time of 3.1 hours. Half of the lairage pens were scored to be contaminated with feces prior to cattle being housed in them.

Overall, 6.3% (50/784) of hide samples collected in the slaughter plant were culture-positive for *E. coli* O157 (Table 5.1). Twenty (50.0%) of the 40 lot-groups had at least one positive hide sample. Within groups that had at least one positive sample, within-lot prevalence for *E. coli* O157 recovery ranged between 5.0% and 35.0% for hide swabs collected in the slaughter plant.

Table 5.1. *E. coli* O157 culture results by sample type

Sample Type	Number Samples	Number Positive	Percent Positive
Loading Chute	40	2	5.0%
Transportation Trailers	146	9	6.2%
Feedlot Cattle Hides	785	48	6.1%
Feedlot Pen Floor Fecals	795	53	6.7%
Lairage Pen	236	18	7.6%
Lairage Pen Waterer	76	4	5.2%
Slaughter Cattle Hides	784	50	6.4%
Slaughter Colon Fecals	798	31	3.9%

Univariable analysis was used to identify potential explanatory variables. Factors meeting inclusion criteria included proportion of *E. coli* O157 positive samples in lairage pen, lairage pen water trough and slaughter fecal samples, feedlot and lairage pen condition, distance transported, time held prior to loading and time in transit, humidity, and temperature (Table 5.2). Behavior variables associated with the outcome included flight zone, slipping during unloading, slipping on way to slaughter, vocalization during unloading, agitation at knock box, behavior during loading, and identification of disease during loading.

Proportion of *E. coli* O157 positive samples in the lairage pen, fecal contamination of lairage pen (yes or no), and distance traveled (short or long haul) were included in the final multivariable model (Table 5.3). In the multivariable model, lots of cattle held in *E. coli* O157-positive lairage pens had eight times the risk of having positive slaughter hide samples for each proportional increase in prevalence compared to cattle held in culture-negative pens. Additionally, lots of cattle that were held in lairage pens contaminated with feces prior to their entry had three times the risk for positive slaughter hide samples when compared to cattle held in clean pens. Lots of cattle transported for long distances

(> 160.9 km) had two times the risk of having positive hide samples at slaughter compared to cattle transported a shorter distance. An interaction term between cleanliness of the lairage pen and having culture positive *E. coli* O157 samples was not

Table 5.2. Univariable analysis of factors associated with *E. coli* O157-positive hide slaughter samples by risk factor.

Factor	P value	Relative Risk	95% Confidence Interval
Proportion Lairage Pen Samples Positive *E. coli* O157	<0.0001	35.84	6.29 - 204.28
Proportion Lairage Water Samples Positive *E. coli* O157	0.0183	7.63	1.41 - 41.25
E. coli O157 Cultured from Lairage Water Samples (N vs Y)	0.0184	2.76	1.19 - 6.43
Proportion Slaughter Fecal Samples Positive *E. coli* O157	0.0482	63.37	1.03 - 3885.47
Feedlot Pen Condition (normal vs standing water)	0.0003	4.56	1.99 - 10.44
Lairage Pen Condition (normal vs heavy fecal contamination)	0.0009	4.81	1.89 - 12.19
Transportation Distance (km)	0.0027	1.01	1.00 - 1.01
Transportation Distance (short vs long)	0.0003	4.01	1.88 - 8.52
Time in Holding Pens Prior to Loading at Feedlot	0.0923	1.14	0.98 - 1.33
Transportation Time (hours)	0.0015	1.67	1.22 - 2.29
Humidity at Time of Feedlot Sampling	0.0039	0.97	0.96 - 0.99
Temperature at Time of Feedlot Sampling	0.0006	1.06	1.03 - 1.10
Cattle Flight Zone (none vs moderate)	0.8660	0.90	0.26 – 3.15
Cattle Flight Zone (none vs extreme)	0.0678	0.22	0.05 - 1.11
Number Cattle Slipping During Unloading	0.0678	1.04	0.99 - 1.09
Number Cattle Slipping on Way to Knock Box	0.0010	1.06	1.02 - 1.09
Number of Cattle Vocalizing During Unloading	0.7569	1.01	0.93 – 1.10
Cattle Agitated at Knock Box (N vs Y)	0.0059	0.31	0.14 - 0.71
Cattle Behavior during Loading (Calm vs Agitated)	0.0243	2.60	1.13 - 5.97
Number Cattle Observed with Pathology During Loading	0.0105	1.45	1.09 - 1.93
Pathology Observed During Loading (N vs Y)	0.0001	3.73	1.89 - 7.35

Table 5.3. Final multivariable model of factors associated with *E. coli* O157-positive hide slaughter samples by risk factor.

Factor	Coefficient	P value	Relative Risk	95% Confidence Interval
Proportion Lairage Pen Samples Positive *E. coli* O157	2.073	0.010	7.95	1.63 – 38.81
Lairage Pen Condition (normal vs heavy fecal contamination)	1.142	0.015	3.13	1.24 – 7.89
Transportation Distance (short vs long)	0.855	0.030	2.35	1.09 – 5.08

statistically significant. The random effects in the final multivariable model were at the feedlot (coefficient 0.061, standard error 0.146) and the slaughter plant (coefficient 0.126, standard error 0.188).

Discussion

Results of the present study indicate that increased distances transported and pathogen or fecal conataminated lairage pens were positively associated with hide contamination by *E. coli* O157 at slaughter. Other studies have been designed to evaluate the effect of transportation on contamination by *E. coli* O157 (*1, 4, 18*). However, these studies either have not found a difference in prevalence after transportation and lairage (*4*) or have not identified potential risk factors or mechanisms for increased prevalence (*1, 18*). The lot level design of this study allowed multiple scenarios to be evaluated and the identification of risk factors for potential intervention points.

Although Reicks et al. (*18*) found a significant increase in *E. coli* O157 levels after transportation, the authors did not think that it was a biologically meaningful difference. The Braham (*4*), Reicks (*18*) and Arthur (*1*) studies only utilized one feedlot and one

slaughter plant so there was not any variability in transportation scenarios for their study to evaluate different circumstances. However, all studies sampled livestock transportation trailers as potential contamination sources. Similar to our study, there was not a definitive association between cleanliness of transportation trailers and prevalence of *E. coli* O157 on hides at slaughter. However, we did find a positive association with fecal contamination of the lairage pens and having *E. coli* O157 culture-positive lairage pens with the prevalence of *E. coli* O157 on hides at slaughter.

Some researchers have hypothesized that stress during transport may affect shedding of pathogens such as *E. coli* O157 (4). Although we did not directly measure stress, use of objective and subjective scoring of behavior is a practical proxy for stress. Many of the behavior variables met the criteria for inclusion but none were significant in the multivariable model. Since *E. coli* O157 is not a pathogen of cattle, it may be less likely to be affected by stress as reflected in this study.

The advantage of using a multilevel model in this study helped to explain some of the random effects in the hierarchical levels. This study enrolled 40 different lots from 18 different feedlots slaughtered at 2 different slaughter facilities. Random effect terms in the model helped to account for some of the unexplained variability related to the slaughter plants or feedlots, but not to the individual lots of cattle in our case. Random effects in the model inherently widen the confidence interval by inflating the standard error of the fixed effects terms (*8*), increasing the difficulty of detecting statistically significant associations. Since lot-level data (not animal-level data) was used, it is not

possible to make conclusions about the effect of transportation and lairage on individual animals. However, since beef cattle are managed and marketed in groups, lot-level analysis was appropriate.

This study demonstrated that the environment is an important avenue for contamination of cattle hides. Prevalence of *E. coli* O157 in feces or on cattle hides measured at the feedlot of origin was not a significant risk factor even in the univariable screening. Previous research has suggested that lots with fecal prevalence greater than 20% at the feedlot will have increased hide and carcass contamination at slaughter when compared to lots of cattle with fecal prevalence less than 20% (*23*). In our study, the within-lot prevalence rarely exceeded 20% (5 out of 40 lots). At this lower within-lot prevalence, environmental contamination may play a more important role in the prevalence of *E. coli* O157 on hides of cattle at slaughter. Although cleanliness and pathogen prevalence in transportation trailers were not significant risk factors, distance transported from the feedlot to the slaughter plant was significant. This may indicate that the longer cattle are held in close confinement together, the greater likelihood there is for cross-contamination between cattle. The most significant finding in this study is the potential role that lairage of cattle prior to slaughter has on contamination of hides. Since an increase in hide prevalence was associated with dirtier or previously *E. coli* O157 contaminated pens, mitigation efforts designed to maintain clean lairage pens may result in reduced hide contamination of *E. coli* O157. Unfortunately, we were not able to sample carcasses during the slaughter process to determine the ultimate effects on carcass contamination. However, it is believed that hide contamination at slaughter is one of the primary risk

factors for carcass contamination (*9*). This study demonstrated that, while risk mitigation is very important within the slaughter plant, there are significant opportunities to manage risk of *E. coli* O157 at the lairage pen as well.

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CHAPTER 6

IMPACT OF TRANSPORATION AND LAIRAGE ON HIDE CONTAMINATION WITH *SALMONELLA ENTERICA* IN FINISHED BEEF CATTTLE

Summary

Transportation of cattle to the slaughter plant could influence hide contamination with *Salmonella enterica.* A study was initiated to investigate the influence of transportation and lairage on shedding and hide contamination of *Salmonella enterica.* Fecal and hide samples were obtained from forty pens of harvest ready beef cattle at the feedlot prior to transport and again at the slaughter plant immediately after slaughter. Potential risk factors for hide contamination at the feedlot, during transport and at slaughter were evaluated. A multilevel Poisson regression model was used to evaluate if transportation and lairage were associated with hide contamination by *Salmonella enterica.* Lots of cattle with positive *Salmonella enterica* hide samples at the feedlot had twice the risk of having positive slaughter hide samples compared to cattle without positive feedlot hide samples (RR, 1.9: 95% CI, 1.2-3.0). Lots of cattle transported in trailers with positive *Salmonella enterica* samples had twice the risk of having positive slaughter hide samples compared to cattle transported in culture negative trailers (RR, 2.3: 95% CI, 1.4-3.6). Lots of cattle transported for long distances had twice the risk of having positive hide samples at slaughter compared to cattle transported shorter distances (RR, 2.3; 95% CI, 1.4-3.7). Lots of cattle held in lairage pens contaminated with feces had twice the risk for positive slaughter hide samples compared to cattle held in clean pens (RR, 1.8; 95%CI, 1.1-3.1). Lots of cattle held off feed longer than 18 hours before loading had twice the

risk of having positive slaughter hide samples compared to cattle held off feed for shorter times (RR, 1.7; 95% CI, 1.0-2.9). Lots of cattle that were agitated during loading had twice the risk of having positive slaughter hide samples compared to cattle that were calm (RR, 2.2; 95% CI, 1.3-3.6). These findings suggest that there are factors present during transportation and lairage that can impact the presence of *Salmonella* on the hides of cattle at slaughter.

Introduction

According to Centers for Disease Control and Prevention (CDC) there are approximately 40,000 reported cases of salmonellosis in the United States each year. Young children are most likely to contract clinical salmonellosis (*5*). The CDC estimates that there are 1.3 million food-borne illness cases every year, although most go unreported (*13*). *Salmonella* Enteritidis and *S.* Typhimurium are responsible for half of the reported cases (*6*). In the Spring of 2002, ground beef was implicated in an outbreak of multidrug-resistant *S.* Newport that was responsible for illness in 47 people in the northeastern US (*1*). Additionally, *Salmonella* has been detected in retail packages of ground beef in several studies (*20, 21, 18*).

Pre-harvest food safety research in the pork industry has focused on *Salmonella* as a food-borne pathogen, with emphasis on the effects of transportation and lairage on *Salmonella* carcass contamination prevalence. Lairage is the period of time that animals are housed at the slaughter plant before they are harvested. Time in lairage and

contamination of the lairage pen with *Salmonella enterica* have been linked with increased prevalence of the pathogen in pork (*10, 11, 12, 16, 19*).

Several characteristics of the transportation and lairage process have potential to influence presence of *Salmonella enterica* on beef cattle hides. Cattle hides may become contaminated from pen-mates due to the proximity of animals in trucks during transportation and in holding pens during lairage. *Salmonella enterica* also may be transferred from contaminated trailers or holding pens to cattle hides. Rivera-Betancourt et al. (*15*) observed that 25 to 52% of lairage pen samples were positive for *Salmonella* spp. in two slaughter plants in the U.S. A UK study of lairage environments found that 6.1% of samples from selected sites along the unloading-to-slaughter routes were culture-positive for *Salmonella* (*17*). In that study, the sites at the slaughter plant that were most frequently contaminated were the holding pen floors, entrance gate to the stun box, and the stun box floor (*17*). These two studies (*15, 17*) identified presence of environmental contamination at slaughter. However, cattle were not sampled at the farm of origin to determine if contamination was present before transportation and lairage. Additionally, it has been reported that the fecal prevalence of *Salmonella enterica* can increase following stress of transportation (*7, 9*).

Several recent studies evaluated effects of transport of beef cattle to slaughter on shedding of enteric pathogens. Beach et al. (*4*) observed a significant increase in *Salmonella* contamination of hides after transport. Reicks et al. (*14*) found a significant increase in prevalence of *Salmonella* following transport. Barham et al. (*2*) found a

significant increase in *Salmonella* spp. prevalence both in feces and on hides after transport.

The objective of this study was to evaluate effects of transport and lairage of beef cattle on recovery of *Salmonella enterica* from hides at the time of slaughter. More specifically, this study examined effects of time, distance, cleanliness of transport and lairage, recovery of *Salmonella enterica* from these environments, and effects of behavior characteristics of the cattle on the likelihood of recovery of *Salmonella enterica* on cattle hides at slaughter.

Materials and Methods

Study design - A cross-sectional design was used for this study of *Salmonella enterica* hide contamination of market-ready beef cattle. Lots of cattle were followed from feedlot environments, through transport, lairage, and into the slaughter plant. Enrollment and biological sampling occurred between June and September.

Study population –Two slaughter plants agreed to participate in the study. Lots of cattle were selected for enrollment based on dates and times of availability provided by the slaughter plants. Ultimately, 40 lots of cattle from 18 feedlots were enrolled. Cattle were housed in open-air, dirt-floored pens typical of commercial feedlot facilities located in Colorado and Nebraska. Twenty animals at the feedlot and at the slaughter plant were selected for sampling.

Data collection – Standardized questionnaires were administered to the feedlot managers in order to capture information about groups of cattle enrolled in the study. Data collected included the estimated mean age, mean weight, days on feed, head count, and gender. Data also were collected regarding the temperature, humidity, feedlot pen condition (dry, damp, wet or standing water) and feedlot water trough condition (clean or contaminated) at the time of biological sampling, and pen dimensions. Trailers used to transport cattle (clean, dry manure, wet manure, or slurry), lairage pens (clean or contaminated), and water troughs (clean or contaminated) were scored for cleanliness prior to loading with cattle. Durations for the time cattle were held in handling facilities before loading, time required for loading onto trucks, transportation time from feedlots to slaughter plants, and duration of holding in lairage pens before slaughter was recorded, as were distances transported from feedlot to slaughter plant. Additionally, a number of handling and behavior indicators were recorded as a proxy to the overall stress that may occur during transportation and lairage. Indicators measured included objective measurement of electrical prod usage, falling and slipping, colliding with fences or other objects, vocalization, and defecation, and subjective assessment of handling (gentle, moderate or rough), and cattle behavior (calm, agitated or excited) were recorded during loading, unloading, and moving to knock box. Additionally, flight zone distance (none, moderate or large) of cattle was subjectively assessed during lairage. Flight zone refers the area around an animal or group of animals that will cause alarm and/or escape behavior if encroached upon. The size of the flight zone is determined by the disposition of the animals. Flight zone was determined by subjectively assessing the cattle's response to the observer approaching the lairage pen. If cattle did not react to the

observer the score was none. If the cattle moved away from the observer the score was moderate. If the cattle moved as far away as the back fence of the pen would allow the score was large.

Sampling protocol – Twenty hide swab samples from each pen of cattle were collected from systematically-random selected individuals while the animals were being loaded for transport to the slaughter plant. At slaughter, another sample of 20 individuals from each of the 40 lots were systematically selected for hide swab sampling at the slaughter plant prior to hide removal for sampling. Sponges moistened with buffered peptone water were used to swab a 100-cm^2 area along the dorsal surface of selected individuals and representative surfaces (floor and walls or rails) of the loading chute, transportation trailers, plus the lairage pens and water troughs. Approximately 10 grams of feces were collected from 20 fresh fecal pats off the feedlot pen floor after cattle had been removed from the pen. The collector walked a Z shaped pattern starting at the front of the pen, walking parallel to the feed-bunk, and then angling across the pen and along the back fence. Each fecal sample was collected with a sterile wooden applicator and placed in a sterile plastic bag. At the slaughter plant, approximately 10 grams of feces were collected from 20 colons after evisceration. Samples were transported in Styrofoam coolers with icepacks to the laboratory for culture.

Culture Protocol – Samples were processed on the day of collection following a previously published protocol (3). Briefly, either 10 g of feces or the hide sponges were suspended in 90 mL of tryptic soy broth and incubated for 2 hr at 25°C, then 6 hr at 42°C,

and left overnight at 4°C. Ten mL of the initial enrichment broth from the fecal or swab sample were transferred to 90 mL of tetrathionate broth and incubated for 24 hours at 37°C. Following enrichment, 100 μL of the suspension was transferred to 10 mL of Rappaport-Vassiliadis broth and incubated for 18 hours at 42°C. A swab of the culture suspension was plated for isolation on an XLT4 agar plate and incubated for 24 hours at 37°C. Up to three suspect colonies were picked and plated to blood agar plates. Following overnight incubation at 37°C, colonies were checked with polyvalent O-grouping antisera for agglutination. Colonies that agglutinated were identified as *Salmonella* by serotyping at the National Veterinary Services Laboratories in Ames, IA.

Statistical analyses – All data were lot-level observations with no individual identification of animals. Data analysis was performed using MLwiN (Center for Multilevel Modeling, Bristol, UK) to account for clustering and random effects that were associated with the hierarchal structure of the data including slaughter plant and feedlot. Clustering in data occurs when some attributes such as environment are shared by some individuals or groups but not others. Groups of cattle that are slaughtered at one slaughter plant will share some unmeasured attributes that cattle at the other slaughter plant were not exposed to, thus violating the assumption of independence. A random effects model assumes that a distribution of effects exists and adds a random variable at each hierarchal level. Multilevel Poisson regression was used to evaluate the relationship between transport, lairage and cattle behavior on hide culture results for *Salmonella enterica* at slaughter. The outcome variable was frequency of positive hide cultures with an offset of the number of samples taken per lot at slaughter. Independent variables

included previously described lot-level exposure data as well as the proportion of positive cultures obtained from feedlot, trailer and lairage pen. A univariable multilevel Poisson regression model was constructed as the first screening process to evaluate which variables should be further considered in multivariable modeling. Fixed effects factors eligible for inclusion in the model were gender, days on feed, temperature, humidity, pen condition, water trough condition, holding, loading, transport and lairage times, distance transported, lairage pen condition, behavioral and handling scoring during loading, unloading and slaughter, and flight zone in pen. Continuous variables that failed to meet the assumption of linearity in the log odds were converted to categorical variables based on quartiles. Univariable relative risks were calculated to determine the magnitude of association and its significance level, using $P \leq 0.2$ as the cutoff for factors to be evaluated for inclusion in the multivariable model. Forward selection was used to construct the multivariable model. Interaction terms were added to evaluate effect modification between variables. Poisson regression modeling yields adjusted relative risk estimates and 95% confidence intervals that relate to average risk of average individuals being positive from a given lot.

Results

A total of 40 lots of cattle were enrolled from 18 different feedlots. The number of lots enrolled varied from 1 to 6 lots per feedlot. Cattle from 18 of the lots were harvested at one slaughter plant and 22 lots at the other. Numbers of cattle in lots ranged from 43 to 443 head, with a mean of 168 head per lot. Cattle were fed a concentrated corn-based ration while at the feedlots, and feeding intervals ranged between 110 and 187 days, with

a mean of 176 days. At the time of sampling, cattle weighed between 500 and 644 kg, with a mean of 579 kg. Seven of the 40 feedlot pens had standing water and 33 were dry or damp. Cattle were transported between 8 and 333 km from the feedlots to the slaughter plants, with a mean transport distance of 125 km. Lairage times at the slaughter plant ranged from 0.2 to 11.8 hours with mean lairage time of 3.1 hours.

Overall, 19.5% (153/784) of hide samples collected in slaughter plants were culture-positive for *Salmonella enterica* (Table 6.1). Thirty-two (80.0%) of the 40 lot-groups had at least one positive hide sample. For groups that had at least one positive sample, within-lot prevalence ranged from 5.0% to 95.0% for hide swabs collected in the slaughter plant.

Table 6.1. *Salmonella enterica* culture results by sample type

Sample Type	**Number Samples**	**Number Positive**	**Percent Positive**
Loading Chute	40	5	12.5%
Transportation Trailers	146	32	21.9%
Feedlot Cattle Hides	785	54	6.9%
Feedlot Pen Floor Fecals	795	37	4.7%
Lairage Pen	236	55	23.3%
Lairage Pen Waterer	76	4	5.3%
Slaughter Cattle Hides	784	153	19.5%
Slaughter Colon Fecals	798	56	7.0%

Two lots were removed from the statistical analysis as outliers because the model was unstable when they were included. These two lots started as the same lot at the feedlot but were split into two drives at the slaughter plant because all of the cattle did not arrive at the slaughter plant at the same time. Therefore, these two groups of cattle had different transportation and lairage scenarios and were treated as individual lots. However, since

we did not know that they were going to split the lot of cattle at the slaughter plant, only half of the samples were collected on each group. Evaluation of the regression model identified these two lots as outliers and, after removal, the standard errors of the coefficients were stable and repeatable.

Univariable analysis was used to screen potential explanatory variables. Factors meeting the inclusion criteria during univariable analysis included presence of *Salmonella enterica* in feedlot hide, transport trailer, lairage pen, lairage pen water trough and slaughter fecal samples, feedlot pen and water trough condition, lairage pen and water trough condition, transport trailer condition, distance transported, length of time from last feeding until loading, time held prior to loading, time in lairage, total time from loading to slaughter, cattle density in feedlot pens, humidity and temperature (Table 6.2). Behavior variables associated with the outcome included flight zone, behavior during loading, identification of disease during loading and crashing into fences during loading, behavior during unloading, identification of disease during unloading, vocalization during unloading and handling during unloading, slipping on the way to slaughter and crashing into fences on the way to slaughter.

Variables that remained in the final model included presence of *Salmonella enterica* in feedlot hide samples and transport trailers, fecal contamination of lairage pen, distance traveled, time from last feeding until loading, and loading behavior (Table 6.3). In the multivariable model, after adjustment for effects of herd and slaughter plant lots of cattle

Table 6.2. Univariable analysis of factors associated with *Salmonella enterica*-positive hide slaughter samples by risk factor.

Factor	P value	Relative Risk	95% CI
Salmonella enterica Cultured from Feedlot Hide Samples (N vs Y)	0.001	2.09	1.34 – 3.28
Salmonella enterica Cultured from Transportation Trailer Samples (N vs Y)	0.001	2.50	1.48 – 4.21
Salmonella enterica Cultured from Lairage Pen Samples (N vs Y)	0.001	0.53	0.36 – 0.77
Salmonella enterica Cultured from Lairage Water Samples (N vs Y)	0.12	0.52	0.22 – 1.19
Salmonella enterica Cultured from Slaughter Colon Samples (N vs Y)	0.009	0.57	0.37 – 0.87
Feedlot Pen Condition (dry vs damp)	<0.001	6.58	3.20 – 13.55
Feedlot Pen Condition (dry vs wet)	0.04	2.73	1.05 – 7.11
Feedlot Pen Condition (dry vs standing water)	0.15	2.04	0.77 – 5.41
Lairage Pen Condition (clean vs fecal contamination)	0.001	2.99	1.56 – 5.72
Feedlot Pen Water Trough Condition (clean vs dirty)	0.001	4.93	1.99 – 12.25
Lairage Pen Water Trough Conditon (clean vs dirty)	0.01	2.20	1.18 – 4.08
Transportation Trailer Condtion (clean or dry manure vs wet manure or slurry)	0.02	1.90	1.10 – 3.27
Transportation Distance (short vs long)	0.11	1.89	0.86 – 4.17
Time Off Feed Prior to Loading (short vs long)	0.20	1.46	0.82 – 2.59
Time in Holding Pens Prior to Loading at Feedlot	0.09	1.02	1.00 – 1.04
Time in Lairage Pens	0.05	0.88	0.77 – 1.00
Total Time from Loading until Slaughter	0.09	0.91	0.81 – 1.01
Density of Cattle in Pen (number cattle / pen area m^2)	0.17	0.04	0.00 – 3.89
Humidity at Time of Feedlot Sampling	0.006	1.03	1.01 – 1.04
Temperature at Time of Feedlot Sampling	0.07	0.97	0.95 – 1.00
Cattle Flight Zone (none vs moderate)	0.003	0.29	0.13 – 0.65
Cattle Flight Zone (none vs extreme)	0.29	0.61	0.25 – 1.52
Cattle Behavior during Loading (Calm vs Agitated)	<0.001	3.38	2.05 – 5.57
Pathology Observed During Loading (N vs Y)	<0.001	3.07	1.87 – 5.03
Number Cattle Crashing into Fences During Loading	0.04	1.20	1.01 – 1.44
Cattle Behavior during Unloading (Calm vs Agitated)	0.02	0.50	0.27 – 0.91
Number Cattle Observed with Pathology During Unloading	0.06	0.96	0.92 – 1.00
Cattle Vocalizing During Unloading (N vs Y)	0.03	1.94	1.05 – 3.59
Handling of Cattle During Unloading (gentle vs moderate)	0.006	2.45	1.29 – 4.67
Handling of Cattle During Unloading (gentle vs rough)	0.001	2.96	1.57 – 5.60
Number Cattle Slipping on Way to Knock Box	<0.001	1.06	1.04 - 1.08
Number Cattle Crashing into fences on Way to Knock Box	0.10	1.03	1.00 – 1.06

with positive *Salmonella enterica*, hide samples at the feedlot had almost two times the risk of having positive slaughter hide samples compared to cattle from lots that did not have positive feedlot hide samples. Additionally, lots of cattle that were transported in trailers with positive *Salmonella enterica* samples had over twice the risk of having positive slaughter hide samples compared to cattle transported in culture-negative trailers. Lots of cattle held in lairage pens contaminated with feces had almost two times the risk of having positive slaughter hide samples when compared to cattle held in clean pens. Lots of cattle transported for long distances (> 160.9 km) had over two times the risk of having positive hide samples at slaughter compared to cattle transported a shorter distance. Lots of cattle for which feed was withdrawn for more than 18 hours before loading had almost two times times the risk of having positive slaughter hide samples compared to cattle held off feed for shorter times. Lots of cattle that were agitated during loading had over twice times the risk of having positive slaughter hide samples compared to cattle that were calm at loading. Random effects in the final multivariable model were 0.078 / 0.078 (coefficient/standard error) at the feedlot and 1.048 / 1.075 (coefficient/standard error) at the slaughter plant.

Discussion

Results of this study demonstrate that the presence of *Salmonella enterica* on hides at the feedlot and in transportation trailers, distance transported, lairage pen environment, time held off feed and behavior at loading were associated with hide contamination at slaughter. Although other studies have evaluated effects of transport on contamination by *Salmonella enterica* (*2, 4, 14*), they have not identified potential risk factors for

increased prevalence. The lot-level design of this study allowed for evaluation of multiple scenarios as well as identification of risk factors for potential interventions.

Table 6.3. Final multivariable model of factors associated with *Salmonella enterica*-positive hide slaughter samples by risk factor.

Factor	Coefficient	P value	Relative Risk	95% CI
Salmonella enterica cultured from feedlot hide samples (N vs Y)	0.635	0.0067	1.89	1.19 – 2.99
Salmonella enterica cultured from transportation trailer samples (N vs Y)	0.813	0.0007	2.25	1.41 – 3.62
Lairage pen condition (clean vs fecal contamination)	0.607	0.0267	1.83	1.07 – 3.14
Transportation distance (short vs long)	0.826	0.0007	2.28	1.41 – 3.69
Time off feed prior to loading (short vs long)	0.550	0.0358	1.73	1.04 – 2.90
Cattle behavior during loading (calm vs agitated)	0.7874	0.0023	2.19	1.32 – 3.62

The Barham (*2*), Beach (*4*) and Reicks (*14*) studies each only utilized one feedlot and one slaughter plant; so there was no variability in transportation scenarios for their study to evaluate different circumstances. All studies sampled livestock transportation trailers as potential sources of contamination. However, unlike our study, an association between culturing *Salmonella enterica* in transportation trailers and the presence of *Salmonella enterica* on hides at slaughter was not present. Additionally, Beach et al. (*4*) did not find a relationship between cleanliness of the lairage pen and prevalence of *Salmonella enterica* on hides at slaughter.

Some researchers have hypothesized that stress during transport may affect shedding of pathogens such as *Salmonella enterica* (*2, 9*). Although we did not directly measure

stress, the use of objective and subjective scoring of behavior is a practical proxy for stress. In the final multivariable model, cattle that were agitated during loading had over twice the risk of having positive slaughter hide samples compared to cattle that were calm at loading. To our knowledge this is the first time that behavior evaluation as a measurement of stress has been identified as risk factor for hide contamination with *Salmonella enterica.*

Using a multilevel model in this study helped to explain some of the random effects in the hierarchical levels. This study enrolled 40 different lots from 18 different feedlots slaughtered at 2 different slaughter facilities. Random effect terms in the model helped to account for variability related to the slaughter plants or feedlots instead of having more unexplained variance attributed to the individual lot level. Random effects in the model inherently widen the confidence interval by inflating the standard error of the fixed effects terms (*8*), increasing the difficulty of detecting statistically significant associations.

This study demonstrated that management of cattle to minimize hide contamination at slaughter with *Salmonella enterica* is all-encompassing. At the feedlot, presence of *Salmonella enterica* on cattle hides, time cattle were held off feed, and behavior during loading were associated with hide contamination at slaughter. Additionally, presence of *Salmonella enterica* in transportation trailers and distance transported also were associated with hide contamination. Finally, cleanliness of the lairage pen was a significant risk factor. The study design did not allow for sampling during the slaughter

process to determine effects on carcass contamination. Since lot-level data were used, it was not possible to make conclusions concerning effects of transportation and lairage on individual animals. However, since beef cattle are managed and marketed in groups, lot-level analyses are appropriate. This study demonstrated that transportation and lairage are associated with hide contamination by *Salmonella enterica* at slaughter. Further evaluation of specific mitigation strategies for these control points is necessary before effective control measures can be instituted.

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CHAPTER 7

OVERALL CONCLUSIONS

Escherichia coli O157 and *Salmonella enterica* are significant causes of foodborne illness throughout the world. Although post-harvest intervention strategies implemented by slaughter plants are extremely effective there continue to be outbreaks of foodborne illness. Understanding the ecology of these pathogens immediately prior to harvest is an important facet of developing further control mechanisms. Identification of risk factors at the feedlot, during transportation or at the slaughter plant is the first step to implementing appropriate intervention strategies.

Feedlot risk factors for fecal prevalence of *E. coli* O157

Geographic location was identified as a risk factor for increased prevalence of *E. coli* O157 in feedlot cattle. The odds of *E. coli* O157 positive fecal samples from pens of cattle from Central Nebraska was 9 times that for pens of cattle from Eastern Colorado. This difference may be attributable to differences in precipitation and humidity, soil composition, management factors, or time from sample collection until culturing.

Brewer's grain in the diet of cattle also was identified as a risk factor for increased prevalence of *E. coli* O157 in feedlot cattle. The odds of *E. coli* O157 positive fecal samples from cattle fed brewer's grains were 6 times that for cattle not fed brewers grains. The mechanism by which diet components influence *E. coli* O157 prevalence is not understood. It is speculated that colon pH is decreased providing a competitive

advantage for *E. coli* O157. However, other diet components that also decrease colon pH do not always positively influence *E. coli* O157.

Genetic relatedness of feedlot and slaughter plant *E. coli* O157 isolates

For hide samples, 64% of isolates at the slaughter plant corresponded to an isolate from the feedlot from which the cattle originated. For carcass samples, 59% of isolates had a corresponding feedlot isolate. Therefore, although the majority of isolates can be linked to the feedlot pen of origin, cattle may be contaminated with new genetic types of *E. coli* O157 between the feedlot and slaughter.

Transportation risk factors for hide contamination with *E. coli* O157

The distance that cattle are transported from the feedlot to the slaughter plant was identified as a risk factor for increased hide prevalence of *E. coli* O157. Cattle that were transported for long distances (> 160.9 km) had twice the risk of having positive hide samples at slaughter compared to cattle transported a shorter distance (RR, 2.4; 95%CI, 1.1-5.1). Longer time in transit probably increases cross-contamination between cattle hides due to the intense density of cattle.

Slaughter plant risk factors for hide contamination with *E. coli* O157

Cleanliness of lairage pens was identified as a risk factor for increased hide prevalence of *E. coli* O157. Cattle that were held in lairage pens contaminated with feces had three times greater risk for positive slaughter hide samples compared to cattle held in clean

pens (RR, 3.1; 95%CI, 1.2-7.9). Fecal contamination in lairage pens is a source of potential pathogens to contaminate cattle hides prior to slaughter.

Presence of *E. coli* O157 in lairage pens was identified as a risk factor for increased hide prevalence of *E. coli* O157. Cattle held in *E. coli* O157 positive lairage pens had eight times greater risk of having positive slaughter hide samples compared to cattle held in culture-negative pens (RR, 8.0; 95%CI, 1.6-38.8).

Feedlot risk factors for hide contamination with *Salmonella enterica*

Presence of *Salmonella enterica* on cattle hides at the feedlot was identified as a risk factor for increased hide prevalence of *Salmonella enterica.* Cattle with positive *Salmonella enterica* hide samples at the feedlot had twice the risk of having positive slaughter hide samples compared to cattle without positive feedlot hide samples (RR, 1.9: 95% CI, 1.2-3.0).

Feed withdrawal before transporting cattle to the slaughter plant was identified as a risk factor for increased hide prevalence of *Salmonella enterica.* Cattle held off feed longer than 18 hours before loading had twice the risk of having positive slaughter hide samples compared to cattle held off feed for shorter times (RR, 1.7; 95% CI, 1.0-2.9). Feed withdrawal may alter the intestinal flora resulting in an increased shedding of *Salmonella enterica.*

The behavior of cattle at loading for transport to the slaughter plant was identified as a risk factor for increased hide prevalence of *Salmonella enterica*. Cattle that were agitated during loading had twice the risk of having positive slaughter hide samples compared to cattle that were calm (RR, 2.2; 95% CI, 1.3-3.6). Agitated cattle may defecate more, increasing the probability of hide contamination.

Transportation risk factors for hide contamination with *Salmonella enterica*

Presence of *Salmonella enterica* in transportation trailers was identified as a risk factor for increased hide prevalence of *Salmonella enterica*. Cattle transported in trailers with positive *Salmonella enterica* samples had twice the risk of having positive slaughter hide samples as cattle transported in culture negative trailers (RR, 2.3: 95% CI, 1.4-3.6).

The distance that cattle are transported from the feedlot to the slaughter plant was identified as a risk factor for increased hide prevalence of *Salmonella enterica*. Cattle transported for long distances (> 160.9 km) had twice the risk of having positive hide samples at slaughter as cattle transported shorter distances (RR, 2.3; 95% CI, 1.4-3.7). Longer time in transit probably increases cross-contamination between cattle hides due to the intense density of cattle.

Slaughter plant risk factors for hide contamination with *Salmonella enterica*

Cleanliness of lairage pens was identified as a risk factor for increased hide prevalence of *Salmonella enterica*. Cattle held in lairage pens contaminated with feces had twice the risk for positive slaughter hide samples as cattle held in clean pens (RR, 1.8; 95%CI, 1.1-

3.1). Fecal contamination in lairage pens is a source of potential pathogens to contaminate cattle hides prior to slaughter.

It is evident that reducing the prevalence of pathogens at the feedlot, maintaining clean transportation trailers and lairage pens and minimizing the distance that cattle are hauled to slaughter are potential control points to decrease the contamination of hides and, subsequently, carcasses of beef cattle during slaughter.

Future directions for research

There are several areas of research that should be pursued based on the results of this work. Additional investigations to further characterize geographic distribution of *E. coli* O157 and associated factors should be conducted. Expand geographic locations evaluated beyond the two states (Colorado and Nebraska). Additionally, identification of factors involved with increased prevalence such as climate, soil composition, management differences, dietary ingredients and source of cattle. Further work also is needed to determine mechanisms for increased prevalence of *E. coli* O257 when cattle are fed fermentation byproducts such as brewer's or distiller's grain.

Investigation at the slaughter plant should be conducted to determine control measures that could increase the cleanliness of lairage pens and the effect of said control measures on reducing carcass contamination by *E. coli* O157 and *Salmonella enterica.*

Further investigation of behavior characteristics and management to reduce agitation of cattle prior to slaughter also should be conducted.