12-8-2017

Epidemiology of Enterohemorrhagic Escherichia Coli: Detection and Ecology in Beef Cattle Systems

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Epidemiology of enterohemorrhagic *Escherichia coli*: detection and ecology in beef cattle systems

By

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A Dissertation
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Veterinary Medical Research
in the Department of Pathobiology and Population Medicine

Mississippi State, Mississippi

December 2017

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Enterohemorrhagic *Escherichia coli* (EHEC) are important foodborne pathogens with a bovine reservoir. For many years, research and regulations have focused on the EHEC serogroup most commonly associated with severe human illness, EHEC O157. However, six additional EHEC serogroups have been identified as important human foodborne pathogens and have been declared adulterants in raw, non-intact ground beef by the United States Department of Agriculture's Food Safety Inspection Service. Collectively these seven organisms are referred to as EHEC-7. With the addition of these six pathogens, epidemiological studies are needed to estimate the probability for cattle to carry them and to identify risk factors associated with their presence in samples of bovine origin. In addition, the potential for pre-harvest control of EHEC-7 in feedlot cattle, particularly by dietary intervention, is a knowledge gap that needs to be addressed. Finally, detection methods of EHEC-7 have not been validated, and there is no "gold-standard" test.

The first study included in this dissertation was a cross-sectional study estimating the prevalence and risk factors associated with hide contamination by EHEC-7 on the

hides of market beef cows at slaughter. The second study was a longitudinal analysis of EHEC-7 from fecal samples from cow-calf herds in Mississippi and Nebraska. The third study was a randomized controlled trial evaluating the effects of fiber from distillers grains on the probability to detect EHEC-7 in samples from the rectoanal mucosa of feedlot steers. The fourth study included in this dissertation was a Bayesian latent class analysis estimating the diagnostic performance of three EHEC-7 detection methods, specifically modeling the performance and prevalence of EHEC O157 in fecal samples from beef cow-calf herds.

DEDICATION

I dedicate this dissertation to my grandfather, Jimmy Lloyd. “Boppa” studied animal husbandry at Mississippi State and obtained a Master’s degree in agriculture. He was always ready to share a story about his devotion to Jesus Christ, love of farming and raising livestock, and memories of his time at Mississippi State University.

ACKNOWLEDGEMENTS

First, I would like to acknowledge my major professor, Dr. Smith. Thank you for all of the time you have spent mentoring me. Thank you for challenging me while also reminding me of your support. Thank you for allowing me to travel all over the country to collect samples, attend workshops and conferences, and report results from our projects. The experiences and lessons learned during the past three years have been truly life changing.

I would also like to thank my committee members: Dr. Hart Bailey, Dr. Galen Erickson, Dr. Carla Huston and Dr. Robert Wills. Thank you for your guidance, encouragement, and informative discussions of food safety, feedlot nutrition, epidemiological methods, biostatistics, and most of all, life!

I owe special gratitude to my husband, Micah, for his unending support and encouragement. Thank you for always believing in me. Also, my newest source of motivation is our son, Micah August Jr. I am forever thankful for all of the sweet snuggles and rocking chair time that we share that gives my mind and body much needed rest and recuperation.

I would also like to thank my mom, and my two older sisters. They have always been and always will be my best friends and greatest confidants. Without the love, prayers, and kindness of my family, the past years would have been much more difficult. Thank you all for seeing me through this journey.

I would also like to acknowledge all of the wonderful graduate students and technicians from MSU and UNL that I had the great privilege of working alongside. Special gratitude goes to Kathleen Barton, Min Wang, Kris Hubbard, Yu-Bin Chou, Uri Donnett, Christina Loftin, and Katie Sanford.

I am grateful to the funding sources for these projects. This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2012-68003-30155."Shiga-toxigenic Escherichia coli (STEC) in the beef chain: Assessing and Mitigating the Risk by Translational Science, Education and Outreach" (STEC CAP).

Finally, I thank my God and savior, Jesus Christ. "Whatever you do, do it all for the glory of the Lord." 1 Cor. 10:31

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CHAPTER I

LITERATURE REVIEW

Escherichia coli

Escherichia coli (*E. coli*) belongs to the Enterobacteriaceae family. *E. coli* is a Gram-negative, rod-shaped, facultative anaerobic bacterium that is highly ubiquitous and diverse. *E. coli* is found in the environment as well as the intestines of humans and animals. *E. coli* is the predominant facultative anaerobe commensal organism of the human large intestine (Nataro & Kaper, 1998). The majority of *E. coli* serotypes are nonpathogenic. Commensal *E. coli* are primary in maintaining the gastrointestinal tract by aiding in food digestion and absorption and play a role in preventing colonization by pathogenic bacteria (Tenaillon et al., 2010); however, certain strains have increased pathogenicity, and even normal "non-pathogenic" strains can cause opportunistic infections in immunocompromised hosts. Pathogenic serotypes typically cause gastrointestinal illness, but can also cause extra-intestinal infections, such as urinary tract infections, sepsis, and meningitis.

EHEC and STEC Defined

Characterization of *E. coli* can be performed by serotyping (Blanco et al., 2004). *E. coli* serotypes are differentiated based on three surface antigens: the somatic (O), capsular (K), and flagellar (H) (Meng & Schroeder, 2007). Strains commonly associated with gastrointestinal illness are subdivided into six major categories based on virulence

mechanisms (Nataro & Kaper, 1998). The groups include enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), diffusely adherent (DAEC), enteroinvasive (EIEC), and enterohemorrhagic (EHEC) (Nataro & Kaper, 1998). For the remainder of this review, we will focus on EHEC pathogens. Historically, Shiga Toxin-producing *E. coli* (STEC) fell into the EHEC category; however, not all STEC organisms are EHEC and capable of producing illness in humans. Literature may also refer to these bacteria as verotoxigenic *E. coli* (VTEC), which will be mentioned in the next section.

Virulence Factors

To adapt to different environments, the genome of *E. coli* has changed by gaining pathogenicity islands, plasmids, genes from phages or by gene subtraction (Maurelli et al. 1998, Leimbach et al., 2013). The acquisition of virulence factors increases pathogenicity of *E. coli* strains. STEC organisms are capable of producing a cytotoxin virulence factor called Shiga toxin (Stx) (O'Brien & LaVeck, 1983), or earlier referred to as verotoxin due to its ability to kill Vero cells (Konowalchuk et al., 1977). There are two types of Shiga toxin genes, Stx1 and Stx2. Shiga toxin 1 is identical to the Shiga toxin produced by *Shigella dysenteriae* type 1 (Nataro & Kaper, 1998). Most STEC O157 strains produce Stx2, which is more divergent from Stx1, with only about 56% homology (Mead & Griffin, 1998). The term EHEC is used to describe STEC strains that cause hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Beutin & Fach, 2014). EHEC organisms have an additional virulence factor, intimin, which allows them to attach and efface the intestinal epithelium, thus causing more severe cases of illness (Kaper et al., 2004, Tarr et al., 2005). Intimin is encoded by the *eae* gene. If the *eae* gene is not present, even bacteria with Stx will not produce human illness. Intimin is the most well-

recognized adhesin, which is involved directly in attachment and effacement of EHECs to intestinal cells (Nataro & Kaper, 1998). Attachment to and effacement of intestinal epithelium is another key factor to HUS pathogenesis. Intimin-negative STEC that have caused HC are called non-classical EHEC.

EHEC Human Illness

Introduction of *E. coli* O157:H7

In 1982 two outbreaks of gastrointestinal illness occurred in Michigan and Oregon which led to the discovery of the EHEC organism, *E. coli* O157:H7 (EHEC O157) (Riley et al., 1983). The outbreak was characterized by severe cramps and hemorrhagic diarrhea, without fever. After microbiological testing on stool samples from affected individuals, a rare serotype of *E. coli* was found. Outbreak investigation indicated that cases were associated with consumption of hamburgers from a particular fast-food chain of restaurants (Riley et al., 1983). In 1993, a multi-state outbreak occurred linked to consumption of under-cooked hamburgers. This large-scale outbreak became known as the Jack in the Box scare. Undercooked hamburgers caused 732 illnesses, 195 hospitalizations, and 3 deaths (Bell et al., 1994, Rangel et al., 2005). The outbreak increased the public recognition of EHEC O157 as an important foodborne pathogen (Bell et al., 1994) and initiated research and regulatory efforts to improve food safety. Since these initial outbreaks, there have been numerous epidemics of gastrointestinal EHEC O157 infections due to the consumption of EHEC contaminated foods or water (Rangel et al., 2005). However, about one-third of all human infections of EHEC O157 are attributed to the consumption of contaminated ground or non-intact beef (Withee et al., 2009).

Symptoms and Complications

Clinical symptoms of enterohemorrhagic *E. coli* include bloody or non-bloody diarrhea, abdominal cramps, and lack of fever (Slutsker et al., 1997). After ingestion of EHEC, onset of symptoms is usually three to seven days (Tarr et al., 2005). Hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) are complications that can occur, and are primarily observed in children. Hemolytic anemia, thrombocytopenia, and renal failure are disease processes observed in patients with HUS (Besser et al., 1999). Antibiotic therapy is not effective or recommended for most EHEC cases; fluid therapy is the recommended treatment (Besser et al., 1999). Non-O157 EHEC infections cause similar symptoms; however, they are less likely to cause severe illness, HUS, and death (CDC, 2013). More severe gastrointestinal infections by non-O157 EHEC are often seen in elderly patients, while O157 most often affects young children. Serious complications, hospitalization, or death occurs primarily in children less than 5 years of age. Humans have vascular receptors for Stx and therefore may become very ill after exposure. Shiga toxins are major contributors in the spread of thrombotic microvascular lesions which form and are seen in HUS cases, and this injury to the endothelial cells is key in pathogenesis of HUS (Richardson et al., 1988). When EHECs are ingested, Stx's are released and absorbed by intestinal epithelium and enter circulation (Hurley et al., 2001) which then initiates further immune response (Proulx et al., 2001). About 15% of children less than 10 years of age diagnosed with EHEC O157:H7 infection will develop HUS (Tarr et al., 2005).

Population at Risk

All humans can be infected by EHEC; however, more severe infections occur in immunocompromised hosts. Children less than 5 years of age have been more commonly affected by HUS and acute renal failure (Gould et al., 2009). Based on data, the U.S. human population, and statistical models, the Centers for Disease Control and Prevention estimated that there are 63,153 illnesses, 2,138 hospitalizations, and 20 deaths in the U.S. each year from domestically acquired foodborne EHEC O157 (Scallan et al., 2011). In addition, each year there are an estimated 112,752 illnesses and 271 hospitalizations due to foodborne non-O157 EHEC pathogens (Scallan et al., 2011). The incidence of laboratory-confirmed EHEC O157 cases in the U.S. in 2012 was 1.12 cases per 100,000 population; however, incidence in children under 5 years of age was 4.71 cases per 100,000 (CDC, 2013). Similarly, the U.S. incidence of EHEC non-O157 illness in children <5 years of age was 4.81 cases per 100,000 children (CDC, 2013). Severity of each EHEC infection case depends on serotype, dose, and host age and health status. The dose required to produce human illness is very low and only 10 to 100 EHEC organisms are needed to produce illness (Hara-Kudo & Takatori, 2011). EHEC is the leading cause of renal failure in children under 5 years of age (Mayer et al., 2012). Mortality from EHEC is highest in people over 60 years of age (Gould et al., 2009). EHEC O157:H7 is associated with the most clinical infections due to EHEC, but EHEC O26, O45, O103, O111, O121, and O145 (EHEC-6) are frequently associated with HC and HUS (Brooks et al., 2005). All people should take necessary precautions when caring for a sick person or handling and preparing food to reduce risk of foodborne illness.

In the U.S., EHEC O157 outbreaks have been more common in northern states compared to southern states, and reasons for this are unknown. It has been suggested that cattle density, temperature, and diet may be factors contributing to this geographical distribution (Heiman et al., 2015).

In addition to severe health risks, EHEC illness is an economically costly disease. In 2003, the annual cost of EHEC O157 illness to the U.S. economy was $405 million (Frenzen et al., 2005). The average cost was $5,515 per patient. This amount increases with increasing severity (Frenzen et al., 2005).

Sources and Transmission to Humans

Infections by EHEC occur via the fecal oral route of transmission. Humans can be exposed to EHEC directly or indirectly. Human exposure occurs largely through contact with contaminated foods, water, the environment, or infected animals or humans (Nataro & Kaper, 1998).

Foodborne infections have been caused by a variety of food products; however, beef products are the most common source. Ground beef is the most common vehicle of human EHEC O157 exposure in the U.S., accounting for 75 of 183 (41%) foodborne outbreaks between 1982 and 2002 (Rangel et al., 2005). Ground beef poses a great risk for human foodborne illness, due to the fact that interior meat may contain living bacteria if it is not heated to an internal temperature high enough to inactivate the organisms. Also, meat in one hamburger patty may come from many cattle, which increases the difficulty of tracing back to one particular carcass. A small fraction of contaminated carcasses can lead to large levels of contaminated ground beef product.

Other food products that have been associated with human illness from EHEC include contaminated potatoes, leafy greens like spinach, alfalfa and clover sprouts, lettuce, and cilantro, cheese, mushrooms, sausage, and shellfish (Rangel et al., 2005, van Overbeek et al., 2014). Liquids including raw milk, unpasteurized apple cider, and water have also contributed to human EHEC illness (Rangel et al., 2005) (Brooks et al., 2005).

Food products are not the only source of human exposure to EHEC. There have been large outbreaks associated with contaminated water supplies (Kondro, 2000, CDC, 1999). There are also numerous environmental exposures including daycare facilities, nursing homes, swimming pools, contact with other sick people, and direct exposure to animal environments such as petting zoos, or livestock exhibitions (Rangel et al., 2005).

USDA FSIS Rulings

Meat sold for human consumption is regulated and inspected by the U.S. Department of Agriculture (USDA). In 1994, human EHEC O157 infection became a notifiable disease following a large outbreak due to undercooked hamburgers (Rangel et al., 2005). In the same year, the USDA Food Safety Inspection Service (USDA-FSIS) declared EHEC O157:H7 adulterants in raw, non-intact beef and began a microbial testing program. Approximately 92.3% of human STEC cases in the U.S. from 2000-2010 were caused by 7 serogroups: O157, O26, O45, O103, O111, O121, and O145 (Gould et al., 2013). Collectively, these seven EHEC serogroups are often referred to as "Top-7" or EHEC-7. In 2011, these six additional serogroups were recognized by USDA-FSIS as adulterants in raw, non-intact beef (2011), and microbial testing was initiated by FSIS in June 2012. The USDA-FSIS has a "zero tolerance" policy on EHEC-7.

Natural Reservoirs of EHEC

Generic *E. coli* are ubiquitous and are often isolated from the gastrointestinal (GI) tracts of humans and animals. Most of these organisms are not harmful and do not cause disease in healthy hosts. However, EHEC have been found in animals that do not appear to be clinically affected. When the first human foodborne outbreak of EHEC O157 in 1982 was linked to ground beef, a bovine reservoir was suspected (Armstrong et al., 1996, Riley et al., 1983). Ruminant animals, such as cattle (Chapman et al., 1997), sheep (Chapman et al., 1997), and deer (Renter et al., 2001), have been found to carry EHEC in their GI tracts, and they have been identified as major reservoirs of EHEC (Gyles, 2007). Additionally EHEC O157 has been isolated from non-ruminant animals such as horses (Hancock et al., 1998), dogs (Beutin et al., 1993, Hancock et al., 1998), pigs (Chapman et al., 1997), rabbits (Garcia & Fox, 2003, Scaife et al., 2006), wild birds (Wallace et al., 1997, Pederson & Clark, 2007), rats (Cizek et al., 1999, Blanco Crivelli et al., 2012), houseflies (Alam & Zurek, 2004), and opossums (Renter et al., 2003). In North America, cattle populations are the most important reservoir of EHEC, especially O157; however, in some countries, sheep are the most significant reservoir (Gyles, 2007). Petting zoos with ruminant animals have been identified as sources of outbreaks (Heuvelink et al., 2002, DebRoy and Roberts, 2006).

Unlike humans, cattle lack vascular receptors to Shiga toxin (Pruimboom-Brees et al., 2000). It was previously thought that EHEC are not capable of producing disease in adult cattle (Sandhu and Gyles, 2002). Although most often cattle are not clinically affected by the EHEC organisms regularly found in their gastrointestinal tracts, there have been cases of EHEC infection causing attaching-effacing lesions and diarrheal

disease in cattle over a year old (Wada et al., 1994, Pearson et al., 1999, Moxley, 2015). Additionally, innate and adaptive immune responses have been seen when cattle have been infected with EHEC O157, which suggests that EHEC O157 is a bovine pathogen (Moxley & Smith, 2010).

Colonization and "Super shedders"

Cattle are known reservoirs for EHEC O157 (Al-Saigh et al., 2004, Chapman et al., 1989, Hancock et al., 1994) and non-O157 EHEC (Barkocy-Gallagher et al., 2003, Geue et al., 2002). The gastrointestinal tract of cattle can become colonized by EHEC. The largest proportion colonize the distal colon, terminal rectal mucosa (TRM) (Naylor et al., 2003, Naylor et al., 2005). This site of colonization is also referred to as the rectoanal junction (RAJ) (Naylor et al., 2003). Colonization may not be limited to this region and the small intestine could support colonization (Keen et al., 2010). The organisms are intermittently shed in the feces, which can be a source of EHECs to other animals or to humans (Sargeant et al., 2000). The magnitude in which EHECs are shed in feces can vary greatly. Fecal shedding of *E. coli* O157, as well as non-O157 EHEC, is transient in nature (Besser et al., 1997, Hancock et al., 1997a, Sargeant et al., 2000, Khaitsa et al., 2003, Menrath et al., 2010). Colonized cattle and have been known to shed EHEC O157 at levels as high as 1.1×10^5 CFU/g feces (Fegan et al., 2004). Cattle shedding EHEC O157 greater than 10^4 CFU/g only represent a small proportion of EHEC O157 positive animals, but are responsible for the majority of environmental contamination (Omisakin et al., 2003, Arthur et al., 2009). These cattle have been termed "super shedders" (Chase-Topping et al., 2008). One study reported that 9 % of the cattle population were super shedders, but they accounted for over 96% of all EHEC O157 shed in the population

(Omisakin et al., 2003.) Additionally, persistently infected cattle may shed EHEC O157 organisms for increased durations (Widiasih et al., 2004). In one study of fecal shedding in calves naturally infected by EHEC O157, duration varied from 1 week to 10 weeks (Widiasih et al., 2004). The prevalence, magnitude, and duration of EHEC O26 shedding was determined to be significantly less than EHEC O157, which indicates serogroup-differences in these factors and associated risk (Widiasih et al., 2004).

Epidemiology of EHEC in Cattle

Prevalence

EHEC carriage by cattle is highly variable and unpredictable. Prevalence is defined as the number of instances of disease in a known population, at a designated time, without distinction between old and new cases (Thrusfield, 2013). Most EHEC literature has focused on measuring prevalence of fecal shedding, hide or carcass carriage of these organisms. In these studies, sampling often occurs at different time points that are convenient to production. Often literature refers to an overall prevalence measure when it would be more appropriate to refer to the proportion of positive samples due to sampling at different times or places. Prevalence is a function of incidence and duration. Incidence of new animals shedding EHEC is very difficult to measure due to the temporal nature of shedding. Cattle shed EHEC in feces for varying durations and at different concentrations (Low et al., 2005, Cobbold et al., 2007, Cernicchiaro et al., 2014). Most studies have focused on measuring prevalence of EHEC or STEC O157, because it causes the majority of human illnesses in the U.S. (Gould et al., 2013). Several studies have reported prevalence of STEC or EHEC in cattle feces from different times or places (Hancock et al., 1994, Smith et al., 2001, Renter et al., 2005, Bosilevac et al., 2013).

Prevalences reported depend highly on the study design and populations sampled. For beef cattle, EHEC O157 prevalence ranged from 0.2% to 27.8% at slaughter (Hussein & Bollinger, 2005). Non-O157 prevalence in beef cattle at slaughter ranged from 2.1% to 70.1% (Hussein & Bollinger, 2005)

Most of the reports available estimating the prevalence of non-O157 EHEC do not discriminate between different serogroups. However, prevalence of fecal shedding and associated risk factors likely varies based on serogroup.

Feedlot

As most of the beef produced in the U.S. comes from steers that have been finished to market weight in intensive feedlot settings, much work has been done to better understand the epidemiology of EHEC in feedlot cattle. In one recent study of EHEC-7 prevalence in one feedlot found that prevalence of EHEC-7 was dependent upon season (Dewsbury et al., 2015). Feedlot environment and diet are additional factors affecting EHEC shedding. Feedlot, feedlot pen, and sampling time have been found to be significant sources of variability in prevalence of STEC fecal shedding (Khaitsa et al., 2003, Smith et al., 2001).

Cow-calf

Hancock et al. (1994) collected rectal swabs from 1,412 cattle from 25 pasture raised beef cattle in Washington state to determine the prevalence of STEC O157 in beef cattle and herds (Hancock et al., 1994). Results showed that STEC O157 was found in 10 (0.71%) individual cattle from 4 (16%) herds (Hancock et al., 1994). In a study which

looked at fresh manure from 10 cow-calf herds from northeast Kansas, 40 of 3,152 (1.3%) fecal samples were positive for STEC O157. In that study, the number of fecal samples collected per animal ranged from 1 to 8, and as the number of samples collected per animal increased, the likelihood of obtaining at least 1 sample with positive results increased significantly ($P < 0.001$). Herd-level prevalence ranged from 0.7 to 2.3% and there was no significant difference by herd (Sargeant et al., 2000).

There are a few studies which have identified animal factors that affect STEC carriage and fecal shedding. Animal age, breed, sex, and stage of production are among the factors analyzed. In a study of a Canadian cow-calf operation, the prevalence of STEC O157 at 1, 3, 5, and 7 weeks after parturition were 25, 27, 23, and 41% for calves and 26, 16, 2, and 18% for cows, respectively (Gannon et al., 2002). In a study conducted on a mixed beef and sheep farm in Scotland, fecal samples were obtained from 49 calves and 45 dams and tested for the presence of serogroups O26, O103, O111, O145, and O157 (Pearce et al., 2004). Results showed that serogroup O26 was found in 17.3% of calf samples and 9.1% of dam samples. Serogroup O103 was found in 5.1% of calf samples and 14.8% of dam samples, and 51% of the calves shed O103 at some time during the sampling period. Serogroup O111 was not found in any samples, while O145 was found in 0.6% of samples and was never positive for presence of Stx. Serogroup O157 was found in 0.6% of calf samples and 6.4% of cow samples. There was significant association with calves shedding O26 and O145 in the same sample (Pearce et al., 2004). These results showed that there were important differences in O groups and age effects. Two studies reported that males have higher prevalence of O157 shedding than females (Tutenel et al., 2002, Yilmaz et al., 2002).

Although beef cows can be culled at any time in production and approximately 10% of all federally inspected beef is from market cows, only one paper has reported the prevalence of EHEC O157 on the hides of culled beef cows at slaughter (Brichta-Harhay et al., 2008). That study reported that prevalence on hides ranged from 39% to 56%, and there was no significant seasonal effect.

It has been suggested that some pre-harvest control of EHEC in the beef chain may include on-farm actions (Sargeant et al., 2007, Smith et al., 2010). However, few studies have been performed to test on-farm factors associated with non-O157 EHEC.

Risk Factors

Season

There have been several epidemiological studies that have identified seasonal changes in the incidence and prevalence of EHEC in cattle feces, on cattle hides, and infections in humans. Fecal shedding of EHEC in cattle increases in warmer weather and is at its peak in the summer months (Hancock et al., 2001, Barkocy-Gallagher et al., 2003). Fecal samples from feedlot pens were collected in the summer and winter months to determine if there were seasonal effects on fecal shedding (Dewsbury et al., 2015). In that study, O26, O103, O145 and O157 were isolated in the summer months, but EHEC O45, O111, and O121 were not. In addition, EHEC O103, O26, O45, and O121 were detected in the winter, but EHEC O111, O145, and O157 were not (Dewsbury, et al., 2015). In 2005, Smith et al. used devices prepared from manila-hemp rope (ROPES) as a pen-level sampling strategy to monitor EHEC O157 prevalence. In their study, prior 7-day mean air temperature was determined to be a significant risk factor for a ROPES-positive result. The odds for a positive ROPES was 1.5 times greater for every 10^0C

increase in mean 7-day air temperature (Smith et al., 2005). In a study conducted at Midwestern U.S. processing plants, hide swabs, fecal samples, and pre- and post-evisceration carcass swabs were collected to determine seasonal prevalence of EHEC O157:H7, non-O157 EHEC, and *Salmonella* (Barkocy-Gallagher et al., 2003). Both *Salmonella* and EHEC O157 prevalence in feces were highest in the summer months ($P < 0.05$); however, non-O157 EHEC and Stx genes were found in more spring and fall fecal samples than in the summer and winter ($P < 0.05$) (Barkocy-Gallagher et al., 2003). In the same study, hide prevalence of STEC O157 was significantly higher in the spring and summer than the fall ($P < 0.05$), and the fall was significantly higher than winter ($P < 0.05$). Prevalence of non-O157 EHEC on hides peaked in the fall ($P < 0.05$) (Barkocy-Gallagher et al., 2003).

From 1983 to 2002, there were 940 non-O157 EHEC isolates from human patients infected with EHEC submitted to the CDC from state public health facilities. The majority of isolates collected were during the summer months of June, July, August, and September; however, the incidence of non-O157 EHEC was highest in the month of August (Brooks et al., 2005). A study which modeled seasonal occurrence of EHEC O157 in live cattle, ground beef, beef consumption, and human illness, found that the seasonal prevalence of ground beef and human illness are nearly proportionally related. The peak in cattle prevalence precedes ground beef and human illness by about one month; however, the pattern of ground beef consumption does not change seasonally. These correlations are interesting and logical pathways to human illness, but they do not show direct causation (Williams et al., 2010).

Temporal effects

It is important to note also that there are large fluctuations in hide contamination and fecal shedding O157 prevalence in both observational and experimental studies (Arthur et al., 2009, Kulow et al., 2012, Khaitsa et al., 2003, Peterson et al., 2007b, Peterson et al., 2007c, Smith et al., 2009a). These changes can occur in a relatively short period of time. In a longitudinal study of EHEC O157 in feedlot cattle, within a 2 week period the prevalence of hide contamination in a feedlot pen of 32 steers increased from 3% of cattle to 94% (Arthur et al., 2009). In a study which looked at the time on feed and shedding of O157, it was observed that the prevalence was significantly higher in pens with cattle on feed for the shortest amount of time (Hancock et al., 1997b).

Region

EHEC O157 is ubiquitous to fed cattle populations (Hancock et al., 1997b, Smith et al., 2001, Sargeant et al., 2003). Regional differences exist in the prevalence of human EHEC illness, and more cases occur in the northern states (Heiman et al., 2015). Literature indicates that there are wide ranges of prevalence estimates in fecal samples from cattle. Two studies evaluated the possible regional effect on fecal shedding of EHEC O157 in feedlots, and did not detect a significant regional difference (Hancock et al., 1997b, Sargeant et al., 2003). However, in a separate study evaluating hide prevalence of O157 in cattle arriving for slaughter at two plants in the north and south of the U.S., there were significant differences in prevalence between the two regions (Rivera-Betancourt et al., 2004). A meta-analysis investigating differences in EHEC O157 distribution found that there was a significant worldwide regional effect on

prevalence (Islam et al., 2014). It is unclear if a regional effect on EHEC-7 prevalence exists in cattle from the U.S.

Pen Conditions

In a study that examined the effects of pen floor conditions on fecal shedding of STEC O157, pen-level prevalence of shedding was higher in cattle housed in muddy or dusty pen floors compared to ideal conditions (moderate dust and minimal mud) (Smith et al., 2001). The median percentage of cattle shedding STEC O157 in pens classified as muddy, normal, or dusty was 22.4, 6.5, and 17.9%, respectively (Smith et al., 2001).

Age

In addition to several environmental factors, there are host factors that also affect EHEC carriage and shedding in cattle. One of these is age of the cattle. A strong effect of age was observed in a study by Nielsen et al. (2002), where cattle between 2 and 6 months of age were more likely to shed EHEC O157 compared to calves less than 2 months or cows (Nielsen et al., 2002). Fecal shedding of O26 has been associated with calves < 7 weeks of age, and as calves aged prevalence decreased over time (Shaw et al., 2004). EHEC-7 were more commonly detected in fecal samples collected in a longitudinal study of 48 steers during the post-weaning period compared to the finishing period or at slaughter (Ekiri et al., 2014).

Production Systems

Cattle production in the United States is a diverse industry. As more research has been done to describe the role of STECs in beef and dairy cattle production systems, it

has been seen that the type of management system affects prevalence of STEC in cattle feces, on hides, and within the environment. Although this dissertation focuses mainly on EHEC in beef cattle populations, there are a variety of review papers that focus solely on STEC in dairy cattle (Hussein & Sakuma, 2005, Farrokh et al., 2013, Kuhnert et al., 2005, Callaway et al., 2009, Callaway et al., 2003). An important difference in the type of cattle production systems lies in the dietary management. Diet has been found to greatly influence fecal shedding of STEC; however, many of the studies that have looked at specific diets or dietary components have conflicting results.

Other risk factors that increase the prevalence of STEC hide contamination include transportation and lairage. In a study which compared pre-transport prevalence of STEC O157 hide contamination to post-transport hide prevalence, it was found that hide prevalence increased from 50.3% (range 21.4%-73.2%) to 94.4% (range 88.9-100%) when sampled prior to loading the truck (pre-transport) and then at slaughter (post-transport) (Arthur et al., 2007).

Diet

One area of *E. coli* research that has been a focus for many years is the diet of cattle. Some of these studies evaluated the effects of diet on generic *E. coli*, generic acid resistant *E. coli,* EHEC O157, and acid resistant EHEC O157. For the purpose of this literature review, we will focus on studies that have looked at dietary effects on STEC or EHEC O157 carriage in cattle.

Several dietary components have been associated with increased EHEC O157 shedding prevalence, one of which is barley. Barley feeding has been positively associated with O157 shedding in feces in both observational and experimental studies

(Dargatz et al., 1997, Berg et al., 2004, Buchko et al., 2000). Cattle fed barley were either more likely to shed EHEC O157 (Dargatz et al., 1997, Buchko et al., 2000) or shed the organism at higher concentrations (Berg *et al*., 2004) compared to cattle fed corn. Additionally, cattle fed barley based diets had significantly higher fecal pH compared to cattle fed corn-based diet, which may have increased EHEC O157 survivability (Buchko et al., 2000).

Another dietary component that has been associated with increased EHEC O157 carriage in feedlot cattle is distillers grains (DG). During World War II, food demand was increased and American farmers improved corn production to levels unseen. In the mid-1950's, after the war had ended, US corn production had reached such a level that supply outweighed demand. Farmers realized that feeding cheap corn to beef cattle could produce high quality beef (Klopfenstein et al., 2008). Corn feeding became well accepted and cattle were finished at a rapid rate compared to forage-based finishing diets. Today beef cattle are still sent to feedlots to be finished on a high concentrate diet prior to harvest; however, this diet has evolved over the last 9 years from a corn-based to distillers grains-based diets (Klopfenstein et al., 2008). Distillers grains are a coproduct of ethanol production and are often used for livestock feed, especially in regions of the U.S. where ethanol production has flourished (Klopfenstein et al., 2008). The solid portion of the "spent" fraction is called wet distillers grain (WDG) and is about 30% dry matter; when this is dehydrated it is referred to as dry distillers grain (DDG) and is about 90% dry matter (Jacob et al., 2008a). In 2007, a rise in ground beef contamination rates in the US led to increased research interest in factors contributing to the change, and the inclusion of distillers grains was a suspected risk factor (U.S. Grains Council, 2012).

Several studies have reported increased fecal shedding or colonization of EHEC O157 in cattle fed diets containing corn distillers grains, specifically at high inclusion rates (>40% dry matter [DM]) in the finishing diet (Dewell et al., 2005, Peterson et al., 2007c, Jacob et al., 2008b, Jacob et al., 2008c, Jacob et al., 2010, Wells et al., 2009, Rich et al., 2010). In a study where steers were fed 0, 20, or 40% corn-based wet distillers grains (WDG) or dried distillers grains (DDG) as percent DM, the cattle fed 40% WDG or DDG had significantly higher fecal prevalence of EHEC O157 than cattle fed 0 or 20% distillers grains (Jacob et al., 2010). In a study where feedlot cattle were fed either 0 or 40% DM wet distillers grains plus solubles (WDGS) in the finishing phase, EHEC O157 prevalence was significantly greater in cattle fed WDGS diets than cattle fed none (Wells et al., 2009). In a previously published trial studying the effects of vaccination against EHEC O157 on fecal shedding and terminal rectal mucosa (TRM) colonization, cattle were fed a diet consisting of 10, 20, 30, 40, or 50% distillers grains on DM basis. Results of TRM colonization by EHEC O157 indicated a significant effect of diet (P = 0.04), in which cattle fed 10, 20, or 30% DG had decreased odds of TRM colonization while cattle fed 40 and 50% DG had increased odds of colonization (Peterson et al., 2007). The mechanism by which this increased carriage of EHEC O157 occurred is not known.

In contrast, there have been some studies that failed to detect differences in fecal shedding of EHEC O157 in cattle fed distillers grains. For example, in a study using a 2 x 2 factorial arrangement in which the factors were 0 or 25% DDGS and 0 or 25% dry-rolled corn (DRC), there was no effect of DDG, DRC, or sampling time on the probability to detect EHEC O157 (Jacob et al., 2009). In another study, cattle fed 25%

WDGS had greater prevalence of EHEC O157 compared to cattle fed steam-flaked corn diets on d122 but not on d136 (Jacob et al., 2008a). Additionally, in a recent feeding study using a 2 x 2 factorial design of 0 or 30% WDGS and direct-fed microbials (DFM) or no DFM, there was very low prevalence of EHEC O157 and no effects of WDGS or DFM were detected on probability of EHEC O157 shedding (Wilson et al., 2016).

Forage-based diets have also been implicated as risk factors for EHEC O157 carriage. In a study comparing an 85% forage diet to a 15% forage diet, cattle fed the 85% forage diet shed higher concentrations of EHEC O157 and for longer duration (Van Baale et al., 2004). Similarly, Wells et al. (2005) found prolonged survival of EHEC O157 in feces from cattle fed bromegrass hay compared with feces from cattle fed corn silage. In a separate study comparing sheep fed grass hay diets to corn-based diets, fecal shedding of EHEC O157 by sheep fed the hay diet occurred in higher concentrations and for twice the duration of sheep fed corn diets (Kudva et al., 1997).

EHEC-7 Interventions

The beef industry has devoted great effort to reducing the risk of EHEC-7 foodborne illness at the pre- and post-harvest level. Currently most of the interventions in use are applied post-harvest at slaughter facilities (Loneragan & Brashears, 2005). However, it has been advised that post-harvest interventions may be overwhelmed when high pathogen loads on hides at slaughter occur (Elder et al., 2000, Arthur et al., 2004). In addition, interventions applied pre-harvest can reduce environmental EHEC exposure.

Pre-harvest Interventions

A few management strategies have targeted the feedlot environment in efforts to decrease EHEC O157 sources or to make the environment less hospitable. These include cleaning water troughs and scraping pen floor surfaces, but they have not been effective methods (LeJeune et al., 2004). Effective pre-harvest interventions include methods to change the gut environment to make it less hospitable for EHEC survival. The use of vaccines, chemicals such as antibiotics and sodium chlorate, and competing bacteria like *Lactobacillus* have been more effective at reducing the probability of EHEC O157 fecal shedding (Smith, 2014).

Vaccines

Vaccinations have been tested for their ability to reduce fecal shedding and colonization of EHEC O157 in cattle populations. The purpose of vaccination against EHEC O157 is to create a gut environment less suitable for colonization, thus decreasing the duration of carriage and fecal shedding (Smith, 2015). There are two types of vaccines for reducing EHEC O157: one that targets the Siderophore Receptor and Porin protein (SRP) and one that targets the Type III secretory proteins (TTSP). Although they target different proteins, they both work to inhibit EHEC O157 survival in the gut. The SRP vaccines inhibit uptake of iron, thus causing nutritional deprivation of EHEC O157 cells (Thornton et al., 2009). The TTSP blocks adherence of the pathogen to the epithelial lining of the intestines (Potter et al., 2004). Both products have been efficacious at reducing the proportion of fecal samples culture positive for EHEC O157 In field trials, two doses of TTSP vaccine effectively reduced EHEC O157 carriage in cattle (Peterson et al., 2007a, Moxley et al., 2009, Smith et al., 2009a, Smith et al., 2009b). A stochastic

simulation estimated that vaccination of cattle against EHEC O157 in the summer could reduce prevalence to levels comparable to winter (Vogstad et al., 2014).

Sodium Chlorate

The use of sodium chlorate as a pre-harvest intervention against EHEC O157 has been studied due to its selective antimicrobial properties (Anderson et al., 2000). When feedlot cattle naturally exposed to generic *E. coli* were supplied sodium chlorate for 1 week prior to harvest, there was decreased carriage in fecal samples (Anderson et al., 2005). Although it may be effective in reducing EHEC , sodium chlorate is not approved for use in cattle (Callaway et al., 2011).

Direct-fed Microbials

Direct-fed microbials (DFM) have been used in feedlot diets to improve growth performance (Callaway et al., 2014). DFM incorporate commensal microbial cultures to improve or balance the gastrointestinal microflora. Most DFM products include a *Lactobacillus*-based strain (Wisener et al., 2014). Significant reductions in EHEC O157 shedding has been observed in studies where cattle were fed DFM utilizing *L. acidophilus* (Brashears et al., 2003, Elam et al., 2003, Peterson et al., 2007b, Vasconcelos et al., 2008, Hanford et al., 2011).

Post-harvest Interventions

Several post-harvest interventions have been implemented and appear to be successful in decreasing human illness due to EHEC O157 since 2000 (Gould et al., 2013). These interventions include hide and carcass washes with hot water and organic acids, steam vacuuming, carcass trimming, and proper sanitation and disinfection of

slaughter equipment (Moxley & Acuff, 2014). These interventions seem to be effective, since studies have shown that prevalence decreases at each step from hide swabbing, pre-evisceration carcass swabs, to post evisceration carcass samples (Elder et al., 2000, Stromberg et al., 2015).

EHEC Detection Methods in Samples of Animal Origin

Detection of EHEC is important to ensure food safety and to evaluate if interventions have been successful at decreasing the presence of the organisms. The USDA FSIS developed standardized methods for EHEC-7 detection in meat (USDA, 2014). These procedures include polymerase chain reaction (PCR) screening of enriched samples for the detection of O-groups, *stx*, and *eae*. Then possible positive samples are plated on chromogenic agar, and individual colonies are tested by PCR, latex agglutination, and biochemical testing procedures. These methods are time-consuming, expensive, and labor-intensive. Therefore, other several methods have been developed to test for EHEC-7. Cytotoxic, immunological, DNA-based and culture-based methods have been used for EHEC detection (Beutin & Fach, 2014).

Methods to detect EHEC O157 have been well established; however, differentiation between serogroups of non-O157 EHEC has been problematic. Because *E. coli* O157 does not ferment sorbitol within 24 hours, Sorbitol MacConkey agar (SMAC) has been used to isolate colonies of *E. coli* O157 from other serogroups (Doyle & Schoeni, 1984, March & Ratnam, 1986). Non-O157 serogroups are able to ferment sorbitol, thus producing pink colonies on SMAC. Agar media that have been developed for non-O157 EHEC have not been validated for effectiveness in isolating EHEC from complex samples, like foods, feces, and hide samples (Stromberg, 2015).

Multiplex PCR (mPCR) allows multiple genes to be targeted within a single assay. This is beneficial because a single test can be used to detect multiple EHEC serogroups, but it is impossible to determine if genes representing an O-group, *eae*, and *stx* are from one or multiple organisms. A mPCR was developed to detect six genes of EHEC O157:H7 (Bai et al., 2010), and a second mPCR was developed to detect EHEC-7 serogroups (Paddock et al., 2012). Together these two assays were modified into an 11-plex PCR detecting EHEC-7 serogroups, *stx1*, *stx2*, *eae*, and *ehxA* (Bai et al., 2012)

Detection of non-O157 EHEC serogroups is problematic. There is a lack of validated culture method to detect and isolate non-O157 EHEC from fecal samples (Noll et al., 2015).

The Absence of a "Gold Standard"

Diagnostic test performance can be evaluated in several ways, but most commonly tests are characterized by diagnostic sensitivity and specificity. Sensitivity is defined as the proportion of true positives that are detected by the method; the specificity of the method is the proportion of true negatives that are detected (Thrusfield, 2013). To calculate sensitivity and specificity, reference populations of diseased and disease-free animals are required. When there is no perfect reference test ("gold-standard") available, such reference populations are impossible to establish (Toft, 2007). Several approaches have been developed to evaluate tests without a gold-standard (Enøe et al., 2000). Latent class analysis is an alternative method to evaluating test performance (Hui and Walter, 1980).

Latent Class Analysis

An alternative approach that can be used in the absence of a "gold-standard" is latent class analysis (LCA). Latent class models are the class of models where the disease status of the individuals is unknown or latent. Latent means that the status exists, but has not been presently realized (Toft, 2005). Hui and Walter (1980) demonstrated that test parameters and true disease prevalence can be estimated using maximum likelihood estimation in latent class models. The assumptions which must be met to accomplish this were: (1) tested individuals are divided into two or more populations with suspected different prevalence of disease; (2) the tests used have the same properties (sensitivity and specificity) regardless of the population; (3) the tests are conditionally independent given the latent disease state. These assumptions can be difficult (or even impossible) to meet; therefore, methods have been suggested to overcome these requirements (Toft et al., 2005).

Different Disease Prevalence

Disease prevalence within populations being studied should be different in order to increase degrees of freedom in the data. If prevalences are not different, it is more appropriate to combine the data into a single population (Toft et al., 2005). Prevalence is rarely known, which makes stratifying into subpopulations difficult. Toft et al (2005) demonstrated that when the difference in prevalence is low, the precision around posterior estimates decreases (thus widening the 95% credible intervals).

Conditional Independence

If the tests in question are not conditionally independent, adjustments must be made in the estimates to account for covariance between tests (Dendukuri & Joseph, 2001, Branscum et al., 2005). Including a covariance when the true state is diseased and a covariance when the true state is non-diseased in the model has been suggested (Vacek, 1985). The assumption of conditional independence can result in biased estimates if the diagnostic tests evaluated are conditionally dependent (Vacek, 1985, Greiner & Gardner, 2000). From covariance, correlation coefficients can be obtained. According to Georgiadis, if correlation coefficients (ρ) are ≤ 0.2, estimates from the conditional dependence and independence models will be similar. If correlation coefficients are > 0.2, the conditional dependence model should be considered.

Model Identifiability

A Bayesian approach requires that the models must be identifiable (Johnson et al., 2001, Andersen, 1997), but performing such analysis allows the researcher to avoid stratifying the population (Joseph et al., 1995). Model identifiability means that data must be able to provide estimates for all of the parameters, requiring that the degrees of freedom in the data is at least equal to the number of parameters (Toft et al., 2005). Model identifiability has been further described and evaluated (Jones et al., 2010). As described by Hui and Walter, if sensitivity and specificity is held constant within all populations, any number of tests (R) and populations (S) can work as long as $S \geq R/(2^{R-1} - 1)$ (Hui & Walter, 1980).

Conclusions from Literature

There is limited information currently available detailing the prevalence and risk factors associated with EHEC-7 contamination on hides of market beef cattle. These culled cows comprise about 10% of all federally inspected beef, and a large proportion would contribute to ground beef production. This led to the objective of our first study, which was to estimate the probability for culled beef cows to have hides contaminated by EHEC-7 at slaughter and to identify risk factors contributing to this probability. In addition, there is minimal literature identifying risk factors contributing to EHEC-7 fecal shedding in beef cow-calf herds.

Pre-harvest interventions for EHEC O157 have been studied for several years and include vaccination, feeding of DFM products, and other chemicals. In addition, feedlot diets may contribute to the gastrointestinal flora and pathogen populations in cattle. The existing literature indicates that feeding high inclusion rates of distillers grains (DGS) to feedlot cattle increases the probability of colonization by and detecting EHEC O157 in feces. The interesting finding in many of these published reports is that feeding lower levels of DGS either had a protective effect or was no different from the corn control diets. The mechanism responsible for this increased shedding at high inclusions is unknown. This knowledge gap led to our third study, which looked at different diets as possible interventions against EHEC-7 carriage.

Finally, testing strategies to detect the seven EHEC serogroups of public health importance are less than perfect. Differentiation between serogroups of non-O157 is problematic. Although there are many methods of detection described in the literature, there is no “gold-standard” method of detection. Based on the literature, the methods

used to detect EHEC-7 have not been validated, nor have estimates of diagnostic performance been published. Our final study utilized Bayesian latent class analysis to evaluate three EHEC-7 diagnostic tests for the detection of EHEC O157 in fecal samples.

References

Al-Saigh, H., C. Zweifel, J. Blanco, J. E. Blanco, M. Blanco, M. A. Usera and R. Stephan, 2004: Fecal shedding of *Escherichia coli* O157, *Salmonella*, and *Campylobacter* in Swiss cattle at slaughter. *J. Food Prot.*, 67, 679-684.

Alam, M. J. and L. Zurek, 2004: Association of *Escherichia coli* O157:H7 with houseflies on a cattle farm. *Appl. Environ. Microbiol*., 70, 7578-7580.

Andersen, S., 1997: Re: "Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard". *Am. J. Epidemiol*., 145, 290-291.

Armstrong, G. L., J. Hollingsworth and J. G. Morris, 1996: Emerging food-borne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.*, 18, 29-51.

Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler and M. Koohmaraie, 2007: Transportation and lairage environment effects on prevalence, numbers, and diversity of *Escherichia coli* O157:H7 on hides and carcasses of beef cattle at processing. *J. Food Pro*t., 70, 280-286.

Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L. Wheeler, M. P. Kent, D. Jaroni, B. Pauling, D. M. Allen and M. Koohmaraie, 2004: *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *J. Food Prot.*, 67, 658-665.

Arthur, T. M., J. E. Keen, J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, X. Nou and M. Koohmaraie, 2009: Longitudinal study of *Escherichia coli* O157:H7 in a beef cattle feedlot and role of high-level shedders in hide contamination. *Appl. Environ. Microbiol*., 75, 6515-6523.

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

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

Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler and M. Koohmaraie, 2003: Seasonal prevalence of Shiga toxin–producing *Escherichia coli*, including O157:H7 and non-O157

serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.*, 66, 1978-1986.

Bell, B. P., M. Goldoft, P. M. Griffin, M. A. Davis, D. C. Gordon, P. I. Tarr, C. A. Bartleson, J. H. Lewis, T. J. Barrett, J. G. Wells, R. Baron and J. Kobayashi, 1994: A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. *JAMA*, 272, 1349-1353.

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

Besser, R. E., P. M. Griffin and L. Slutsker, 1999: *Escherichia coli* O157:H7 gastroenteritis and the hemolytic uremic syndrome: an emerging infectious disease. *Annu. Rev. Med.*, 50, 355-367.

Besser, T. E., D. D. Hancock, L. C. Pritchett, E. M. McRae, D. H. Rice and P. I. Tarr, 1997: Duration of detection of fecal excretion of *Escherichia coli* O157:H7 in cattle. *J. Infect. Dis.*, 175, 726-729.

Beutin, L. and P. Fach, 2014: Detection of Shiga toxin-producing *Escherichia coli* from nonhuman sources and strain typing. *Microbiol. Spectrum* 2, 1-23.

Beutin, L., D. Geier, H. Steinruck, S. Zimmermann and F. Scheutz, 1993: Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. *J. Clin. Microbiol.*, 31, 2483-2488.

Blanco Crivelli, X., M. V. Rumi, J. C. Carfagnini, O. Degregorio and A. B. Bentancor, 2012: Synanthropic rodents as possible reservoirs of shigatoxigenic *Escherichia coli* strains. *Front. Cell. Infect. Microbiol.*, 2, 134.

Blanco, M., J. E. Blanco, A. Mora, G. Dahbi, M. P. Alonso, E. A. Gonzalez, M. I. Bernardez and J. Blanco, 2004: Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (eae-xi). *J. Clin. Microbiol.*, 42, 645-651.

Bosilevac, J. M., B. E. Luedtke, T. M. Arthur, N. Kalchayanand, J. W. Schmidt, S. D. Shackelford and T. L. Wheeler, 2013: Identification of bovine reservoirs of human pathogenic non-O157 Shiga toxin-producing *Escherichia coli*. *American Meat Institute Foundatio*n.

Branscum, A. J., I. A. Gardner and W. O. Johnson, 2005: Estimation of diagnostic-test sensitivity and specificity through Bayesian modeling. *Prev. Vet. Med*, 68, 145-163.

Brashears M. M., M. L. Galyean, G. H. Loneragan, J. E. Mann, K. Killinger-Mann, 2003: Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. *J. Food Prot.*, 66:5, 748-54.

Brichta-Harhay, D. M., M. N. Guerini, T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler and M. Koohmaraie, 2008: *Salmonella* and *Escherichia coli* O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States: an evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. *Appl. Environ. Microbiol*., 74, 6289-6297.

Brooks, J. T., E. G. Sowers, J. G. Wells, K. D. Greene, P. M. Griffin, R. M. Hoekstra and N. A. Strockbine, 2005: Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. *J. Infect. Dis*., 192, 1422-1429.

Buchko, S. J., R. A. Holley, W. O. Olson, V. P. Gannon and D. M. Veira, 2000: The effect of different grain diets on fecal shedding of *Escherichia coli* O157:H7 by steers. *J. Food Prot.*, 63, 1467-1474.

Callaway, T. R., M. A. Carr, T. S. Edrington, R. C. Anderson and D. J. Nisbet, 2009: Diet, *Escherichia coli* O157:H7, and cattle: A review after 10 years. *Curr. Issues Mol. Biol.*, 11, 67-79.

Callaway, T. R., J. A. Carroll, J. D. Arthington, T. S. Edrington, M. L. Rossman, M. A. Carr, N. A. Krueger, S. C. Ricke, P. Crandall and D. J. Nisbet, 2011: *Escherichia coli* O157:H7 populations in ruminants can be reduced by orange peel product feeding. *J. Food Prot.*, 74, 1917-1921.

Callaway, T. R., R. O. Elder, J. E. Keen, R. C. Anderson and D. J. Nisbet, 2003: Forage feeding to reduce preharvest *Escherichia coli* populations in cattle, a review. *J. Dairy Sci.*, 86, 852-860.

Callaway, T. R., T. S. Edrington, and D. J. Nisbet, 2014: Meat science and muscle biology symposium: Ecological and dietary impactors of foodborne pathogens and methods to reduce fecal shedding in cattle. *J Anim Sci*, 92, 1356-1365.

CDC, 1999: Outbreak of *Escherichia coli* O157:H7 and *Campylobacter* among attendees of the Washington County Fair-New York, 1999. *Morb Mortal Wkly Rep*, 48, 803-805.

CDC, 2013: Incidence and trends of infection with pathogens transmitted commonly through food— Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 1996–2012. *Morb. Mortal. Wkly. Rep.*, 62, 283-287.

Chapman, P. A., C. A. Siddons, A. T. Gerdan Malo and M. A. Harkin, 1997: A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. *Epidemiol. Infect.*, 119, 245-250.

Chapman, P. A., D. J. Wright and P. Norman, 1989: Verotoxin-producing *Escherichia coli* infections in Sheffield: cattle as a possible source. *Epidemiol. Infect.*, 102, 439-445.

Cizek, A., P. Alexa, I. Literak, J. Hamrik, P. Novak and J. Smola, 1999: Shiga toxin-producing *Escherichia coli* O157 in feedlot cattle and Norwegian rats from a large-scale farm. *Lett. Appl. Microbiol.*, 28, 435-439.

Dargatz, D. A., S. J. Wells, L. A. Thomas, D. D. Hancock and L. P. Garber, 1997: Factors associated with the presence of *Escherichia coli* O157 in feces of feedlot cattle. *J. Food Prot.*, 60, 466-470.

Dendukuri, N. and L. Joseph, 2001: Bayesian approaches to modeling the conditional dependence between multiple diagnostic tests. *Biometrics*, 57, 158-167.

Dewell, G. A., J. R. Ransom, R. D. Dewell, K. McCurdy, I. A. Gardner, A. E. Hill, J. N. Sofos, K. E. Belk, G. C. Smith and M. D. Salman, 2005: Prevalence of and risk factors for *Escherichia coli* O157 in market-ready beef cattle from 12 U.S. feedlots. *Foodborne Pathog. Dis.*, 2, 70-76.

Dewsbury, D. M., D. G. Renter, P. B. Shridhar, L. W. Noll, X. Shi, T. G. Nagaraja and N. Cernicchiaro, 2015: Summer and winter prevalence of Shiga toxin-producing *Escherichia coli* (STEC) O26, O45, O103, O111, O121, O145, and O157 in feces of feedlot cattle. *Foodborne Pathog. Dis.*, 12, 726-732.

Doyle, M. P. and J. L. Schoeni, 1984: Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. *Appl. Environ. Microbiol.*, 48, 855-856.

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

Elam NA, J. F. Gleghorn, J. D. Rivera, M. L. Galyean, P. J. Defoor, M. M. Brashears, and S. M. Younts-Dahl, 2003: Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium fruendenreichii* on performance,

carcass, and intestinal characteristics, and *Escherichia coli* strain O157 shedding of finishing beef steers. *J. Anim. Sci.* 81, 2686–2698.

Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie and W. W. Laegreid, 2000: Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. U. S. A*, 97, 2999-3003.

Enøe, C., M. P. Georgiadis and W. O. Johnson, 2000: Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown. *Prev. Vet. Med*, 45, 61-81.

Farrokh, C., K. Jordan, F. Auvray, K. Glass, H. Oppegaard, S. Raynaud, D. Thevenot, R. Condron, K. De Reu, A. Govaris, K. Heggum, M. Heyndrickx, J. Hummerjohann, D. Lindsay, S. Miszczycha, S. Moussiegt, K. Verstraete and O. Cerf, 2013: Review of Shiga-toxin-producing *Escherichia coli* (STEC) and their significance in dairy production. *Int. J. Food Microbiol.*, 162, 190-212.

Fegan, N., P. Vanderlinde, G. Higgs and P. Desmarchelier, 2004: The prevalence and concentration of *Escherichia coli* O157 in faeces of cattle from different production systems at slaughter. *J. Appl. Microbiol*., 97, 362-370.

Frenzen, P. D., A. Drake and F. J. Angulo, 2005: Economic cost of illness due to *Escherichia coli* O157 infections in the United States. *J. Food Prot.*, 68, 2623-2630.

Gannon, V. P. J., T. A. Graham, R. King, P. Michel, S. Read, K. Ziebell and R. P. Johnson, 2002: *Escherichia coli* O157:H7 infection in cows and calves in a beef cattle herd in Alberta, Canada. *Epidemiol. Infect*., 129, 163-172.

Garcia, A. and J. G. Fox, 2003: The rabbit as a new reservoir host of enterohemorrhagic *Escherichia coli*. *Emerg. Infect*. Dis., 9.

Geue, L., M. Segura-Alvarez, F. J. Conraths, T. Kuczius, J. Bockemuhl, H. Karch and P. Gallien, 2002: A long-term study on the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) on four German cattle farms. *Epidemiol. Infect*., 129, 173-185.

Gould, L. H., L. Demma, T. F. Jones, S. Hurd, D. J. Vugia, K. Smith, B. Shiferaw, S. Segler, A. Palmer, S. Zansky and P. M. Griffin, 2009: Hemolytic uremic syndrome and death in persons with *Escherichia coli* O157:H7 infection, foodborne diseases active surveillance network sites, 2000-2006. *Clin. Infect. Dis.*, 49, 1480-1485.

Gould, L. H., R. K. Mody, K. L. Ong, P. Clogher, A. B. Cronquist, K. N. Garman, S. Lathrop, C. Medus, N. L. Spina, T. H. Webb, P. L. White, K. Wymore, R. E.

Gierke, B. E. Mahon and P. M. Griffin, 2013: Increased recognition of non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States during 2000-2010: epidemiologic features and comparison with *E. coli* O157 infections. *Foodborne Pathog. Dis.*, 10, 453-460.

Greiner, M. and I. A. Gardner, 2000: Application of diagnostic tests in veterinary epidemiologic studies. *Prev. Vet. Med*, 45, 43-59.

Gyles, C. L., 2007: Shiga toxin-producing Escherichia coli: an overview. *J. Anim. Sci*., 85, E45-62.

Hancock, D., T. Besser, J. Lejeune, M. Davis and D. Rice, 2001: The control of VTEC in the animal reservoir. *Int. J. Food Microbiol.*, 66, 71-78.

Hancock, D. D., T. E. Besser, M. L. Kinsel, P. I. Tarr, D. H. Rice and M. G. Paros, 1994: The prevalence of *Escherichia coli* O157.H7 in dairy and beef cattle in Washington State. *Epidemiol. Infect*., 113, 199-207.

Hancock, D. D., T. E. Besser, D. H. Rice, E. D. Ebel, D. E. Herriott and L. V. Carpenter, 1998: Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the Northwestern USA. *Prev. Vet. Med*, 35, 11-19.

Hancock, D. D., T. E. Besser, D. H. Rice, D. E. Herriott and P. I. Tarr, 1997a: A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. *Epidemiol. Infect*., 118, 193-195.

Hancock, D. D., D. H. Rice, L. A. Thomas, D. A. Dargatz and T. E. Besser, 1997b: Epidemiology of *Escherichia coli* O157 in feedlot cattle. *J. Food Prot*., 60, 462-465.

Hanford, K. J., W. M. Kreikemeier, D. R. Ware, 2011: The effect of Bovamine on feedlot performance of finishing cattle: A meta-analysis. *Proceedings of the American Dairy Science Association and American Society of Animal Science Conference*; July 10-14; New Orleans, Louisiana. *J. Anim. Sci.*, 89, 25.

Hara-Kudo, Y. and K. Takatori, 2011: Contamination level and ingestion dose of foodborne pathogens associated with infections. *Epidemiol. Infect*., 139, 1505-1510.

Heiman, K. E., R. K. Mody, S. D. Johnson, P. M. Griffin and L. H. Gould, 2015: *Escherichia coli* O157 outbreaks in the United States, 2003–2012. *Emerg. Infect. Dis*., 21, 1293-1301.

Hui, S. L. and S. D. Walter, 1980: Estimating the error rates of diagnostic tests. *Biometrics*, 36, 167-171.

Hurley, B. P., C. M. Thorpe and D. W. Acheson, 2001: Shiga toxin translocation across intestinal epithelial cells is enhanced by neutrophil transmigration. *Infect. Immun.*, 69, 6148-6155.

Hussein, H. S. and L. M. Bollinger, 2005: Prevalence of Shiga toxin-producing *Escherichia coli* in beef cattle. *J. Food Prot.*, 68, 2224-2241.

Hussein, H. S. and T. Sakuma, 2005: Prevalence of Shiga toxin-producing *Escherichia coli* in dairy cattle and their products. *J. Dairy Sci.*, 88, 450-465.

Islam, M. Z., A. Musekiwa, K. Islam, S. Ahmed, S. Chowdhury, A. Ahad and P. K. Biswas, 2014: Regional variation in the prevalence of *E. coli* O157 in cattle: A meta-analysis and meta-regression. *PLoS. ONE*, 9, e93299.

Jacob, M. E., J. T. Fox, J. S. Drouillard, D. G. Renter and T. G. Nagaraja, 2008a: Effects of dried distillers' grain on fecal prevalence and growth of *Escherichia coli* O157 in batch culture fermentations from cattle. *Appl. Environ. Microbiol.*, 74, 38-43.

Jacob, M. E., J. T. Fox, J. S. Drouillard, D. G. Renter and T. G. Nagaraja, 2009: Evaluation of feeding dried distiller's grains with solubles and dry-rolled corn on the fecal prevalence of *Escherichia coli* O157:H7 and *Salmonella* spp. in cattle. *Foodborne Pathog. Dis.*, 6, 145-153.

Jacob, M. E., J. T. Fox, S. K. Narayanan, J. S. Drouillard, D. G. Renter and T. G. Nagaraja, 2008b: Effects of feeding wet corn distillers grains with solubles with or without monensin and tylosin on the prevalence and antimicrobial susceptibilities of fecal foodborne pathogenic and commensal bacteria in feedlot cattle. *J. Anim. Sci.*, 86, 1182-1190.

Jacob, M. E., Z. D. Paddock, D. G. Renter, K. F. Lechtenberg and T. G. Nagaraja, 2010: Inclusion of dried or wet distillers' grains at different levels in diets of feedlot cattle affects fecal shedding of *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.*, 76, 7238-7242.

Jacob, M. E., G. L. Parsons, M. K. Shelor, J. T. Fox, J. S. Drouillard, D. U. Thomson, D. G. Renter and T. G. Nagaraja, 2008c: Feeding supplemental dried distiller's grains increases faecal shedding of *Escherichia coli* O157 in experimentally inoculated calves. *Zoonoses Public Health*, 55, 125-132.

Johnson, W. O., J. L. Gastwirth and L. M. Pearson, 2001: Screening without a "gold standard": the Hui-Walter paradigm revisited. *Am. J. Epidemiol.*, 153, 921-924.

Jones, G., W. O. Johnson, T. E. Hanson and R. Christensen, 2010: Identifiability of models for multiple diagnostic testing in the absence of a gold standard. *Biometrics*, 66, 855-863.

Joseph, L., T. W. Gyorkos and L. Coupal, 1995: Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard. *Am. J. Epidemiol*., 141, 263-272.

Kaper, J. B., J. P. Nataro and H. L. T. Mobley, 2004: Pathogenic *Escherichia coli*. *Nat. Rev. Micro.*, 2, 123-140.

Keen, J. E., W. W. Laegreid, C. G. Chitko-McKown, L. M. Durso and J. L. Bono, 2010: Distribution of Shiga-toxigenic *Escherichia coli* O157 in the gastrointestinal tract of naturally O157-shedding cattle at necropsy. *Appl. Environ. Microbiol*., 76, 5278-5281.

Khaitsa, M. L., D. R. Smith, J. A. Stoner, A. M. Parkhurst, S. Hinkley, T. J. Klopfenstein and R. A. Moxley, 2003: Incidence, duration, and prevalence of *Escherichia coli* O157:H7 fecal shedding by feedlot cattle during the finishing period. *J. Food Prot.*, 66, 1972-1977.

Klopfenstein, T. J., G. E. Erickson and V. R. Bremer, 2008: Use of Distillers Co-products in Diets Fed to Beef Cattle. In: B. A. Babcock, D. J. Hayes and J. D. Lawrence (eds.), Using Distillers Grains in the U.S. and International Livestock and Poultry Industries. Midwest Agribusiness Trade Research and Information Center, Ames, Iowa.

Kondro, W., 2000: *E. coli* outbreak deaths spark judicial inquiry in Canada. Lancet, 355, 2058.

Konowalchuk, J., J. I. Speirs and S. Stavric, 1977: Vero response to a cytotoxin of *Escherichia coli*. *Infect. Immun.*, 18, 775-779.

Kudva, I. T., C. W. Hunt, C. J. Williams, U. M. Nance and C. J. Hovde, 1997: Evaluation of dietary influences on *Escherichia coli* O157:H7 shedding in sheep. *Appl. Environ. Microbiol*., 63, 3878-3886.

Kuhnert, P., C. R. Dubosson, M. Roesch, E. Homfeld, M. G. Doherr and J. W. Blum, 2005: Prevalence and risk-factor analysis of Shiga toxigenic *Escherichia coli* in faecal samples of organically and conventionally farmed dairy cattle. *Vet. Microbiol*, 109, 37-45.

Kulow, M. J., T. K. Gonzales, K. M. Pertzborn, J. Dahm, B. A. Miller, D. Park, R. Gautam, C. W. Kaspar, R. Ivanek and D. Dopfer, 2012: Differences in

colonization and shedding patterns after oral challenge of cattle with three *Escherichia coli* O157:H7 strains. *Appl. Environ. Microbiol.,* 78, 8045-8055.

LeJeune, J. T., T. E. Besser, D. H. Rice, J. L. Berg, R. P. Stilborn and D. D. Hancock, 2004: Longitudinal study of fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle: predominance and persistence of specific clonal types despite massive cattle population turnover. *Appl. Environ. Microbiol*., 70, 377-384.

Loneragan, G. H. and M. M. Brashears, 2005: Pre-harvest interventions to reduce carriage of *E. coli* O157 by harvest-ready feedlot cattle. *Meat Sci.*, 71, 72-78.

March, S. B. and S. Ratnam, 1986: Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J. Clin. Microbiol*., 23, 869-872.

Mayer, C. L., C. S. Leibowitz, S. Kurosawa and D. J. Stearns-Kurosawa, 2012: Shiga toxins and the pathophysiology of hemolytic uremic syndrome in humans and animals. *Toxins*, 4, 1261-1287.

Mead, P. S. and P. M. Griffin, 1998: *Escherichia coli* O157:H7. *The Lancet*, 352, 1207-1212.

Meng, J. and C. M. Schroeder, 2007: *Escherichia coli*. In: S. Simjee (ed.), *Foodborne Dis.*. Humana Press, Totowa, NJ.

Menrath, A., L. H. Wieler, K. Heidemanns, T. Semmler, A. Fruth and N. Kemper, 2010: Shiga toxin producing *Escherichia coli*: identification of non-O157:H7-Super-shedding cows and related risk factors. *Gut Pathog*., 2, 7.

Moxley, R. A. and G. R. Acuff, 2014: Peri- and postharvest factors in the control of Shiga toxin-producing *Escherichia coli* in beef. *Microbiol. Spectrum*, 2.

Moxley, R. A. and D. R. Smith, 2010: Attaching-effacing *Escherichia coli* infections in cattle. *Vet. Clin. North Am. Food Anim. Pract.*, 26, 29-56.

Moxley, R. A., D. R. Smith, M. Luebbe, G. E. Erickson, T. J. Klopfenstein and D. Rogan, 2009: *Escherichia coli* O157:H7 vaccine dose-effect in feedlot cattle. *Foodborne Pathog. Dis.*, 6, 879-884.

Nataro, J. P. and J. B. Kaper, 1998: Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.*, 11, 142-201.

Naylor, S. W., J. C. Low, T. E. Besser, A. Mahajan, G. J. Gunn, M. C. Pearce, I. J. McKendrick, D. G. E. Smith and D. L. Gally, 2003: Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of

enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infect. Immun.*, 71, 1505-1512.

Naylor, S. W., A. J. Roe, P. Nart, K. Spears, D. G. Smith, J. C. Low and D. L. Gally, 2005: *Escherichia coli* O157 : H7 forms attaching and effacing lesions at the terminal rectum of cattle and colonization requires the LEE4 operon. *Microbiol.*, 151, 2773-2781.

Nielsen, E. M., C. Tegtmeier, H. J. Andersen, C. Grønbæk and J. S. Andersen, 2002: Influence of age, sex and herd characteristics on the occurrence of verocytotoxin-producing *Escherichia coli* O157 in Danish dairy farms. *Vet. Microbiol*, 88, 245-257.

Noll, L. W., P. B. Shridhar, D. M. Dewsbury, X. Shi, N. Cernicchiaro, D. G. Renter and T. G. Nagaraja, 2015: A comparison of culture- and PCR-based methods to detect six major non-O157 serogroups of Shiga toxin-producing *Escherichia coli* in cattle feces. *PLoS. ONE*, 10, e0135446.

O'Brien, A. D. and G. D. LaVeck, 1983: Purification and characterization of a *Shigella dysenteriae* 1-Like toxin produced by *Escherichia coli*. *Infect. Immun.*, 40, 675-683.

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

Pearce, M. C., C. Jenkins, L. Vali, A. W. Smith, H. I. Knight, T. Cheasty, H. R. Smith, G. J. Gunn, M. E. J. Woolhouse, S. G. B. Amyes and G. Frankel, 2004: Temporal shedding patterns and virulence factors of *Escherichia coli* serogroups O26, O103, O111, O145, and O157 in a cohort of beef calves and their dams. *Appl. Environ. Microbiol.*, 70, 1708-1716.

Pederson, K. and L. Clark, 2007: A review of Shiga toxin *Escherichia coli* and Salmonella enterica in cattle and free-ranging birds: potential association and epidemiological links. *Human-Wildlife Conflicts*, 1, 68-77.

Peterson, R. E., T. Klopfenstein, R. Moxley, G. E. Erickson and S. Hinkley, 2007a: Efficacy of dose regimen and observation of herd immunity from a vaccine against *Escherichia coli* O157:H7 for feedlot cattle. *J. Food Prot.*, 70, 2561-2567.

Peterson, R. E., T. J. Klopfenstein, G. E. Erickson, J. Folmer, S. Hinkley, R. A. Moxley and D. R. Smith, 2007b: Effect of *Lactobacillus acidophilus* strain NP51 on *Escherichia coli* O157H7 fecal shedding and finishing performance in beef feedlot cattle. *J. Food Prot.*, 70, 287-291.

Peterson, R. E., T. J. Klopfenstein, R. A. Moxley, G. E. Erickson, S. Hinkley, G. Bretschneider, E. M. Berberov, D. Rogan and D. R. Smith, 2007c: Effect of a vaccine product containing type III secreted proteins on the probability of *Escherichia coli* O157:H7 fecal shedding and mucosal colonization in feedlot cattle. *J. Food Prot.*, 70, 2568-2577.

Potter, A. A., S. Klashinsky, Y. Li, E. Frey, H. Townsend, D. Rogan, G. Erickson, S. Hinkley, T. Klopfenstein, R. A. Moxley, D. R. Smith and B. B. Finlay, 2004: Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. *Vaccine*, 22, 362-369.

Proulx, F., E. G. Seidman and D. Karpman, 2001: Pathogenesis of Shiga Toxin-Associated Hemolytic Uremic Syndrome. *Ped. Research*, 50, 163-171.

Pruimboom-Brees, I. M., T. W. Morgan, M. R. Ackermann, E. D. Nystrom, J. E. Samuel, N. A. Cornick and H. W. Moon, 2000: Cattle lack vascular receptors for *Escherichia coli* O157:H7 Shiga toxins. *Proc. Natl. Acad. Sci. U. S. A*, 97, 10325-10329.

Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin and D. L. Swerdlow, 2005: Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg. Infect. Dis*., 11, 603-609.

Renter, D. G., G. Morris, J. M. Sargeant, L. L. Hungerford, J. Berezowski, T. Ngo, K. Williams and D. W. K. Acheson, 2005: Prevalence, risk factors, O serogroups, and virulence profiles of Shiga toxin-producing bacteria from cattle production environments. *J. Food Prot.*, 68, 1556-1565.

Renter, D. G., J. M. Sargeant, S. E. Hygnstrom, J. D. Hoffman and J. R. Gillespie, 2001: *Escherichia coli* O157:H7 in free-ranging deer in Nebraska. *J. Wildl. Dis*., 37 755-760.

Renter, D. G., J. M. Sargeant, R. D. Oberst and M. Samadpour, 2003: Diversity, frequency, and persistence of *Escherichia coli* O157 strains from range cattle environments. *Appl. Environ. Microbiol*., 69, 542-547.

Rich, A. R., A. N. Jepson, M. K. Luebbe, G. E. Erickson, T. J. Klopfenstein, D. R. Smith and R. A. Moxley, 2010: Vaccination to reduce the prevalence of *Escherichia coli* O157:H7 in feedlot cattle fed wet distillers grains plus solubles. *Nebraska Beef Cattle Reports.*

Richardson, S. E., M. A. Karmali, L. E. Becker and C. R. Smith, 1988: The histopathology of the hemolytic uremic syndrome associated with verocytotoxin-producing *Escherichia coli* infections. *Hum. Pathol.*, 19, 1102-1108.

Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake and M. L. Cohen, 1983: Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.*, 308, 681-685.

Rivera-Betancourt, M., S. D. Shackelford, T. M. Arthur, K. E. Westmoreland, G. Bellinger, M. Rossman, J. O. Reagan and M. Koohmaraie, 2004: Prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in two geographically distant commercial beef processing plants in the United States. *J. Food Prot.*, 67, 295-302.

Sargeant, J. M., M. R. Amezcua, A. Rajic and L. Waddell, 2007: Pre-harvest interventions to reduce the shedding of *E. coli* O157 in the faeces of weaned domestic ruminants: a systematic review. *Zoonoses Public Health*, 54, 260-277.

Sargeant, J. M., J. R. Gillespie, R. D. Oberst, R. K. Phebus, D. R. Hyatt, L. K. Bohra and J. C. Galland, 2000: Results of a longitudinal study of the prevalence of *E. coli* on cow-calf farms. *AJVR*, 61, 1375-1379.

Sargeant, J. M., M. W. Sanderson, R. A. Smith and D. D. Griffin, 2003: *Escherichia coli* O157 in feedlot cattle feces and water in four major feeder-cattle states in the USA. *Prev. Vet. Med*, 61, 127-135.

Scaife, H. R., D. Cowan, J. Finney, S. F. Kinghorn-Perry and B. Crook, 2006: Wild rabbits (*Oryctolagus cuniculus*) as potential carriers of verocytotoxin-producing *Escherichia coli*. *Vet. Rec*, 159, 175-178.

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. Widdowson, S. L. Roy, J. L. Jones and P. M. Griffin, 2011: Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.*, 17, 7-15.

Shaw, D., C. Jenkins, M. Pearce, T. Cheasty, G. Gunn, G. Dougan, H. Smith, M. Woolhouse and G. Frankel, 2004: Shedding patterns of verocytotoxin-producing *Escherichia coli* strains in a cohort of calves and their dams on a Scottish beef farm. *Appl. Environ. Microbiol.*, 70, 7456-7465.

Slutsker, L., A. A. Ries, K. D. Greene, J. G. Wells, L. Hutwagner and P. M. Griffin, 1997: *Escherichia coli* O157:H7 diarrhea in the United States: clinical and epidemiologic features. *Ann. Intern. Med.*, 126.

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

Smith, D. R., 2014: Cattle production systems: Ecology of existing and emerging *Escherichia coli* types related to foodborne illness. *Ann. Rev. Anim. Biosci.*, 2, 445-468.

Smith, D. R., 2015: Vaccination of Cattle against *Escherichia coli* O157:H7. In: V. Sperandio and C. J. Hovde (eds.), Enterohemorrhagic *Escherichia coli* and other Shiga toxin-producing *E. coli*. ASM Press, Washington, DC.

Smith, D. R., R. A. Moxley, S. L. Clowser, J. D. Folmer, S. Hinkley, G. E. Erickson and T. J. Klopfenstein, 2005: Use of rope devices to describe and explain the feedlot ecology of *Escherichia coli* O157:H7 by time and place. *Foodborne Pathog. Dis.*, 2, 50-60.

Smith, D. R., R. A. Moxley, T. J. Klopfenstein and G. E. Erickson, 2009a: A randomized longitudinal trial to test the effect of regional vaccination within a cattle feedyard on *Escherichia coli* O157:H7 rectal colonization, fecal shedding, and hide contamination. *Foodborne Pathog. Dis.*, 6, 885-892.

Smith, D. R., R. A. Moxley, R. E. Peterson, T. J. Klopfenstein, G. E. Erickson, G. Bretschneider, E. M. Berberov and S. Clowser, 2009b: A two-dose regimen of a vaccine against type III secreted proteins reduced *Escherichia coli* O157:H7 colonization of the terminal rectum in beef cattle in commercial feedlots. *Foodborne Pathog. Dis.*, 6, 155-161.

Smith, D. R., K. Novotnaj and G. Smith, 2010: Preharvest food safety: what do the past and the present tell us about the future? *J. Agromedicine*, 15, 275-280.

Stromberg, Z. R., 2015: Detection methods and intestinal adherence of non-O157 Shiga toxin-producing *Escherichia coli*. (Doctoral dissertation). University of Nebraska-Lincoln, Lincoln, NE.

Stromberg, Z. R., N. W. Baumann, G. L. Lewis, N. J. Sevart, N. Cernicchiaro, D. G. Renter, D. B. Marx, R. K. Phebus and R. A. Moxley, 2015: Prevalence of enterohemorrhagic *Escherichia coli* O26, O45, O103, O111, O121, O145, and O157 on hides and preintervention carcass surfaces of feedlot cattle at harvest. *Foodborne Pathog. Dis.*, 12, 631-638.

Tarr, P. I., C. A. Gordon and W. L. Chandler, 2005: Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *The Lancet*, 365, 1073-1086.

Thornton, A. B., D. U. Thomson, G. H. Loneragan, J. T. Fox, D. T. Burkhardt, D. A. Emery and T. G. Nagaraja, 2009: Effects of a siderophore receptor and porin proteins-based vaccination on fecal shedding of *Escherichia coli* O157:H7 in experimentally inoculated cattle. *J. Food Prot.*, 72, 866-869.

Thrusfield, M., 2013: Veterinary Epidemiology. Wiley.

Toft, N., E. Jørgensen and S. Højsgaard, 2005: Diagnosing diagnostic tests: evaluating the assumptions underlying the estimation of sensitivity and specificity in the absence of a gold standard. *Prev. Vet. Med.*, 68, 19-33.

Tutenel, A. V., D. Pierard, J. Uradzinski, E. Jozwick, M. Pastuszczak, J. Van Hende, M. Uyttendaele, J. Debevere, T. Cheasty, J. Van Hoof and L. De Zutter, 2002: Isolation and characterization of enterohemorrhagic *Escherichia coli* O157:H7 from cattle in Belgium and Poland. *Epidemiol. Infect.*, 129, 41-47.

U.S. Grains Council, 2012: Is there a connection between feeding DDGS and *E. coli* O157:H7 shedding in beef cattle? In: A Guide to Distiller's Dried Grains with Solubles (DDGS).

USDA-FSIS, 2011: Shiga Toxin-Producing *Escherichia coli* in Certain Raw Beef Products. *Fed. Regist.*, Washington, DC.

Vacek, P. M., 1985: The Effect of Conditional Dependence on the Evaluation of Diagnostic Tests. *Biometrics*, 41, 959-968.

Van Baale, M. J., J. M. Sargeant, D. P. Gnad, B. M. DeBey, K. F. Lechtenberg and T. G. Nagaraja, 2004: Effect of forage or grain diets with or without monensin on ruminal persistence and fecal *Escherichia coli* O157:H7 in cattle. *Appl. Environ. Microbiol.*, 70, 5336-5342.

Van Overbeek, L. S., J. van Doorn, J. H. Wichers, A. van Amerongen, H. J. W. van Roermund and P. T. J. Willemsen, 2014: The arable ecosystem as battleground for emergence of new human pathogens. *Front. Microbiol.*, 5, 104.

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

Vogstad, A. R., R. A. Moxley, G. E. Erickson, T. J. Klopfenstein and D. R. Smith, 2014: Stochastic simulation model comparing distributions of STEC O157 faecal shedding prevalence between cattle vaccinated with type III secreted protein vaccines and non-vaccinated cattle. *Zoonoses Public Health*, 61, 283-289.

Wallace, J. S., T. Cheasty and K. Jones, 1997: Isolation of Vero cytotoxin-producing *Escherichia coli* O157 from wild birds. *J. Appl. Microbiol.*, 82, 399-404.

Wells, J. E., S. D. Shackelford, E. D. Berry, N. Kalchayanand, M. N. Guerini, V. H. Varel, T. M. Arthur, J. M. Bosilevac, H. C. Freetly, T. L. Wheeler, C. L. Ferrell and M. Koohmaraie, 2009: Prevalence and level of *Escherichia coli* O157:H7 in feces and on hides of feedlot steers fed diets with or without wet distillers grains with solubles. *J. Food Prot.*, 72, 1624-1633.

Widiasih, D. A., N. Ido, K. Omoe, S. Sugii and K. Shinagawa, 2004: Duration and magnitude of faecal shedding of Shiga toxin-producing *Escherichia coli* from naturally infected cattle. *Epidemiol. Infect*., 132, 67-75.

Williams, M. S., J. L. Withee, E. D. Ebel, N. E. Bauer, Jr., W. D. Schlosser, W. T. Disney, D. R. Smith and R. A. Moxley, 2010: Determining relationships between the seasonal occurrence of *Escherichia coli* O157:H7 in live cattle, ground beef, and humans. *Foodborne Pathog. Dis.*, 7, 1247-1254.

Wilson, B. K., B. P. Holland, D. L. Step, M. E. Jacob, D. L. VanOverbeke, C. J. Richards, T. G. Nagaraja and C. R. Krehbiel, 2016: Feeding wet distillers grains plus solubles with and without a direct-fed microbial to determine performance, carcass characteristics, and fecal shedding of *Escherichia coli* O157:H7 in feedlot heifers. *J. Anim. Sci*., 94, 297-305.

Wisener, L. V., J. M. Sargeant, A. M. O'Connor, M. C. Faires, S. K. Glass-Kaastra, 2015: The use of direct-fed microbials to reduce shedding of *Escherichia coli* O157 in beef cattle: A systematic review and meta-analysis. *Zoonoses Public Health,* 62, 75-89.

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

Yilmaz, A., H. Gun and H. Yilmaz, 2002: Frequency of *Escherichia coli* O157:H7 in Turkish cattle. *J. Food Prot.*, 65, 1637-1640.

CHAPTER II

CROSS-SECTIONAL STUDY TO ESTIMATE THE PREVALENCE OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* ON HIDES OF MARKET BEEF COWS AT HARVEST

(Submitted to Zoonoses and Public Health)

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Abstract

Cattle hides are an important source of enterohemorrhagic Escherichia coli (EHEC) carcass contamination at slaughter. The objective of this study was to estimate the probability for hide contamination with seven EHEC serogroups among U.S. market beef cows at slaughter and to test the effects of season and geographic region on prevalence of hide contamination. Hides (n = 800) of market cows were swabbed at slaughter immediately after stunning and prior to hide removal. Cows were sampled from two geographically distinct beef packing plants during 4 seasons of 2015. Cattle source was categorized by northern or southern region. Samples were tested for EHEC by a molecular screening assay. The effects of region, season, and their interaction on the probability of hide contamination by each EHEC serogroup were tested in separate multilevel multivariable logistic regression models, accounting for the random effect of clustering by plant. Statistical significance was set $\alpha = 0.05$. Out of 800 total samples, at least one EHEC was detected on 630 (79%) hides. EHEC O26 was detected on 129 (16%) of all hides sampled, EHEC O45 on 437 (55%), EHEC O103 on 289 (36%), EHEC O111 on 189 (24%), EHEC O121 on 140 (18%), EHEC O145 on 171 (21%), and EHEC O157 on 89 (11%). Detection of EHEC O26 and EHEC O121 was associated with season. Season and region were associated with detecting EHEC O45 and EHEC O157. Season by region interactions were associated with the outcome of detecting EHEC O103, EHEC O111, and EHEC O145. Season, region of origin, and the interaction of these factors affect hide contamination of market beef cattle at slaughter by EHEC, and each serogroup responds to these factors uniquely.

Introduction

Enterohemorrhagic *Escherichia coli* (EHEC) are human foodborne pathogens. Cattle populations are important reservoirs of EHEC O157 (Hancock et al., 1994, Laegreid et al., 1999, Chapman et al., 1993) and non-O157 EHEC (Barkocy-Gallagher et al., 2003, Geue et al., 2002). EHEC of seven specific serogroups are classified as adulterants in raw, non-intact beef by the USDA Food Safety and Inspection Service (USDA-FSIS), and collectively are referred to as EHEC-7 in the present study. These serogroups include EHEC O26, O45, O103, O111, O121, O145, and O157 (USDA-FSIS, 2012). Ground beef has been implicated as a source of food-borne EHEC O157 and non-O157 outbreaks (Bell et al., 1994, Rangel et al., 2005, Riley et al., 1983, Robbins et al., 2014).

Culled beef cows and bulls are removed from cow-calf production and may be marketed for beef, often through auction markets. In annual slaughter reports, the US Department of Agriculture (USDA) differentiates beef cows from dairy cows by referring to them as “other cows”. According to slaughter reports from 2006 to 2015, approximately 80% of federally inspected beef slaughtered were fed steers and heifers. Dairy cows, other cows, and bulls comprised the remaining approximately 20%. Market beef cows alone comprised 9.8% of all federally inspected beef during these years. (USDA-NASS, 2007, USDA-NASS, 2008, USDA-NASS, 2009, USDA-NASS, 2010, USDA-NASS, 2011, USDA-NASS, 2013, USDA-NASS, 2012, USDA-NASS, 2014, USDA-NASS, 2015, USDA-NASS, 2016).

Cattle hides can be contaminated by EHEC through direct contact with feces, contact with other cattle or animals, dust, or insects (Keen et al., 2002). Hides serve as a

source of further animal-animal transfer of EHEC, as well as hide-carcass transfer, which poses a human health risk. Carcasses become contaminated during the removal of hides during processing (Arthur et al., 2002, Barkocy-Gallagher et al., 2003, Arthur et al., 2004, Brichta-Harhay et al., 2008). Hide contamination by EHEC O157 has been associated with subsequent carcass contamination (Elder et al., 2000, Arthur et al., 2002, Keen et al., 2002, Barkocy-Gallagher et al., 2003, Arthur et al., 2004, Koohmaraie et al., 2005, Schmidt et al., 2012). Hide prevalence has been used as a pre-harvest measure of EHEC contamination (Arthur et al., 2004, Barkocy-Gallagher et al., 2003, Brichta-Harhay et al., 2008, Wells et al., 2009, Smith et al., 2009, Stromberg et al., 2015b, Stromberg et al., 2016). Additionally, reduction in the prevalence of EHEC O157 on the hides at harvest is correlated to reduction in carcass contamination prevalence (Nou et al., 2003, Bosilevac et al., 2004). Seasonal variation of EHEC O157 contamination has been observed on hides of fed cattle at slaughter, with greater prevalence in the summer months compared to winter (Barkocy-Gallagher et al., 2003). In the same study, non-O157 EHEC were more likely in the fall; however, these serogroups were not individually assessed to determine if there were unique seasonal differences (Barkocy-Gallagher et al., 2003). To our knowledge, there have been no studies to address potential seasonal or regional effects on hide contamination by individual non-O157 EHEC serogroups in market beef cows at slaughter.

Therefore, the primary objective of this study was to estimate the probability for hide contamination with EHEC-7 among U.S. market beef cows at slaughter. The secondary objective was to evaluate the effects of geographic region of origin and season on EHEC-7 hide contamination of market cows at slaughter.

Materials and Methods

Sampling Methods

The cross-sectional study was conducted from February to November, 2015 at two U.S. cattle slaughter facilities, one located in the southern plains (Plant A) and one in the northern plains (Plant B). Both facilities slaughter a variety of cattle types including fed steers and heifers, market dairy cows, and market beef cows and bulls. Plant A processes approximately 1,700 cattle per day, and Plant B averages 1,750 head per day. Sampling occurred in the months of February, May, August, and November which represented the seasons of winter, spring, summer, and fall, respectively. During each season, both plants were visited within the same week. At each visit, 100 market beef cattle were sampled systematically in line order. The first market beef cow on the line after researchers' arrival was selected and every subsequent third market beef cow on the line was flagged for sampling. Dairy breed, dairy-cross breed, and cattle from feedlots were excluded from sampling. A total of 800 hide swabs were collected over the study period to determine the prevalence of EHEC-7 hide contamination in market beef cows at slaughter.

Four hundred samples from each geographical location gave 85% power to detect a difference in overall proportion of positive samples (for each of the EHEC-7 serogroup) between 20% and 12%, assuming $\alpha = 0.05$.

Hide surface samples were collected from cattle after exsanguination and prior to hide removal. Cattle were not subjected to chemical washes before samples were collected. However, at both slaughter facilities, live cattle were sprayed in lairage with

water to reduce dust. Hide samples were collected using sponges (Whirl-Pak® Speci-Sponge®, Nasco, Fort Atkinson, WI) suspended in 35 mL buffered peptone water (BPW). Prior to sampling, excess BPW was squeezed back into the Whirl-Pak bag. An area of at least 1,000 cm^2 behind the shoulder, approximately 15 cm from the midline was swabbed by 10 two-direction strokes across the area. After the first 5 strokes, the sponge was turned over to use both sides, and the sponge was then returned to the Whirl-Pak bag. Samples were then returned to the cooler and kept on ice packs. Samples were shipped to the laboratory to be received within 24 hr of collection.

To keep up with line speed, samples were obtained collectively by a team of three technicians. One technician identified cows to be sampled, collected back tag numbers or other identifying information, and tagged the animals using either spray paint or flagging ribbon to identify which cattle were sampled. The second technician carefully opened and closed Whirl-Pak bags to decrease cross-contamination potential between samples. The third technician was responsible for swabbing each hide and returning the used swab to its pre-labeled Whirl-Pak bag. New gloves were worn for every sample collected.

Cattle were scored for hide cleanliness at the time that the backtag information was obtained. There were two scores assigned to each cow. The first score was binary, indicating whether there was the presence of fresh manure on the hide. The second score was based on a scoring method previously described (Hauge et al., 2012). An ordinal scoring system categorized the amount of dried manure on hides where 1, 2, and 3 represented cows with no or very little dried manure, moderate amount of dried manure, and cattle with heavy amount of dried manure on the hide, respectively.

Region of Origin

Backtag information was obtained for the cattle, when available, to identify the state where they were last sold (salebarn state). When backtag information was not available, the lot and animal numbers were recorded, and the origin information was collected from the plant managers. State of origin was then categorized into northern or southern regions of the US. An arbitrary classification of region was determined to be Interstate 70, which runs east to west in the central US, approximating 39° latitude. Cattle originating north of Interstate-70 were categorized as "northern", and cattle from south of it were categorized as "southern".

Sample Processing

Hide sponge samples were processed within 24 h after collection. Sponge bags were manually mixed thoroughly upon arrival to the laboratory. Two-1 mL aliquots were removed and each placed in a 1.5 mL microcentrifuge tube, one containing 0.5 mL 50% Glycerol (diluted in BHI). Both aliquots were stored at -80°C. Ninety mL of *Escherichia coli* broth (EC; Oxoid Ltd., Hampshire, UK) was added to each hide sponge sample. The contents of this sample were manually mixed by squeezing the bag 5 times. Each EC broth-BPW suspension was incubated at 40°C for 6 h and held at 4°C until the following morning according to validated protocols (Stromberg et al., 2015a). A 1 mL aliquot of post-enrichment culture was frozen at -80°C in 0.5 mL 50% Glycerol (diluted in BHI). Finally, 250 µL of a 1 mL aliquot of post-enrichment culture was submitted to GeneSeek® (Lincoln, NE) for analysis. The remaining post-enrichment aliquot was stored at -80°C.

Detection of EHEC

The mass spectrometry-based NeoSEEK™ STEC Detection and Identification test (Neogen® Corp.) was used to detect EHEC in samples. The NeoSEEK™ test is a highly multiplexed (89 independent target) EHEC PCR assay that utilizes matrix-assisted laser desorption/ionization (MALDI)-time-of-flight (TOF) mass spectroscopy on a Sequenom® platform. NeoSEEK identified the genes for Shiga toxin (*stx*) and intimin (*eae*) as well as EHEC-7 based on *eae* subtypes and proprietary O group single nucleotide polymorphisms. If both the specific O group genes and *stx* were present within a sample, it was identified as Shiga toxin-producing *E. coli* (STEC). Further, the results of the NeoSEEK test were interpreted as positive for EHEC O26 when positive for O26, *stx*, and *eae*-Beta; positive for EHEC O45, O103, and O121 when positive for the respective O group genes, *stx*, and *eae*-Epsilon; positive for EHEC O111 when positive for O111, *stx*, and *eae*-Gamma2; and positive for EHEC-O145 and O157 when positive for the respective O group genes, *stx*, and *eae*-Gamma1. From these interpretations, samples were correspondingly recorded as positive or negative for each of the EHEC serogroups.

Statistical Analysis

Descriptive statistics were performed using Microsoft Excel, 2013 (Microsoft Corporation, Redmond, WA). The probability of detecting each EHEC-7 serogroup was modeled in generalized linear mixed models using PROC GLIMMIX in SAS 9.4 (SAS

Institute Inc., Cary, NC) with logit link and binomial distribution. Separate models were developed for the outcome of each EHEC-7 serogroup. Manual forward model selection was utilized to evaluate the fixed effects of season, region, presence of fresh manure on the hides, and degree of dried manure on hides. The interaction effect of season and region was included in the model selection. The effect of plant was included as a random effect. If observations were missing information, such as region of origin or hide scores, they were excluded from statistical analysis. Seasons without any positive samples detected were excluded from analysis to allow for model convergence. Therefore, in the model for the outcome of EHEC O111, the winter season was excluded from the analysis, and for the outcome of EHEC O157, the spring season was excluded. Additionally, in the model for the outcome of EHEC O121, region was included in the model, despite its insignificance, because it allowed for model convergence. Differences in least squares means were determined for outcomes with significant effects using the LSMEANS statement and the DIFF option. To account for the effect of multiple comparisons, the simulate adjustment was used. Model-adjusted predicted probabilities to detect each of the EHEC-7 were obtained using the ILINK function in the LSMEANS statement. Generalized chi-square to degrees of freedom (df) ratios were assessed to determine model fit. Statistical significance was set at $\alpha = 0.05$.

Results

EHEC-7

Six hundred and thirty of the 800 (79%) hide samples collected were positive for at least one serogroup of EHEC-7. Of the 400 samples each obtained at Plants A and B, 370 (93%) and 260 (65%) were positive for at least one EHEC-7 serogroup, respectively. There were 56 samples obtained from cattle without region of origin information and 27 without either hide score due to sampling error. Table 2.1 includes the unadjusted proportion of hides that tested positive for each EHEC-7 serogroup for each season, region, and separate hide scores. Table 2.2 includes the final multivariable logistic regression models developed for each serogroup. Of the 800 cattle sampled, 365 (46%) were from the northern region, 379 (47%) were from the southern region, and 56 (7%) cattle were missing origin information. At Plant A 79, 271, and 50 cattle were sampled from the northern, southern, and unknown regions, respectively. At Plant B 286, 108, and 6 cattle were sampled from the northern, southern, and unknown regions, respectively.

Table 2.1 Crude proportion of positive hide samples detected for each EHEC serogroup by season, region, and hide scores.

		EHEC O26		EHEC O45		EHEC O103		EHEC O111		EHEC O121		EHEC O145		EHEC O157	
Season	n	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive
Winter	200	1	0.5	97	48.5	75	37.5	0	0	1	0.5	36	18.0	43	21.5
Spring	200	14	7.0	95	47.5	59	29.5	99	49.5	133	66.5	60	30.0	0	0
Summer	200	42	21.0	125	62.5	77	38.5	67	33.5	5	2.5	24	12.0	16	8.0
Fall	200	72	36.0	120	60.0	78	39.0	23	11.5	1	0.5	51	25.5	30	15.0
Region	n	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive
Northern	365	49	13.4	194	53.2	127	34.8	50	13.7	63	17.3	64	17.5	22	6.0
Southern	379	77	20.3	218	57.5	136	35.9	111	29.3	69	18.2	85	22.4	62	16.4
Fresh Manure	n	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive
0	645	121	18.8	349	54.1	223	34.6	159	24.7	114	17.7	131	20.3	65	10.0
1	128	8	6.3	67	52.3	46	35.9	29	22.7	22	17.2	35	27.3	20	15.6
Dry Manure	n	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive
1	664	127	19.1	360	54.2	239	36.0	175	26.4	114	17.2	146	22.0	72	10.8
2	88	2	0.02	46	52.3	26	29.5	12	13.6	88	20.5	18	20.5	11	12.5
3	21	0	0	10	47.6	4	19.0	1	4.8	21	19.0	2	9.5	2	9.5

n: Total number of samples collected; denominator

x: Number of positive samples; numerator

Table 2.2 Separate final logistic regression models for hide contamination by each EHEC-7 serogroup.

Serogroup	Effect	Estimate	St. Err.	OR	95% CI	DF	P-value
EHEC O26	Intercept	-5.69	1.4			1	
	Season						<0.0001
	Winter	0	.	.		.	
	Spring	2.74	1.04	15.6	1.1, 211	795	
	Summer	4.10	1.02	60.5	4.7, 779	795	
	Fall	4.98	1.02	145.1	11.3, >999	795	
EHEC O45	Intercept	-0.45	0.58			1	
	Season						0.001
	Winter	0	.	.	.	.	
	Spring	0.39	0.22	1.5	0.83, 2.6	738	
	Summer	0.84	0.22	2.3	1.3, 4.1	738	
	Fall	0.61	0.21	1.8	1.1, 3.2	738	
	Region						0.009
	Northern	0.48	0.18	1.6	1.1, 2.3	738	
	Southern	0	.			.	
EHEC O103	Intercept	-0.12	0.65			1	
	Season						0.16
	Winter	0	.	.	.	.	
	Spring	-1.43	0.36	.	.	735	
	Summer	-0.75	0.31	.	.	735	
	Fall	-0.77	0.34	.	.	735	
	Region						0.004
	Northern	-1.09	0.37	.	.	735	
	Southern	0	.	.	.	.	
	Season × Region						<0.0001
	Winter N	0	.	0.34	0.11, 1.0	.	
	Winter S	0	.	.	.	.	
	Spring N	2.64	0.50	4.7	1.4, 15.2	735	
	Spring S	0	.	.	.	.	
	Summer N	2.27	0.47	3.3	1.1, 9.3	735	
	Summer S	0	.	.	.	.	
	Fall N	1.81	0.47	2.0	0.79, 5.3	735	
	Fall S	0	.			.	
EHEC O111	Intercept	-1.12	0.51			1	
	Season						<0.0001
	Spring	1.87	0.31	.	.	557	
	Summer	0	.	.	.	.	
	Fall	-1.35	0.42	.	.	557	
	Region						0.40
	Northern	1.18	0.37	.	.	557	
	Southern	0	.	.	.	.	
	Season × Region						<0.0001
	Spring N	-3.67	0.55	0.08	0.02, 0.33	557	
	Spring S	0	.	.	.	.	
	Summer N	0	.	3.26	1.1, 9.4	.	
	Summer S	0	.	.	.	.	
	Fall N	-0.58	0.58	1.8	0.5, 7.0	557	
	Fall S	0	.	.	.	.	

Table 2.2 (continued)

EHEC O121	Intercept	-5.32	1.03			1	
	Season						<0.0001
	Winter	0	.	.	.	.	
	Spring	6.37	1.02	582	43.9, >999	738	
	Summer	1.59	1.11	4.9	0.3, 80.3	738	
	Fall	-0.09	1.42	0.9	0.03, 32.9	738	
	Region						0.53
	Northern	0.21	0.33	1.2	0.64, 2.36	738	
	Southern	0	.	.	.	.	
EHEC O145	Intercept	-1.52	0.78			1	
	Season						0.02
	Winter	0	.	.	.	.	
	Spring	1.23	0.40	.	.	732	
	Summer	0.008	0.42	.	.	732	
	Fall	0.35	0.44	.	.	732	
	Region						0.62
	Northern	0.54	0.44	.	.	732	
	Southern	0	.	.	.	.	
	Season × Region						0.008
	Winter N	0	.	1.7	0.46, 6.4	.	
	Winter S	0	.	.	.	.	
	Spring N	-1.53	0.64	0.37	0.08, 1.7	732	
	Spring S	0	.	.	.	.	
	Summer N	-0.61	0.71	0.93	0.15, 5.6	732	
	Summer S	0	.	.	.	.	
	Fall N	0.52	0.55	2.9	0.97, 8.5	732	
	Fall S	0	.	.	.	.	
	Fresh hide score						0.03
	0	-0.65	0.29	0.5	0.3, 0.9	732	
	1	0	.	.	.	.	
EHEC O157	Intercept	-1.62	1.66			1	
	Season						<0.0001
	Winter	0	.	.	.	.	
	Summer	-1.64	0.36	0.19	0.08, 0.45	574	
	Fall	-0.62	0.31	0.54	0.30, 1.1	574	
	Region						0.04
	Northern	-0.64	0.31	0.53	0.29, 0.98	574	
	Southern	0	.			.	

For season × region interactions, odds ratios (OR) are comparing regional effects within each season.

Multivariable Model for EHEC O26

In total, 129 of the 800 (16%) hide samples were positive for EHEC O26. Season was associated with probability for hide contamination by EHEC O26 ($P < 0.0001$) (Fig.

2.1). Compared to the winter, odds for detecting O26 on hides were greater in the spring (OR = 15.6, 95% CI: 1.1, 211.8), summer (OR = 60.5, 95% CI: 4.7, 779.3), and fall (OR = 145.1, 95% CI: 11.3, >999). The chi-square to df ratio was equal to 1.82.

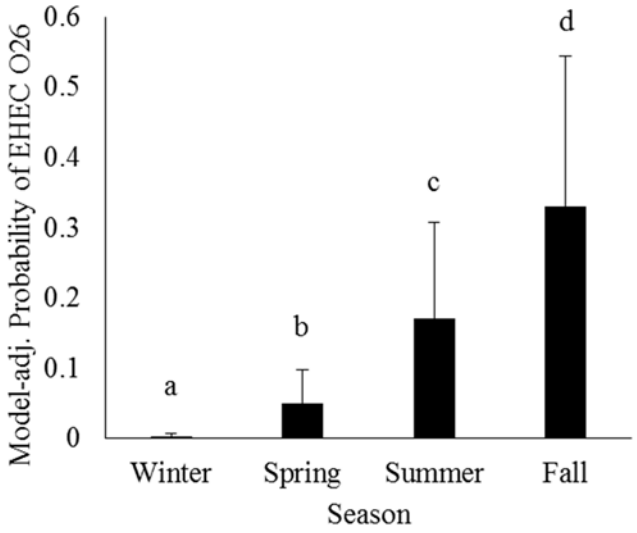

Figure 2.1 The model-adjusted probability for detecting EHEC O26 on the hides of beef cows at slaughter for each season.

Differing superscripts are significantly different at $\alpha = 0.05$. Error bars represent one standard error.

Multivariable Model for EHEC O45

Of the 800 total hide samples, 437 (55%) were positive for EHEC O45. The main effects of season ($P = 0.001$) and region ($P = 0.009$) were associated with the probability for hides to test positive for EHEC O45 in the multivariable logistic regression model. The seasonal effects are shown in figure 2.2. Compared to the winter, hide contamination

by EHEC O45 was more likely in the fall (OR = 1.8, 95% CI: 1.2, 2.8) and summer (OR = 2.3, 95% CI: 1.5, 3.6). Compared to winter, there was no difference in probability for EHEC O45 hide contamination in the spring (OR = 1.4, 95% CI: 0.95, 2.3). Hide contamination by EHEC O45 was more likely in cattle from the northern region than cattle from the southern region (OR = 1.6, 95% CI: 1.1, 2.3). The chi-square to df ratio was equal to 1.01.

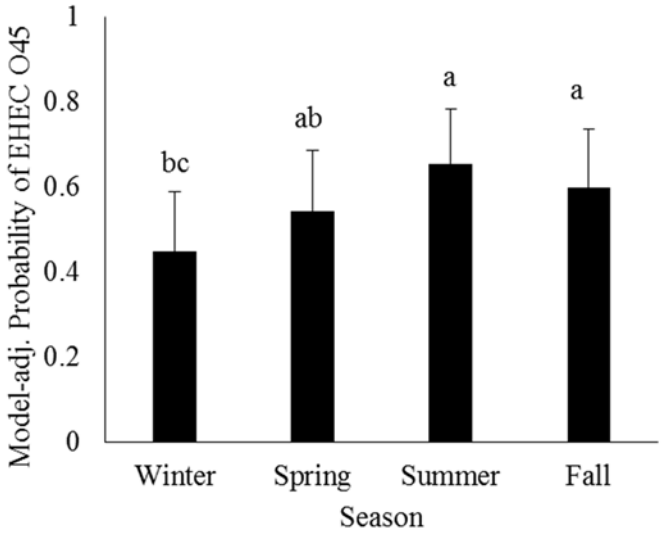

Figure 2.2 The model-adjusted probability for detecting EHEC O45 on the hides of beef cows at slaughter for each season.

Differing superscripts are significantly different at $\alpha = 0.05$. Error bars represent one standard error.

Multivariable Model for EHEC O103

In total, 289 of the 800 (36%) hide samples were positive for EHEC O103. The interaction effect of season and region was associated with the probability for hides to

test positive for EHEC O103 (Fig. 2.3). In the spring (OR = 4.7, 95% CI: 1.4, 15.2) and summer (OR = 3.3, 95% CI: 1.1, 9.3) cattle from the northern region had increased odds to have hides contaminated by EHEC O103 compared to southern cattle. However, in winter or fall there were no regional differences in odds for cattle to have hides contaminated by EHEC O103. The chi-square to df ratio was equal to 1.01.

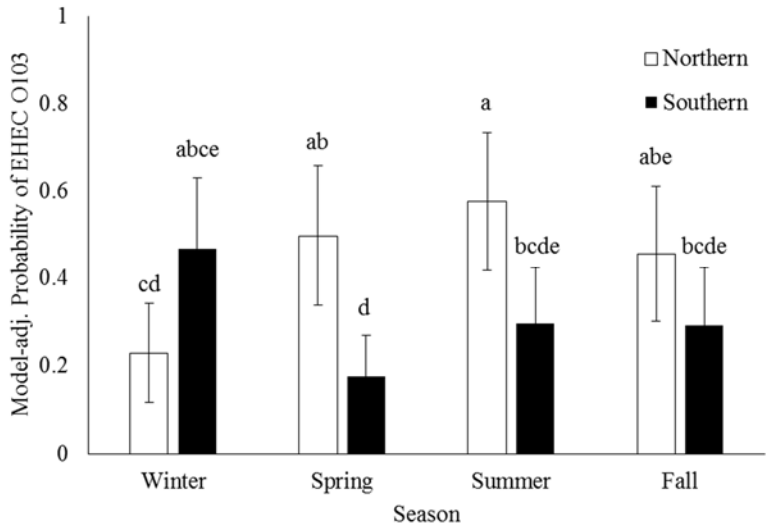

Figure 2.3 The model-adjusted probability for detecting EHEC O103 on the hides of beef cows at slaughter for the season by region interaction.

Differing superscripts are significantly different at $\alpha = 0.05$. Error bars represent one standard error.

Multivariable Model for EHEC O111

In total, 189 of the 800 (24%) hide samples were positive for EHEC O111. The interaction of season and region was associated with odds for market cattle to have EHEC

O111 on hides at slaughter (Fig. 2.4). In the spring cattle from the north were less likely (OR = 0.08, 95% CI: 0.02, 0.33) to have hides contaminated compared to cattle from the south; however in the summer, cattle from the north had increased odds (OR = 3.3, 95% CI: 1.1, 9.4) to be contaminated by EHEC O111. In the fall there was no difference in odds for hide contamination by EHEC O111 between cattle from the northern or southern regions. The chi-square to df ratio was equal to 1.01.

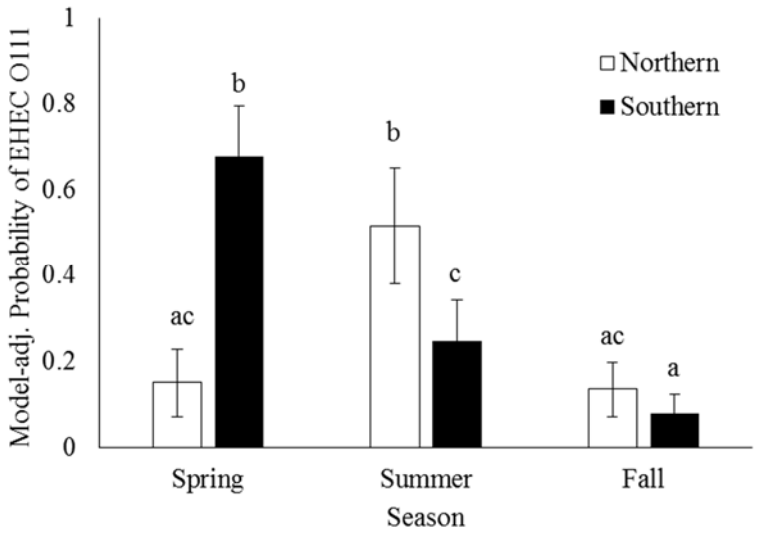

Figure 2.4 The model-adjusted probability for detecting EHEC O111 on the hides of beef cows at slaughter for the season by region interaction.

The winter season is excluded due to no positive samples being detected. Different superscripts within a given season indicate a significant difference by region at $\alpha = 0.05$. Error bars represent one standard error.

Multivariable Model for EHEC O121

In total, 140 of the 800 (18%) hide samples were positive for EHEC O121.

Season was associated with the probability to detect EHEC O121 on hides of market beef

cattle. In the spring, there was greater probability for cattle to have contaminated hides by EHEC O121 than during any other season. Compared to odds for hide contamination in the spring, there were protective effects during winter (OR = 0.002, 95% CI: <0.001, 0.02), summer (OR = 0.008, 95% CI: 0.003, 0.03), and fall (OR = 0.002, 95% CI: <0.001, 0.02). Region was included in the model because it allowed for convergence, although it was not associated with probability to detect EHEC O121 ($P = 0.53$). The chi-square to df ratio was equal to 1.04.

Multivariable Model for EHEC O145

In total, 171 of the 800 (21%) hide samples were positive for EHEC O145. The interaction effect of season and region ($P = 0.008$) (Fig. 2.5) and the presence of fresh manure on the hides ($P = 0.03$) were associated with the detection of EHEC O145. For the season by region interaction, there were no regional differences within each season. However, cattle from the south had significantly greater odds to have contaminated hides in the spring than in the winter (OR = 3.4, 95% CI: 1.0, 11.5) or summer (OR = 3.4, 95% CI: 1.2, 9.3). Cattle that had visible fresh manure on the hides at the time of swabbing were more likely (OR = 1.9, 95% CI: 1.1, 3.4) to have hides contaminated by EHEC O145 than cattle that did not have fresh manure on the hides. The chi-square to df ratio was equal to 1.01.

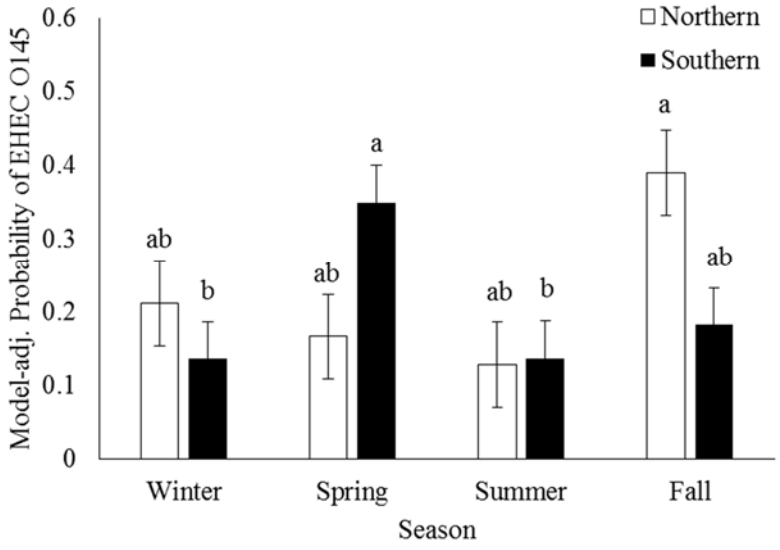

Figure 2.5 The model-adjusted probability for detecting EHEC O145 on the hides of beef cows at slaughter for the season by region interaction.

Different superscripts within a given season indicate a significant difference by region at $\alpha = 0.05$. Error bars represent one standard error.

Multivariable Model for EHEC O157

In total, 89 of the 800 (11%) hide samples were positive for EHEC O157. The effects of season ($P = 0.0001$) and region of origin ($P = 0.04$) were associated with the detection of EHEC O157 on hides of market beef cattle at slaughter. There were decreased odds to detect EHEC O157 on hides in the summer compared to the winter (OR = 0.19, 95% CI: 0.08, 0.45) or fall (OR = 0.36, 95% CI: 0.15, 0.85) (Fig. 2.6). Odds for EHEC O157 hide contamination were greater for cattle from the south (OR = 1.9, 95% CI: 1.0, 3.5) compared to cattle from the north. The chi-square to df ratio was equal to 0.89.

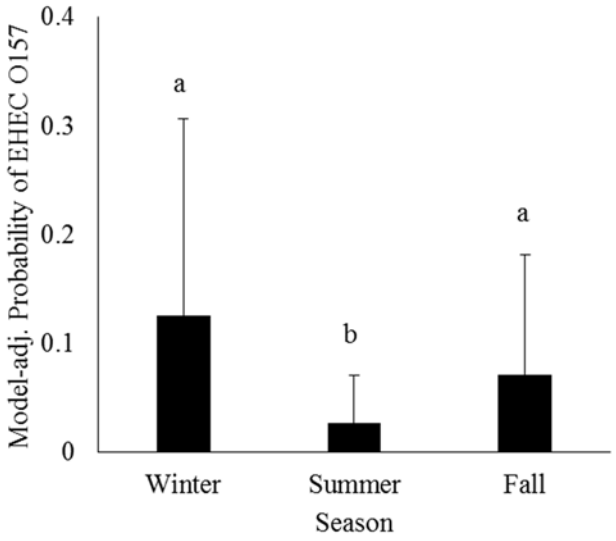

Figure 2.6 The model-adjusted probability for detecting EHEC O157 on the hides of beef cows at slaughter for each season

The spring season is excluded due to no positive samples being detected. Differing superscripts are significantly different at $\alpha = 0.05$. Error bars represent one standard error.

Discussion

To the best of our knowledge, this is the first study to report the prevalence of individual EHEC-7 serogroups in hide samples from market beef cattle at slaughter. The prevalence of EHEC O157 at slaughter in this study was lower than that detected in previous studies (Brichta-Harhay et al., 2008, Barkocy-Gallagher et al., 2003). One study reported that the prevalence of *E. coli* O157:H7 hide contamination of cull beef and dairy cattle at slaughter ranged from 39% to 56%, with no significant effect of season (Brichta-Harhay et al., 2008). The prevalence estimate of EHEC O157 on hides of market beef cattle in the present study more closely resembled that of a study of 355 hides of beef cattle slaughtered at Midwestern packing plants, in which the reported prevalence was

10.7% (Elder et al., 2000). In another study of EHEC prevalence on hides of fed cattle, collective non-O157 EHEC prevalence was highest in the fall at 77.7% (258 of 332) (Barkocy-Gallagher et al., 2003). In our study, we identified individual non-O157 EHEC serogroups and individually identified risk factors, such as season, associated with positive hide samples. Further, the EHEC serogroups we detected in hide samples are classified as adulterants in raw, non-intact beef by the USDA-FSIS. Interestingly, for several EHEC serogroups, the interaction of season by region was associated with the probability to detect positive hide samples. Therefore, the effect of season depended on the cattle's region of origin.

In our study, there were differences in prevalence of EHEC-7 between the two packing plants. Of course, there were many unmeasured differences between the two plants related to management, physical facilities, and employee practices. The purpose of this study was not to evaluate how these various plant-level factors affect EHEC detection. The inclusion of plant in the models as a random effect accounts for the lack of independence between cattle sampled within the two plants due to plant-level factors.

Visual cleanliness of hides was not predictive of hide contamination by EHEC serogroups, except for EHEC O145, where visible fresh manure increased the odds for detection of the organism. The association between visual hide cleanliness scores and microbial contamination has been inconsistent (Van Donkersgoed et al., 1997, Antic et al., 2010, Smith, et al., 2005, Nastasijevic et al. 2008). In a study where 100 cattle hides were sampled at slaughter, the highest prevalence of *E. coli* O157 was found on hides and carcasses of the dirtiest ordinal visual hide scores. However, there was not a linear

relationship between visual hide scores and the presence of EHEC O157 in that study (Blagojevic et al., 2012). In our study, there were numerically lower probabilities to detect EHEC O26, O103, and O111 on hides with higher scores for presence of dried manure. Although the score for dried manure was not significant in the multivariable models, this inverse relationship is worth noting. Others have reported that sites on the hides with the greatest amount of visible fecal contamination had the lowest relative EHEC O157 isolation rates (Keen et al., 2002). In our study, scoring visual hide cleanliness was not a good predictor of hide contamination by most EHEC-7 serogroups.

The NeoSEEK™ STEC Detection and Identification Test has been utilized to detect "top six" non-O157 and O157 EHEC in samples of bovine feces, hides, and dehided carcass surfaces (Bosilevac et al., 2013; Wang et al., 2014; Stromberg et al., 2015, 2016a; Agga et al., 2017). However, validation of the NeoSEEK test was performed on beef trim samples, not on the above-mentioned sample types. It is possible that within a sample, molecular markers could have originated from more than one bacterial cell, causing a false-positive result. Nevertheless, there is no adequate agar plating isolation method for non-O157 EHEC, and these culture methods are insensitive, and prone to false negative results and under-reporting of prevalence (Bosilevac et al., 2013; Wang et al., 2014; Noll et al., 2015; Stromberg et al., 2015, 2016a, 2016b; Agga et al., 2017).

Interpretation of the results of this study requires consideration that our results were obtained by sampling cattle on eight days in four seasons in a single year. There are important temporal effects on EHEC carriage by cattle (Khaitsa et al., 2003, Pearce et al.,

2004). Therefore, our specific results could have differed depending on the day of sampling. Rather than considering the prevalence estimates for any EHEC serogroup as absolute season and region parameters, we believe it is the relationships discovered in the regression models that are important. For example, that detection of each EHEC serogroup may differ by season or region, or possibly interact by conditions of season and region. Most importantly, we note that these relationships differed by serogroup.

The effect of season on EHEC O157 carriage in cattle has been studied for many years. Our study is the first to test the effects of season and region of origin on the probability to detect individual EHEC-7 serogroups in market beef cows. The relationship between season and region was different for each EHEC serogroup. The various season-by-region interactions for each EHEC serogroup may represent differences in the production environments of cow-calf operations in the northern and southern US. However, it is more likely these results demonstrate that conditions that differ over time and place affect the risk that cattle carry each EHEC-7 serogroup differently.

Previously, risk factors for hide contamination by non-O157 EHEC, have not been evaluated at the serogroup level (Barkocy-Gallagher et al., 2003). However, our findings suggest that risk factors might best be evaluated for each EHEC serogroup separately because each EHEC is uniquely affected by conditions associated with season and region.

References

Antic, D., B. Blagojevic, M. Ducic, I. Nastastijevic, R. Mitrovic, and S. Buncic, 2010: Distribution of microflora on cattle hides and its transmission to meat via direct contact. *Food Control* 21, 1025-1029.

Arthur, T. M., G. A. Barkocy-Gallagher, M. Rivera-Betancourt, and M. Koomaraie, 2002: Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* on carcasses in commercial beef cattle producing plants. *Appl. Environ. Microbiol.* 68, 4847-4852.

Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L. Wheeler, M. P. Kent, D. Jaroni, B. Pauling, D. M. Allen and M. Koohmaraie, 2004: *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *J. Food Prot.* 67, 658-665.

Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler and M. Koohmaraie, 2003: Seasonal prevalence of Shiga toxin–producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66, 1978-1986.

Bell, B. P., M. Goldoft, P. M. Griffin, M. A. Davis, D. C. Gordon, P. I. Tarr, C. A. Bartleson, J. H. Lewis, T. J. Barrett, J. G. Wells, R. Baron, and J. Kobayashi, 1994: A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. *JAMA,* 272**,** 1349-1353. doi: 10.1001/jama.1994.03520170059036

Blagojevic, B., D. Antic, M. Ducic, and S. Buncic, 2012: Visual cleanliness scores of cattle at slaughter and microbial loads on the hides and carcases. *Vet. Rec.*, 170, 563.

Bosilevac, J. M., T. M. Arthur, T. L. Wheeler, S. D. Shackelford, M. Rossman, J. O. Reagan, and M. Koohmaraie, 2004: Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and *Enterobacteriaceae* are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. *J. Food Prot.,* 67, 646-650.

Brichta-Harhay, D. M., M. N. Guerini, T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler and M. Koohmaraie, 2008: *Salmonella* and *Escherichia coli* O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States: an evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. *Appl. Environ. Microbiol.,* 74**,** 6289-6297.

Chapman, P. A., C. A. Siddons, D. J. Wright, P. Norman, J. Fox and E. Crick, 1993: Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiol. Infect.,* 111, 439-447.

Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie and W. W. Laegreid, 2000: Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97, 2999-3003. doi: 10.1073/pnas.060024897

Geue, L., M. Segura-Alvarez, F. J. Conraths, T. Kuczius, J. Bockemuhl, H. Karch and P. Gallien, 2002: A long-term study on the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) on four German cattle farms. *Epidemiol. Infect.* 129**,** 173-185.

Hancock, D. D., T. E. Besser, M. L. Kinsel, P. I. Tarr, D. H. Rice and M. G. Paros, 1994: The prevalence of *Escherichia coli* O157.H7 in dairy and beef cattle in Washington State. *Epidemiol. Infect.* 113, 199-207.

Hauge, S. J., O. Nafstad, O.-J. Røtterud and T. Nesbakken, 2012: The hygienic impact of categorisation of cattle by hide cleanliness in the abattoir. *Food Control* 27, 100-107.

Keen, J. E. and R. O. Elder, 2002: Isolation of shiga-toxigenic *Escherichia coli* O157 from hide surfaces and the oral cavity of finished beef feedlot cattle. *J. Am. Vet. Med. Assoc.* 220, 756-763.

Khaitsa, M. L., D. R. Smith, J. A. Stoner, A. M. Parkhurst, S. Hinkley, T. J. Klopfenstein, and R. A. Moxley, 2003: Incidence, duration, and prevalence of *Escherichia coli* O157:H7 fecal shedding by feedlot cattle during the finishing period. *J. Food Prot.* 66, 1972-1977.

Koohmaraie, M., T. M. Arthur, J. M. Bosilevac, M. Guerini, S. D. Shackelford, and T. L. Wheeler, 2005: Post-harvest interventions to reduce/eliminate pathogens in beef. *Meat Sci.*, 71, 79-91.

Laegreid, W. W., R. O. Elder and J. E. Keen, 1999: Prevalence of *Escherichia coli* O157:H7 in range beef calves at weaning. *Epidemiol. Infect.* 123, 291-298.

Nastasijevic, I., R. Mitrovic, and S. Buncic, 2008: Occurrence of *Escherichia coli* O157 on hides of slaughtered cattle. *Lett. Appl. Microbiol.* 46, 126-131.

Nou, X., M. Rivera-Betancourt, J. M. Bosilevac, T. L. Wheeler, S. D. Shackelford, B. L. Gwartney, J. O. Reagan, and M. Koohmaraie, 2003: Effect of chemical dehairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and enterobacteriaceae on carcasses in a commercial beef processing plant. *J. Food Prot.*, 66, 2005-2009.

Pearce, M. C., C. Jenkins, L. Vali, A. W. Smith, H. I. Knight, T. Cheasty, H. R. Smith, G. J. Gunn, M. E. J. Woolhouse, S. G. B. Amyes, G. Frankel, 2004: Temporal shedding patterns and virulence factors of *Escherichia coli* serogroups O26, O103, O111, O145, and O157 in a cohort of beef calves and their dams. *Appl. Env. Microbiol.* 70, 1708-1716.

Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin and D. L. Swerdlow, 2005: Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg. Infect. Dis.* 11**,** 603-609.

Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake and M. L. Cohen, 1983: Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New Eng. J. Med.* 308, 681-685.

Robbins, A., M. Anand, D. C. Nicholas, J. S. Egan, K. A. Musser, S. Giguere, H. Prince, H. E. Beaufait, S. D. Sears, J. Borda, D. Dietz, T. Collaro, P. Evans, S. A. Seys and B. W. Kissler, 2014: Ground beef recall associated with non-O157 Shiga toxin-producing *Escherichia coli*, United States. *Emerg. Infect. Dis.* 20, 165-167.

Schmidt, J. W., T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, and T. L. .Wheeler, 2012: Detection of *Escherichia coli* O157:H7 and *Salmonella enterica* in air and droplets at three U.S. commercial beef processing plants. *J. Food Prot.* 75, 2213-2218.

Smith, D. R., R. A. Moxley, S. L. Clowser, J. D. Folmer, S. Hinkley, G. E. Erickson, and T. J. Klopfenstein, 2005: Use of rope devices to describe and explain the feedlot ecology of *Escherichia coli* O157:H7 by time and place. *Foodborne Pathog. Dis.* 2, 50-60.

Smith, D. R., R. A. Moxley, T. J. Klopfenstein and G. E. Erickson, 2009: A randomized longitudinal trial to test the effect of regional vaccination within a cattle feedyard on *Escherichia coli* O157:H7 rectal colonization, fecal shedding, and hide contamination. *Foodborne Pathog. Dis.* 6, 885-892. doi: 10.1089/fpd.2009.0299

Stromberg, Z. R., G. L. Lewis, D. B. Marx, and R. A. Moxley, 2015a: Comparison of enrichment broths for supporting growth of Shiga toxin-producing *Escherichia coli*. *Curr. Microbiol.,* 71, 214-219. doi: 10.1007/s00284-015-0824-8

Stromberg, Z. R., N. W. Baumann, G. L. Lewis, N. J. Sevart, N. Cernicchiaro, D. G. Renter, D. B. Marx, R. K. Phebus and R. A. Moxley, 2015b: Prevalence of enterohemorrhagic *Escherichia coli* O26, O45, O103, O111, O121, O145, and O157 on hides and preintervention carcass surfaces of feedlot cattle at harvest. *Foodborne Patho. Dis.,* 12**,** 631-638. doi: 10.1089/fpd.2015.1945

Stromberg, Z. R., G. L. Lewis, S. S. Aly, T. W. Lehenbauer, J. M. Bosilevac, N. Cernicchiaro and R. A. Moxley, 2016: Prevalence and level of enterohemorrhagic *Escherichia coli* in culled dairy cows at harvest. *J. Food Prot.,* 79**,** 421-431. doi: 10.4315/0362-028X.JFP-15-368

USDA-FSIS, 2012: Shiga Toxin-Producing *Escherichia coli* in Certain Raw Beef Products. Available at https://www.fsis.usda.gov/OPPDE/rdad/FRPubs/2010-0023FRN.pdf (accessed 06 August 2015).

USDA-National Agricultural Statistics Service, 2007: Livestock Slaughter 2006 Summary. Available at: http://usda.mannlib.cornell.edu/usda/nass/LiveSlauSu//2000s/2007/LiveSlauSu-03-02-2007.pdf (accessed 14 November 2015).

USDA-NASS, 2008: Livestock Slaughter 2007 Summary. Available at: http://usda.mannlib.cornell.edu/usda/nass/LiveSlauSu//2000s/2008/LiveSlauSu-03-07-2008_revision.pdf (accessed 14 November 2015).

USDA-NASS, 2009: Livestock Slaughter 2008 Summary. Available at: http://usda.mannlib.cornell.edu/usda/nass/LiveSlauSu//2000s/2009/LiveSlauSu-03-06-2009.pdf (accessed 14 November 2015).

USDA-NASS, 2010: Livestock Slaughter 2009 Summary. Available at: http://usda.mannlib.cornell.edu/usda/nass/LiveSlauSu//2010s/2010/LiveSlauSu-04-29-2010_new_format.pdf (accessed 14 November 2015).

USDA-NASS, 2011: Livestock Slaughter 2010 Summary. Available at: http://usda.mannlib.cornell.edu/usda/nass/LiveSlauSu//2010s/2011/LiveSlauSu-04-25-2011.pdf (accessed 14 November 2015).

USDA-NASS, 2012: Livestock Slaughter 2011 Summary. Available at: http://usda.mannlib.cornell.edu/usda/nass/LiveSlauSu//2010s/2012/LiveSlauSu-04-23-2012.pdf (accessed 14 November 2015).

USDA-NASS, 2013: Livestock Slaughter 2012 Summary. Available at: http://usda.mannlib.cornell.edu/usda/nass/LiveSlauSu//2010s/2013/LiveSlauSu-04-22-2013.pdf (accessed 14 November 2015).

USDA-NASS, 2014: Livestock Slaughter 2013 Summary. Available at: http://usda.mannlib.cornell.edu/usda/nass/LiveSlauSu//2010s/2014/LiveSlauSu-04-21-2014.pdf (accessed 14 November 2015).

USDA-NASS, 2015: Livestock Slaughter 2014 Summary. Available at: http://usda.mannlib.cornell.edu/usda/nass/LiveSlauSu//2010s/2015/LiveSlauSu-04-27-2015.pdf (accessed 14 November 2015).

USDA-NASS, 2016: Livestock Slaughter 2015 Summary. Available at: http://usda.mannlib.cornell.edu/usda/nass/LiveSlauSu//2010s/2016/LiveSlauSu-04-20-2016.pdf (accessed 14 November 2015).

Van Donkersgoed, J., K. W. F. Jericho, H. Grogan, and B. Thorlakson, 1997. Preslaughter hide status of cattle and the microbiology of carcasses. *J. Food Prot.* 60, 1502-1508.

Wells, J. E., S. D. Shackelford, E. D. Berry, N. Kalchayanand, M. N. Guerini, V. H. Varel, T. M. Arthur, J. M. Bosilevac, H. C. Freetly, T. L. Wheeler, C. L. Ferrell and M. Koohmaraie, 2009: Prevalence and level of *Escherichia coli* O157:H7 in feces and on hides of feedlot steers fed diets with or without wet distillers grains with solubles. *J. Food Prot.,* 72**,** 1624-1633.

CHAPTER III

A STUDY OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* IN MISSISSIPPI AND NEBRASKA BEEF COW-CALF HERDS

(Prepared for submission to Zoonoses and Public Health)

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Abstract

The objective of this study was to describe the probability of detecting seven serogroups of enterohemorrhagic *Escherichia coli* (EHEC-7) of public health importance in fecal samples from beef cow-calf herds, and to test for factors associated with their detection. Fresh fecal samples (n = 85) from two Mississippi herds and two Nebraska herds were collected in each of 4 seasons. Samples were tested for each EHEC-7 serogroup by a molecular screening assay. Separate management groups within herds were sampled, and group-level factors were recorded. To measure the effects of factors on fecal shedding of EHEC-7, separate multivariable logistic regression models were used, accounting for the random effect of clustering by group. Statistical significance was set $\alpha = 0.05$. In total, 59 samples (4.3%) were positive for EHEC O26, and samples from Nebraska herds were more likely to be positive than Mississippi samples (OR = 16.4, 95% CI: 1.2, 200). EHEC O45 was present in 44 samples (3.2%). For every 1°C temperature increase, odds for detection increased (OR = 1.1, 95% CI: 1.0, 1.2). Odds for EHEC O45 detection decreased if a precipitation event occurred (OR = 0.05, 95% CI: 0.004, 0.52). EHEC O103 was detected in 66 samples (4.9%) with greatest probability to be detected in the summer. EHEC O111 was detected in 71 samples (5.2%). Odds of O111 detection were increased in Nebraska compared to Mississippi (OR = 7.8, 95% CI: 1.3, 47.6), and odds for detection increased (OR = 1.3, 95% CI: 1.1, 1.6) with temperature. In total, 43 samples (3.2%) were positive for EHEC O145, and the greatest probability for detection was in the summer. These results indicate that there is great spatial and temporal variation in the probability to detect EHEC-7 in cow-calf herds. Factors that vary by time

and place, such as precipitation, ambient temperature, region, and season, are associated with the probability to detect EHEC-7 in fresh feces collected from cow-calf herds.

Introduction

Enterohemorrhagic *Escherichia coli* (EHEC) are foodborne pathogens with a bovine reservoir (Chapman et al., 1993, Hancock et al., 1994, Armstrong et al., 1996, Laegreid et al., 1999). EHEC-contaminated beef continues to be an important source of human EHEC infection (Riley et al., 1983, Bell et al., 1994, Rangel et al., 2005, Robbins et al., 2014). Seven EHEC serogroups (EHEC-7) are classified as adulterants in raw, non-intact beef: O26, O45, O103, O111, O121, O145, and O157 (USDA-FSIS, 2012). These seven serogroups were responsible for 92.3 % of all human EHEC cases from 2000-2010 (Gould et al., 2013).

Animal and environmental risk factors associated with the carriage of EHEC O157 have been well documented (Smith et al., 2001, Gannon et al., 2002, Smith et al., 2005, Renter et al., 2004, Renter et al., 2005, Callaway et al., 2009). These factors include age, season, diet, and feedlot pen-floor conditions. However, there is only limited published information regarding the ecology and prevalence of non-O157 EHEC, especially in beef cow-calf herds (Baltasar et al., 2014, Pearce et al., 2004). Mature beef cows marketed for slaughter comprised approximately 8% of all federally inspected cattle slaughtered in 2015 (USDA-NASS, 2016), and they often enter the food chain as ground beef, an important source of EHEC foodborne illness. Pre-harvest control of EHEC might prevent market cows from carrying the pathogens into the abattoir (Sargeant et al., 2007, Smith et al., 2010). Therefore, the objective of this study was to describe the probability of detecting EHEC O157 and non-O157 EHEC in the feces of beef cows and to identify explanatory factors.

Materials and Methods

This longitudinal study was conducted from February to September 2016 at 2 cow-calf herds each in Mississippi and Nebraska, for a total of 4 herds. Sampling was performed seasonally where February, April, July, and September represented the seasons of winter, spring, summer, and fall, respectively. Mississippi and Nebraska herds utilized rotational grazing where smaller subsets of cows and calves were moved from pasture to pasture to meet nutritional demands. Therefore, multiple management groups within a single herd were sampled each day, and there was no guarantee that the groups were maintained over the entire year.

Sample collection

A gloved technician collected approximately 20-30 g of fresh fecal material from fecal pats on the ground from adult cows. Samples were placed into pre-labeled sterile plastic specimen containers using new, clean plastic spoons. Containers were stored in a cooler until sampling was completed. After all samples were collected, contents from each container were stirred for approximately 30 seconds using 2 foam tipped swabs (catalog no. 89031-280; VWR International, Buffalo Grove, IL). Each swab contained approximately 1 g of fecal material. The two swabs were aseptically broken off into a pre-labeled 15 mL conical tube containing 10 mL chilled (5-10°C) EC broth (Oxoid). The suspended fecal swabs were transported or shipped overnight to the laboratory on ice and processed within 24 h.

Sample processing, handling, and EHEC detection

Samples were processed according to described methodology (Schneider et al., 2017a). Briefly, fecal swabs were left in the tubes of EC broth and incubated at 40°C for 6 h followed by a 4°C hold in a programmable incubator until further processed. Further processing included a 10 s vortexing, 1 min settling time, followed by removal of one 1 ml aliquot for DNA preparation. A 250 µl subsample of the 1 ml aliquot was submitted to GeneSEEK™ (Lincoln, NE) for NeoSEEK™ STEC testing.

Detection of EHEC-7 was performed by the mass spectrometry-based NeoSEEK™ STEC Detection and Identification test (Neogen® Corp.). Information regarding the use and validation of the NeoSEEK test have been previously described (Schneider et al., 2017a). In short, analysis included identification of *stx* and *eae* genes as well as top 6 non-O157 EHEC and EHEC O157 detection based on *eae* subtypes and proprietary O group single nucleotide polymorphisms.

Variables recorded at sampling

Group-level factors were recorded at the time of sampling. These factors included the season, region (MS vs NE), calving season, presence of calves within group at the time of sampling, diet, water source, high temperature (°C) on the day of sampling, and if a precipitation event occurred within 7 days prior to sampling.

Statistical methods

Descriptive statistics were performed using Microsoft Excel (2013, Microsoft Corporation, Redmond, WA). Group-level prevalence was the proportion of positive samples detected from within the group at the time of sampling. The probability of detecting each EHEC-7 serogroup was modeled in generalized linear mixed models using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC) with logit link and binomial distribution, where "events" were the number of EHEC-positive fecal samples and "trials" were the total number of fecal samples collected on each respective sampling day. Separate models were developed for the response variable of each EHEC-7 serogroup. Manual forward model selection was utilized to test the main effects. Clustering by management group was accounted for as a random effect. Statistical significance was set $\alpha = 0.05$. Differences in least squares means were determined for outcomes with significant effects using the LSMEANS statement and the DIFF option. Model-adjusted predicted probabilities to detect each of the EHEC-7 were obtained using the ILINK function in the LSMEANS statement. Generalized chi-square to degrees of freedom (df) ratios were assessed to determine model fit. Statistical significance was set at $\alpha = 0.05$.

Results

Over the entire study, 196 (14.4%) of the 1,357 fecal samples collected were positive for at least one EHEC-7 serogroup. Numerically, the greatest prevalence of detection of every EHEC serogroup occurred in the summer samples (Fig. 3.1). Table 3.1 presents the total of samples collected and tested positive for each EHEC serogroup by season and

farm. Table 3.2 shows the final logistic regression models developed for the outcome of each EHEC serogroup.

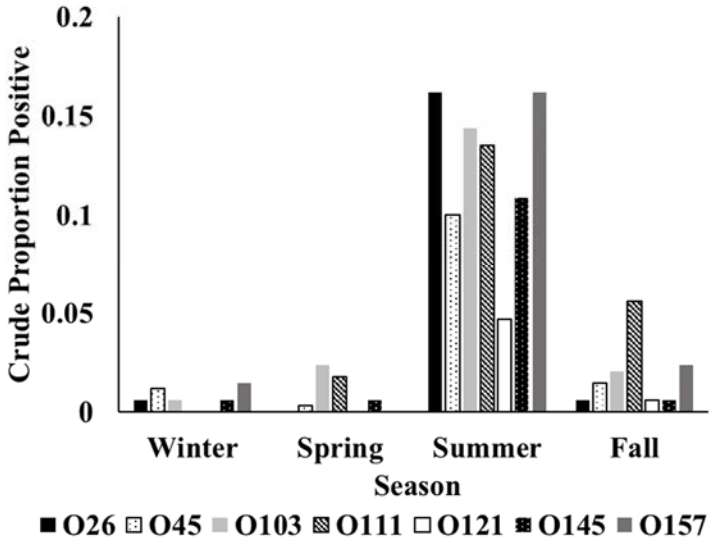

Figure 3.1 Proportion of positive fecal samples collected from beef cow-calf herds by season.

Table 3.2 Proportion of positive fecal samples detected for each EHEC serogroup by season and farm.

		EHEC O26		EHEC O45		EHEC O103		EHEC O111		EHEC O121		EHEC O145		EHEC O157	
	n	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive	x	% positive
Winter	**340**	**2**	**0.6**	**4**	**1.2**	**2**	**0.6**	**0**	**0**	**0**	**0**	**2**	**0.6**	**5**	**1.5**
Farm A	85	0		0		2	0.6	0		0		1	1.2	5	5.9
Farm B	85	1	1.2	0				0		0		0		0	
Farm C	85	1	1.2	0				0		0		0		0	
Farm D	85	0		4	4.7			0		0		1	1.2	0	
Spring	**337**	**0**		**1**	**0.3**	**8**	**2.4**	**6**	**1.8**	**0**		**2**	**0.6**	**0**	
Farm A	85	0		1	1.2	2	2.4	0		0		0		0	
Farm B	83	0		0		6	7.1	6	7.2	0		2	2.4	0	
Farm C	84	0		0		0		0		0		0		0	
Farm D	85	0		0		0		0		0		0		0	
Summer	**340**	**55**	**16.2**	**34**	**10.0**	**49**	**14.4**	**46**	**13.5**	**16**	**4.7**	**37**	**10.9**	**55**	**16.2**
Farm A	85	0		4	4.7	18	21.2	4	4.7	0		4	4.7	0	
Farm B	85	0		1	1.2	4	4.7	12	14.1	0		0		0	
Farm C	85	12	14.1	11	12.9	10	11.8	7	8.2	0		31	36.5	18	21.2
Farm D	85	43	50.6	18	21.2	17	20.0	23	27.1	16	18.8	0		37	43.5
Fall	**340**	**2**	**0.6**	**5**	**1.5**	**7**	**2.1**	**19**	**5.6**	**2**	**0.6**	**2**	**0.6**	**8**	**2.4**
Farm A	85	2	2.4	4	4.7	3	3.5	0		0		0		3	3.5
Farm B	85	0		0		4	4.7	0		0		0		5	5.9
Farm C	85	0		1	1.2	0		9	10.6	2	2.4	2	2.4	0	
Farm D	85	0		0		0		10	11.8	0		0		0	

n: number of samples collected

x: number of positive samples detected

Farm A and B were located in Mississippi. Farms C and D were located in Nebraska.

Table 3.3 Separate final logistic regression models for detection of each EHEC-7 serogroup in fecal samples from cow-calf herds accounting for the random effect of group.

Serogroup	Effect	Estimate	St. Err.	OR	95% CI	DF	P-value
EHEC O26	Intercept	-6.00	0.90			32	
	Group	4.9	2.02				
	Region						0.04
	MS	0	.	.		.	
	NE	2.80	1.25	16.5	1.2, 220	21	
EHEC O45	Intercept	-9.98	1.82			33	
	Group	2.3	1.11				
	Rain						0.02
	No	3.10	1.17	22.2	1.9, 257	19	
	Yes	0	.	.	.	.	.
	TempHigh						0.05
	1° increase	0.06	0.03	1.1	1.0, 1.12	.	
EHEC O103	Intercept	-5.11	0.77			33	
	Group	1.8	0.74				
	Season						0.002
	Winter	0	.	.	.	.	
	Spring	1.36	0.89	3.9	0.6, 25.2	18	
	Summer	2.78	0.77	16.1	3.2, 80.5	18	
	Fall	0.96	0.86	2.6	0.4, 15.8	18	
EHEC O111	Intercept	-18.6	4.32			32	
	Group	2.7	1.10				
	Region						0.03
	MS	0	.	.	.	.	
	NE	2.06	0.86	7.8	1.3, 47.0	20	
	TempHigh						0.003
	1° increase	0.16	0.05	1.2	1.1, 1.3	20	
EHEC O145	Intercept	-5.38	0.85			33	
	Group	2.6	1.13				
	Season						0.05
	Winter	0	.	.	.	.	.
	Spring	-0.19	1.07	0.8	0.1, 7.8	18	
	Summer	2.13	0.92	8.4	1.2, 57.5	18	
	Fall	0.14	1.06	1.2	0.1, 10.6	18	

EHEC O26

In total, 59 fecal samples (4.3%) were positive for EHEC O26, ranging from 0 to 88% within management groups. Accounting for clustering by group, region was associated with the probability to detect EHEC O26 in fecal samples. Odds for detecting EHEC O26 from Nebraska samples were 16 times as great as Mississippi samples (OR = 16.4, 95% CI: 1.2-200).

EHEC O45

Forty-four (3.2%) samples were positive for EHEC O45 throughout the entire study, ranging from 0 to 49% of samples collected within management groups. In the multivariable model, the presence of precipitation event and the high temperature on the day of sampling were associated with EHEC O45 detection in manure samples. Odds for detection of EHEC O45 in manure samples decreased if a precipitation event occurred within 7 days prior to sampling (OR = 0.05, 95% CI: 0.004-0.5). For every 1° C increase in ambient temperature, odds to detect EHEC O45 increased (OR = 1.1, 95% CI: 1.0-1.2).

EHEC O103

Of 1,357 fecal samples, 66 (4.9%) were positive for EHEC O103 throughout the entire study. Prevalence within a group ranged from 0 to 41% at a given sampling time. Season was associated with probability to detect EHEC O103 in fecal samples. Compared to the summer, there were decreased odds of detecting EHEC O103 in the winter (OR = 0.06,

95% CI: 0.01-0.31), spring (OR = 0.24, 95% CI: 0.07-0.84), and fall (OR = 0.16, 95% CI: 0.05-0.49).

EHEC O111

EHEC O111 was detected in 71 (5.2%) samples over the entire study, ranging from 0 to 51% within groups at a point in time. Region and high temperature were associated with the probability to detect EHEC O111. Odds for detecting EHEC O111 were increased in Nebraska compared to Mississippi (OR = 7.8, 95% CI: 1.3-47.6). As temperature increased by 1° C, odds for detection increased (OR = 1.3, 95% CI: 1.1-1.6).

EHEC O145

Of 1,357 fecal samples, 43 (3.2%) were positive for EHEC O145 throughout the entire study. Prevalence ranged from 0 to 69% within management groups. Season was associated with the probability to detect EHEC O145 in manure samples. Compared to the summer, there were decreased odds for detection in the winter (OR = 0.12, 95% CI: 0.02-0.83), spring (OR = 0.10, 95% CI: 0.01-0.77), and fall (OR = 0.14, 95% CI: 0.02-0.91).

EHEC O121 and O157

Eighteen (1.3%) samples were positive for EHEC O121 throughout the entire study, with prevalence ranging from 0 to 37% within management groups. EHEC O157 was detected in 68 (5.0%) samples throughout the entire study. Prevalence within management groups

ranged from 0 to 86%. After adjusting for clustering by management group, we failed to detect any significant factors associated with the detection of EHEC O121 or EHEC O157.

Discussion

This study was one of the first to describe the probability of detecting EHEC-7 in fecal samples from cow-calf herds. Point prevalence in this study had large variation based on EHEC serogroup, region, season, and management group. This is likely due to the temporal nature of EHEC carriage by cattle, with detection of these organisms largely dependent upon time and place (Khaitsa et al., 2003, Pearce et al., 2004). Similar to our findings, a longitudinal study of cows and calves from a Scotland beef farm sampled for EHEC found shedding to be sporadic and random (Pearce et al., 2004).

Season was associated with the detection of several EHEC serogroups and the greatest numerical prevalence of all EHECs occurred in the summer. The seasonal impact on EHEC O157 fecal shedding has been well-documented, however, only a few studies have evaluated the effect of season on carriage of non-O157 EHEC in cattle (Dewsbury et al., 2015, Barkocy-Gallagher et al., 2003). Collectively, non-O157 EHEC hide contamination of fed cattle at slaughter was most prevalent in the fall season, but there was no discrimination between different serogroups (Barkocy-Gallagher et al., 2003). Dewsbury et al. collected pen-floor fecal samples from feedlots in the summer months and winter months to determine seasonal prevalence of EHEC-7. In that study, O26, O103, O145 and O157 were isolated in the summer months, but EHEC O45, O111, and O121 were not. In addition, EHEC O103, O26, O45, and O121 were detected in the

winter, but EHEC O111, O145, and O157 were not (Dewsbury, et al., 2015). In our study, all serogroups were detected in the summer and had numerically highest prevalence in the summer sampling. This seasonal difference was significant for EHEC O103 and EHEC O145. In contrast to the Dewsbury study, we did detect EHEC O145 and O157 in winter samples. The differences between our seasonal findings and the Dewsbury paper could be due to differences in production environment. Renter et al. (2005) reported significant differences by production environment in the prevalence of Shiga Toxin-producing (Stx) bacteria within cattle populations (Renter et al., 2005). Significantly greater odds to detect Stx-producing bacteria occurred in cows on pasture than any other production environment cohort (Renter et al., 2005). Seasonal effects on fecal shedding may affect the probability of EHEC transmission or survival in the environment.

Presence of EHEC O26 and O111 were associated with region and were more likely in samples from Nebraska than Mississippi. Each EHEC-7 serogroup, except EHEC O121, was detected in at least one sample from every farm, which illustrates how widespread the presence of these EHEC organisms is in beef cow-calf herds. However, EHEC O121 was only detected in samples from farms C and D, which represented the Nebraska farms. Regional differences in prevalence of EHEC in cattle have been observed in some studies (Islam et al., 2014, Schneider et al., 2017b). In Schneider et al., culled beef cattle were sampled at slaughter for EHEC hide contamination. In that study, there was a significant season by region interaction associated with detection of EHEC O111. In the summer, cattle from the north were more likely to carry EHEC O111 on hides than cattle from the south. This is similar to our finding of increased odds of

detection in NE farms. The current study only included sampling from 2 farms within each region at 4 time points. It would be beneficial to include additional years of sampling to assess if regional differences remain consistent for these organisms.

Our study is the first to our knowledge to assess the seasonality of EHEC-7 fecal shedding within beef cow-calf herds in the US. This study provides important data needed to populate a quantitative microbial risk assessment. One limitation to the current study is that our sampling occurred at each farm only one day within a given season. Also, this study only occurred during one year. However, this study provides insight to factors associated with the probability to detect these organisms in cow-calf herds, as well as prevalence estimates for each EHEC serogroup. This also illustrates the temporal and group effects of EHEC fecal shedding in cow-calf herds. Time and place remain important factors to consider when evaluating the ecology and distribution of EHEC-7 in cattle populations.

References

Armstrong, G. L., J. Hollingsworth and J. G. Morris, 1996: Emerging food-borne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.*, 18, 29-51.

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

Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler and M. Koohmaraie, 2003: Seasonal prevalence of Shiga Toxin–producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.*, 66, 1978-1986.

Bell, B. P., M. Goldoft, P. M. Griffin, M. A. Davis, D. C. Gordon, P. I. Tarr, C. A. Bartleson, J. H. Lewis, T. J. Barrett, J. G. Wells, R. Baron and J. Kobayashi, 1994: A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. *J.A.M.A.*, 272, 1349-1353.

Callaway, T. R., M. A. Carr, T. S. Edrington, R. C. Anderson and D. J. Nisbet, 2009: Diet, *Escherichia coli* O157:H7, and cattle: A review after 10 years. *Curr. Issues Mol. Biol.*, 11, 67-79.

Chapman, P. A., C. A. Siddons, D. J. Wright, P. Norman, J. Fox and E. Crick, 1993: Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiol. Infect.*, 111, 439-447.

Dewsbury, D. M., D. G. Renter, P. B. Shridhar, L. W. Noll, X. Shi, T. G. Nagaraja and N. Cernicchiaro, 2015: Summer and winter prevalence of Shiga Toxin-producing *Escherichia coli* (STEC) O26, O45, O103, O111, O121, O145, and O157 in feces of feedlot cattle. *Foodborne Pathog. Dis.*, 12, 726-732.

Gannon, V. P. J., T. A. Graham, R. King, P. Michel, S. Read, K. Ziebell and R. P. Johnson, 2002: *Escherichia coli* O157:H7 infection in cows and calves in a beef cattle herd in Alberta, Canada. *Epidemiol. Infect.*, 129, 163-172.

Gould, L. H., R. K. Mody, K. L. Ong, P. Clogher, A. B. Cronquist, K. N. Garman, S. Lathrop, C. Medus, N. L. Spina, T. H. Webb, P. L. White, K. Wymore, R. E. Gierke, B. E. Mahon and P. M. Griffin, 2013: Increased recognition of non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States during 2000-2010: Epidemiologic features and comparison with *E. coli* O157 infections. *Foodborne Pathog. Dis.*, 10, 453-460.

Hancock, D. D., T. E. Besser, M. L. Kinsel, P. I. Tarr, D. H. Rice and M. G. Paros, 1994: The prevalence of *Escherichia coli* O157.H7 in dairy and beef cattle in Washington State. *Epidemiol. Infect*., 113, 199-207.

Islam, M. Z., A. Musekiwa, K. Islam, S. Ahmed, S. Chowdhury, A. Ahad and P. K. Biswas, 2014: Regional variation in the prevalence of *E. coli* O157 in cattle: A meta-analysis and meta-regression. *PLoS. ONE*, 9, e93299.

Khaitsa, M. L., D. R. Smith, J. A. Stoner, A. M. Parkhurst, S. Hinkley, T. J. Klopfenstein and R. A. Moxley, 2003: Incidence, duration, and prevalence of *Escherichia coli* O157:H7 fecal shedding by feedlot cattle during the finishing period. *J. Food Prot.*, 66, 1972-1977.

Laegreid, W. W., R. O. Elder and J. E. Keen, 1999: Prevalence of *Escherichia coli* O157:H7 in range beef calves at weaning. *Epidemiol. Infect*., 123, 291-298.

Pearce, M. C., C. Jenkins, L. Vali, A. W. Smith, H. I. Knight, T. Cheasty, H. R. Smith, G. J. Gunn, M. E. J. Woolhouse, S. G. B. Amyes and G. Frankel, 2004: Temporal shedding patterns and virulence factors of *Escherichia coli* serogroups O26, O103, O111, O145, and O157 in a cohort of beef calves and Their Dams. *Appl. Environ. Microbiol*., 70, 1708-1716.

Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin and D. L. Swerdlow, 2005: Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg. Infect. Dis*., 11, 603-609.

Renter, D. G., G. Morris, J. M. Sargeant, L. L. Hungerford, J. Berezowski, T. Ngo, K. Williams and D. W. K. Acheson, 2005: Prevalence, risk factors, O serogroups, and virulence profiles of Shiga toxin-producing bacteria from cattle production environments. *J. Food Prot.*, 68, 1556-1565.

Renter, D. G., J. M. Sargeant and L. L. Hungerford, 2004: Distribution of *Escherichia coli* O157:H7 within and among cattle operations in pasture-based agricultural areas. *Am. J. Vet. Res.*, 65, 1367-1376.

Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake and M. L. Cohen, 1983: Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.*, 308, 681-685.

Robbins, A., M. Anand, D. C. Nicholas, J. S. Egan, K. A. Musser, S. Giguere, H. Prince, H. E. Beaufait, S. D. Sears, J. Borda, D. Dietz, T. Collaro, P. Evans, S. A. Seys and B. W. Kissler, 2014: Ground beef recall associated with non-O157 Shiga toxin-producing *Escherichia coli*, United States. . *Emerg. Infect. Dis*., 20, 165-167.

Sargeant, J. M., M. R. Amezcua, A. Rajic and L. Waddell, 2007: Pre-harvest interventions to reduce the shedding of *E. coli* O157 in the faeces of weaned domestic ruminants: a systematic review. *Zoonoses Public Health*, 54, 260-277.

Schneider, L. G., T. J. Klopfenstein, Z. R. Stromberg, G. L. Lewis, G. E. Erickson, R. A. Moxley and D. R. Smith, 2017a: A randomized controlled trial to evaluate the effects of dietary fibre from distillers grains on enterohemorrhagic *Escherichia coli* detection from the rectoanal mucosa and hides of feedlot steers. *Zoonoses Public Health*.

Schneider, L. G., Z. R. Stromberg, G. L. Lewis, R. A. Moxley and D. R. Smith, 2017b: Cross-sectional study to estimate the prevalence of enterohemorrhagic *Escherichia coli* on hides of market beef cows at harvest. Manuscript submitted for publication.

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

Smith, D. R., R. A. Moxley, S. L. Clowser, J. D. Folmer, S. Hinkley, G. E. Erickson and T. J. Klopfenstein, 2005: Use of rope devices to describe and explain the feedlot ecology of Salmonella by time and place. *Foodborne Pathog. Dis.*, 2, 61-69.

Smith, D. R., K. Novotnaj and G. Smith, 2010: Preharvest food safety: what do the past and the present tell us about the future? *J. Agromedicine*, 15, 275-280.

USDA-FSIS, 2012: Shiga Toxin-Producing *Escherichia coli* in Certain Raw Beef Products. Available at https://www.fsis.usda.gov/OPPDE/rdad/FRPubs/2010-0023FRN.pdf (accessed 06 August 2015).

USDA-NASS, 2016: Livestock Slaughter 2015 Summary. Available at: http://usda.mannlib.cornell.edu/usda/nass/LiveSlauSu//2010s/2016/LiveSlauSu-04-20-2016.pdf (accessed 14 November 2015).

CHAPTER IV

A RANDOMIZED CONTROLLED TRIAL TO EVALUATE THE EFFECTS OF DIETARY FIBER FROM DISTILLERS GRAINS ON ENTEROHEMORRHAGIC *ESCHERICHIA COLI* DETECTION FROM THE RECTOANAL MUCOSA AND HIDES IN FEEDLOT STEERS

(Accepted by Zoonoses and Public Health)

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Abstract

Feeding high levels (≥ 40% dry matter) of distillers grains may increase the risk for cattle to carry enterohemorrhagic *Escherichia coli* (EHEC) O157. The mechanism for the increased risk is not known, nor whether non-O157 EHEC are similarly affected. Our objective was to test if the fiber content or other components of modified distillers grains plus solubles (MDGS) affects the probability for cattle to carry EHEC serogroups of public health importance. A 2 x 2 plus 1 factorial treatment arrangement within a randomized block design was utilized. Within each of 4 blocks, 25 feedlot pens (n = 8 steers/pen) were assigned randomly to (1) corn-based control diet; (2) 20% dry matter (DM) MDGS; (3) 40% DM MDGS; (4) corn bran added to corn-based diet to match fiber of 20% MDGS or (5) 40% MDGS. Rectoanal mucosa swabs (RAMS) were collected on day (d)0, d35, d70, and d105; hide swabs were collected on the last feeding day. Samples were tested for EHEC by a molecular screening assay. The effects of fiber source and fiber level on EHEC carriage were tested using multi-level logistic regression (generalized linear mixed models; $\alpha = 0.05$). EHEC O45 RAMS detection was associated with fiber level, source, and sampling day. EHEC O103 RAMS detection increased by feeding 40% MDGS but not the corresponding corn bran diet. Hide contamination by EHEC O45 or O103 was less likely in cattle fed MDGS compared to corn bran diets. EHEC O111 RAMS detection decreased by feeding 40% MDGS but not by feeding the corresponding corn bran diet. Detection of EHEC O157 or O145 was not associated with dietary factors. Feeding 40% MDGS increased the probability for carriage of some EHEC serogroups but decreased probability of others, which indicated that EHEC

serogroups have different risk factors associated with feeding MDGS and little association with dietary fiber.

Introduction

Cattle populations are important reservoirs for enterohemorrhagic *Escherichia coli* (EHEC), and these foodborne pathogens are capable of causing severe human illness (Chapman et al., 1993; Hancock et al., 1994; Armstrong et al., 1996; Laegreid et al., 1999). Contaminated beef products have been implicated as an important source of human EHEC illness (Riley et al., 1983; Bell et al.,1994; Rangel et al., 2005; Robbins et al., 2014). Thus, seven serogroups of EHEC (EHEC-7) commonly associated with human illness have been declared adulterants in raw non-intact beef and raw intact beef intended for use in raw non-intact product, including: EHEC O26, O45, O103, O111, O121, O145, and O157 (USDA-FSIS, 2012).

There is growing interest in feedlot diets and their effects on EHEC fecal shedding. Due to increased availability in regions where ethanol production has flourished, corn-based distillers grains, an ethanol fermentation co-product derived from corn, have been increasingly used in feedlot diets (Klopfenstein et al., 2008). In 2007, a rise in ground beef contamination rates in the US led to increased research interest in factors contributing to the change, and the inclusion of distillers grains was a suspected risk factor (U.S. Grains Council, 2012). Several studies have reported increased fecal shedding or colonization of EHEC O157 in cattle fed diets containing corn distillers grains, specifically at high inclusion rates (>40% dry matter [DM]) in the finishing diet (Dewell et al., 2005; Peterson et al., 2007; Jacob et al., 2008a, 2008b, 2010; Wells et al., 2009; Rich et al., 2010). The mechanism by which this occurred is not well understood. Corn is approximately two-thirds starch. When starch from corn is fermented to produce

ethanol, the remaining fiber, fat, and protein are concentrated in the distillers grains by a factor of three (Klopfenstein et al., 2008; NASEM, 2016).

In a study comparing an 85% forage diet to a 15% forage diet, cattle fed the 85% forage diet shed higher concentrations of EHEC O157 and for longer duration (Van Baale et al., 2004). Similarly, Wells et al. (2005) found prolonged survival of EHEC O157 in feces from cattle fed bromegrass hay compared with feces from cattle fed corn silage. In a separate study comparing sheep fed grass hay diets to corn-based diets, fecal shedding of EHEC O157 by sheep fed the hay diet occurred in higher concentrations and for twice the duration of sheep fed corn diets (Kudva et al., 1997). These studies led to our hypothesis that the increased concentration of neutral detergent fiber (NDF) provided in modified distillers grains plus solubles (MDGS) diets could be the component causing increased fecal shedding observed when MDGS diets are fed at high levels of DM.

Therefore, the objective of this study was to determine whether the level of fiber or source of fiber in the diet affects the probability of detecting seven serogroups of EHEC in rectoanal mucosa swabs and hides of feedlot steers.

Materials and Methods

Experimental Animals

The study was performed at the University of Nebraska-Lincoln Eastern Nebraska Research and Extension Center feedlot with all procedures approved by the Institutional Animal Care and Use Committee. Eight hundred crossbred steers were obtained in the fall of 2014 and maintained on cornstalks over the winter. Then steers were fed a forage-based diet on dry lot until 5 days prior to the study start. Cattle were limit-fed a diet of

50% alfalfa and 50% Cargill Sweet Bran®, a corn gluten feed product, at 2% of body weight (BW) to reduce variation in gastrointestinal fill prior to two-day weigh-in. (Watson et al., 2013). Initial BW were calculated as the average weight over 2 consecutive days prior to the first feeding day on treatment diets (Stock et al., 1983).

Adaptation to finishing diets allows cattle to gradually adjust to increased starch in the diet. Cattle in this study were adapted to their respective finishing diets over a 5-step, 21 day period where alfalfa was replaced with increasing inclusion of dry-rolled corn. Steers were placed in their respective treatment pens on d0 of the trial (19 May 2015) and were concurrently started on the first of the adaptation diets. Steers completed the adaptation diets and were first fed their complete respective experimental diets on day 21 (9 June 2015) of the study.

Study Design

The study design was a 2 x 2 plus 1 factorial arrangement of treatments within a randomized complete block experimental design. There were two levels of fiber source (MDGS or corn fiber isolate), two levels of fiber concentration (17 % NDF or 22% NDF), and a corn control diet. Prior to the start of the study, steers were assigned to four blocks based on BW. The heaviest 200 cattle were assigned to block 1. Subsequent blocks were assigned 200 steers by decreasing BW, so that the lightest weight steers were the final group to go to slaughter. Pens within blocks were started on finishing diets at weekly intervals. Based on first-day weights, steers were stratified by BW for assignment to pens so that pens within blocks had similar average BW. Within each of 4 blocks, 25 feedlot pens (n=8 steers/pen) were assigned randomly using a random number generator in a balanced manner to the following corn-based diets: (1) control diet (CON); (2) 20%

DM MDGS (20MDGS); (3) 40% DM MDGS (40MDGS); (4) corn bran and solvent extract germ meal added to match NDF of 20% MDGS (20FIB) or (5) 40% MDGS (40FIB; Table 1). Dietary NDF of each diet was measured monthly by laboratory analysis (Van Soest et al., 1991).

Rectoanal mucosa swabs (RAMS) of each steer were collected d0, d35, d70, and d105. Pre-harvest hide swabs were collected in the feedyard the day before slaughter as cattle were loaded onto trucks for transport. Due to sampling error, hide samples from block 1 were not used in the analysis. Cattle from blocks 1, 2, and 3 were on feed for 134 days, while block 4 steers were fed for 148 days to reach market weight. Slaughter dates were September 30, October 7, 14, and November 4, for blocks 1, 2, 3, and 4, respectively. All steers were fed a beta$_2$ adrenergic receptor agonist, ractopamine hydrochloride (Optaflexx®, Elanco Animal Health; Greenfield, IN), at 300 mg/steer daily, for the last 28 days in the feedyard. One steer from the 40MDGS treatment of block 1 was removed from the trial on day 86 due to repeated bloating, one d105 sample was missing from a steer in the 40MDGS treatment of block 2, and one steer from the CON treatment of block 4 died on day 147.

Table 4.1 Composition (% of diet DM) of dietary treatments fed to 800 yearling steers on a study to test the effect of fiber level or fiber source on EHEC carriage.

	Treatment[1]				
Ingredient	CON	20MDGS	40MDGS	20FIB	40FIB
Dry-rolled corn	68.5	51.5	31.5	60	51.5
High-moisture corn	12	12	12	12	12
MDGS[2]	-	20	40	-	-
SEM[3]	-	-	-	1.5	3
Wet Corn Bran	-	-	-	7	14
Corn Silage	8	8	8	8	8
Alfalfa Hay	3.5	3.5	3.5	3.5	3.5
Supplement	8	5	5	8	8
Nutrient Composition, % of DM					
CP	14.1	15.1	19.8	14.1	13.3
NDF	11.0	16.7	22.0	16.6	22.2
ADF	4.5	6.6	8.6	6.0	7.5
Lignin	1.7	2.3	2.9	1.9	2.2

[1] Treatments included CON-control; 20MDGS-20% modified distillers grains plus solubles; 40MDGS-40% modified distillers grains plus solubles; 20FIB-fiber fed from concentrated ingredients to mimic fiber provided by 20MDGS; 40FIB-fiber fed from concentrated ingredients to mimic fiber provided by 40MDGS.
[2] MDGS: Modified distillers grains plus solubles,
[3] SEM: solvent extracted germ meal

Sample Size

During the study design phase we used a simulation model to estimate that 20 pens of 8 steers each per dietary treatment gave 80% power to detect differences in the

proportion of positives between 30% to 16%, at alpha = 0.05 and assuming moderate pen-level clustering effect.

Blinding

Sample containers were identified with barcodes and were cross-referenced to animal identification at the cattle handling chute. Laboratory results were recorded by barcode number. The dietary treatment information was maintained separately from the laboratory database and combined only at the end of the study. In this manner, all research personnel collecting feces and hide samples in the feedlot were blind to previous microbiological results, and laboratory personnel were blind to animal identification, dietary treatments, and previous results. Feedlot personnel were not blind to which pens received respective diets, but were not involved in any microbial sample collection.

Rectoanal Mucosa Collection and Methodology

Cattle were restrained in a squeeze chute for sample collection. RAMS were collected from each steer by inserting a foam-tipped swab (catalog no. 89031-280; VWR International, Buffalo Grove, IL) 3-5 cm into the anus and swabbing the mucosal surface. Immediately following RAMS collection, the swab was aseptically broken off into a conical tube containing 5 ml chilled (5-10°C) EC broth (Oxoid). RAMS were transported to the laboratory on ice and processed within 24 h.

Rectoanal Mucosa Swab Processing and Handling

Upon arrival in the laboratory, each RAMS in the 15 ml tube was vortexed at high speed for 10 s and any debris was allowed to settle for 1 min (Bosilevac et al., 2013; Agga et al., 2017). Two 1 ml aliquots were removed: 1 ml for prevalence DNA

preparation and 1 ml for future culture-based work; to the latter, 0.5 ml 50% sterile BHI-glycerol was added. These pre-enrichment aliquots were stored at -80°C. The RAMS were left in the tubes of EC broth and incubated at 40°C for 6 h followed by a 4°C hold in a programmable incubator until further processed. Further processing included a 10 s vortexing, 1 min settling time, followed by removal of one 1 ml aliquot for prevalence DNA use and one 1 ml aliquot for future culture-based work. To the latter 0.5 ml 50% sterile BHI-glycerol was added, and this post enrichment aliquot was stored at -80°C. A 250 µl subsample of the 1 ml prevalence DNA aliquot was submitted to GeneSEEK™ (Lincoln, NE) for NeoSEEK™ STEC testing (as described below).

Hide Sample Collection and Methodology

Prior to loading cattle onto trucks to go to the abattoir, hide surface samples were collected from the dorsal midline by a gloved worker swabbing an approximately 1,000 cm^2 area with a 10 × 23-cm sponge (Speci-Sponge®; Nasco, Fort Atkinson, WI) originally suspended in 35 ml of buffered peptone water (BPW). Sponges were then returned to the Whirl-Pak® bag, placed on ice, and transported to the laboratory for processing within 24 h of collection.

Hide Sample Processing and Handling

Upon arrival at the laboratory, a 1ml aliquot of the BPW-sample was removed and placed in a microcentrifuge tube containing 0.5 ml 50% glycerol (diluted in BHI) and frozen at -80°C. An additional 1 ml aliquot of the BPW-sample was transferred to a microcentrifuge tube and stored at -80°C. To the remaining BPW-sample in the bag, 90 ml of EC was added, leaving the sponge in the bag to retain bacteria that might be held

within its porous surfaces. The contents of this sample were manually mixed by squeezing the bag five times. The samples were enriched in the EC-BPW suspension at 40°C for 6 h and held at 4°C until the following morning. A 1 ml aliquot of post-enrichment culture was frozen at -80°C in 0.5 mL 50% glycerol (diluted in BHI). Two hundred fifty µl of a second 1 ml aliquot was removed and submitted to GeneSEEK™ for NeoSEEK™ STEC testing (as described below). The remaining post-enrichment aliquot was stored at -80°C.

Detection of EHEC

DNA prepared from 250 µl of each of the prevalence DNA aliquots (previously prepared from enriched RAMS or hide sponges and stored at 4°C) was tested for EHEC-7 by the mass spectrometry-based NeoSEEK™ STEC Detection and Identification test (Neogen® Corp.). The NeoSEEK™ test is a highly multiplexed (89 independent target) EHEC PCR assay that utilizes matrix-assisted laser desorption/ionization (MALDI)-time-of-flight (TOF) mass spectroscopy on a Sequenom® platform. This test was validated on studies involving beef trim, but has been used for EHEC detection in samples of bovine feces, hides, and carcass surfaces (Bosilevac et al., 2013; Stromberg et al., 2015, 2016; Wang et al., 2014; Agga et al., 2017). Analysis included identification of *stx* and *eae* genes as well as top 6 non-O157 EHEC and EHEC O157 detection based on *eae* subtypes and proprietary O group single nucleotide polymorphisms. Samples were identified as Shiga toxin-producing *E. coli* (STEC) if the specific O group and *stx* genes were present. Using the NeoSEEK™ test, EHEC were defined as STEC O26 and *eae*-Beta; STEC O45, O103, and O121 and *eae*-Epsilon; STEC O111 and *eae*-Gamma2; and STEC-O145 and

O157 and *eae*-Gamma1. Samples were recorded as positive or negative for each of the EHEC serogroups.

Statistical Analysis

A commercially available statistical software, SAS 9.4, was utilized to perform analyses (SAS Institute Inc., Cary NC). Separate analyses using generalized linear mixed models (PROC GLIMMIX of SAS) were performed on RAMS and hide results for each serogroup. For all analyses, statistical significance was defined at α equal to 0.05. Multilevel, multivariable logistic regression accounting for random effects of clustering by pen within block, block, and the autoregressive correlation structure defining the repeated measure of animal identification over time was used test the effects of fiber level and fiber source on the probability of detecting each EHEC-7 serogroup from RAMS or hide samples. Because of the dietary adaptation period, which lasted from d0 to d21 of the study, only d70 and d105 samples (n = 1,278) were included in these analyses. Models were selected using manual forward selection. The source of fiber was a categorical variable, MDGS or corn bran. Dietary fiber level (NDF) was tested as a continuous and as a categorical variable, in each serogroup model to determine the best model-fit. In the final analysis, the level of NDF within diets was included in all models as a categorical variable because the levels fed were discrete, and model fit was either improved or was not different compared to models including NDF level as a continuous variable. Variables tested as fixed effects included source, NDF level, and sample period, as well as the 2-way interactions of source × NDF level, source × sampling period, and NDF level × sampling period. Differences in least squares means were determined for outcomes with significant effects using the LSMESTIMATE statement, and the simulate

adjustment option was used to account for multiple comparisons. Model-adjusted predicted probabilities to detect each EHEC-7 serogroup were obtained using the ILINK option in the LSMEANS statement. In similar fashion, separate univariable logistic regression analyses were performed for each serogroup to test if there was an effect of sample period on the probability to detect EHEC from RAMS using data from all sampling periods. Finally, multivariable multilevel logistic regression was used to test if there were differences in the probability to detect each EHEC-7 serogroup for each treatment compared to the corn control diet. The random effects of pen within block, block, and the autoregressive correlation structure defining the repeated measure of animal identification over time were included. Samples (n = 1,598) from d 70 and d105 from all steers were included in the analyses. Differences in the least squares means for specific comparisons of each diet to the control were determined using the LSMESTIMATE statement in SAS and the simulate adjustment for multiple comparisons.

Results

RAMS Samples

In total 3,198 RAMS samples were submitted for EHEC detection and identification. There were no significant differences in prevalence for each EHEC serogroup between treatment groups, pens, or blocks at d0. Figure 4.1 includes the proportion of steers RAMS positive for each EHEC serogroup throughout the study based on NeoSEEK STEC analysis.

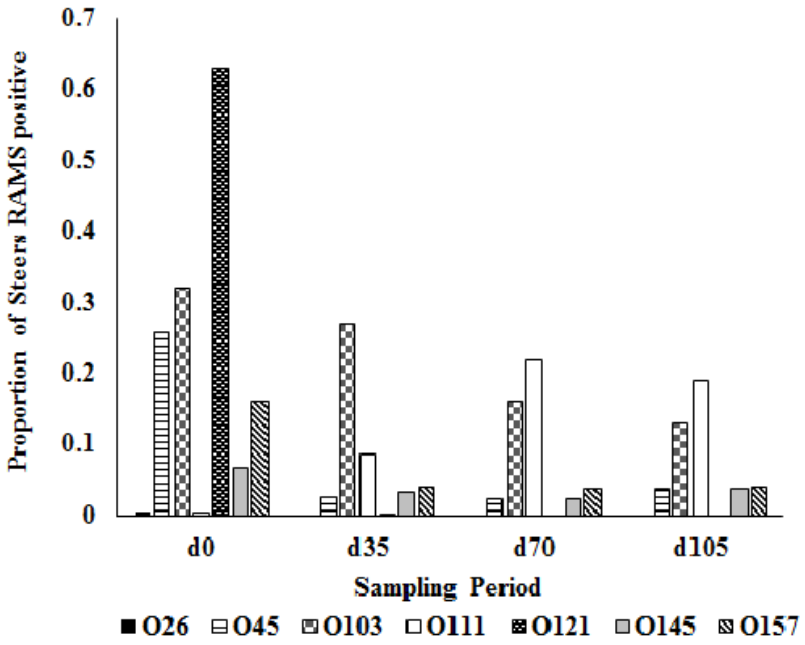

Figure 4.1 The proportion of EHEC-7 positive rectoanal mucosa swabs (RAMS) from 800 feedlot cattle on a study to test the effect of fiber level or fiber source on EHEC carriage during each sampling period.

EHEC O26

There were 4 (0.13%) EHEC O26 positive RAMS samples out of 3,198 throughout the entire study. All of these samples were collected at d0, therefore no analysis was performed to determine factors associated with EHEC O26 RAMS detection.

EHEC O45

A total of 275 (8.6%) samples were positive for EHEC O45. At d0, 206 (25.8%) of 800 steers tested positive for EHEC O45. This was greater than any other sample

period ($P < 0.0001$). There were 18 (2.3%) and 31 (3.9%) RAMS positive steers at d70 and d105, respectively. In the multivariable model, there were significant main effects of sample period ($P = 0.0007$), NDF level ($P < 0.0001$), and NDF source ($P = 0.006$). The interaction of NDF level and source ($P = 0.26$) was not associated with the probability to detect EHEC O45. The effect of sample period is shown in Figure 4.2. Compared to d70, the odds of detecting EHEC O45 in RAMS were 2.1 times greater at d105 (95% CI: 1.4-3.1). The effect of NDF level is shown in Figure 4.3. Compared to 17% NDF, the odds of detecting O45 was 9.1 times greater in steers fed 22% NDF (95% CI: 3.1-26.3). Figure 4.4 shows the effect of fiber source on probability to detect EHEC O45 in RAMS samples. Feeding MDGS increased the probability to detect O45 in RAMS samples compared to corn fiber isolate diets (OR = 4.1, 95% CI: 1.5-11.1).

Compared to the probability to detect EHEC O45 in cattle fed the corn control diet, cattle fed the 40% MDGS diet were more likely to have EHEC O45 positive RAMS ($P = 0.005$). Odds for detecting EHEC O45 in cattle fed 40% MDGS were 10.6 times as great as cattle fed the corn control diet (OR = 10.6, 95% CI: 1.7-66.7).

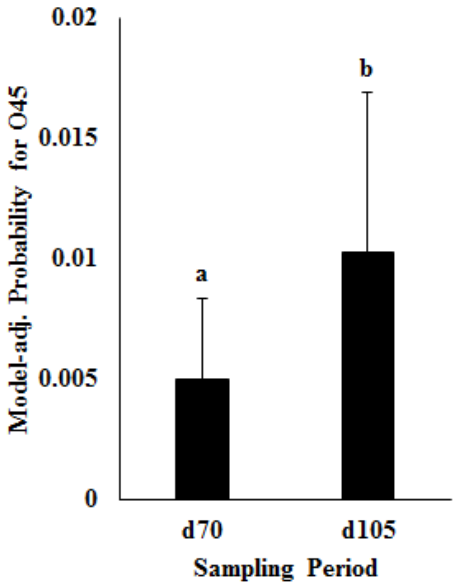

Figure 4.2 The effect of sampling period on the probability for detecting EHEC O45 from rectoanal mucosa swabs (RAMS) of 800 feedlot cattle on a study to test the effect of fiber level or fiber source on EHEC carriage.

The model-adjusted probability to detect EHEC O45 was greater at day 105 compared to day 70. Error bars represent one SE of the mean.

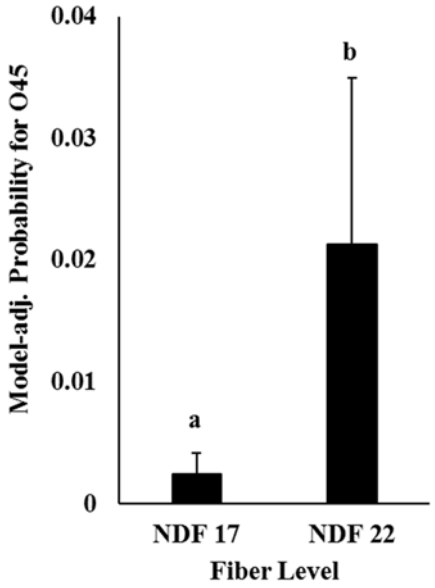

Figure 4.3 The effect of fiber level on the probability for detecting EHEC O45 from rectoanal mucosa swabs (RAMS) of 800 feedlot cattle on a study to test the effect of fiber level or fiber source on EHEC carriage.

Error bars represent one SE of the mean.

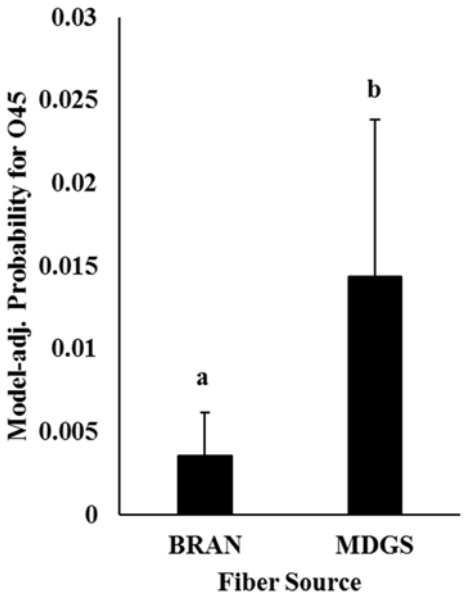

Figure 4.4 The effect of fiber source on the probability for detecting EHEC O45 from rectoanal mucosa swabs (RAMS) of 800 feedlot cattle on a study to test the effect of fiber level or fiber source on EHEC carriage.

Error bars represent one SE of the mean.

EHEC O103

A total of 703 (22.0%) samples were positive for EHEC O103 over the entire study. At d0, 256 (32%) were positive for O103. Probability to detect EHEC O103 was greater at d0 than any other sampling day ($P < 0.0001$). At d70 and d105, there was a total of 231 (14.5%) RAMS positive samples out of 1,598. In the multivariable model, the interactions of NDF level × source ($P = 0.02$) and source × sampling period ($P = 0.03$) were associated with the detection of EHEC O103 in RAMS samples. The interaction of NDF level and source is shown in Figure 4.5. At 17% NDF there was no

difference between probability to detect EHEC O103 in cattle fed MDGS and cattle fed the corn bran diet ($P = 0.68$). At 22% NDF, there was increased probability to detect EHEC O103 from RAMS samples from cattle fed MDGS compared to cattle fed the corn bran diet ($P < 0.0001$). For the interaction of source × sampling period, there was no difference in EHEC O103 detection between sampling periods for cattle fed the corn bran diets ($P = 0.89$). However, when MDGS was fed there was increased probability to detect EHEC O103 at d70 compared to d105 (OR = 1.8, 95% CI: 1.2-2.6; $P = 0.03$).

When comparing the probability to detect EHEC O103 in cattle fed the corn control diet to the probability in cattle fed other diets, only cattle fed the 40% MDGS diet were different ($P < 0.0001$). Odds for detecting EHEC O103 in RAMS from cattle fed 40% MDGS were 3.6 times as great as cattle fed the corn diet (95% CI: 1.8-7.1).

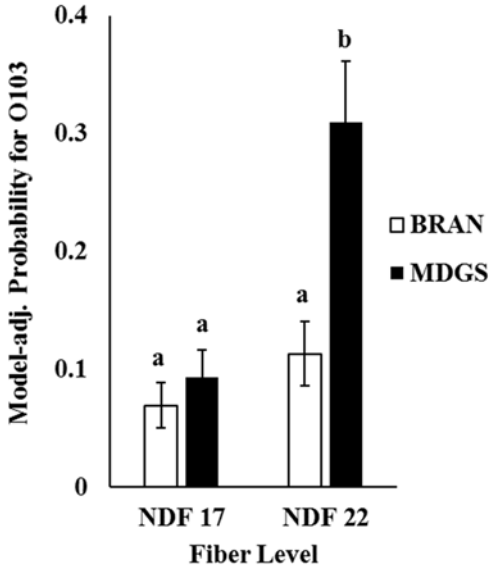

Figure 4.5 The interaction effect of fiber level and fiber source on probability to detect EHEC O103 from rectoanal mucosa swabs (RAMS) of 800 feedlot cattle.

Error bars represent one SE of the mean.

EHEC O111

In total, there were 399 (12.5%) RAMS samples positive for EHEC O111. Unlike other serogroups, EHEC O111 was more likely to be detected at d70 or d105 than d0 or d35 ($P < 0.0001$). Of the 399 positive samples, 327 (82%) were from d70 or d105. The interaction of NDF level × source was associated with the probability to detect EHEC O111 from RAMS samples ($P = 0.03$) (Fig. 4.6). At 17% NDF, the inclusion of MDGS did not affect probability of EHEC O111 shedding ($P = 0.59$). However, when NDF was 22% and MDGS was the source of fiber, probability of detecting EHEC O111 in feces decreased compared to the matched corn bran diet (OR = 0.4, 95% CI: 0.3-0.6; $P = 0.0002$).

Compared to cattle fed the control corn diet, cattle fed the 40% MDGS diet had lower probability for detection of EHEC O111 in RAMS ($P = 0.03$). Cattle fed the 40% MDGS diet had 0.5 times the odds to have EHEC O111 positive RAMS samples than cattle fed the corn control diet (95% CI: 0.3-0.96).

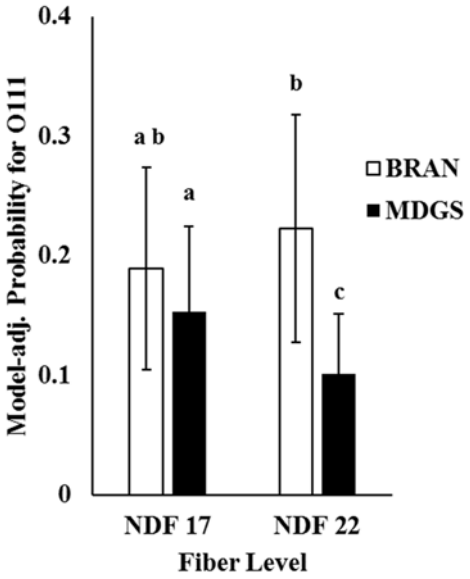

Figure 4.6 The interaction effect of fiber level and fiber source on probability to detect EHEC O111 from rectoanal mucosa swabs (RAMS) of 800 feedlot cattle.

Error bars represent one SE of the mean.

EHEC O121

There were 504 (15.8%) total RAMS samples positive for EHEC O121, and 503 (99.8%) of those occurred at d0. Only 1 sample at d35 was positive for EHEC O121 and none were positive at d75 or d105. Therefore, no statistical analysis was performed to determine dietary effects on RAMS detection.

EHEC O145

In total, there were 128 (4.0%) RAMS samples positive for EHEC O145. At d0, 53 out of 800 samples were positive, which was a greater proportion than any other sampling day ($P < 0.01$). The percent of RAMS samples positive for EHEC O145 was

2.3% and 3.8% at d70 and d105. We were unable to analyze the effects of NDF level and source on the probability to detect EHEC O145 because the model failed to converge.

EHEC O157

There was a total of 225 (7.0%) positive RAMS samples for EHEC O157 throughout the study. At d0 129 (16.1%) of 800 samples were positive for EHEC O157. This was a greater prevalence than any other sample day ($P < 0.0001$). The percent of RAMS samples positive for EHEC O157 was 3.8% and 4.1% at d70 and d105, respectively. We failed to detect any significant explanatory variables: source ($P = 0.42$), NDF level ($P = 0.87$), and sample period ($P = 0.80$). There was no interaction between NDF level and source ($P = 0.31$) (Fig. 4.7).

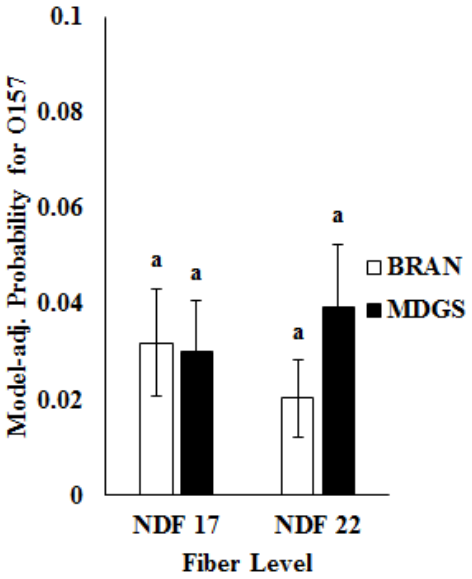

Figure 4.7 The interaction effect of fiber level and fiber source on probability to detect EHEC O157 from rectoanal mucosa swabs (RAMS) of 800 feedlot cattle.

The interaction was not associated with probability to detect EHEC O157 ($P = 0.31$). Error bars represent one SE of the mean.

Hide Samples

Hide samples (n = 589) were submitted for EHEC detection. Figure 4.8 provides the proportions of hide swabs positive for each EHEC serogroup the day before slaughter.

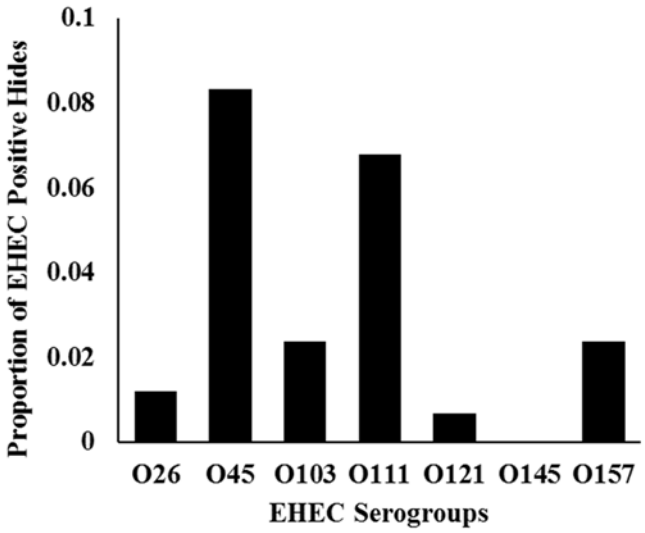

Figure 4.8 Crude proportion of EHEC-7 positive hide swabs on the day prior to slaughter from 589 feedlot cattle on a study to test the effect of fiber level or fiber source on EHEC carriage.

EHEC O45 and O103

The main effect of source was associated with the detection of EHEC O45 (P = 0.04) and EHEC O103 (P = 0.04) on the hides at loadout. The inclusion of MDGS in the diet had a protective effect where the likelihood of detecting EHEC O45 (OR = 0.3, 95%

CI: 0.1-0.97) or EHEC O103 (OR = 0.1, 95% CI: 0.01-0.9) on hides was decreased compared to cattle fed diets of corn bran fiber.

EHEC O26, O111, O121, O157

Overall prevalence of EHEC O26, O111, O121, and O157 was low (≤ 6.8%) at the feedyard on the day before slaughter. In each of the separate models for these organisms, we failed to detect any significant explanatory variables associated with the probability to detect these serogroups on hides at loadout.

Discussion

The fiber level of the diet was associated with the probability of detecting EHEC O45 from RAMS samples. However, this was the only serogroup that was associated with the main effect of fiber level. An interaction between fiber level and fiber source was associated with EHEC O103 RAMS detection. This interaction indicated that cattle fed the 40% MDGS diet had significantly greater probability to have RAMS positive for EHEC O103 compared to cattle fed the matched fiber diet. Interestingly, EHEC O111 was affected oppositely compared to EHEC O103. The inclusion of MDGS at 22% NDF (40% MDGS diet) had a protective effect on EHEC O111 shedding and decreased the probability to detect the organism from RAMS. These interactions are indications that the probability to detect EHEC O103 and O111 from RAMS were not due to fiber concentration of the diet, but rather to some other component of distillers grains.

We observed no change in the risk to detect EHEC O157 from cattle at any level of corn bran inclusion, but this may have been due to the low probability to detect EHEC O157 throughout the study. Although not statistically significant, there was numerically

greater probability to detect EHEC O157 in steers fed 40% distillers grains diets, which is consistent with several published studies. In a study where steers were fed 0, 20, or 40% corn-based wet distillers grains (WDG) or dried distillers grains (DDG), the cattle fed 40% WDG or DDG had higher fecal prevalence of EHEC O157 than cattle fed 0 or 20% distillers grains (Jacob et al., 2010). In a study where feedlot cattle were fed either 0 or 40% wet distillers grains plus solubles (WDGS) in the finishing phase, EHEC O157 prevalence was greater in cattle fed WDGS diets than cattle fed none (Wells et al., 2009). In a previously published trial studying the effects of vaccination against EHEC O157 on fecal shedding and terminal rectal mucosa (TRM) colonization, cattle were fed a diet consisting of 10, 20, 30, 40, or 50% distillers grains on DM basis. Results of TRM colonization by EHEC O157 indicated an effect of diet ($P = 0.04$), in which cattle fed 10, 20, or 30% DG had decreased odds of TRM colonization while cattle fed 40 and 50% DG had increased odds of colonization (Peterson et al., 2007).

Similar to the results of the present study, other studies have not detected significant differences in fecal shedding of EHEC O157 in cattle fed distillers grains. For example, in a study using a 2 x 2 factorial arrangement in which the factors were 0 or 25% DDGS and 0 or 25% dry-rolled corn (DRC), there was no effect of DDGS, DRC, or sampling time on the probability to detect EHEC O157 (Jacob et al., 2009). In another study, cattle fed 25% WDGS had greater prevalence of EHEC O157 compared to cattle fed steam-flaked corn diets on d122 but not on d136 (Jacob et al., 2008a). Additionally, in a recent feeding study using a 2 x 2 factorial design of 0 or 30% WDGS and direct-fed microbials (DFM) or no DFM, there was very low prevalence of EHEC O157 and no effects of WDGS or DFM were detected on probability of EHEC O157 shedding (Wilson

et al., 2016). The level of dietary inclusion of distillers grains in these studies was lower than the level of inclusion of this current study.

The proportion of hides with contamination in this study was lower than expected. In the current study, the probability to detect any EHEC serogroup on hides was less than 8.3%. Recent studies using a similar molecular test method for cattle at harvest found EHEC on 94% of veal calf hides (Wang et al., 2014) and on 65% of cull dairy cow hides (Stromberg et al., 2016). In contrast to the above-mentioned studies, we sampled hides as cattle were loaded onto trucks for transport to slaughter instead of at the packing plant. Transport and lairage can significantly increase the prevalence and concentration of EHEC O157 on hides of cattle (Arthur et al., 2007; Smith et al., 2009). In a vaccine trial, EHEC O157 hide contamination was more likely at the abattoir before hide removal than the day prior in the feedyard (Smith et al., 2009).

Hide contamination by EHEC O157 has been found to be more likely in cattle fed 40% distillers grains compared to cattle fed a dry-rolled corn based diet with 0% distillers grains (Wells et al., 2009, 2011). The prevalence of hide contamination for most EHECs in the current study was too low for meaningful interpretation of risk factors. However, feeding distillers grains was protective of hide contamination by EHEC O45 and O103. These findings might be spurious or it may be that feeding MDGS affects the survivability of these organisms on hides, in the environment, or in the rumen, which could be a source of hide contamination through grooming.

The NeoSEEK™ STEC test was originally validated by the Neogen® Corp. on samples of beef trim, but has subsequently been used to detect “top six” non-O157 and

O157 EHEC in samples of bovine feces, hides and dehided carcass surfaces (Bosilevac et al., 2013; Wang et al., 2014; Stromberg et al., 2015, 2016a; Agga et al., 2017). As opposed to artificial inoculation, the results of these studies are a validation of this test's utility on these types of samples. As with any PCR method conducted on DNA extracted from enrichment cultures, false positive results may have potentially contributed to the results of the present and other studies. However, there is no adequate agar plating isolation method for non-O157 EHEC, and these culture methods are insensitive, and prone to false negative results and under-reporting of prevalence (Bosilevac et al., 2013; Wang et al., 2014; Noll et al., 2015; Stromberg et al., 2015, 2016a, 2016b; Agga et al., 2017).

The highest proportion of RAMS detection of all EHEC serogroups, except O111, was observed at d0. There were also effects of sampling period on the detection of EHEC O45 and EHEC O103. Large temporal effects have been observed for fecal shedding of EHEC O157 (Smith et al., 2001; Khaitsa et al., 2003; Smith et al., 2005; Alam & Zurek, 2006), and this variation over time may apply to other EHEC serogroups.

This is the first study to look at specific dietary components and their effects on individual EHEC-7 serogroup carriage. In an earlier study, we demonstrated that EHEC O157 and *Salmonella* spp, carriage by feedlot cattle differed by management and environmental factors that change over time and place (Smith et al., 2005). We hypothesized in this study that non-O157 EHEC organisms might not share the same risk factors as the often studied EHEC O157. The results of this study provide evidence that EHEC serogroups are independently and uniquely affected by dietary components, levels,

or their interactions. In conclusion, each EHEC serogroup was uniquely affected by dietary factors and sampling day, which indicates that these serogroups should be evaluated separately.

References

Agga, G. E., T. M. Arthur, S. Hinkley, and J. M. Bosilevac, 2017: Evaluation of rectoanal mucosal swab sampling for molecular detection of enterohemorrhagic *Escherichia coli* in beef cattle. *J. Food Prot.* 80, 661-667.

Alam, M. J. and L. Zurek, 2006: Seasonal prevalence of *Escherichia coli* O157:H7 in beef cattle feces. *J. Food Prot.* 69, 3018-3020. doi: 10.4315/0362-028x-69.12.3018

Armstrong, G. L., J. Hollingsworth and J. G. Morris, 1996: Emerging food-borne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.* 18, 29-51.

Arthur, T. M., X. Nou, N. Kalchayanand, J. M. Bosilevac, T. Wheeler, and M. Koohmaraie, 2011: Survival of *Escherichia coli* O157:H7 on cattle hides. *Appl. Environ. Microbiol.* 77, 3002-3008.

Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie, 2007: Transportation and lairage environment effects on prevalence, numbers, and diversity of *Escherichia coli* O157:H7 on hides and carcasses of beef cattle at processing. *J. Food Prot.* 70, 280-286.

Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L. Wheeler, M. P. Kent, D. Jaroni, B. Pauling, D. M. Allen, and M. Koohmaraie, 2004: *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, Enterobacteriaceae, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *J. Food Prot.* 67, 658-665.

Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie, 2003: Seasonal prevalence of Shiga toxin–producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66, 1978-1986.

Bell, B. P., M. Goldoft, P. M. Griffin, M. A. Davis, D. C. Gordon, P. I. Tarr, C. A. Bartleson, J. H. Lewis, T. J. Barrett, J. G. Wells, R. Baron, and J. Kobayashi, 1994: A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. *JAMA,* 272**,** 1349-1353. doi: 10.1001/jama.1994.03520170059036

Berry, E. D., J. E. Wells, and V. H. Varel, 2014: Persistence of *Escherichia coli* O157:H7 in feces from cattle fed diets with or without wet distillers grains with solubles. *J. Anim. Sci.*, 922 (E-Suppl.2), 525.

Bosilevac, J. M., B. L. Luedtke, T. M. Arthur, N. Kalchayanand, J. W. Schmidt, S. D. Shackelford, and T. L. Wheeler, 2013: Identification of bovine reservoirs of human pathogenic non-O157 Shiga toxin-producing *Escherichia coli*. Final report submitted to the American Meat Institute Foundation, April 30, 2013.

Chapman, P. A., C. A. Siddons, D. J. Wright, P. Norman, J. Fox, and E. Crick, 1993: Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiol. Infect.,* 111, 439-447.

Dewell, G. A., J. R. Ransom, R. D. Dewell, K. McCurdy, I. A. Gardner, A. E. Hill, J. N. Sofos, K. E. Belk, G. C. Smith, and M. D. Salman, 2005: Prevalence of and risk factors for *Escherichia coli* O157 in market-ready beef cattle from 12 U.S. feedlots. *Foodborne Pathog. Dis.* 2, 70-76. doi: 10.1089/fpd.2005.2.70

Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid, 2000: Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97, 2999-3003. doi: 10.1073/pnas.060024897

Hancock, D. D., T. E. Besser, M. L. Kinsel, P. I. Tarr, D. H. Rice, and M. G. Paros, 1994: The prevalence of *Escherichia coli* O157.H7 in dairy and beef cattle in Washington State. *Epidemiol. Infect.* 113, 199-207.

Jacob, M. E., J. T. Fox, S. K. Narayanan, J. S. Drouillard, D. G. Renter, and T. G. Nagaraja, 2008a: Effects of feeding wet corn distillers grains with solubles with or without monensin and tylosin on the prevalence and antimicrobial susceptibilities of fecal foodborne pathogenic and commensal bacteria in feedlot cattle. *J. Anim. Sci.* 86, 1182-1190. doi: 10.2527/jas.2007-0091

Jacob, M. E., G. L. Parsons, M. K. Shelor, J. T. Fox, J. S. Drouillard, D. U. Thomson, D. G. Renter, and T. G. Nagaraja, 2008b: Feeding supplemental dried distiller's grains increases faecal shedding of *Escherichia coli* O157 in experimentally inoculated calves. *Zoonoses Public Health* 55, 125-132. doi: 10.1111/j.1863-2378.2008.01115.x

Jacob, M. E., Z. D. Paddock, D. G. Renter, K. F. Lechtenberg and T. G. Nagaraja, 2010: Inclusion of dried or wet distillers' grains at different levels in diets of feedlot cattle affects fecal shedding of *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 76, 7238-7242. doi: 10.1128/AEM.01221-10

Jacob, M. E., J. T. Fox, J. S. Drouillard, D. G. Renter, and T. G. Nagaraja. 2009: Evaluation of feeding dried distiller's grains with solubles and dry-rolled corn on the fecal prevalence of *Escherichia coli* O157:H7 and *Salmonella* spp. in cattle. *Foodborne Pathog. Dis.* 6, 145-153. doi: 10.1089/fpd.2008.0159

Keen, J. E., and R. O. Elder, 2002: Isolation of shiga-toxigenic *Escherichia coli* O157 from hide surfaces and the oral cavity of finished beef feedlot cattle. *J. Am. Vet. Med. Assoc.* 220, 756-763.

Khaitsa, M. L., D. R. Smith, J. A. Stoner, A. M. Parkhurst, S. Hinkley, T. J. Klopfenstein, and R. A. Moxley, 2003: Incidence, duration, and prevalence of *Escherichia coli* O157:H7 fecal shedding by feedlot cattle during the finishing period. *J. Food Prot.* 66, 1972-1977.

Klopfenstein, T. J., G. E. Erickson, and V. R. Bremer, 2008: Use of Distillers Co-products in Diets Fed to Beef Cattle. In B. A. Babcock, D. J. Hayes and J. D. Lawrence (Eds.), *Using Distillers Grains in the U.S. and International Livestock and Poultry Industries.* Aimes, Iowa: Midwest Agribusiness Trade Research and Information Center.

Koohmaraie, M., T. M. Arthur, J. M. Bosilevac, M. Guerini, S. D. Shackelford, and T. L. Wheeler, 2005: Post-harvest interventions to reduce/eliminate pathogens in beef. *Meat Sci.* 71, 79-91.

Kudva, I. T., C. W. Hunt, C. J. Williams, U. M. Nance, and C. J. Hovde, 1997: Evaluation of dietary influences on *Escherichia coli* O157:H7 shedding in sheep. *Appl. Environ. Microbiol.* 63, 3878-3886.

Laegreid, W. W., R. O. Elder, and J. E. Keen, 1999: Prevalence of *Escherichia coli* O157:H7 in range beef calves at weaning. *Epidemiol. Infect.* 123, 291-298.

NASEM, National Academies of Sciences, Engineering, and Medicine, 2016: Nutrient Requirements of Beef Cattle. Eighth Revised Edition. Washington, DC: The National Academies Press. doi: 10.17226/19014.

Noll, L. W., P. B. Shridhar, D. M. Dewsbury, X. Shi, N. Cernicchiaro, D. G. Renter, and T. G. Nagaraja, 2015: A comparison of culture- and PCR-based methods to detect six major non-O157 serogroups of Shiga toxin-producing *Escherichia coli* in cattle feces. *PLoS One* 10:e0135446.

Peterson, R. E., T. J. Klopfenstein, R. A. Moxley, G. E. Erickson, S. Hinkley, G. Bretschneider, E. M. Berberov, D. Rogan, and D. R. Smith, 2007: Effect of a vaccine product containing type III secreted proteins on the probability of *Escherichia coli* O157:H7 fecal shedding and mucosal colonization in feedlot cattle. *J. Food Prot.* 70, 2568-2577.

Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin, and D. L. Swerdlow, 2005: Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg. Infect. Dis.* 11**,** 603-609.

Rich, A. R., A. N. Jepson, M. K. Luebbe, G. E. Erickson, T. J. Klopfenstein, D. R. Smith, and R. A. Moxley, 2010: Vaccination to reduce the prevalence of *Escherichia coli* O157:H7 in feedlot cattle fed wet distillers grains plus solubles. *Neb. Beef Cattle Report.* 580, 94-95.

Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen, 1983: Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New Eng. J. Med.* 308, 681-685.

Robbins, A., M. Anand, D. C. Nicholas, J. S. Egan, K. A. Musser, S. Giguere, H. Prince, H. E. Beaufait, S. D. Sears, J. Borda, D. Dietz, T. Collaro, P. Evans, S. A. Seys, and B. W. Kissler, 2014: Ground beef recall associated with non-O157 Shiga toxin-producing *Escherichia coli*, United States. *Emerg. Infect. Dis.* 20, 165-167.

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

Smith, D. R., R. A. Moxley, S. L. Clowser, J. D. Folmer, S. Hinkley, G. E. Erickson, and T. J. Klopfenstein, 2005: Use of rope devices to describe and explain the feedlot ecology of *Escherichia coli* O157:H7 by time and place. *Foodborne Patho. Dis.* 2, 50-60. doi: 10.1089/fpd.2005.2.50

Smith, D. R., R. A. Moxley, T. J. Klopfenstein, and G. E. Erickson, 2009: A randomized longitudinal trial to test the effect of regional vaccination within a cattle feedyard on *Escherichia coli* O157:H7 rectal colonization, fecal shedding, and hide contamination. *Foodborne Patho. Dis.* 6, 885-892. doi: 10.1089/fpd.2009.0299

Stock, R., T. J. Klopfenstein, D. Brink, S. Lowry, D. Rock, and S. Abrams, 1983: Impact of weighing procedures and variation in protein degradation rate on measured performance of growing lambs and cattle. *J. Anim. Sci.* 57, 1276-1285.

Stromberg, Z. R., N. W. Baumann, G. L. Lewis, N. J. Sevart, N. Cernicchiaro, D. G. Renter, D. B. Marx, R. K. Phebus, and R. A. Moxley, 2015: Prevalence of enterohemorrhagic *Escherichia coli* O26, O45, O103, O111, O121, O145, and O157 on hides and pre-intervention carcass surfaces of feedlot cattle at harvest. *Foodborne Pathog. Dis.* 12, 631-638.

Stromberg, Z. R., G. L Lewis, S. S. Aly, T. W. Lehenbauer, J. M. Bosilevac, N. Cernicchiaro, and R. A. Moxley, 2016a: Prevalence and concentration of enterohemorrhagic *Escherichia coli* in culled dairy cattle at harvest. *J. Food Prot.* 79, 421-431.

Stromberg, Z. R., G. L. Lewis, and R. A. Moxley, 2016b: Comparison of agar media for the detection and quantification of Shiga toxin-producing *Escherichia coli* O26, O45, O013, O111, O121, O145, and O157 in cattle feces. *J. Food Prot.* 79, 939-949.

U.S. Grains Council, 2012: Is There a Connection Between Feeding DDGS and *E. coli* O157:H7 Shedding in Beef Cattle? In *A Guide to Distiller's Dried Grains with Solubles (DDGS).* Available at http://www.grains.org/sites/default/files/ddgs-handbook/Complete%202012%20DDGS%20Handbook.pdf (accessed on 30 November 2015).

USDA-FSIS, 2012: Shiga Toxin-Producing *Escherichia coli* in Certain Raw Beef Products. Available at https://www.fsis.usda.gov/OPPDE/rdad/FRPubs/2010-0023FRN.pdf (accessed 06 August 2015).

Van Baale, M. J., J. M. Sargeant, D. P. Gnad, B. M. DeBey, K. F. Lechtenberg, and T. G. Nagaraja, 2004: Effect of forage or grain diets with or without monensin on ruminal persistence and fecal *Escherichia coli* O157:H7 in cattle. *Appl. Environ. Microbiol.* 70, 5336-5342. doi: 10.1128/AEM.70.9.5336-5342.2004

Van Soest, P. J., J. B. Robertson, and B. A. Lewis, 1991: Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583-3597.

Wang, R., M. Koohmaraie, B. E. Luedtke, T. L. Wheeler, and J. M. Bosilevac, 2014: Effects of in-plant interventions on reduction of enterohemorrhagic *Escherichia coli* and background indicator microorganisms on veal calf hides. *J. Food Prot.* 77, 745-751. doi: 10.4315/0362-028X.JFP-13-388

Watson, A. K., B. L. Nuttelman, T. J. Klopfenstein, L. W. Lomas, and G. E. Erickson, 2013: Impacts of limit-feeding procedure on variation and accuracy of cattle weights. *J. Anim. Sci.* 91, 5507-5517. doi: 10.2527/jas.2013-6349

Wells, J. E., E. D. Berry, and V. H. Varel, 2005: Effects of common forage phenolic acids on *Escherchia coli* O157:H7 viability in bovine feces. *Appl. Environ. Microbiol.* 71, 7974-7979. doi:10.1128/AEM.71.12.7974-7979.2005

Wells, J. E., S. D. Shackelford, E. D. Berry, N. Kalchayanand, J. M. Bosilevac, and T. L. Wheeler, 2011: Impact of reducing the level of wet distillers grains fed to cattle prior to harvest on prevalence and levels of *Escherichia coli* O157:H7 in feces and on hides. *J. Food Prot.* 74, 1611-1617. doi: 10.4315/0362-028X.JFP-11-160

Wells, J. E., S. D. Shackelford, E. D. Berry, N. Kalchayanand, M. N. Guerini, V. H. Varel, T. M. Arthur, J. M. Bosilevac, H. C. Freetly, T. L. Wheeler, C. L. Ferrell, and M. Koohmaraie, 2009: Prevalence and level of *Escherichia coli* O157:H7 in feces and on hides of feedlot steers fed diets with or without wet distillers grains with solubles. *J. Food Prot.* 72, 1624-1633.

Wilson, B. K., B. P. Holland, D. L. Step, M. E. Jacob, D. L. VanOverbeke, C. J. Richards, T. G. Nagaraja, and C. R. Krehbiel, 2016: Feeding wet distillers grains plus solubles with and without a direct-fed microbial to determine performance, carcass characteristics, and fecal shedding of *Escherichia coli* O157:H7 in feedlot heifers. *J. Anim. Sci.* 94, 297-305.

CHAPTER V

BAYESIAN ESTIMATION OF THE PERFORMANCE OF THREE METHODS USED TO DETECT ENTEROHEMORRHAGIC *ESCHERICHIA COLI* O157 IN CATTLE FECES

(Prepared for submission to Zoonoses and Public Health)

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Abstract

Detection methods for enterohemorrhagic *Escherichia coli* in complex samples of bovine origin have not been validated and thus there is no true perfect reference test. The objective of this study was to estimate test characteristics of three commonly utilized detection methods, as well as estimate the true prevalence of EHEC O157 in fecal samples obtained from beef cow-calf herds. Freshly defecated fecal samples (n=1,024) were collected from herds in Mississippi and Nebraska and each sample was tested in parallel for EHEC O157 with three separate methods: enriched culture, NeoSEEK STEC Detection and Identification (NS), and multiplex polymerase chain reaction (mqPCR). Bayesian latent class analysis was performed to estimate test performance and prevalence of O157 fecal shedding in herds. Sensitivity analysis was utilized to determine the influence of priors on posterior estimates. Conditional dependence models were evaluated and correlation coefficients between tests were calculated. Culture was estimated to be 55.4% (95% credible interval (CrI): 41.7-69.1) sensitive and 99.8% (95% CrI: 99.4-100) specific. The NS test was 77.5% sensitive (95% CrI: 58.7-95.3) and 99.7% (95% CrI: 99.1-99.9) specific. Sensitivity for the mqPCR was 18.0% (95% CrI: 10.3-28.2) and specificity was 98.5% (95% CrI: 97.6-99.2). There was considerable conditional dependence between culture and mqPCR for positive fecal samples. According to our estimates, the NS test was the most sensitive EHEC O157 detection method, and culture or NS were the more specific tests.

Introduction

Enterohemorrhagic *Escherichia coli* are important foodborne pathogens with a cattle reservoir (Chapman et al., 1993, Hancock et al., 1994, Geue et al., 2002, Barkocy-Gallagher et al., 2003). Cattle lack vascular receptors for Shiga toxin (Pruimboom-Brees et al., 2000) and are often subclinically infected by EHEC (Cray & Moon, 1995). EHEC are shed in cattle feces (Sargeant et al., 2000), and may colonize the terminal rectum of cattle (Naylor et al., 2003, Naylor et al., 2005). Cattle hides may be contaminated by EHEC at the time of slaughter and hide contamination is correlated to carcass contamination (Arthur et al., 2004). Because cattle populations serve as an important source of the bacteria to their environment and to humans, much effort has gone into investigating the prevalence, risk factors associated with detection, and efficacy of pre- and post-harvest intervention strategies.

For many years EHEC O157 was the primary serogroup of research and regulatory interest; however, in more recent years, 6 additional serogroups have been identified as commonly associated sources of human illness and thus have been listed as adulterants in raw, non-intact beef by USDA FSIS (USDA-FSIS, 2012). Collectively these seven serogroups of EHEC are responsible for 92.3% of all human EHEC illness (Gould et al., 2013), and together they are referred to as EHEC-7.

Strategies to detect the seven EHEC serogroups of public health importance are less than perfect. USDA FSIS developed standardized methods for EHEC-7 detection in meat products (USDA-FSIS, 2014). These procedures include PCR screening of enriched samples for the detection of O-groups, *stx*, and *eae*. Then possible positive samples are plated on chromogenic agar, and individual colonies are tested by PCR, latex

agglutination, and biochemical testing procedures. These methods are time-consuming, expensive, and labor-intensive. Therefore, several additional methods have been developed to test for EHEC-7 in samples of animal origin. Differentiation between serogroups of non-O157 is difficult. Although there are many methods described in the literature, there is no reference standard and performance has not been estimated. Three methods utilized in several pre-harvest EHEC studies include: enriched culture (Stromberg et al., 2016b, Stromberg et al., 2015b), NeoSEEK STEC Detection and Identification test (Neogen® Corp)(Bosilevac et al., 2013, Wang et al., 2014, Stromberg et al., 2015a, Stromberg et al., 2016a, Agga et al., 2017, Schneider et al., 2017a, Schneider et al., 2017b), and multiplex polymerase chain reaction (Noll et al., 2015b, Shridhar et al., 2016).

Diagnostic test performance is usually characterized by the sensitivity and specificity. In our study, the sensitivity is the proportion of EHEC contaminated samples that test positive, and the specificity is the proportion of non-EHEC contaminated samples to test negative. To calculate these measures, the true status of EHEC contamination must be known. Latent class analysis (LCA) is a method for evaluating test performance (Hui & Walter, 1980). Latent class analysis is a class of models where the disease status of the individuals is unknown (Toft et al., 2005); however, LCA does assume that each individual can be classified dichotomously (i.e. positive or negative). Each sample is tested used multiple diagnostic methods, and a Bayesian approach combines test results with external prior information (Berkvens et al., 2006).

The objective of the study was to utilize Bayesian latent class analysis to estimate the sensitivity and specificity of three EHEC O157 detection methods: enriched culture,

the commercially available NeoSEEK^{TM} STEC Detection and Identification test (NS), and multiplex RT-PCR (mqPCR).

Materials and Methods

Fecal Sample Collection and Methodology

Freshly defecated fecal samples were collected for a separate study from two beef cow-calf herds in Nebraska and Mississippi during each of the four seasons in 2016. Briefly, samples were collected directly from manure pats on the ground using a clean disposable spoon and clean gloves. Samples were collected into a specimen cup and stirred for approximately 10 seconds with two foam-tipped swabs (catalog no. 89031-280; VWR International, Buffalo Grove, IL) to obtain a homogenous sample. The swabs were inserted into a 15 mL conical tube (Falcon) containing 10 mL chilled (5-10 ºC) EC broth (EC, Oxoid). Samples were processed within 24 h after collection.

Sample Processing and EHEC Testing

All samples were submitted to the University of Nebraska-Lincoln for enrichment and processing. Upon arrival in the laboratory, fecal swabs in the 15 mL tubes were vortexed at high speed for 10 sec and any debris was allowed to settle for 1 min. Two 1 mL aliquots were removed: 1 mL for NS DNA preparation and a 1 mL aliquot for culture-based work was added to 0.5 mL 50% sterile BHI-glycerol. These pre-enrichment aliquots were stored at -80ºC. The fecal swabs were left in the tubes of EC and incubated at 40ºC for 6 h followed by a 4ºC hold in a programmable incubator until further processed. Further processing included a 10 sec vortexing, 1 min settling time, followed

by removal of one 1 mL aliquot for DNA preparation and one 1 mL aliquot for future culture based work which was added to 0.5 mL 50% sterile BHI-glycerol. These post enrichment aliquots were stored at -80ºC. The remaining enrichment was frozen until shipped overnight on dry ice to the Kansas State University laboratory for mqPCR analysis.

Blinding

All personnel at the participating laboratories were blind to results from other detection methods. Results from each method were sent directly to the epidemiology lab at Mississippi State University's College of Veterinary Medicine, where records were stored. At the end of the study, data from the three methods were combined and analyzed.

Enriched Culture

Three immunomagnetic separation (IMS) treatments were used on each sample. One treatment consisted of using Dynal anti-*E.coli* O157 IMS beads, the second treatment used a pool of Abraxis® (Warminster, PA) O103, O111, O145 IMS beads and the third, a pool of Abraxis® (Warminster, PA) O26, O45 and O121 IMS beads. Using the KingFisher 96 deep-well, all IMS beads (Abraxis and Dynal) were incubated with a 490 µL sample diluted in 490 µL Wash Buffer (PBS-Tween) for 15 minutes, and then underwent four washes (2.0 min each) with Abraxis wash buffer. After this, beads were dropped into 1.0 mL of BPW. Fifty µL of each of the non-O157 bead suspensions was spiral-plated onto mPossé2 medium (Stromberg et al., 2015a) using the Eddy Jet 2 using E-mode 50 µL, yielding two plates per sample. Fifty µL of each of the O157 bead

suspensions was spiral-plated onto CCT-CHROMagar O157 using the Eddy Jet 2 using E-mode 50 µL. Plates were incubated at 37°C for 18 h.

Five mauve colonies from CCT-CHROMagar O157 and 20 phenotypically correct colonies from each of the Modified Possé 2 plates (10 from each of the mPossé 2 plates) were randomly picked and streaked for isolation individually onto quartered blood agar plates (Remel; BAP). All plates were incubated at 37°C for 15 h.

Isolated colonies from the above BAP were picked into 50 µL of Ultra-Pure Water (UPW) each and prepared for use as the DNA template to be screened by PCR that targets the *stx* genes. Ten µL of each DNA template prep, representing six of the original colonies, was pooled for the stx PCR screening process. Samples were considered positive for EHEC serogroups depending on PCR confirmation.

NeoSEEK

The NeoSEEK™ test is a highly multiplexed (89 independent target) EHEC PCR assay that utilizes matrix-assisted laser desorption/ionization (MALDI)-time-of-flight (TOF) mass spectroscopy on a Sequenom® platform. The NS test identified the genes for Shiga toxin (*stx*) and intimin (*eae*) as well as EHEC-7 based on *eae* subtypes and proprietary O group single nucleotide polymorphisms. If both the specific O group genes and stx were present within a sample, it was identified as Shiga toxin-producing *E. coli* (STEC). Further, the results of the NS test were interpreted as positive for EHEC O26 when positive for O26, *stx*, and *eae*-Beta; positive for EHEC O45, O103, and O121 when positive for the respective O group genes, *stx*, and *eae*-Epsilon; positive for EHEC O111 when positive for O111, *stx*, and *eae*-Gamma2; and positive for EHEC-O145 and O157 when positive for the respective O group genes, *stx*, and *eae*-Gamma1. From these

interpretations, samples were correspondingly recorded as positive or negative for each of the EHEC serogroups.

mqPCR

The mqPCR assays have been previously described in great detail (Shridhar et al., 2016, Noll et al., 2015b). In brief, three separate mqPCR assays (mqPCR1, mqPCR2, and mqPCR3) were developed to detect O26, O45, O103, O111, O121, O145, and O157. The serogroups targeted by mqPCR1 were O26, O103, and O111, and the serogroups targeted by mqPCR2 were O45, O121, and O145. Serogroup O157, as well as the three major virulence genes (*eae*, *stx*$_1$, and *stx*$_2$), were targeted in mqPCR3. Together the three assays were used to determine if a sample was positive or negative for each of the EHEC-7 serogroups and the major virulence genes. If a sample contained genes encoding for the O-group 157, *stx*, and *eae*, it was called EHEC O157 positive.

Statistical Analysis

Latent class analyses were performed to estimate the sensitivity and specificity of each of three EHEC O157 detection methods. Additionally, prevalence estimates were made for each population of cattle sampled. A Bayesian latent class analysis was performed in a freeware program OpenBUGS 3.2.3 (Lunn et al., 2000) using adaptations of models described (Toft et al., 2007, Rahman et al., 2013).

A latent class approach can be utilized to evaluate diagnostic tests when a gold standard does not exist (Hui and Walter, 1980). One of the assumptions which must be met to perform the analysis as described by Hui and Walter is that the tests are conditionally independent. If diagnostic tests are based on similar techniques, conditional independence

cannot be assumed. Ignoring potential conditional independence can lead to bias in the test parameter estimates (Vacek, 1985). Therefore, models including conditional covariance should be assessed.

Modeling Conditional Dependence

Because culture was confirmed by PCR, and both NS and mqPCR were also DNA-based, conditional independence could not be assumed. The conditional independence model was used in comparison to evaluate conditional dependence between every pair of tests: culture and NS, culture and mqPCR, and NS and mqPCR. Additionally, models were developed with covariance between the pairs: culture-NS and culture-mqPCR, culture-NS and NS-mqPCR, and culture-mqPCR and NS-mqPCR. Lastly, a model including covariance between all of the above pairs was constructed. To evaluate goodness of fit of the models, Deviance Information Criterion (DIC) and parameters effectively estimated (*pD*) were assessed. Models with a smaller DIC are preferred. When comparing any two models, a significant reduction in DIC is at least 3 units less (Spiegelhalter et al., 2002). If the difference is less than 3 units, models were considered similar and other criteria were evaluated to determine the better model.

Conditional covariance between pairs of tests among infected animals and among non-infected animals were obtained, and correlation coefficients were calculated (Georgiadis et al., 2003). According to Georgiadis, if correlation coefficients (ρ) are ≤ 0.2, estimates from the conditional dependence and independence models will be similar. If correlation coefficients are > 0.2, the conditional dependence model should be considered. Median posterior estimates and their 95% credible intervals (95% CrI) were obtained. The 95%

CrI shows the range of values of 95% of the samples obtained from the posterior distribution in all of the models.

Prior Distributions for Parameters

Literature regarding the test characteristics of the three detection methods was limited. Therefore, prior information was obtained by expert elicitation. For each parameter, experts were asked to give the most likely value (mode), a value of certainty, and a value that the parameter is below or above. Beta Buster© (downloadable from http://www.epi.ucdavis.edu/diagnostictests/betabuster.html) software was used to obtain alpha and beta parameters for each prior. Alpha and beta values obtained from Beta Buster were averaged to give a composite prior for each parameter (Burgman et al., 2011).

Model Diagnostics

All models were run using three chains, a burn-in period of 10,000, and 50,000 iterations to obtain posterior estimates. Convergence was diagnosed using trace plots, autocorrelation plots, and Brooks, Gelman, and Rubin plots to compare chains with different starting values.

Sensitivity of Priors

Sensitivity analyses were performed to determine the influence of prior information on the estimates by using uniform priors. Similar to the sensitivity analysis in Rahman et al., the following sets of priors were included: 1. uniform prior (UP) for prevalence (Pr) and informative priors (IP) for sensitivities (Se) and specificities (Sp); 2. UP for Pr and Se, IP

for Sp; 3. UP for Pr and Sp, IP for Se; 4. IP for Pr and UP for Se and Sp; 5. IP for Pr and Se, UP for Sp; 6. IP for Pr (Rahman et al., 2013).

For each set of alternative priors, models were run using the same number of chains, iterations, and diagnostics. Posterior estimates and their 95% CrI were compared to determine influence of prior information.

Results

Cross-classified results for each of the three detection methods are presented in Table 5.1.

Table 5.1 Cross-classified results for EHEC O157 fecal shedding in beef cow-calf herds based on culture, NeoSEEK, and mqPCR

Test Combination[1]	**Population**							
C, NS, mqPCR[2]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
111	0	0	0	2	0	0	10	0
110	5	0	0	0	0	0	26	0
101	0	0	0	0	0	0	0	0
100	1	0	0	0	0	0	1	0
011	0	0	0	0	0	0	0	0
010	2	0	0	1	0	0	19	0
001	0	0	4	0	4	0	1	0
000	120	128	124	125	124	128	71	128

[1]1: positive test result; 0: negative test result
[2]C: culture, NS: NeoSEEK, mqPCR: multiplex quantitative PCR

Conditional dependence

Table 5.2 presents the models along with *pD* and DIC values. The DIC for the conditional independence model was 124.7. All models with conditional dependence between culture and mqPCR had significantly lower DICs (116.4-118.2) compared to the conditional independence model. The model with a low DIC and with correlation coefficients greater than 0.2 was selected. Other models with equally low DIC values

included correlation coefficients with 95% credible intervals encompassing 0. Therefore, the model accounting only for conditional dependence between culture and mqPCR (DIC= 116.6) was selected as the final model. The true prevalence of EHEC O157 fecal shedding in the eight populations, sensitivity, specificity and dependence coefficients for the three tests are shown in table 5.3. Sensitivity and specificity of culture were estimated as 62.9% (95% CrI: 51.4-73.7) and 99.8% (95% CrI: 99.5-100), respectively. The NS test was 92.3% sensitive (95% CrI: 81.9-98.0) and 99.7% (95% CrI: 99.1-99.9) specific. Sensitivity and specificity for the mqPCR was 20.0% (95% CrI: 12.0-30.2) and 98.6% (95% CrI: 97.7-99.2), respectively. There was considerable correlation between culture and mqPCR in infected animals.

Table 5.2 Different conditional dependence models used to estimate the prevalence of EHEC O157 fecal shedding in beef cow-calf herds and the sensitivity and specificity of three EHEC detection methods.

Models	***pD***	**DIC**	**ρ_a[1] (95%CrI)**	**ρ_b[2] (95%CrI)**
Conditional independence	5.11	124.7	-	-
CD[3] between C[4] and NS[5]	5.17	125.2	0.34 (-0.08, 0.61)	0.38 (0.02, 0.87)
CD between C and mqPCR[6]	**5.18**	**116.6**	**0.31 (0.10, 0.31)**	**0.13 (0.003, 0.42)**
CD between NS and mqPCR	5.43	126.4	-0.02 (-0.35, 0.16)	0.21 (0.005, 0.54)
CD between C and NS;	5.17	116.4	0.36 (-0.05, 0.60);	0.34 (0.02, 0.85);
C and mqPCR			0.33 (0.13, 0.47)	0.13 (0.001, 0.42)
CD between C and NS;	5.06	125.2	0.38 (-0.08,0.63);	0.38 (0.02,0.87);
NS and mqPCR			0.10 (-0.21, 0.28)	0.22 (0.004, 0.57)
CD between C and mqPCR;	5.56	118.2	0.32 (0.12, 0.45);	0.12 (0.002, 0.40);
NS and mqPCR			-0.06 (-0.41, 0.14)	0.20 (0.004, 0.53)
CD among all pairs of tests:	5.43	116.9		
C and NS;			0.42 (-0.06, 0.65);	0.33 (0.02, 0.85);
C and mqPCR;			0.33 (0.13, 0.47);	0.12 (0.0008, 0.40);
NS and mqPCR			0.11 (-0.23, 0.29)	0.21 (0.004, 0.54)

The bolded model was determined the most appropriate and was used in the final analyses.

[1]ρ_a: Correlation coefficient for positive samples

[2]ρ_b: Correlation coefficient for negative samples

[3]CD: Conditional dependence

[4]C: Culture

[5]NS: NeoSEEK STEC Detection and Identification test

[6]mqPCR: multiplex quantitative polymerase chain reaction assay

Table 5.3 Median posterior estimates of prevalence, sensitivity, and specificity of culture, NeoSEEK, and mqPCR.

Parameter	Median	95% Credible Interval
P_1	0.07	[0.03, 0.12]
P_2	0.016	[0.0033, 0.046]
P_3	0.016	[0.0033, 0.046]
P_4	0.036	[0.013, 0.076]
P_5	0.016	[0.0033, 0.046]
P_6	0.016	[0.0033, 0.046]
P_7	0.41	[0.33, 0.50]
P_8	0.016	[0.0033, 0.046]
$Se_{culture}$	0.63	[0.51, 0.74]
$Sp_{culture}$	0.999	[0.995, 0.999]
Se_{NS}	0.92	[0.82, 0.98]
Sp_{NS}	0.997	[0.991, 0.999]
Se_{mqPCR}	0.20	[0.12, 0.30]
Sp_{mqPCR}	0.986	[0.977, 0.992]

P: Prevalence estimate in each of 8 subpopulations
Se: Sensitivity estimate of each detection method
Sp: Specificity estimate of each detection method

Sensitivity analysis

The final conditional dependence model was used for sensitivity analyses. Each of the different models run for sensitivity of priors yielded posterior estimates that were similar. The prevalence, sensitivity, and specificity parameters from the different models of sensitivity analyses were similar since their 95% CrI overlapped. This indicated that the models were robust and that the posterior estimates were driven by the data regardless of whether informative or uninformative priors were used.

Discussion

Bayesian latent class analysis can be used to combine data and prior information to estimate the performance characteristics of the diagnostic tests being compared. This study utilized Bayesian latent class modeling to estimate sensitivity and specificity of three detection methods used to identify EHEC O157 in fecal samples from cow-calf herds: enriched culture, NeoSEEK^{TM}, and mqPCR.

Our analysis indicated that the NS test was the most sensitive, followed by culture, and mqPCR. All three tests were highly specific. To our knowledge, there is no existing literature available reporting the sensitivity or specificity of any of the tested detection methods. In general, we expected culture to be the least sensitive test, because it requires viable bacteria to be present in the sample to yield a positive test. In contrast, the mqPCR and NS tests are DNA-based and do not require live bacteria for a positive result. Therefore, increased diagnostic sensitivity would be expected. A few papers have compared culture-based and various DNA-based EHEC detection methods, either in spiked samples or in samples from naturally shedding cattle (Stromberg et al., 2015a, Stromberg et al., 2016a, Noll et al., 2015b, Noll et al., 2015a, Bosilevac et al., 2013). In one study, fair agreement ($\kappa = 0.40$) was reported between culture and the NS test for the detection of EHEC O157 in fecal, hide, and carcass samples collected from culled dairy cattle at harvest (Stromberg et al., 2016a). However, in a separate study of feedlot cattle, significant disagreement between NS and culture was observed (Stromberg et al., 2015a). In addition, when culture-based detection has been compared to multiplex PCR using spiked fecal samples, fair agreement ($\kappa = 0.27$) was observed (Noll et al., 2015b).

When testing conditional dependence models, the most important covariance was observed between culture and mqPCR. This was not very surprising, since the culture results were confirmed using multiplex PCR. The assumption of conditional independence can result in biased estimates if the diagnostic tests evaluated are conditionally dependent (Vacek, 1985, Greiner & Gardner, 2000). If conditional independence is not appropriate, adjustments must be made in the estimates to account for covariance between tests (Dendukuri & Joseph, 2001, Branscum et al., 2005) In our analyses, we saw that compared to the conditional independence model, our estimates from the final model were not significantly different (overlapping CrI). Still, the final model was selected based on a lower DIC, *pD*, and important correlation between culture and mqPCR for infected (sensitivity). Other models with equally low DIC values included correlation coefficients that were unimportant (< 0.20) (Georgiadis et al., 2003).

A sensitivity analysis was performed using combinations of informative and uninformed priors to determine their influence on posterior estimates. Posterior estimates for sensitivity and specificity were data driven, and only exhibited slight, unimportant changes in the median estimates depending on if priors were informed or uninformed. When Se, Sp, or both Se and Sp were informed and Pr was uninformed, the Pr estimates for each subpopulation were decreased and 95% CrI broadened, compared to models where Pr priors were informed. Again, these were not important changes since the 95% CrI overlapped. Overall, our models were data-driven and the use of prior inputs was most important to allow for an identifiable model.

The results of our study give an estimate of the sensitivity and specificity of enriched culture, NS, and mqPCR to detect EHEC O157 in fecal samples from beef cow-

calf herds. The NS test had not previously been validated in complex microbial samples, like hides or feces. Neogen® Corp. performed a validation using spiked beef trim samples and determined that the test was 100% sensitive and 100% specific (Hosking & Petrik, 2013). Although our posterior estimates did not indicate that the NS test was perfect, it was the test with the greatest accuracy. NS provided the most sensitive detection method, and thus may be a good EHEC detection method in bovine fecal samples to decrease likelihood of obtaining a false negative result. All three methods were highly specific, therefore a positive result on any of the three methods could be used to rule in the presence of EHEC O157 within a sample.

References

Agga, G. E., T. M. Arthur, S. Hinkley and J. M. Bosilevac, 2017: Evaluation of rectoanal mucosal swab sampling for molecular detection of enterohemorrhagic *Escherichia coli* in beef cattle. *J. Food Prot*., 80, 661-667.

Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L. Wheeler, M. P. Kent, D. Jaroni, B. Pauling, D. M. Allen and M. Koohmaraie, 2004: *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae,* and *Escherichia coli* O157 at various steps in commercial beef processing plants. *J. Food Prot*., 67, 658-665.

Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler and M. Koohmaraie, 2003: Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot*., 66, 1978-1986.

Berkvens, D., N. Speybroeck, N. Praet, A. Adel and E. Lesaffre, 2006: Estimating disease prevalence in a Bayesian framework using probabilistic constraints. *Epidemiology*, 17, 145-153.

Bosilevac, J. M., B. E. Luedtke, T. M. Arthur, N. Kalchayanand, J. W. Schmidt, S. D. Shackelford and T. L. Wheeler, 2013: Identification of bovine reservoirs of human pathogenic non-O157 Shiga toxin-producing *Escherichia coli*. *American Meat Institute Foundation*.

Branscum, A. J., I. A. Gardner and W. O. Johnson, 2005: Estimation of diagnostic-test sensitivity and specificity through Bayesian modeling. *Prev. Vet. Med*, 68, 145-163.

Burgman, M. A., M. McBride, R. Ashton, A. Speirs-Bridge, L. Flander, B. Wintle, F. Fidler, L. Rumpff and C. Twardy, 2011: Expert status and performance. *PLoS. ONE*, 6, e22998.

Chapman, P. A., C. A. Siddons, D. J. Wright, P. Norman, J. Fox and E. Crick, 1993: Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiol. Infect*., 111, 439-447.

Cray, W. C. and H. W. Moon, 1995: Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. Appl. Environ. *Microbiol*., 61, 1586-1590.

Dendukuri, N. and L. Joseph, 2001: Bayesian approaches to modeling the conditional dependence between multiple diagnostic tests. *Biometrics*, 57, 158-167.

Georgiadis, M. P., W. O. Johnson, I. A. Gardner and R. Singh, 2003: Correlation-adjusted estimation of sensitivity and specificity of two diagnostic tests. *J. R. Stat. Soc. Ser. C (Appl Stat)* 52, 63-76.

Geue, L., M. Segura-Alvarez, F. J. Conraths, T. Kuczius, J. Bockemuhl, H. Karch and P. Gallien, 2002: A long-term study on the prevalence of shiga toxin-producing *Escherichia coli* (STEC) on four German cattle farms. *Epidemiol. Infect.*, 129, 173-185.

Gould, L. H., R. K. Mody, K. L. Ong, P. Clogher, A. B. Cronquist, K. N. Garman, S. Lathrop, C. Medus, N. L. Spina, T. H. Webb, P. L. White, K. Wymore, R. E. Gierke, B. E. Mahon and P. M. Griffin, 2013: Increased recognition of non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States during 2000-2010: epidemiologic features and comparison with *E. coli* O157 infections. *Foodborne Pathog. Dis.*, 10, 453-460.

Greiner, M. and I. A. Gardner, 2000: Application of diagnostic tests in veterinary epidemiologic studies. *Prev. Vet. Med*, 45, 43-59.

Hancock, D. D., T. E. Besser, M. L. Kinsel, P. I. Tarr, D. H. Rice and M. G. Paros, 1994: The prevalence of *Escherichia coli* O157.H7 in dairy and beef cattle in Washington State. *Epidemiol. Infect.*, 113, 199-207.

Hosking, E. and D. Petrik, 2013: NeoSEEK approach to STEC detection and identification In: GeneSeek (ed.).

Hui, S. L. and S. D. Walter, 1980: Estimating the error rates of diagnostic tests. *Biometrics*, 36, 167-171.

Lunn, D. J., A. Thomas, N. Best and D. Spiegelhalter, 2000: WinBUGS - A Bayesian modelling framework: Concepts, structure, and extensibility. *Statistics and Computing*, 10, 325-337.

Naylor, S. W., J. C. Low, T. E. Besser, A. Mahajan, G. J. Gunn, M. C. Pearce, I. J. McKendrick, D. G. E. Smith and D. L. Gally, 2003: Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infect. Immun.*, 71, 1505-1512.

Naylor, S. W., A. J. Roe, P. Nart, K. Spears, D. G. Smith, J. C. Low and D. L. Gally, 2005: *Escherichia coli* O157 : H7 forms attaching and effacing lesions at the terminal rectum of cattle and colonization requires the LEE4 operon. *Microbiology*, 151, 2773-2781.

Noll, L. W., P. B. Shridhar, D. M. Dewsbury, X. Shi, N. Cernicchiaro, D. G. Renter and T. G. Nagaraja, 2015a: A comparison of culture- and PCR-based methods to

detect six major non-O157 serogroups of Shiga Toxin-producing *Escherichia coli* in cattle feces. *PLoS. ONE*, 10, e0135446.

Noll, L. W., P. B. Shridhar, X. Shi, B. An, N. Cernicchiaro, D. G. Renter, T. G. Nagaraja and J. Bai, 2015b: A four-plex real-time PCR assay, based on rfbe, stx1, stx2, and eae genes, for the detection and quantification of Shiga Toxin-producing *Escherichia coli* O157 in cattle feces. *Foodborne Pathog Dis*, 12, 787-794.

Pruimboom-Brees, I. M., T. W. Morgan, M. R. Ackermann, E. D. Nystrom, J. E. Samuel, N. A. Cornick and H. W. Moon, 2000: Cattle lack vascular receptors for *Escherichia coli* O157:H7 Shiga toxins. *Proc. Natl. Acad. Sci. U. S. A,* 97, 10325-10329.

Rahman, A. K., C. Saegerman, D. Berkvens, D. Fretin, M. O. Gani, M. Ershaduzzaman, M. U. Ahmed and A. Emmanuel, 2013: Bayesian estimation of true prevalence, sensitivity and specificity of indirect ELISA, Rose Bengal Test and Slow Agglutination Test for the diagnosis of brucellosis in sheep and goats in Bangladesh. *Prev. Vet. Med*, 110, 242-252.

Sargeant, J. M., J. R. Gillespie, R. D. Oberst, R. K. Phebus, D. R. Hyatt, L. K. Bohra and J. C. Galland, 2000: Results of a longitudinal study of the prevalence of *E. coli* on cow-calf farms. *A. J. V. R.*, 61, 1375-1379.

Schneider, L. G., T. J. Klopfenstein, Z. R. Stromberg, G. L. Lewis, G. E. Erickson, R. A. Moxley and D. R. Smith, 2017a: A randomized controlled trial to evaluate the effects of dietary fibre from distillers grains on enterohemorrhagic *Escherichia coli* detection from the rectoanal mucosa and hides of feedlot steers. *Zoonoses Public Health*.

Schneider, L. G., Z. R. Stromberg, G. L. Lewis, R. A. Moxley and D. R. Smith, 2017b: Cross-sectional study to estimate the prevalence of enterohemorrhagic *Escherichia coli* on hides of market beef cows at harvest. Manuscript submitted for publication.

Shridhar, P. B., L. W. Noll, X. Shi, B. An, N. Cernicchiaro, D. G. Renter, T. G. Nagaraja and J. Bai, 2016: Multiplex quantitative PCR assays for the detection and quantification of the six major non-O157 *Escherichia coli* Serogroups in cattle feces. *J. Food Prot*., 79, 66-74.

Spiegelhalter, D. J., N. G. Best, B. P. Carlin and A. van der Linde, 2002: Bayesian measures of model complexity and fit. *J. R. Statist. Soc.* , 64, 583-639.

Stromberg, Z. R., N. W. Baumann, G. L. Lewis, N. J. Sevart, N. Cernicchiaro, D. G. Renter, D. B. Marx, R. K. Phebus and R. A. Moxley, 2015a: Prevalence of enterohemorrhagic *Escherichia coli* O26, O45, O103, O111, O121, O145, and O157 on hides and pre-intervention carcass surfaces of feedlot cattle at harvest. *Foodborne Pathog. Dis.*, 12, 631-638.

Stromberg, Z. R., G. L. Lewis, S. S. Aly, T. W. Lehenbauer, J. M. Bosilevac, N. Cernicchiaro and R. A. Moxley, 2016a: Prevalence and level of enterohemorrhagic *Escherichia coli* in culled dairy cows at harvest. *J. Food Prot*., 79, 421-431.

Stromberg, Z. R., G. L. Lewis, D. B. Marx and R. A. Moxley, 2015b: Comparison of enrichment broths for supporting growth of Shiga Toxin-producing *Escherichia coli*. Current *Microbiology*, 71, 214-219.

Stromberg, Z. R., G. L. Lewis and R. A. Moxley, 2016b: Comparison of Agar Media for Detection and quantification of Shiga Toxin–producing *Escherichia coli* in cattle feces. *J. Food Prot*., 79, 939-949.

Toft, N., J. Akerstedt, J. Tharaldsen and P. Hopp, 2007: Evaluation of three serological tests for diagnosis of Maedi-Visna virus infection using latent class analysis. *Vet. Microbiol*, 120, 77-86.

Toft, N., E. Jørgensen and S. Højsgaard, 2005: Diagnosing diagnostic tests: evaluating the assumptions underlying the estimation of sensitivity and specificity in the absence of a gold standard. *Prev. Vet. Med*, 68, 19-33.

USDA-FSIS, 2012: Shiga Toxin-Producing Escherichia coli in Certain Raw Beef Products. Available at https://www.fsis.usda.gov/OPPDE/rdad/FRPubs/2010-0023FRN.pdf (accessed 06 August 2015).

USDA-FSIS, 2014: Microbiology Laboratory Guidebook 5B.05., Detection and isolation of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) from meat products and carcass and environmental sponges.

Vacek, P. M., 1985: The effect of conditional dependence on the evaluation of diagnostic tests. *Biometrics*, 41, 959-968.

Wang, R., M. Koohmaraie, B. E. Luedtke, T. L. Wheeler and J. M. Bosilevac, 2014: Effects of in-plant interventions on reduction of enterohemorrhagic *Escherichia coli* and background indicator microorganisms on veal calf hides. *J. Food Prot*., 77, 745-751.

CHAPTER VI

CONCLUSIONS

Although there are now seven EHEC serogroups that are of primary foodborne public health importance, literature has focused largely on EHEC O157 because it was the first, and remains the most common, cause of significant EHEC foodborne illness in humans. The first chapter of this dissertation summarized existing literature pertaining to EHEC-7 human illness, epidemiology in cattle populations, interventions, and detection methods. This review of the literature demonstrated areas that were lacking information regarding non-O157 EHEC in beef cattle populations. This dissertation addressed several of these knowledge gaps, primarily yielding prevalence and risk factors associated with EHEC-7 carriage in beef cows on pasture and at slaughter, dietary factors contributing to EHEC-7 shedding in the finishing period, and an estimation of the sensitivity and specificity for detecting EHEC O157 using three EHEC-7 detection methods. .

In the second chapter, a cross-sectional study was conducted to determine the probability for market beef cows to have hides contaminated by EHEC-7 at slaughter. This study was conducted at two geographically distinct slaughter facilities and seasonal sampling was performed. We concluded that the probability to detect each EHEC serogroup was associated uniquely with season, region of origin, or the interaction effect of season by region of origin. This was the first study to perform seasonal sampling for EHEC-7 on hides of market beef cows at slaughter.

The third chapter describes a longitudinal study of EHEC-7 fecal shedding in cow-calf herds from Mississippi and Nebraska. After performing the first study, we wanted to determine if there were on-farm factors contributing to EHEC-7 carriage in beef cows. The results of this study revealed that EHEC O26 and O111 were associated with region, and samples from Nebraska herds were more likely to be positive than samples from Mississippi herds. The greatest prevalence of detection for every EHEC-7 serogroup occurred in the summer sampling period. This was an interesting finding, because previous studies in fed cattle indicated that carriage of non-O157 EHEC was greatest in the fall season. Also, our results were different from one paper describing summer and winter fecal prevalence of EHEC-7 in feedlot cattle. This difference may demonstrate a possible season by production environment interaction where seasonal occurrence of EHEC-7 is dependent upon the stage of production. In addition, this chapter illustrates the temporality of EHEC-7 detection in cattle environments.

The fourth chapter describes a randomized controlled trial performed in a research feedlot where steers were one of five diets. The study was a 2 x 2 +1 factorial arrangement of treatments where there were two levels of fiber source (MDGS or corn fiber isolate), two levels of fiber concentration (17 % NDF or 22% NDF), and a corn control diet. Individual steers were sampled for EHEC-7 using rectoanal mucosa swabs (RAMS) at different sampling days throughout the feeding period. This study revealed that EHEC O45 RAMS detection was associated with fiber level, source, and sampling day. EHEC O103 RAMS detection increased by feeding 40% MDGS but not the corresponding corn bran diet. Hide contamination by EHEC O45 or O103 was less likely in cattle fed MDGS compared to corn bran diets. EHEC O111 RAMS detection

decreased by feeding 40% MDGS but not by feeding the corresponding corn bran diet. Detection of EHEC O157 or O145 was not associated with dietary factors. Feeding 40% MDGS increased the probability for carriage of some EHEC serogroups but decreased probability of others, which indicated that EHEC serogroups have different risk factors associated with feeding MDGS and little association with dietary fiber.

My final project (chapter 5) was a Bayesian latent class analysis to estimate the sensitivity, specificity of three EHEC-7 detection methods and estimate true prevalence in 8 subpopulations. This study estimated that the NeoSEEK test was highly sensitive and specific for detecting EHEC O157 in fecal samples. Also, culture and mqPCR were conditionally dependent tests and less sensitive than NS for the detection of EHEC O157. The performance of these methods for each non-O157 EHEC serogroup remains to be seen.

In summary, the projects included in this dissertation have demonstrated that each EHEC-7 serogroup has unique risk factors. In the past, many studies have not discriminated between the non-O157 EHEC serogroups. This work demonstrates that when performing studies to evaluate the epidemiology of EHEC-7, each serogroup should be individually identified and separate analyses performed to determine associations. For the past few years, significant time and funding has been devoted to the development of rapid, cost-effective detection methods that can identify individual EHEC-7 serogroups in samples of bovine origin. Until now, the performance of these tests was unknown. Future work will include performing Bayesian latent class analyses to estimate performance of the methods on detecting each non-O157 EHEC. The work described in this dissertation contributes to the body of literature, has provided

epidemiological data that will be utilized to populate a quantitative microbial risk assessment, and furthers our understanding of detection of non-O157 EHEC in beef cattle populations.