EPIDEMIOLOGY OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* IN THE BOVINE RESERVOIR: SEASONAL PREVALENCE AND GEOGRAPHIC DISTRIBUTION

by

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Abstract

Cattle shed Shiga toxin-producing *Escherichia coli* (STEC) in their feces. Therefore, cattle pose a risk to contaminate produce, water, and beef products intended for human consumption. The United States Department of Agriculture Food Safety and Inspection Service consider seven STEC serogroups (O26, O45, O103, O111, O121, O145, and O157) as adulterants in raw, non-intact beef products. Contrary to O157, the frequency and distribution of non-O157 serogroups and virulence genes have not been well-established in cattle. Therefore, the objectives of my thesis research were: 1) to appraise and synthesize data from peer-reviewed literature on non-O157 serogroup and virulence gene prevalence, and 2) to determine the prevalence of seven STEC in feedlot cattle feces across seasons. A systematic review and meta-analysis of published literature were conducted to gather, summarize, and interpret the existent data regarding non-O157 serogroup and virulence gene prevalence in cattle. Random-effects meta-analyses were used to obtain pooled non-O157 fecal prevalence estimates for continents worldwide and meta-regression analyses were conducted to evaluate effects of specific factors on between-study heterogeneity. Results indicated that non-O157 serogroup and virulence gene fecal prevalence significantly differed ($P < 0.05$) by geographic region, with North America yielding the highest pooled prevalence estimate worldwide. While previous research has demonstrated a strong seasonal shedding pattern of STEC O157, data regarding the seasonality of non-O157 STEC shedding in cattle is very limited. A repeated cross-sectional study was conducted to obtain serogroup and virulence gene prevalence data for the seven STEC in pre-harvest cattle feces, in summer and winter. We found that non-O157 serogroups were recovered in fecal samples collected in both seasons but virulence genes, thus STEC, were

rarely detected in summer and undetected in winter. In conclusion, non-O157 STEC are present in cattle feces at very low frequencies, but STEC O103 and O157 significantly differed ($P < 0.05$) between seasons. Overall, the research described in this thesis greatly contributes to the limited body of data regarding non-O157 serogroup and virulence gene distribution in cattle and provides a better understanding of two major risk factors, season and geographic distribution, associated with STEC fecal shedding in cattle.

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Dedication

I am honored to dedicate this thesis to my mom, Tamara Dewsbury, and to my grandpa, Graham Dewsbury. Thank you for your constant encouragement, never-ending support, and unconditional love.

Preface

The second and third chapters in this thesis, entitled "Prevalence of Non-O157 Shiga Toxin-producing *Escherichia coli* Serogroups (O26, O45, O103, O111, O121, and O145) and Virulence Genes in Feces, Hides, and Carcasses of Cattle in North America and Worldwide: A Systematic Review and Meta-analysis of Published Literature" and "Summer and Winter Prevalence of Shiga Toxin-producing *Escherichia coli* (STEC) O26, O45, O103, O111, O121, O145, and O157 in Feces of Feedlot Cattle", will be submitted and were submitted for publication in *Animal Health Research Reviews* and *Foodborne Pathogens and Disease*, respectively; therefore, these chapters were formatted according to the respective journal specifications.

Chapter 1: Review of the Epidemiology of Shiga Toxin-producing *Escherichia coli* and the Bovine Reservoir

Introduction

Shiga toxin-producing *Escherichia coli* (*E. coli*) (STEC) are foodborne pathogens of public health importance in the United States. Members of the *Enterobacteriaceae* family, STEC are Gram-negative, rod-shaped, facultative anaerobic organisms. Currently, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) declare seven STEC—O26, O45, O103, O111, O121, O145, and O157 (STEC-7)—as adulterants in raw, non-intact beef products (e.g., ground beef, mechanically tenderized steak). The STEC-7 is comprised of the Shiga toxin-producing *E. coli* most commonly associated with human illness. In the United States, there were 5,763 laboratory-confirmed human STEC illnesses reported to the CDC in 2011 (CDC, 2013). The STEC-7 accounted for 73.5% of total human STEC illnesses reported; STEC serogroups O157, O26, O103, O111, O121, O45, and O145 caused 41.1, 10.6, 9.5, 5.6, 3.1, 2.3 and 1.3% of human illnesses, attributed to STEC, respectively (CDC, 2013).

Human STEC infections are acquired through fecal-oral contact; direct or indirect contact with contaminated human and animal feces lead to STEC infections in humans (Evans and Evans, 1996). Once ingested, STEC have the ability to evade human defenses allowing for bacterial colonization and, potentially, human illness. In humans, STEC infections range in severity depending on the immune status of the infected individual, in addition to the amount of bacteria consumed. In comparison to healthy adults, high risk

groups such as the young, the elderly, and the immunocompromised are more likely to suffer severe illnesses and life threatening complications when infected with STEC.

Human infections can be acquired from a wide variety of sources: environment, infected humans, contaminated food and/or water, and animals. Although STEC can survive in the environment and non-bovine mammals, cattle serve as the primary reservoir of STEC. Shiga toxin-producing *E. coli* are commensal organisms in the gastrointestinal tract of cattle; consequently, these enteric pathogens are shed in their feces—serving as a source of contamination of water and food products. Cattle feces have been the source of contamination in human illness outbreaks; cattle have most commonly been implicated as the reservoir responsible for ground beef STEC contamination (Rangel *et al.,* 2005; Williams *et al.,* 2010; USDA, 2014). Prevalence of STEC in cattle feces and on hides, prior to slaughter, offer a proxy of risk for potential beef contamination. Data regarding prevalence of non-O157 STEC in the cattle reservoir are lacking in the United States (Pihkala *et al.,* 2010); data are needed to populate quantitative microbial risk assessments of these pathogens as non-O157 STEC human illnesses are becoming increasingly recognized nationwide (Brooks *et al.,* 2005; Pihkala *et al.,* 2012).

Escherichia coli O157, the most common STEC, has been widely researched in cattle following its declaration as an adulterant, approximately 20 years ago. Prevalence of *E. coli* O157 in cattle has been shown to be associated with human illness outbreaks, in the United States, demonstrating the importance of the cattle reservoir in human illness (Williams *et al.,* 2010). In cattle, a transient, or intermittent, shedding pattern has been established for *E. coli* O157 (Besser *et al.,* 1997; Hancock *et al.,* 1997a; Sargeant *et al.,* 2000). *Escherichia coli* O157 shedding in the bovine reservoir has been shown to be influenced by

environmental factors, including but not limited to season and geographic location, as well as host factors: diet, age, and type (Barkocy-Gallagher *et al*., 2003; Smith *et al*., 2005; Berry *et al*., 2006; Edrington *et al*., 2006; Fox *et al*., 2007; Jacob *et al*., 2009; Callaway *et al*., 2009, 2013; Jeon *et al*., 2013; Ekiri *et al*., 2014; Islam *et al*., 2014). While non-O157 STEC are contributing to human illnesses (cases, hospitalizations, and deaths) (Crim *et al.,* 2014), the current data regarding the prevalence of non-O157 STEC in the bovine reservoir is limited in the United States (Cernicchiaro *et al.,* 2013; Dargatz *et al.,* 2013; Ekiri *et al.,* 2014). The majority of data regarding non-O157 STEC are in regards to prevalence in food products, such as ground beef and dairy products, while data on the prevalence and risk factors of non-O157 STEC in cattle is lacking (Pihkala *et al.,* 2012). Non-O157 STEC prevalence in the bovine reservoir needs to be further evaluated in order to assess the environmental and host factors influencing non-O157 STEC shedding in cattle, in addition to evaluating the importance of cattle as a reservoir for non-O157 STEC.

Pathogens of Public Health Importance

Shiga toxin-producing *Escherichia coli*—the bacteria. *Escherichia coli* are ubiquitous organisms, most of which do not cause human illness. However, there are six pathotypes of *E. coli* that cause diarrhea in humans: Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETEC), Diffusely Adherent *E. coli* (DAEC), and Shiga toxigenic *E. coli* (STEC) (Croxen *et al.,* 2013). Based on the Kauffman classification scheme, the O (somatic/lipopolysaccharide), H (flagellar), and sometimes K (capsular) antigens are determined (Croxen *et al.,* 2013), and then *E. coli* are

classified into their respective pathotypes. Commonly, *E. coli* are classified in regard to their O:H antigen combination, or serotype. Shiga toxin-producing *E. coli,* or Shiga toxigenic *E. coli,* is the most important pathotype of public health importance worldwide. A subset of STEC that is commonly associated with human illness is referred to as Enterohemorrhagic *E. coli* (EHEC). The STEC pathotype refers to *E. coli* that harbor a Shiga toxin gene (*stx*1 and/or *stx*2), whereas EHEC refers to *E. coli* that harbor at least one Shiga toxin gene and the *eae* gene—which encodes essential proteins for the bacterial attachment necessary in causing human disease. The most common EHEC serotype associated with human disease worldwide is *E. coli* O157:H7 (Croxen *et al.,* 2013). In the United States, *E. coli* O157:H7 was classified as an adulterant in raw, non-intact beef products by USDA-FSIS in 1994.

More recently, in addition to *E. coli* O157, other *E. coli* serogroups are becoming increasingly recognized as important human pathogens (CDC, 2013). In 2012, six additional serogroups—O26, O45, O103, O111, O121, and O145—were declared adulterants in raw, non-intact beef products by FSIS; 70% of the non-O157 STEC associated illnesses in the United States are attributed to these six serogroups (Brooks *et al.,* 2005). The USDA-FSIS defines a sample (e.g., ground beef) to be adulterated by STEC if it harbors an O antigen of public health importance (O26, O45, O103, O111, O121, O145, or O157), a Shiga toxin gene (*stx*1 and/or *stx*2), and an *eae* gene. This adulterant case definition represents EHEC, a subset of STEC, due to the presence of the *eae* gene. Shiga toxigenic *E. coli* will only cause human disease if they harbor the *eae* gene needed to encode for proteins essential for bacterial colonization in the large intestine.

Bacterial mechanism of disease. The pathogenesis of Shiga toxigenic *E. coli* is very complex; a general overview has been adapted from Gyles 2007 and Gragg 2014. Shiga toxin-producing *E. coli* are ingested via an indirect vehicle, such as contaminated food and/or water, or by directly ingesting fecal material. These pathogens have the ability to survive the low pH in the human stomach allowing them to reach their target—the large intestine—where they have the potential to colonize. Shiga toxigenic *E. coli* can only attach to the host epithelial cells in the large intestine provided they harbor the *eae* gene. The *eae* gene encodes for the protein intimin, which is necessary for bacterial attachment; the *eae* gene is essential for STEC to cause human illness. Intimin is encoded on a pathogenicity island on the bacterial chromosome termed the locus of enterocyte effacement, or LEE island. Once the *E. coli* cells reach the large intestine, intimin allows the bacterial cell to attach to the intestinal epithelial cells, or enterocytes. Following the adherence to the host cell, the bacterial cell uses a type III secretion system where a protein, tir, is injected into the host cell. Tir is a receptor for intimin and is encoded by the *tir* gene. The tir protein allows the STEC cell to be tightly bound to the host cell. After this strong, intimate attachment occurs, the bacterial cell manipulates and rearranges the actin present in the host cell to form a pedestal—creating the characteristic attachment and effacement lesion on the microvilli in the intestine of the host. Once the pedestal is formed, STEC cells begin to export virulence factors, notably—Shiga toxin(s). Virulence genes will not be expressed if the cell does not have the ability to attach (i.e., the cell lacks the *eae* gene). Shiga toxins exported from the bacterial cell in the large intestine can directly damage blood vessels in the colon, leading to gastroenteritis and hemorrhagic colitis (Gyles, 2007). Shiga toxins may also be absorbed by the large intestine and circulated in the blood causing systemic

infections primarily in the target areas—the central nervous system and kidneys—where toxin receptors, globotriaosylceramide or Gb3, are abundant (Gyles, 2007; Croxen *et al.,* 2013).

Shiga toxin, a cytotoxic enterotoxin, is the main virulence factor in human STEC infection severity, leading to life threatening complications such as HUS and TTP. There are two types of Shiga toxins (encoded by the *stx* gene) produced by *E. coli*, Shiga toxin 1 (*stx*1) and Shiga toxin 2 (*stx*2); subtypes of these toxins include: *stx*1_a, *stx*1_c, *stx*1_d, *stx*2_a, *stx*2_b, *stx*2_c, *stx*2_d, *stx*2_e, *stx*2_f, and *stx*2_g (Croxen *et al.,* 2013). Shiga toxin-producing *E. coli* can carry *stx*1, *stx*2, both *stx*1 and *stx*2, or a combination of *stx*2 genes (Croxen *et al.,* 2013). Shiga toxins have an A_1B_5 structure and are encoded on a prophage inserted into the bacterial chromosome. Shiga toxin 1 produced by STEC is closely related to the Shiga toxin produced by *Shigella dysenteriae* serotype 1 (O'Brien *et al.,* 1982; Johannes and Roemer, 2010). Shiga toxin-producing *E. coli* are believed to have acquired *stx* production by horizontal gene transfer from *S. dysenteriae.* Through bacterial evolution, STEC have evolved to produce *stx*2, in addition to *stx*1, which results in a more severe human illness (Gyles, 2007; Gould *et al.,* 2013). Shiga toxin-producing *E. coli* can also harbor other virulence factors such as hemolysin (*ehx*) that also have the potential to increase disease severity; hemolysin is a protein that is responsible for lysing red blood cells and is also potentially cytotoxic to endothelial cells (Croxen *et al.,* 2013). Although Shiga toxin and intimin are the key virulence factors in human disease, there are many other bacterial factors aiding in the complex pathogenesis of Shiga toxigenic *E. coli* infections; however, the disease pathogenesis is also influenced by host factors.

Human illness. The severity of human STEC infections depends on the serotype, dose, and the infected individual's age and health status. The infectious dose necessary to cause human illness is very low; only 10 to 100 cells are needed to cause disease. However, ingestion of fewer than 10 cells of *E. coli* O157:H7 has been reported to cause human infection (Hara-Kudo and Takatori, 2011). The incubation period of STEC can range from 24 hours to 10 days, with symptoms usually occurring 3 to 4 days after exposure, depending on the amount of bacteria ingested and the current immune status of the infected individual (CDC, 2014). High-risk groups such as young children, elderly adults, and immunocompromised individuals tend to suffer more severe infections. Shiga toxin-producing *E. coli* human illness severity can range from: no clinical signs (asymptomatic), mild to severe watery diarrhea (gastroenteritis), bloody diarrhea (hemorrhagic colitis), and severe life threatening complications such as hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenia purpura (TTP). Symptoms of HUS include low platelet count (thrombocytopenia), low red blood cell count (hemolytic anemia), and acute renal failure (Mayo Clinic, 2012). Acute renal failure occurs when an individual's kidneys become impaired and lose the ability to filter blood; blood accumulates waste products previously filtered by kidneys leading to severe complications and death (Mayo Clinic, 2012). Hemolytic uremic syndrome is more commonly associated with young children and is the leading cause of acute renal failure in children (Siegler and Oakes, 2005; Kuter, 2014). Shiga toxin-producing *E. coli* O157:H7 is a major cause of HUS worldwide (Croxen *et al.,* 2013). The other life threatening complication associated with STEC is TTP, which is more commonly associated with adults. Thrombotic thrombocytopenia purpura is characterized by the same symptoms as HUS except individuals suffering from TTP are less likely to

suffer kidney failure and more likely to suffer neurological symptoms such as confusion and coma—which aren't commonly associated with HUS (Kuter, 2014). Shiga toxin-producing *E. coli* can cause debilitating illnesses and have the potential to be lethal with the ingestion of a very low dose.

According to FoodNet active surveillance data, there were a total of 19,056 infections, 4,200 hospitalizations, and 80 deaths due to foodborne pathogens reported in 2013 (Crim *et al.,* 2014). Shiga toxin-producing *E. coli* (non-O157 and O157) are among the top foodborne bacterial pathogens reported to the Center for Disease Control and Prevention (CDC) in addition to *Salmonella, Campylobacter, Shigella, Listeria, Vibrio,* and *Yersinia. Salmonella* and *Campylobacter* are the most frequently reported bacterial pathogens associated with foodborne disease annually; in 2013, *Salmonella* and *Campylobacter* were the causative agent in 7,277 and 6,621 cases, 2,003 and 1,010 hospitalizations, and 27 and 12 deaths, respectively, as reported by FoodNet (Crim *et al.,* 2014). Shiga toxin-producing *E. coli* account for a fraction of the total foodborne illnesses annually; 1,113 cases, 286 hospitalizations, and 4 deaths were attributed to STEC in 2013 (Crim *et al.,* 2014). However, foodborne illnesses are believed to be underreported (Scallan *et al.,* 2011). Each year it is estimated that 63,153 STEC O157 and 112,752 non-O157 STEC illnesses occur; 68% and 82% of STEC O157 and non-O157 STEC infections, respectively, are foodborne (Scallan *et al.,* 2011).

Sources of bacterial transmission. Shiga toxin-producing *E. coli* infections are transmitted via the fecal-oral route (Evans and Evans, 1996). There are many sources of human exposure to STEC pathogens that have been linked to human illnesses including, but

not limited to: daycares, elementary schools, nursing homes, correctional facilities, swimming pools, lake water, contaminated food or water, contact with animal or human feces, and direct contact with animals and animal environments (Pihkala *et al.,* 2012). Additionally, STEC infections can be transmitted by human-to-human contact which tends to be more common in facilities where immunocompromised individuals reside or poor-hygiene practices may be common (e.g., daycare, nursing home). In the United States, between 1990 and 2010, non-O157 STEC outbreaks have been attributed to the following sources of transmission: foodborne (45%), human-to-human contact (39%), and the remaining outbreaks (16%) were attributed to water, contact with animals, contact with animals and ill humans, and unknown sources of transmission (Luna-Gierke *et al.,* 2014). Similarly, *E. coli* O157:H7 outbreaks in the United States, between 1982 and 2002, were comprised of the following sources of transmission: foodborne (52%), human-to-human contact (14%), waterborne (9%), contact with animals (3%), and unknown sources (21%) (Rangel *et al.,* 2005). Although there are many sources of STEC transmission that have been linked to human illness, the primary source of transmission, for both non-O157 and O157 STEC human illness outbreaks, is foodborne (Rangel *et al.,* 2005; Luna-Gierke *et al.,* 2014).

Implicated food products. There are a variety of food products that have been linked to STEC illness in humans such as milk, cheese, beef sausage, ground beef, lettuce, spinach, sprouts, apple cider, apple juice, coleslaw, berries, and grapes (Rangel *et al.,* 2005; Pihkala *et al.,* 2012). Food products become contaminated with STEC through various transmission cycles, ultimately with a common source—feces. Animal feces, from bovine and other

species, have been shown to directly contaminate irrigation water, municipal water, and field crops, subsequently causing human illness outbreaks (Anonymous, 2000; Jay *et al.,* 2007; Oliveira *et al.,* 2012; Laidler *et al.,* 2013). Proper manure management by cattle producers (e.g., feedlots, dairies) must be employed to minimize environmental contamination and potential human exposure and illness; however, uncontrollable environmental factors may aid in the spread of STEC organisms. In 2000, Canadians suffered a large gastroenteritis outbreak due to contamination of the municipal water supply in Ontario; evidence suggests *E. coli* O157:H7 was the etiologic agent and bovine manure—the source (Anonymous, 2000). Drainage from surrounding cattle farms were likely the source of contamination of this large outbreak in Ontario; the spread of *E. coli* through rainfall has also been displayed elsewhere (Anonymous, 2000; Ferguson *et al.,* 2007).

Irrigation with contaminated water is a source of contamination of field crops including fruits and vegetables. Shiga toxin-producing *E. coli* can persist in the environment aiding in the contamination of subsequent produce (e.g., lettuce, spinach) grown in contaminated soil. Crops grown in soil with *E. coli* O157:H7 present have been shown to become contaminated due to bacterial transport via the root system of plants (i.e., lettuce); soil contaminated with higher amounts of *E. coli* O157:H7 were associated with a higher prevalence of lettuce leaf contamination (Solomon *et al.,* 2002; Oliveira *et al.,* 2012). Although there is no scientific evidence for non-O157 STEC, it can be hypothesized that bacterial transport via plant root systems would occur similarly for non-O157 STEC; however, data are needed to support this hypothesis. On the other hand, in the dairy cattle environment, STEC can contaminate soil, and other bedding, which can contaminate the

udders of dairy cattle leading to contamination of milk and other dairy products if proper sanitation practices aren't employed during milking and milk is consumed raw. This cross-contamination in the cattle environment, due to the lack of proper sanitation during the milking process, poses a threat to unpasteurized dairy products. While Shiga toxin-producing *E. coli* have been implicated as a causative agent in many foodborne illness outbreaks, ground beef is a commonly implicated source of STEC leading to foodborne illness in humans in the United States.

The USDA-FSIS estimates that approximately one-third of human illnesses are attributed to beef, based on *E. coli* O157:H7 outbreak data from 2000 to 2006 (Withee *et al.,* 2009). Similarly, between 1982 and 2002, ground beef was the source of human illness in 41% of the foodborne outbreaks attributed to *E. coli* O157:H7 in the United States (Rangel *et al.,* 2005). In 2013, the USDA-FSIS reported five class I recalls due to STEC contamination (USDA, 2014). A class I recall indicates that agents (STEC) with the potential to cause health problems or death when the product is consumed are present in the food product (USDA, 2014). All recalled products due to STEC contamination were labelled either "ground beef products" or "beef products" (USDA, 2014). Four of the STEC recalls were attributed to STEC O157 while a single recall was attributed to non-O157 STEC; in total, 1,840,533 pounds of beef were recalled in 2013 due to STEC contamination (USDA, 2014). Nearly two-million pounds of beef were recalled in 2013, resulting in a huge loss for the beef industry. The average price of ground beef in 2013 was $3.40, these five recalls due to STEC contamination result in roughly a $6,257,812.20 economic loss to the beef industry (US Department of Labor, 2014). The importance of detecting and mitigating

the risk of STEC in the beef supply is at an all-time high, both for the consumers and the producers.

While it is important to note that Shiga toxin-producing *E. coli* can be inactivated when food products are properly cooked, many consumers are unaware of how to properly cook meat products although aware of the risk associated with consuming under-cooked beef. A study by Røssvoll *et al.,* found that only 8.6% (65 out of 755) of survey participants stated they knew what the core temperature should be in a hamburger and only 0.2% (2 out of 768) of survey participants measured the inside temperature of a hamburger to decide if the hamburger was "sufficiently cooked" (Røssvoll *et al.,* 2014). Ground beef must be cooked to an internal temperature of 160° F (71°C) to inactivate bacterial pathogens, including STEC, which may be present. Contrary to their current cooking habits, 94.5% (966 out of 1,022) of the survey participants were aware that raw ground beef may contain harmful bacteria (Røssvoll *et al.,* 2014). Due to the improper cooking techniques practiced by the majority of consumers, the beef industry cannot rely on the consumers to ensure elimination of potential pathogens present. In order to reduce STEC infections attributed to beef products, the presence of these pathogens in the cattle reservoir must be considered.

The primary source of cross-contamination of beef at the harvest facility is cattle hides (Loneragan and Brashears, 2005). Cattle fecal material contaminates hides of cattle and subsequently poses a threat to cross-contaminate beef carcasses and beef products at the harvest facility. Therefore, cattle fecal and hide prevalence of STEC can be indicators of potential cross-contamination of beef carcasses prior to harvest (Renter *et al.,* 2008). In a mathematical model, fecal and hide prevalence of *E. coli* O157 in cattle were statistically

associated (Loneragan and Brashears, 2005). Feces are ultimately the source of STEC organisms and subsequent hide and beef contamination. Fecal prevalence of STEC in the cattle reservoir provides an estimate of risk of beef contamination at slaughter and the subsequent potential for human illnesses.

Due to USDA-FSIS and industry standards, the beef industry has employed many interventions to decrease the bacterial load of STEC, and other pathogens, in cattle (pre- and post-harvest); therefore, reducing entry and subsequent contamination from these organisms in the food supply. There have been a wide variety of interventions employed by the beef industry to mitigate the risk of *E. coli* O157 in beef. Pre-harvest interventions include, but are not limited to: management practices to maintain a clean environment including clean water and feed, rations including prebiotics and/or probiotics, vaccination regimen, hide washes, and use of bacteriophage while post-harvest interventions often include hide and carcass washes (hot water and organic acids (e.g., lactic acid)), steam vacuuming, carcass trimming, and proper sanitation and disinfection of equipment at the harvest facility (Koohmaraie *et al.,* 2005; LeJeune and Wetzel, 2007). These implementations in the cattle industry, pre- and post-harvest, and in other food production systems seem to be successful in decreasing human illnesses attributed to *E. coli* O157 since 2000 (Gould *et al.,* 2013). However, due to the recent declaration of these foodborne pathogens, this trend has not been established for non-O157 STEC. The incidence of human illnesses attributed to non-O157 STEC has increased in the United States; however, the incidence likely appears inflated due to a recent increase in testing and development of detection methods for these non-O157 STEC (Gould *et al.,* 2013).

Detection of Shiga Toxin-producing *Escherichia coli*

Diagnostic methods have been well established in the past for *E. coli* O157; however, due to biochemical differences between *E. coli* O157 and non-O157 serogroups, differentiating these STEC serogroups poses a challenge. *Escherichia coli* O157 does not ferment sorbitol within 24 hours, therefore Sorbitol MacConkey agar (SMAC) can be used to easily distinguish potential *E. coli* O157 colonies from other serogroups, as they appear clear or gray on this medium (March and Ratnam, 1986). In contrast, the non-O157 serogroups ferment sorbitol making them difficult to distinguish using SMAC for culture isolation as non-O157 serogroups appear pink. However, chromogenic media is available to differentiate the non-O157 serogroups by color phenotype based on biochemical properties (Possé *et al.,* 2008; Tillman *et al.,* 2012; Wylie *et al.,* 2013). Enrichment and immunomagnetic separation (IMS) culture techniques paired with the use of various culture methodology have been shown to improve the detection of STEC, thus increasing apparent prevalence estimates obtained in the cattle reservoir, when utilized (Smith, 2014). Molecular detection methods like polymerase chain reaction (PCR) are available to identify O antigens and virulence genes (Bai *et al.,* 2010; Bai *et al.,* 2012; Paddock *et al.,* 2012). The use of commercially available antisera is used to serotype isolates, usually after the O gene has been identified (Guinée *et al.,* 1972).

The IMS procedure, which consists of antibody coated magnetic beads, is employed to increase detection of a specific organism while decreasing the background organisms that may be present. The use of IMS has been shown to increase diagnostic test sensitivity compared to other methods where IMS is not employed for STEC O157 in cattle feces (Chapman *et al.,* 1994; Islam *et al.,* 2014). Recently, the use of culture-based detection

methods, including an IMS step, has been shown to increase the apparent prevalence of non-O157 STEC detected in cattle feces when compared to direct PCR (Cernicchiaro *et al.,* 2013). The serogroup-specificity of the beads used to detect specific *E. coli* O groups of interest also influences the prevalence estimates obtained. Non-specific binding between IMS beads and their target serogroup of interest has been shown (Cernicchiaro *et al.,* 2013). This poorly understood phenomena may result in unequal test sensitivities among the STEC serogroups of interest due to the lack of IMS bead specificity; this issue needs to be further evaluated to assess diagnostic test sensitivity and specificity. The diagnostic methods employed directly impact the prevalence estimates obtained.

Even though there are assays available to aid in detection of STEC of public health importance, there is a lack of a formal standardized method to identify these pathogens and virulence genes in the live cattle reservoir. This lack of standardization makes it difficult to interpret and compare prevalence estimates among published literature, even within the same matrix (e.g., feces, hide, carcass), and over the years with advances in detection (e.g., enrichment, IMS). Detection methods have been well established for detecting *E. coli* O157 in bovine feces; however, diagnostic methods for non-O157 STEC are still being explored and evaluated. Evaluation of current literature suggests that culture-based detection methods employing enrichment and IMS techniques increases the diagnostic test sensitivity (Chapman *et al.,* 1994; Cernicchiaro *et al.,* 2013); however, standardized diagnostic methods for identifying STEC in cattle feces are still needed. Cattle fecal prevalence is a potential indicator STEC contamination risk at the harvest facility and valid standardized procedures would provide the estimates necessary to understand the burden of STEC in cattle prior to harvest.

While methods of detection have not been standardized for identifying STEC in cattle feces, hides, or carcasses, the FSIS presently has a standard operating procedure in place for detecting these adulterants in ground beef. Currently the FSIS uses the DuPont™ BAX® system for detecting these pathogens in retail raw ground beef (Wasilenko *et al.,* 2014). Briefly, the DuPont™ BAX® system is comprised of the following methodology: sample preparation and enrichment, real-time PCR screening procedure for *eae* and *stx* genes—if samples test positive to the initial screen they then undergo IMS and the bead suspensions are subsequently plated and colonies are then serologically agglutinated and confirmed by BAX® Real-time PCR STEC Suite (for non-O157 confirmation) or BAX® Real-time PCR assay for *E. coli* O157:H7 (Wasilenko *et al.,* 2014). In the future, there should be steps taken to outline a standard methodology for STEC in other matrices besides ground beef. Standardization in detection methodology for STEC would lead to a more conclusive and consistent estimate of STEC risk between matrices, allowing more robust comparisons to be made between studies while assessing the risk of these pathogens prior to their entry into the food supply.

Non-human Sources of Shiga Toxin-producing *Escherichia coli*

In addition to cattle, STEC serogroups have been identified in other animals such as domestic animals—goats, sheep, poultry, swine, cats, and dogs, and wild animals—deer, elk, coyotes, feral swine, birds, opossums, raccoons, and bison (Asakura *et al.,* 1998; Shere *et al.,* 1998; Zschöck et al*.,* 2000; Renter *et al.,* 2001*,* 2004; Bentancor *et al.,* 2007; Bettelheim, 2007; Jay *et al.,* 2007; Cooley *et al.,* 2013; Laidler *et al.,* 2013; Callaway *et al.,*

2014; Swirski *et al.,* 2014). Domestic animals, more commonly ruminants (cattle, sheep, and goats), pose a direct risk to humans, especially high-risk groups, at petting zoos, agricultural fairs, rodeos, and other events where humans are in direct contact with animals or the animal environment and proper hygiene practices aren't employed. Wild animals also have the potential to contaminate produce, animal feed, field crops, and the environment by shedding these pathogens in their excrement and indirectly causing human illness. Feral swine and deer have been implicated as sources of STEC outbreaks in the United States (Jay *et al.,* 2007; Laidler *et al.,* 2013). In 2006, feral swine feces contaminated baby spinach farms in California which resulted in a multi-state *E. coli* O157:H7 outbreak resulting in 183 illnesses, 95 hospitalizations, 29 HUS cases, and one death (CDC, 2006; Jay *et al.,* 2007). More recently, in 2011, fresh strawberries were implicated as a vehicle of 15 *E. coli* O157:H7 illnesses, six hospitalizations, and four HUS cases—two of which resulted in death; deer feces were the source of strawberry contamination in Oregon (Laidler *et al.,* 2013).

In addition to animal reservoirs, insects, such as stable flies (*Stomoxys calcitrans*) and house flies (*Musca domestica*), have been shown to carry STEC (Bailey *et al.,* 1973; Alam and Zurek, 2004; Castro *et al.,* 2013). Stable flies have the potential to spread STEC long distances—up to 18 miles in 24 hours, potentially aiding in STEC transmission between cattle and within the cattle environment (Bailey *et al.,* 1973; Castro *et al.,* 2013). House flies (*Musca domestica*) have demonstrated a potential role in transmission of *E. coli* O157:H7 between cattle and the surrounding environment (Alam and Zurek, 2004). Viable bacterial cells and virulence genes have been previously isolated from the mouth, body surface, and intestinal contents of the stable fly, further indicating that these insects may

spread STEC (Castro *et al.,* 2013). While STEC have been isolated from many animal sources, with respect to the beef industry—cattle remain the primary reservoir of concern regarding human STEC infections in the United States.

Biology of Shiga Toxin-producing *Escherichia coli* in Cattle

Escherichia coli, including commensal and pathogenic organisms, are mesophiles and prefer the animal gastrointestinal (GI) tract due to the favorable growth conditions provided—anaerobic environment with a temperature between 20 to 45˚C. Shiga toxigenic *E. coli* are commensal organisms in the gastrointestinal tract of cattle, as well as other animals, and these human pathogens are shed in their feces. Cattle are the principal reservoir for STEC in the United States. Shiga toxin-producing *E. coli* have the ability to colonize cattle at any age (Baehler and Moxley, 2000). The primary site for STEC colonization is the recto-anal junction, or the distal colon, in the cattle hindgut (Naylor *et al.,* 2003). Similar to human disease pathogenesis, colonization requires STEC organisms to possess the necessary genes to successfully attach to the host epithelial cells; this attachment yields the same attaching and effacing lesions found in human patients (Baehler and Moxley, 2000).

Once the STEC colonize the hindgut of cattle they are shed in feces; these organisms are shed intermittently (Sargeant *et al.,* 2000). Studies show that cattle can shed STEC for approximately a month; although cattle are often not persistently infected, re-infection is common when cattle are in close contact (e.g., feedlot, transport) (Besser *et al.,* 1997; Khaitsa *et al.,* 2003). Although inflammation and immune responses occur following *E. coli*

O157 colonization in cattle, adult cattle do not show clinical symptoms of STEC infection (Moxley and Smith, 2010). In the event that STEC organisms do not colonize the hindgut, the organisms will still be shed in their feces, although for a shorter duration (e.g., days).

The transient nature of *E. coli* O157 fecal shedding in cattle has been well established (Besser *et al.,* 1997; Hancock *et al.,* 1997a; Sargeant *et al.,* 2000), intermittent shedding has also been observed for non-O157 STEC (Menrath *et al.,* 2010). In cattle populations, there are long periods of low prevalence and short periods of high prevalence within herds (Renter and Sargeant, 2002); this has been demonstrated for *E. coli* O157 in cattle feces (Hancock *et al.,* 1997a; Renter and Sargeant, 2002). Cattle shedding *E. coli* O157:H7 at a concentration greater than 10^4 colony forming units (CFU) per gram of feces are termed "super-shedders" (Chase-Topping *et al.,* 2008). Most cattle shed *E. coli* O157 below this super-shedder threshold; however, these small populations of super-shedder cattle have been documented to shed the majority of the *E. coli* in the herd (Omisakin *et al.,* 2003; Chase-Topping *et al.,* 2007). In the United Kingdom, 9% of the cattle population tested were super-shedders, this small proportion of cattle accounted for over 96% of *E. coli* O157 shed in the entire population at slaughter (Omisakin *et al.,* 2003); similar results were also established in Scotland (Chase-Topping *et al.,* 2007).

More recently, this super-shedding phenomenon has been observed in non-O157 serogroups as well; cattle in a herd with super-shedders were twice as likely to be positive by PCR in comparison to cattle in a herd with no super-shedders present (Menrath *et al.,* 2010). Identifying super-shedder cattle at harvest could exponentially decrease the risk of STEC exposure to other cattle and subsequent food products at that specific point-in-time. The temporality component of STEC in cattle populations makes it difficult to identify and

control super-shedders due to the transient nature of fecal shedding in cattle. Super-shedding in cattle is intermittent, therefore cattle are not super-shedders the duration of their lives, rather they are termed super-shedders when observed to shed STEC at concentrations greater than 10^4 CFU/g. Although representative of a small part of the cattle population, super-shedders are hypothesized to play a key role in STEC exposure and transmission within cattle cohorts; this super-shedding status poses a serious risk to beef contamination due to the high concentrations of bacteria harbored at slaughter (Omisakin *et al.,* 2003; Chase-Topping *et al.,* 2007). In addition to the transient shedding patterns of STEC observed in cattle, the prevalence of STEC in the bovine reservoir can be altered by many cattle risk factors, such as the environment, diet, and age.

Cattle Risk Factors Affecting Shiga Toxin-producing *Escherichia coli* Fecal Prevalence

Environment. Although the primary habitat of *E. coli* organisms is in the gastrointestinal tract of animals, *E. coli* can survive in the environment in secondary habitats like water and soil, aiding in the transmission of STEC between cattle. *Escherichia coli* can survive outside of the host animal in the environment for long periods of time, which poses a persistent problem as this allows for re-infection within a herd, or introduction to a new cohort, aiding in the transmission of STEC in cattle populations. In manure, *E. coli* O157:H7 has been shown to survive for up to 21 months, although uncommon (Kudva *et al.,* 1998). Environmental temperature and fecal moisture content have been shown to influence the survivability of fecal bacteria present in manure (Wang *et al.,* 2004). Though extreme, *E. coli* O157:H7 has been shown to survive subzero conditions (-20°C) in bovine manure for

at least 100 days, demonstrating the ability of *E. coli* to endure harsh environmental conditions allowing for its survival in the cattle environment (Kudva *et al.,* 1998). In the cattle environment, water troughs harbor STEC and are a vehicle for transmission between animals sharing a common water source (Midgley and Desmarchelier, 2001; Callaway *et al.,* 2013). Fecal-contaminated feed bunks and cattle grooming behaviors are also plausible routes of horizontal STEC transmission between cattle (McGee *et al.,* 2004). In addition to fomites, migratory birds, particularly European starlings, have been shown to spread *E. coli* O157:H7 between farms, demonstrating starlings as a potential mechanical and biological vector of STEC transmission into the cattle environment (Cernicchiaro *et al.,* 2012; Callaway *et al.,* 2014; Swirski *et al.,* 2014).

In feedlots, a commercial environment of beef cattle in the United States, pen-floor conditions have been shown to influence the prevalence of STEC O157 shedding in cattle. In Smith *et al.* 2001, within-pen prevalence of *E. coli* O157:H7 was higher in cattle housed in pens with muddy or dusty floors compared to cattle housed in an intermediate or normal pen condition (Smith *et al.,* 2001). However, Stanford *et al.* suggests that hide contamination may play a more important role in *E. coli* transmission between cattle compared to pen-floor contamination (Stanford *et al.,* 2011). Increased cattle density has been associated with an increased prevalence of fecal-contaminated hides (Renter *et al.,* 2008; Stanford *et al.,* 2011; Callaway *et al.,* 2013). During transport to the harvest facility, cattle are transported on large trailers and are in close quarters—this is a potential route of pre-harvest contamination of hides with feces and transmission of STEC within a cohort. The contamination of the cattle environment paired with the survivability and transmissibility of STEC within the bovine reservoir and the environment leads to the

continuous cycle of STEC transmission in feedlots through various cohorts of cattle (Midgley and Desmarchelier, 2001). In addition to the numerous routes of transmission, constant re-exposure, and asymptomatic carriage of STEC in cattle, there are also manageable as well as uncontrollable cattle risk factors that have been shown to influence fecal shedding in the bovine reservoir. Shedding patterns and STEC prevalence in cattle can be altered by a variety of factors, most of which the mechanism of action are poorly understood. In addition to the cattle environment, cattle diet, cattle age, and cattle type are a few of the risk factors that have been shown to influence fecal shedding.

Diet. In the gastrointestinal tract of cattle, *E. coli* O157 colonization and subsequent shedding can be influenced by a variety of factors in the gut, including but not limited to pH, volatile fatty acids (VFA), and competitor organisms (Reviewed in Jacob *et al.,* 2009). Diet has been associated with *E. coli* O157 prevalence in cattle (Reviewed in Callaway *et al.,* 2009). Distiller's grains, an ethanol by-product and common feedstuff in commercial operations, have been shown to be associated with higher levels of *E. coli* O157 fecal shedding in cattle (Jacob *et al.,* 2010). Forage-fed cattle typically have lower fecal concentrations of *E. coli,* including *E. coli* O157:H7, when compared to grain-fed cattle (Callaway *et al.,* 2013). Grain form has also been shown to impact fecal shedding of *E. coli* O157. Fox *et al.,* 2007 showed that cattle fed diets containing steam-flaked grains rather than dry-rolled grains increased *E. coli* O157 fecal shedding in commercial feedlot cattle (Fox *et al.,* 2007). A plausible hypothesis of the contribution of grain form to *E. coli* O157 fecal shedding is that dry-rolling of grains, opposed to steam-flaking, allows more starch to be passed to the large intestine and colon of cattle where it is fermented to produce VFA's

consequently reducing *E. coli* O157 populations shed in feces (Fox *et al.,* 2007). Other dietary inclusions such as probiotics (e.g., direct-fed microbials), beta-agonists, ionophores, and other feedstuffs have been shown to affect fecal shedding (Reviewed in Callaway *et al.,* 2009). The impact of diet on fecal shedding of STEC O157 in cattle is not consistent across studies, demonstrating the complexity of microbial ecology and *E. coli* shedding in cattle (Jacob *et al.,* 2009). While cattle diet has been widely researched for *E. coli* O157 fecal shedding, data are needed to assess the role of diet in non-O157 STEC shedding in cattle.

Age. In addition to environmental and management factors, host factors, such as age, also play a role in *E. coli* shedding in cattle. Zhao *et al.,* 2013 reported a significant increase of STEC shedding in the youngest group of calves when compared to their dams (Zhao *et al.,* 2013). In a study by Nielsen *et al.,* 2002, a strong effect of age was observed; cattle between two and six months of age shed significantly higher levels of STEC O157 than cows (Nielsen *et al.,* 2002). Shaw *et al.,* 2004 demonstrated an association between STEC O26 fecal shedding and very young calves (less than 7 weeks of age); as the calves aged, STEC O26 shedding prevalence decreased over the 21-week study period (Shaw *et al.,* 2004). Non-O157 and O157 STEC were most common in feces of calves post-weaning compared their prevalence observed during the finishing period and at slaughter (Ekiri *et al.,* 2014). The associations found may be a function of calf age, but it may also be attributed to change in diet, immunity, or environment (Nielsen *et al.,* 2002; Shaw *et al.,* 2004; Zhao *et al.,* 2013; Ekiri *et al.,* 2014). The colonization of *E. coli* in calves may be due to a naïve microbial population in the gut attributed to calf age and immune status. Therefore, when calves are introduced to STEC in the environment, from their dam, or exposed to stressors (e.g.,

weaning), they are easily colonized and shed these pathogens at higher levels than cows with well-established gut microbiota. It is important to note that some studies are inconsistent with the conclusions regarding STEC fecal shedding influenced by cattle age, this inconsistency could be due to various contributing factors (e.g., environment, diet) or confounding by unknown variables.

Type. Cattle genetics have been the motivation for testing hypotheses regarding *E. coli* fecal shedding (Berry *et al.,* 2006; Jeon *et al.,* 2013). In a comparison of *Bos taurus* and *Bos indicus* cattle, no differences were identified in generic *E. coli* carriage based on cattle genotypes (Berry *et al.,* 2006). However, more recently, genetic factors have been shown to affect *E. coli* O157 colonization. Jeon *et al.,* demonstrated that Brahman cattle (*Bos indicus*) were more resistant to *E. coli* O157 colonization than Angus (*Bos taurus*) and Angus-Brahman cross-bred cattle (Jeon *et al.,* 2013). Cattle genetics and their influence on fecal shedding of *E. coli* are contradictory, elucidating the need for more data to support either hypothesis. Similarly, cattle type, beef or dairy, is also a plausible risk factor influencing shedding of *E. coli* in cattle. A meta-analysis by Islam *et al.,* 2014 reported overall, worldwide *E. coli* O157:H7 prevalence estimates of 1.75%, 6.84%, and 19.58% for dairy, beef, and feedlot cattle, respectively, significant differences were observed between dairy and feedlot cattle (Islam *et al.,* 2014). While differences based on cattle genetics and cattle type may be observed, the prevalence outcome is confounded within a multitude of factors, primarily production system.

In the United States, beef cattle are raised in a pasture-based system and/or a feedlot setting. In a pasture-based production system, cattle are often housed at a lower

stocking density compared to a feedlot operation where animals are more densely stocked. The stocking density, or concentration of animals per area, is vastly different between pasture and feedlot systems in the United States. Increased cattle density allows for disease transmission to occur between cattle more frequently. Cattle density has been positively associated with prevalence of contaminated hides, a route for beef contamination (Renter *et al.,* 2008; Stanford *et al.,* 2011; Callaway *et al.,* 2013); this association demonstrates the increase of STEC exposure with increasing number of animals housed together. Additionally, in a feedlot setting, animals of all ages are constantly entering and leaving the feedyard, allowing for more disease exposure and susceptible individuals to enter a population. In contrast, dairy cattle are raised in similar settings as feedlot cattle however, the turnover rate is lower (Sanderson, 2015). Dairy cattle stay in the production setting longer than beef cattle in a feedyard, thus reducing the introduction of STEC and other agents into the herd. In the *E. coli* O157 fecal prevalence meta-analysis findings by Islam *et al.,* the lack of a statistically significant difference in fecal shedding between dairy and beef cattle may indicate this similarity in production system, while fecal prevalence in feedlot cattle is observed to be significantly higher (Islam *et al.,* 2014).

While cattle genetics and cattle type may be risk factors influencing fecal shedding, the production environment impact on shedding offers a more plausible hypothesis. The function of the cattle environment, management, and other practices associated with different production systems offer a reasonable hypothesis influencing the epidemiological triad (host-agent-environment) of STEC in cattle populations (Sanderson, 2015). Cattle risk factors, both known and unknown, affect *E. coli* shedding in cattle, additionally,

extrinsic factors such as season and geographic region have been shown to be associated with STEC shedding in the bovine reservoir and human illnesses.

Seasonality of Shiga Toxin-producing *Escherichia coli*

Escherichia coli O157:H7 has a strong seasonal pattern. In summer months, *E. coli* O157 is the most prevalent serogroup shed by cattle (Barkocy-Gallagher *et al.,* 2003; Williams *et al.,* 2010). While *E. coli* O157 reaches peak prevalence in the cattle reservoir during summer months, *E. coli* O157 is rarely isolated from cattle during winter months (Barkocy-Gallagher *et al.,* 2003; Smith *et al.,* 2005; Edrington *et al.,* 2006); demonstrating that significant differences between summer and winter *E. coli* O157 shedding in cattle exist. In contrast, a study evaluating the seasonal trend of non-O157 STEC observed a different pattern; non-O157 STEC were more prevalent in spring and fall rather than summer and winter in cattle (Barkocy-Gallagher *et al.,* 2003). Although a seasonal trend has been well established for *E. coli* O157, the seasonality of non-O157 STEC has not been widely researched in the cattle reservoir.

The seasonality component of STEC shedding in cattle is not completely understood; however, there are some plausible hypotheses believed to contribute to cattle fecal shedding such as the effect of day length and ambient temperature (Edrington *et al.,* 2006). Edrington *et al.,* suggests that day length and subsequent physiological changes that occur within the cattle reservoir may be a more important contributing factor to the seasonality component of *E. coli* O157:H7 in cattle, opposed to ambient temperature alone (Edrington *et al.,* 2006). Day length may alter seasonally secreted hormones (e.g., melatonin) in cattle,

subsequently modifying bacterial populations, shedding patterns, and/or immunity which contribute to the observed seasonal trend of *E. coli* O157:H7 shedding in cattle, in addition to ambient temperature (Edrington *et al.,* 2006, 2008). Contrary to this suggested hypothesis, ambient temperature seems to be the most plausible and widely-accepted hypothesis. Increased ambient temperature may favor bacterial growth and increase STEC survival in the cattle environment (e.g., feed, water, soil) and cattle reservoir (e.g., feces, hides), allowing for increased disease transmissibility between cattle, leading to subsequent beef contamination and human illnesses (Smith *et al.,* 2005). While there are multiple hypotheses regarding the etiology of seasonal STEC shedding in the cattle reservoir, the mechanism is poorly understood.

The seasonality of STEC shedding observed in cattle correlates to human illnesses attributed to *E. coli* O157:H7 (Williams *et al.,* 2010). This correlation demonstrates the importance of the cattle reservoir in human illness. As illustrated in a mathematical model, the supply of ground beef remains fairly constant year-round; however, in summer months, *E. coli* O157:H7 prevalence in cattle peaks in June followed by a peak in ground beef contamination and human illness attributed to *E. coli* O157:H7 one month later (Williams *et al.,* 2010). The observed one-month delay between peak in cattle prevalence and human illness is reasonable as it may take a month, on average, for beef products to be processed, shipped, and consumed (Williams *et al.,* 2010). In addition to STEC O157, human illnesses attributed to non-O157 STEC outbreaks are also more common in the summer months. Most of the non-O157 STEC outbreaks reported (58%) in the United States occurred in four months—between June and September (Luna-Gierke *et al.,* 2014); these outbreaks encompassed all reported outbreaks from various sources and haven't been associated

with cattle prevalence (Luna-Gierke *et al.,* 2014). Prevalence and seasonality of non-O157 STEC in the cattle reservoir must be further evaluated to assess the correlation, or lack thereof, of non-O157 STEC in cattle and subsequent human illnesses attributed to non-O157 STEC.

Alternate hypotheses regarding the seasonality of human illnesses attributed to STEC exist. While food handling, preparation, and storage are all key factors in mitigating foodborne illness year-round, some argue that an increase in poor food handling and storage practices occur during the warmer months, as picnics and barbeques are more common. This hypothesis is plausible as these are key factors influencing any foodborne pathogen and resulting illness. However, the evidence of prevalence in the bovine reservoir peaking prior to beef contamination, constant consumption of beef year-round, and increased human illness in warmer months indicate the role of cattle in this transmission process. While the increase of poor food handling and preparation techniques likely increase foodborne illnesses, including those attributed to STEC, cattle feces are responsible for initially contaminating the food product. Although the seasonality component of STEC pathogens is not fully understood in the bovine reservoir, there is a strong correlation present between prevalence of STEC shed by cattle and subsequent human illnesses attributed to these foodborne pathogens.

Geography of Shiga Toxin-producing *Escherichia coli*

In addition to seasonality, geographic location may impact Shiga toxigenic *E. coli* prevalence in cattle and consequently, the frequency of human infection. Surveillance by

the CDC shows that the laboratory-confirmed incidence rate for STEC infections, both O157 and non-O157 serogroups, tend to be geographically distributed in the upper Midwestern United States (CDC, 2013). However, this geographic distribution has not been correlated to prevalence in cattle (Hancock *et al.,* 1997b). Although regional differences in *E. coli* O157 shed by cattle have not been established in feedlots within the United States (Hancock *et al.,* 1997b), a meta-analysis by Islam *et al.* demonstrates that *E. coli* O157 fecal prevalence in cattle significantly differs between geographic regions worldwide (Islam *et al.,* 2014). Worldwide *E. coli* O157 cattle prevalence was estimated to be 5.68% (95% CI 5.16-6.20%); however, *E. coli* O157 prevalence in cattle from North America (Canada, United States, Mexico) was higher than other regions—7.35% (95% CI 6.44-8.26%) (Islam *et al.,* 2014). In North America, the estimate was fairly robust, including 46 studies and 110,641 cattle sampled (Islam *et al.,* 2014). In further analysis, the United States was the region of highest prevalence in North America—7.6% (Islam *et al.,* 2014). The increased *E. coli* O157 burden in the United States demonstrates the importance of the cattle reservoir to public health in this country.

The six non-O157 STEC of public health importance in the United States have also been identified in cattle feces worldwide, including the following regions: Asia, Europe, North America, and South America (Zschöck et al*.,* 2000; Kobayashi *et al.,* 2001; Jenkins *et al.,* 2002; Khan *et al.,* 2002; Meichtri *et al.,* 2004; Padola *et al.,* 2004; Shaw *et al.,* 2004; Bonardi *et al.,* 2005; Kijima-Tanaka *et al.,* 2005; Zweifel *et al.,* 2005; Jeon *et al.,* 2006; Pearce *et al.,* 2006; Bonardi *et al.,* 2007; Renter *et al.,* 2007; Karama *et al.,* 2008; Joris *et al.,* 2011; Monaghan *et al.,* 2011; Sasaki *et al.,* 2011; Lynch *et al.,* 2012; Thomas *et al.,* 2012; Cernicchiaro *et al.,* 2013; Sasaki *et al.,* 2013a, 2013b; Baltasar *et al.,* 2014). Similar to *E. coli*

O157, evaluation of the literature indicates cattle in the United States to have the highest non-O157 STEC burden reported in North America. Non-O157 STEC cattle fecal prevalence by region ranged from undetected to: 17% in North America, 12% in South America, 4% in Asia, and 2.4% in Europe (Padola *et al.,* 2004; Shaw *et al.,* 2004; Jeon *et al.,* 2006; Cernicchiaro *et al.,* 2013). Although non-O157 STEC fecal prevalence in cattle has been the primary objective of studies in Australia and Africa, pathogenic non-O157 STEC have not been detected in cattle feces from these regions (Cobbold and Desmarchelier, 2001; Musa *et al.,* 2013). While geographical differences in cattle type, production system, cattle diet, season, and laboratory methodology employed must also be taken into account to assess true geographic differences, it appears that *E. coli* prevalence in the bovine reservoir may be influenced by region.

Conclusion

The many risk factors affecting the epidemiology of Shiga toxin-producing *E. coli* shedding in cattle poses a challenge when attempting to establish the true prevalence of these foodborne pathogens in the bovine reservoir. Fecal prevalence estimates obtained from cattle offer a proxy for subsequent risk of beef contamination and human illnesses. This literature review regarding the epidemiology of Shiga toxigenic *E. coli* and the bovine reservoir reveals some of the major inconsistencies and data gaps that exist in the published literature to date, especially for non-O157 STEC. In order to assess and mitigate the risk of these non-O157 STEC pathogens in the beef supply, their presence in the cattle reservoir must be further evaluated. Data regarding non-O157 STEC in cattle are lacking;

therefore, this thesis will focus on presenting data, both empirical and gathered from the body of literature, on the prevalence of non-O157 STEC (O26, O45, O103, O111, O121, and O145), in addition to STEC O157, in the bovine reservoir.

This thesis is structured as follows:

- Chapter 2 systematically reviews non-O157 STEC peer-reviewed published literature worldwide with respect to the cattle reservoir. Three matrices (fecal, hide, and carcass) were included in the search. Non-O157 fecal prevalence data was analyzed by region using random-effects meta-analyses models to obtain pooled prevalence estimates and meta-regression techniques to explore factors contributing to between-study heterogeneity. Hide and carcass data were presented descriptively as very few articles were retrieved.
- Chapter 3 examines the seasonality of the STEC-7 in cattle during summer and winter months. This cross-sectional study evaluates the fecal prevalence of STEC in pre-harvest commercial feedlot cattle in the central United States during summer and winter months—known high and low prevalence seasons of *E. coli* O157 fecal shedding in cattle.
- Chapter 4 reflects on the conducted research, identifying key conclusions and additional research questions that need to be further evaluated regarding STEC in pre-harvest cattle.

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Chapter 2: Prevalence of Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) Serogroups (O26, O45, O103, O111, O121, and O145) and Virulence Genes in Feces, Hides, and Carcasses of Cattle in North America and Worldwide: A Systematic Review and Meta-analysis of Published Literature

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Abstract

The objective of this study was to gather, integrate, and interpret scientific data on prevalence and concentration of non-O157 Shiga toxin-producing *Escherichia coli* serogroups (O26, O45, O103, O111, O121, and O145) and virulence genes (*stx* and *eae*) in fecal, hide, and carcass samples in pre- and peri-harvest cattle. Following formal systematic review methodology, four electronic databases (Agricola, Web of Science, PubMed, and Food Safety and Technology Abstracts) were used to retrieve peer-reviewed articles of interest. The search retrieved 2,365 articles; however, only 105 articles

qualified for the risk of bias assessment. Sixty articles failed the quality criteria, thus only 45 articles were eligible for meta-analysis inclusion. Based on the number of articles retrieved, fecal (n=42) prevalence data were synthesized quantitatively using random-effects meta-analyses, whereas hide (n=4) and carcass (n=3) prevalence, and concentration (n=1) data were summarized descriptively. Meta-analysis results indicate that global non-O157 fecal prevalence significantly differs ($P < 0.01$) between geographic regions (Africa, Asia, Australia, Europe, North America, and South America). Meta-regression analyses were conducted to assess the effect of specific factors (e.g., continent, specimen type) on between-study heterogeneity for each outcome classification. Non-O157 serogroup and virulence gene pooled prevalence estimates were highest for North America.

Key-words: cattle; concentration; *Escherichia coli*; non-O157; pre-harvest; prevalence; review; Shiga toxin; STEC

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens that can cause severe human illness and even death. The United States Centers for Disease Control and Prevention (CDC) reported that six non-O157 STEC (O26, O45, O103, O111, O121, and O145) are responsible for over 70% of non-O157 STEC-associated human illnesses in the United States (Brooks *et al.,* 2005; Scallan *et al.,* 2011). Cattle, a known reservoir of STEC, shed these pathogens in their feces; therefore, posing a risk to contaminate produce, water, and beef products intended for human consumption (Bettelheim, 2000; Pihkala *et al.,* 2012). Currently, the United States Department of Agriculture Food Safety and Inspection

Service (USDA-FSIS) considers seven STEC (O26, O45, O103, O111, O121, O145, and O157) as adulterants in raw, non-intact beef products. The USDA-FSIS defines a beef product (e.g., ground beef) to be adulterated by STEC if an isolate harboring an O antigen (O26, O45, O103, O111, O121, O145, or O157), a Shiga toxin gene (*stx*1 and/or *stx*2), and an *eae* gene is detected.

The primary source of beef contamination at the harvest facility is via fecal contamination through cattle hides (Loneragan and Brashears, 2005). Therefore, cattle fecal and hide STEC prevalence estimates offer a proxy of the potential STEC risk at slaughter (Renter *et al.,* 2008), whereas concentration estimates provide a quantification of the risk these pathogens represent. Prevalence and concentration estimates of non-O157 pathogens are crucial to assess the frequency and bacterial load of these bacteria in the bovine reservoir and to better understand mitigation strategies aiming to lower the risk of these foodborne pathogens present in the host in order to decrease risks through multiple mechanisms of human exposure (e.g., water, produce, beef, direct contact). *Escherichia coli* O157, the most common STEC in North America, has been extensively studied in cattle since it was declared an adulterant in 1994; however, the prevalence and distribution of the non-O157 serogroups have not been well-established in cattle. In order to mitigate the risk of these pathogens in food products, we must evaluate pathogen frequency in the bovine reservoir.

Despite the apparent increase in human clinical cases in North America due to non-O157 STEC, data in cattle, a known STEC reservoir, are lacking. Most of the literature regarding non-O157 STEC reports prevalence of STEC in food products (e.g., ground beef and dairy products) and is rarely reported as serogroup-specific estimates. Epidemiology

of non-O157 serogroups and virulence genes in the cattle reservoir are not well-understood as the frequency and distribution have not been well-established in cattle and their environment, especially in North America. Therefore, the objective of this study was to gather, integrate, and interpret peer-reviewed data on the prevalence and concentration of non-O157 STEC serogroups (O26, O45, O103, O104, O111, O121, O145) and virulence genes in fecal, hide, and carcass samples in pre- and peri-harvest adult cattle using research synthesis methods (systematic review, meta-analysis, and meta-regression) to yield a comprehensive summary of peer-reviewed scientific literature published across the globe.

Materials and Methods

Systematic Review

Study Question

The original research question formulated was—What is the prevalence and concentration of non-O157 serogroups (O26, O45, O103, O111, O121, O145) and virulence genes (*stx*1, *stx*2, *eae*) in fecal, hide, and carcass samples in pre- and peri-harvest North American (USA, Mexico, and Canada) cattle? Pre-harvest cattle were defined as cattle (8 months of age and older) housed at a feedlot/farm up until loading onto transport to the harvest facility; while peri-harvest was defined as the time after the adult cattle leave the farm or feedlot until after stunning and hide removal but prior to the application of any carcass interventions at the processing plant. Due to the low number of publications originating from North America, the research question and search were refined to include peer reviewed literature from any region of the world.

In order to generate a complete list of all primary research that could answer our research question, search terms were created to account for the population and outcomes of interest. The search algorithm included the following terms: "(Beef OR Dairy OR Cattle OR Cow) AND (*Escherichia coli* OR STEC OR Shiga toxin OR Shiga toxin producing OR non-O157) AND (hide OR fecal OR carcass) AND (prevalence OR concentration)". The actual Boolean expressions used (i.e., format), varied depending on the search engine. Protocols and tools were developed, pre-tested, and implemented for each step of the review process using spreadsheets created in Excel (Microsoft Windows, 2010). The relevance screening was pre-tested using a set of ten abstracts that were reviewed for relevance by two reviewers (DD and NC) to determine reproducibility.

Search Strategy

Electronic databases accessed through the Kansas State University Library in July 2014 included: Agricola, Web of Science, PubMed, and Food Safety and Technology Abstracts (FSTA). After all relevant references were identified they were imported into a bibliographic management program (EndNote, Thomas Reuters). In addition, peer-reviewed articles retrieved by three epidemiologists (DR, MS, and NC) who have collected literature over the last 20 years on *Escherichia coli*, were also reviewed for inclusion.

Relevance Screening

The title and abstract of articles identified through electronic databases and hand searches were screened for relevance by a trained reviewer (DD) based on preset inclusion and exclusion criteria (Table 2.1). A second reviewer (NC) validated the first reviewer's work. Literature pertaining to experimental studies, in vitro experiments, statistical models, or non-primary research (e.g., literature reviews, short communications) was

excluded, in addition to non-peer reviewed and grey literature. Although the original search did not restrict articles based on publication year, we only included articles published after 2000, because diagnostic protocols used in articles published prior to 2000 were generally very different than the methods currently used. Studies considered relevant based on eligibility criteria advanced to the risk of bias assessment. Subsequently, the full versions of papers written in English were retrieved. No language restrictions were set on the original search in July 2014; however, after the retrieval of full-text articles, articles were excluded if they were not available in English, due to budgetary constraints that prevented us from using translation services.

Risk of Bias Assessment

Two reviewers independently evaluated the risk of bias of all retrieved full-text articles using a set of quality criteria (Table 2.2). Disagreements were resolved by consensus or a third reviewer's opinion. The risk of bias assessment tool was pre-evaluated using a sample of ten full-text articles. A set of seven quality criteria were designed to assess internal and external validity factors from primary studies. Internal validity (i.e., bias) factors assessed included study design and study population (cattle type). External validity (i.e., generalizability) factors appraised were animal production setting and study catchment area. Criteria were created based on guidelines described by Sargeant *et al.* (2006) and Higgins and Green (2011). Four of the quality criteria (2, 3, 5, and 7) were necessary for an article to advance to data extraction as they were deemed crucial to address the research question and extract relevant data, while meeting internal and external validity characteristics. In some instances, cattle type (criterion two) was not explicitly stated but if there was enough information (e.g., breed, age, diet, housing)

provided to indicate that the study population referred to healthy, adult cattle, the article was still considered for data extraction. If authors stated a specific breed, reviewers assigned the breed to a cattle type category. Criterion three posed a challenge regarding articles published from countries where animal production practices were not familiar to the reviewers; therefore, unless the authors specifically stated that the animals were housed in a research farm, it was assumed that animals were housed in representative field conditions for that country. Articles meeting at least those four criteria underwent data extraction.

A second risk of bias assessment consisted of subgroup analysis conducted to explore the study characteristics as covariates in meta-regression models (see below in section "Data Analysis: Meta-analysis and Evaluation of Heterogeneity").

Data Extraction

A data extraction spreadsheet tool was developed in Microsoft Excel (2010), where each column represented a field of interest when extracting data from the full-text papers. The data extraction form was pre-tested by all reviewers using a sample of ten full-text articles. Data extraction was performed independently by two reviewers. Disagreements were resolved by consensus or a third reviewer's opinion. Data were extracted for the different outcomes of interest (O26, O45, O103, O111, O121, and O145) reported at the various hierarchical levels (e.g., sample, animal, pen, feedlot, lot, processing plant). Outcomes of interest were further classified into three case definitions (or outcome classifications)—serogroup, Shiga toxin-producing *E. coli* (STEC), or Enterohemorrhagic *E. coli* (EHEC), to assess the prevalence of serogroup and virulence gene combinations. The three outcome classifications were defined as follows: 1) "serogroup" refers to samples

that tested positive for an *E. coli* serogroup gene of interest (O26, O45, O103, O111, O121, or O145), 2) "STEC", refers to samples that tested positive for a specific *E. coli* O serogroup and at least one Shiga toxin (*stx*1 and/or *stx*2) gene, and 3) "EHEC" refers to samples that tested positive for an *E. coli* O serogroup, at least one Shiga toxin gene, and the intimin (*eae*) gene. Data extracted from each article included the following: author, title, year of study, month of study, country of study, continent of study, cattle type (beef, dairy, unknown), breed, age, specimen type (cecal, pen-floor, rectal grab, rectal swab), and time of harvest (pre- or peri-harvest). Variables related to the outcome measures that were extracted included: number of positive samples, number of samples tested, prevalence (as reported by authors or calculated) or proportion positive, standard error of the prevalence (provided or calculated), outcome classification (serogroup, STEC, or EHEC), non-O157 gene of interest (O26, O45, O103, O111, O121, and O145), diagnostic methodology (use of immunomagnetic separation (IMS) or other methods), and hierarchical level of data reported (sample, animal, pen, feedlot, lot, processing plant). If data from a study were not explicitly presented but enough information was available (e.g., prevalence and number of samples tested), reviewers conducting the data extraction computed the required values. If the authors stated that they tested for serogroups and/or virulence genes of interest but did not detect them, it was recorded as a data point equal to zero for the respective case definition with the provided denominator.

The data were extracted as unique events (hereafter defined as "datasets") and individual rows were included, from the same article in the data extraction form, when articles presented information on prevalence or concentration for different outcome definitions. Therefore, an article (a peer-reviewed publication describing prevalence or

concentration of non-O157 in bovine fecal samples eligible for data extraction) could contain more than one dataset. Each entry (dataset) into the database reflected one outcome classification (e.g., serogroup O26, STEC O45, EHEC O103), at a single time point (e.g., day, month, season, year), as classified by a diagnostic test, representing one cattle type (dairy, beef), at different hierarchical levels.

Data Analysis

Fecal prevalence results were summarized quantitatively, whereas hide and carcass data were summarized descriptively. Only results presented at the sample-level were included in the meta-analysis; data presented at different hierarchical levels (e.g., pen-, feedlot-, farm-, plant-level) were excluded from the analyses. Using EpiTools (Sergeant, 2015), prevalence estimates obtained from pooled fecal samples were adjusted to compute individual sample-level prevalence estimates using the pooled prevalence calculator for fixed pool size (assuming a perfect test). Non-pooled sample-level data were not adjusted; only crude estimates were used in the analysis. All data were analyzed using STATA 12.0 (StatCorp LP, College Station, Texas, USA).

Meta-analysis and Evaluation of Heterogeneity

Fecal prevalence values and their standard errors were logit transformed using the following formulae: $logit\ prevalence = \ln\left[\frac{p}{1-p}\right]$ and $logit\ standard\ error = \sqrt{\frac{1}{np} + \frac{1}{n(1-p)}}$ where p = proportion positives of the study population for the respective outcome classification/serogroup and n = sample size (Sanchez *et al.,* 2007; Lambert *et al.,* 2015). Data entries equivalent to zero number of positive values (i.e., n = 0) were replaced with 0.5 prior to the logit transformation. Random-effects meta-analyses were conducted to

estimate the prevalence of non-O157 serogroup, STEC, and EHEC outcome classifications in bovine fecal samples using the logit prevalence and logit standard error computed from primary studies. Pooled fecal prevalence estimates and 95% confidence intervals were obtained using the DerSimonian-Laird random-effects method (DerSimonian and Laird, 1986) in meta-analysis models using the "metan" command in STATA.

Between-study heterogeneity was quantified using the Cochrane's chi-square test of homogeneity (*Q*) and the I^2 statistic (Higgins *et al.,* 2003; Higgins and Thompson, 2004). Cochrane's Q statistic was used to evaluate whether the variation between studies exceeds that expected by chance and is used to compute the I^2 statistic; $I^2 = \left[\frac{Q - degrees\ of\ freedom}{Q}\right] * 100$ (Higgins *et al.,* 2003). *P*-values less than 0.10 indicated significant between-study heterogeneity. The I^2 statistic represents the percentage of variation across studies that are due to heterogeneity rather than chance (Higgins *et al.,* 2003). Using the scale suggested by Higgins *et al.,* I^2 values between 25-50%, 50-75%, and ≥75% indicate low, moderate, and high degrees of heterogeneity, respectively (Higgins *et al.,* 2003). Causes of heterogeneity were explored using subgroup analysis and meta-regression techniques. Worldwide pooled fecal prevalence estimates were obtained by stratifying by continent, and by outcome classification (serogroup, STEC, and EHEC). Additionally, meta-analyses were conducted to obtain serogroup-specific pooled fecal prevalence estimates stratified by outcome classification in North America. The final pooled logit results (including their 95% confidence intervals) obtained in the meta-analyses models were back-transformed using the formula $p = \frac{e^{logit}}{e^{logit}+1}$ and were expressed as percentages (Sanchez *et al.,* 2007; Lambert *et al.,* 2015).

Meta-regression models were used to explore additional potential sources of between-study heterogeneity among articles reporting fecal prevalence using the "metareg" command. Univariable and multivariable meta-regression models were built to explore variables contributing to the between-study heterogeneity for the worldwide pooled fecal prevalence estimates obtained for each outcome classification. Explanatory variables included in the meta-regression models were: time of harvest (pre- or peri-harvest), cattle type (beef, dairy, or unknown), use of IMS (yes or no), specimen type (cecal, rectal grab, pen-floor, rectal swab, or unknown), and continent (Asia, Europe, North America, or South America). Initially, univariable meta-regression models were built to explore the unconditional associations between each of the explanatory variables and the fecal prevalence for each outcome classification. Variables with $P < 0.10$ in the univariable screen were included in the multivariable meta-regression models. The final pooled logit regression coefficients (and their 95% confidence intervals) were back-transformed and expressed as percentages. *P*-values less than 0.05 were deemed significant. Due to the limited number of articles retrieved, meta-regression analyses were not conducted to explore heterogeneity between studies from articles reporting prevalence within North America.

Assessment of Publication Bias

Funnel plots were generated using the "metafunnel" command specifying the "egger" option to assess potential publication bias. Although subjective, funnel plots allow visual interpretation of whether the association between prevalence estimates and a measure of study size (e.g., standard error) is greater than what may be expected to occur by chance (Sterne *et al.,* 2000). The Egger's regression asymmetry test was used to

evaluate the presence of small study effects for non-O157 serogroup, STEC, and EHEC outcome classifications worldwide (Egger *et al.,* 1997). Bias coefficients were generated using the "metabias" command specifying "egger". *P*-values less than 0.05 indicated funnel plot asymmetry. This regression-based test for detection of skewness determined whether the intercept deviated significantly from zero in a regression of standardized prevalence estimates (on a logit scale) against their precision (Steichen, 1998).

Results

Systematic Review

The number of research articles retrieved in each step of the process is presented in Figure 2.1. Initially, a total of 2,365 articles were obtained from four online databases. Nine hundred and seventy-three articles were duplicates, 977 were excluded based on the title and abstract screening, and 292 articles were excluded because they were published prior to the year 2000. One hundred and twelve full-text articles were retrieved (11 articles could not be retrieved as they were not accessible through the Kansas State University Library); however, twenty-nine were excluded as they did not meet our inclusion criteria (Table 2.1). Twenty-two additional articles, considered relevant, were identified from the authors' collections. A total of 105 articles were subjected to the risk of bias assessment (Table 2.2). Based on an a priori decision, full-text articles underwent a risk of bias assessment evaluating a set of seven criteria related to internal and external validity factors. Sixty articles did not meet the risk of bias assessment criteria while 45 articles did and thus proceeded to data extraction. The majority of the articles were excluded based on failure to meet criterion seven as serogroup-specific data were often not

reported. Many articles combined all non-O157 serogroups into one estimate or combined all serogroups, including O157 and other non-O157 serogroups, into a single estimate.

Our systematic review identified a moderate number of articles reporting the prevalence of non-O157 serogroups and virulence genes in cattle feces (n = 42; Table 2.3) and very few pertaining to hide (n = 4; Midgley and Desmarchelier, 2001; Monaghan *et al.,* 2012; Thomas *et al.,* 2012; Svoboda *et al.,* 2013) or carcass (n = 3; Breum *et al.,* 2010; Thomas *et al.,* 2012; Svoboda *et al.,* 2013) prevalence worldwide. A few articles (n = 3) provided data for more than one matrix of interest (Midgley and Desmarchelier, 2001; Thomas *et al.,* 2012; Svoboda *et al.,* 2013). Concentration data were scarce for all matrices: only one article (Thomas *et al.,* 2012) was retrieved, thus, concentration data were summarized in the text.

Fecal Prevalence

Worldwide Fecal Prevalence Estimates

Forty-two articles from six continents (Europe, n = 14; North America, n = 9; Asia, n = 10; South America, n = 6; Australia, n = 2; Africa, n = 1) were eligible for inclusion in the fecal prevalence meta-analysis (Table 2.3). Nineteen countries were included in the analysis, with the following distribution of countries within each continent: Europe (Belgium, n = 1; France, n = 1; Germany, n = 1; Ireland, n = 3; Italy, n = 2; Scotland, n = 3; Serbia, n = 1; Spain, n = 1; Switzerland, n = 1), North America (United States, n = 6; Canada, n = 3), Asia (Bangladesh, n = 1; India, n = 2; Japan, n = 5; Korea, n = 2), South America (Argentina, n = 3; Brazil, n = 3), Australia (n = 2), and Africa (Nigeria, n = 1). Pooled prevalence estimates significantly differed among continents for the serogroup, STEC, and EHEC outcome classifications (Table 2.4).

The worldwide non-O157 serogroup meta-analysis was comprised of 12 articles including a total of 111 datasets. Given the scarcity of articles from Africa, Australia, and South America, these continents were omitted from the worldwide serogroup meta-analysis; thus, datasets from only three continents (Asia, Europe, and North America) were included in the analysis. The worldwide pooled non-O157 serogroup prevalence in cattle feces was estimated to be 4.33% (95% CI = 3.22-5.81%). Pooled fecal prevalence was highest for North America (5.68%), followed by Asia (5.01%) and Europe (2.34%). There was evidence of moderate to high between-study heterogeneity in the worldwide random-effects meta-analysis model based on the I^2 statistics. Univariable meta-regression identified continent, time of harvest, cattle type, and specimen type as factors significantly ($P < 0.10$) contributing to between-study heterogeneity of non-O157 serogroup fecal prevalence estimates in cattle worldwide (Table 2.5). All variables (continent, time of harvest, cattle type, IMS, and specimen type) were included in the multivariable meta-regression. Cattle type and specimen type were identified as significant ($P < 0.05$) factors contributing to between-study heterogeneity of non-O157 serogroup prevalence estimates in cattle worldwide. The covariates (e.g., cattle type, specimen type) included in the multivariable meta-regression model explain 37.82% (adjusted R^2) of serogroup between-study heterogeneity.

The meta-analysis conducted to summarize non-O157 STEC fecal prevalence included 30 articles and 144 datasets worldwide and yielded a non-O157 STEC pooled prevalence estimate of 1.01% (95% CI = 0.78-1.32%) (Table 2.6). Pooled non-O157 STEC fecal prevalence estimates were computed for Asia, Europe, North America, and South America; no data were obtained for Africa or Australia. As with the serogroup outcome, the

non-O157 STEC pooled fecal prevalence estimate was the highest for North America, 3.27% (95% CI = 2.46-4.35%). The pooled non-O157 STEC prevalence estimates for Asia, Europe, and South America were 0.82, 0.31, and 0.62%, respectively. Due to evidence of between-study heterogeneity (I^2 statistic), meta-regression analyses were conducted (Table 2.6). Univariable meta-regression models identified all factors (continent, time of harvest, cattle type, IMS, and specimen type) to significantly ($P < 0.10$) contribute to between-study heterogeneity. In the multivariable meta-regression model, all factors except cattle type remained significant ($P < 0.05$) thus contributing to between-study heterogeneity; this multivariable model explained 55.84% (adjusted R^2) of the STEC between-study heterogeneity.

Lastly, a meta-analysis comprising 97 unique datasets was conducted to obtain pooled fecal EHEC prevalence estimates by continent (Table 2.6). No data were obtained for Africa, therefore only data from five continents (Asia, Australia, Europe, North America, and South America) were included in the worldwide meta-analysis. Two articles (3 datasets) were identified containing non-O157 EHEC prevalence estimates in Australian cattle; however, the prevalence values extracted were equal to zero. Due to data replacement where zero values were present (i.e., no samples tested positive), the meta-analysis produced an EHEC estimate for Australia despite the fact EHEC was not detected in Australia. The worldwide non-O157 EHEC fecal pooled prevalence was 0.55% (95% CI = 0.40-0.77%). North America (2.17%, 95% CI = 1.32-3.54%) was the region with the highest non-O157 EHEC fecal prevalence in cattle. Pooled non-O157 fecal EHEC prevalence estimates obtained for Asia, Australia, Europe, and South America were 0.81, 0.09, 0.33, and 0.66%, respectively. Evidence of heterogeneity was identified between-studies of all

continents with the exception of Australia based on the I^2 statistic. Continent, specimen type, and cattle type were identified as factors significantly ($P < 0.10$) contributing to between-study heterogeneity in the univariable meta-regression analyses; however, in the multivariable meta-regression model, time of harvest and specimen type were the only significant factors contributing to between-study heterogeneity (Table 2.7). Covariates (e.g., time of harvest, specimen type) in the multivariable meta-regression models explained 47.26% (adjusted R^2) of EHEC between-study heterogeneity.

Asymmetry in the funnel plots for serogroup, STEC, and EHEC outcomes worldwide indicated potential publication bias (data not shown). Bias coefficients using the Egger's test indicated that small study effects were present. Bias coefficients (*P*-values) for serogroup, STEC, and EHEC outcomes were -2.14 ($P = 0.02$), -3.10 ($P < 0.01$), and -3.43 ($P < 0.01$), respectively, indicating that the effect (i.e., fecal prevalence) estimated from the smaller studies was less than the effect estimated from the larger studies.

North America Fecal Prevalence Estimates

Overall, North America yielded the highest pooled fecal prevalence estimates for all outcomes worldwide, 5.68, 3.27, and 2.17% for non-O157 fecal pooled serogroup, STEC, and EHEC prevalence estimates, respectively. Nine articles included fecal prevalence estimates for North American cattle and were included in the meta-analyses models (Schurman *et al.,* 2000; Thran *et al.* 2001; Renter *et al.,* 2007; Karama *et al.,* 2008; Paddock *et al.,* 2012; Cernicchiaro *et al.,* 2013; Dargatz *et al.,* 2013; Baltasar *et al.,* 2014; Ekiri *et al.,* 2014). Although articles from the United States and Canada were included in the STEC and EHEC analyses (no studies from Mexico were available), only data from the United States were eligible for serogroup analysis. The pooled fecal prevalence estimates for specific

non-O157 serogroup, STEC, and EHEC outcomes in North America are presented in Table 2.8. Across all outcome classifications, O26 and O103 were the two O-genes most frequently detected from cattle feces in North America. The serogroup meta-analysis included 39 datasets from three articles. Pooled fecal prevalence estimates for serogroup O26 and O103 were 19.25 and 11.85%, respectively. The meta-analysis for the STEC outcome in North America included 60 datasets from six articles; fecal pooled prevalence estimates for STEC O26 and O103 were 5.84, and 4.20%, respectively. Lastly, a meta-analysis including 18 datasets from four articles was conducted for EHEC fecal prevalence in North America; estimates remained highest for EHEC O26 (3.75%) and O103 (4.46%) in cattle feces.

In order to further explore fecal prevalence in North American cattle, random-effects meta-analyses were conducted to obtain pooled fecal prevalence estimates for the United States and Canada for each outcome classification. These analyses included six articles from the United States (Thran *et al.* 2001; Paddock *et al.,* 2012; Cernicchiaro *et al.,* 2013; Dargatz *et al.,* 2013; Baltasar *et al.,* 2014; Ekiri *et al.,* 2014) and three articles from Canada (Schurman *et al.,* 2000; Renter *et al.,* 2007; Karama *et al.,* 2008). Serogroup outcome data remained unchanged as the United States was the only country represented in the meta-analysis for North America. However, STEC and EHEC outcomes were significantly ($P < 0.05$) different between the United States and Canada. In the meta-analysis of STEC by country, 51 datasets from four articles from the United States and nine datasets from two articles from Canada were included in the analysis. Pooled fecal STEC prevalence estimates (95% CI) obtained for the United States and Canada were 5.23% (4.01-6.79%) and 0.17% (0.09-0.33%), respectively. Meta-analysis conducted for non-

O157 EHEC included ten datasets from two articles from the United States and eight datasets from two articles from Canada. Pooled fecal EHEC prevalence estimates (95% CI) obtained for the United States and Canada were 4.94% (3.03-7.94%) and 0.14% (0.06-0.33%), respectively. Overall, non-O157 fecal prevalence estimates obtained for North America were highest for the United States compared to Canada. Although there was evidence of between-study heterogeneity in North America for the different outcome classifications, due to the limited number of studies, meta-regression analysis was not attempted.

Hide Prevalence and Concentration

Non-O157 serogroup and virulence gene prevalence and concentration data were extremely limited for cattle hides: only four articles were retrieved (Midgley and Desmarchelier, 2001; Monaghan *et al.,* 2012; Thomas *et al.,* 2012; Svoboda *et al.,* 2013), thus results were reported descriptively. All four articles contained data on peri-harvest beef cattle, two articles utilized IMS (Thomas *et al.,* 2012; Svoboda *et al.,* 2013), and three countries were represented (Australia, Ireland, and the United States).

Two articles (five datasets) provided data on non-O157 serogroups O26, O103, O111, and O145 (Thomas *et al.,* 2012; Monaghan *et al.,* 2012). These non-O157 serogroups were detected on peri-harvest beef cattle hides ranging from 0.0 to 27.1% (Monaghan *et al.,* 2012; Thomas *et al.,* 2012); no data were extracted for serogroups O45 or O121. The two predominately isolated serogroups from beef cattle hides were serogroups O26 and O103, with reported prevalence estimates of 6.0 (109/402) and 27.1% (24/402), respectively (Thomas *et al.,* 2012). Furthermore, Thomas *et al.,* quantified serogroup O103 on cattle hides (n = 130) at harvest yielding estimates for six samples between 10 and 110 CFU/cm^2,

the other 124 samples contained colony counts too low to estimate by direct plating methods (Thomas *et al.,* 2012).

Hide prevalence estimates were obtained for all six non-O157 STEC of interest in three articles (Monaghan *et al.,* 2012; Thomas *et al.,* 2012; Svoboda *et al.,* 2013). Non-O157 STEC hide prevalence estimates in peri-harvest beef cattle ranged from 0.0 to 0.2% (Monaghan *et al.,* 2012; Thomas *et al.,* 2012; Svoboda *et al.,* 2013). Only STEC O26 and O103 were detected on cattle hides; reported prevalence estimates for both STEC O26 and O103 were 0.2% (1/402) (Thomas *et al.,* 2012). Other non-O157 STEC (O45, O111, O121, and O145) were investigated, but were undetected on peri-harvest cattle hides (Monaghan *et al.,* 2012; Thomas *et al.,* 2012; Svoboda *et al.,* 2013).

Three articles were identified containing non-O157 EHEC hide data (Midgley and Desmarchelier, 2001; Monaghan *et al.,* 2012; Thomas *et al.,* 2012). Prevalence estimates reported for EHEC O26 ranged from 0.0 to 4.0% on cattle hides; EHEC O111, O103, and O145 were investigated, but were undetected on cattle hides prior to harvest (Midgley and Desmarchelier, 2001; Monaghan *et al.,* 2012; Thomas *et al.,* 2012). No prevalence estimates were identified for EHEC O45 and O121 on cattle hides.

Carcass Prevalence

Non-O157 serogroup prevalence estimates on beef cattle carcasses were obtained from two articles (Thomas *et al.,* 2012; Svoboda *et al.,* 2013). Prevalence of the six non-O157 serogroups ranged from 0.0 to 13.8% on carcass samples from cattle (Thomas *et al.,* 2012; Svoboda *et al.,* 2013). Serogroup O26 prevalence ranged from 0.5 (2/402) to 4.9% (10/203) on beef cattle carcass samples. Svoboda *et al.,* estimated serogroup O45 and O121 carcass prevalence to be 13.8 (28/203) and 10.8% (22/203), respectively (Svoboda

et al., 2013). Serogroup O103 carcass prevalence ranged from 5.5 (22/402) to 11.8% (24/203) (Thomas *et al.,* 2012; Svoboda *et al.,* 2013). Interestingly, in the study by Thomas *et al.* (2012), serogroup O103 was quantified from cattle hides; however, no quantifiable concentrations of serogroup O103 were detected on the corresponding cattle carcasses (Thomas *et al.,* 2012). Serogroup O145 prevalence in carcass samples ranged from 0.5 (2/402) to 1.5% (3/203) (Thomas *et al.,* 2012; Svoboda *et al.,* 2013). Serogroup O111 was not detected in the two studies (Thomas *et al.,* 2012; Svoboda *et al.,* 2013). All prevalence estimates reported for STEC and EHEC O26, O103, O111, and O145 were zero (Breum *et al.,* 2010; Thomas *et al.,* 2012). STEC and EHEC data were not retrieved for O45 and O121 on cattle carcasses.

Discussion

This study gathered and synthetized estimates of prevalence and concentration of Shiga toxin-producing *E. coli* non-O157 serogroups and virulence genes in fecal, hide, and carcass samples from pre- and peri-harvest cattle from North America and across the globe. Besides summarizing measures of pathogen frequency and concentration from the existent body of work, this study demonstrated estimates of potential risks and identified some of the factors responsible for between-study heterogeneity as well as important knowledge gaps in published literature. Collectively, data regarding non-O157 serogroups and virulence genes in cattle is very limited. In regards to our initial study objective, data are still needed for non-O157 serogroup and virulence gene frequency and distribution in North American (Canada, United States, and Mexico) cattle.

Data retrieved for North America comprised fecal prevalence estimates in pre-harvest beef cattle populations. Sources of between-study heterogeneity were not assessed for North America due to the limited sample size. In order to further understand the epidemiology of non-O157 serogroups and virulence genes, more data are needed from North American cattle populations. Important knowledge gaps that need to be addressed include the potential difference in fecal shedding and overall bacterial load between cattle types (dairy and beef cattle). Fecal, hide, and carcass non-O157 serogroup and virulence gene data from peri-harvest cattle are needed to estimate the potential of fecal contamination at harvest. Comprehensive knowledge of non-O157 pathogen frequency gathered from the existing literature can be incorporated with experimental and observational data into quantitative microbial risk assessment models.

The systematic review process identified 45 articles worldwide, most of which represented non-O157 serogroup and virulence gene prevalence data in pre-harvest beef cattle feces. Results from the worldwide meta-analyses by non-O157 serogroup, STEC, and EHEC fecal outcomes indicated that cattle harbor and shed non-O157 serogroups and virulence genes in very low frequencies. In terms of global fecal prevalence, North America yielded the highest estimate of pooled fecal prevalence for non-O157 serogroup, STEC, and EHEC outcome classifications, demonstrating the importance of cattle as a reservoir of non-O157 organisms, especially in the United States. Estimates of serogroup prevalence are less representative of the frequency of foodborne pathogens shed by cattle, as they only denote the presence of the O serogroup gene but not of the genes responsible for pathogenicity in humans. However, their potential to acquire virulence genes substantiate their assessment in bovine matrices. The most common non-O157 serogroups in the

articles included in this study were O26 and O103, in both fecal and hide samples. Non-O157 pathogens most commonly associated with human illnesses reported in the United States are O26 and O111 (Luna-Gierke et al., 2014). However, in our study, EHEC O111 was the second least frequent O group detected in cattle in the United States. A worldwide standardized case definition for reporting pathogenic STEC, in terms of serogroup and virulence gene profiles, is needed to accurately represent the risk of these foodborne pathogens throughout the beef continuum globally. Although, beyond the scope of this review, the association between human illness outbreaks attributed to non-O157 pathogens from cattle sources needs to be further elucidated.

There were a few challenges encountered in this search and review process. Firstly, there was no clear and consistent case definition for STEC and/or pathogenic STEC (i.e., EHEC) reported in the literature. Therefore, case definitions were classified by reviewers into three outcome definitions as they pertained to serogroup, STEC, and EHEC data. Many articles retrieved in the search included collective estimates of "STEC" or "non-O157 STEC" and therefore were not included in our analyses as they did not meet the criteria defined for the risk of bias assessment.

Non-primary research (literature reviews, short communications, abstract-only, conference proceedings), non-peer reviewed, and grey literature were not included in our systematic review. In general, these types of literature rarely contain sufficient information to allow data extraction of relevant information. In addition, we aimed to obtain or calculate measures of disease frequency and their standard errors, and these publications generally lack that level of detailed information. Searching through government reporting websites (i.e., Current Research Information System (CRIS-USDA))

produced several reports, however, most of the ones deemed relevant contained data published in peer-reviewed journals, hence, to avoid potential duplication, we decided to only extract data from peer-reviewed articles. We did use a hand search of our collections of peer-reviewed papers and reference lists from review papers on the subject to validate the results of the electronic search. Some articles identified in the hand search that were included in the systematic review and meta-analysis, were not found in the electronic search. Overall, there were limited reports pertaining to fecal prevalence in certain regions, few articles reporting hide and carcass prevalence and a single peer-reviewed manuscript on concentration of non-O157 serogroups and virulence genes in feces, hides, and carcass samples. Due to low statistical precision because of the small number of studies included in some of the subgroup analysis and meta-regression models, estimates should be interpreted with discretion. Likewise, very few articles reported model-adjusted prevalence estimates after accounting for the hierarchical structure of the data or the study design features. In all but those cases, the precision of the estimates may be overestimated. To avoid such methodological differences, only raw data were included as well as sole information from one of the many organizational levels (i.e., animal-level).

Heterogeneity in this study could not be attributed to a particular source of bias. Besides publication bias, many other sources of selection bias such as location and language bias could be present, along with poor study quality or design, true heterogeneity, and/or chance (Egger *et al.,* 1997; Sterne *et al.,* 2000; Chan *et al.,* 2004; Higgins and Green, 2011; Sterne *et al.,* 2011; O'Connor *et al.,* 2014). It is possible that empirical data produced in certain geographical locations may be published in local reporting systems or journals in the native language rather than in international, peer-reviewed journals, due to cost

(publication costs, translation services, or similar), and restricted institutional or personal subscription access. Similarly, there is potential for language bias given we excluded articles that were not published in English.

Internal and external validity factors also could have biased the estimates of the present review, and as such they were incorporated in the risk of bias assessment step. Our risk of bias assessment included a set of criteria that were based on similar questions proposed by Sargeant *et al.* (2006) and Higgins and Green (2011). Although, we recognize that excluding articles based on this stage can introduce bias in our estimates, some of the criteria were deemed crucial to address the research question and extract relevant data, while meeting internal and external validity criteria. Some of the inclusion and exclusion criteria pre-determined in the relevance screening could potentially have introduced bias due to exclusion based on publication year. By excluding articles published before 2000 we tried to minimize the variability in diagnostic methods and their corresponding sensitivity of detection. Specifically, we wanted to incorporate studies that employed an immunomagnetic separation (IMS) step, as this procedure has improved the sensitivity of culture-based methods (Chapman *et al.,* 1994; Cernicchiaro *et al.,* 2013); however, the majority of articles relied on molecular testing and only twelve of the forty-five articles reported using IMS (Bonardi *et al.,* 2005; Jeon *et al.,* 2006; Pearce *et al.,* 2006; Bonardi *et al.,* 2007; Joris *et al.,* 2011; Sasaki *et al.,* 2011; Lynch *et al.,* 2012; Paddock *et al.,* 2012; Thomas *et al.,* 2012; Cernicchiaro *et al.,* 2013; Svoboda *et al.,* 2013; Ekiri *et al.,* 2014). Nevertheless, publication year did not necessarily reflect study year as some of the studies published in early 2000 were conducted in mid or late 90s, and as such, some of their diagnostic protocols are not comparable to the ones currently used. The type of diagnostic methods

used was found to significantly explain some of the between-study heterogeneity in univariable meta-regression models for all outcome classifications, and for multivariable meta-regression models for non-O157 STEC prevalence. Since apparent prevalence estimates are directly impacted by the accuracy of the detection protocols used, the estimates of the present analysis may be biased; however, given the diversity of detection protocols employed and their different accuracy, it would be difficult to predict the directionality of the potential bias.

The differences in animal and farm management in addition to production systems in different regions likely also contributed to the between-study heterogeneity. Although we attempted to classify cattle type into beef and dairy, many studies did not describe their study population; therefore, distinguishing between these cattle types was not possible for 14 articles (Table 2.3). Production systems and management practices differ worldwide; thus, we assigned the extracted fecal prevalence data into geographical regions to minimize variability in production and management. However, between-study heterogeneity remained high and while covariates (e.g., specimen type, time of harvest) partially explained some between-study heterogeneity it appears that additional data on factors such as season, cattle age, and cattle diet may be necessary to further explain the observed variability among studies and prevalence estimates. Season, cattle age and diet are factors that are known to influence *E. coli* O157 fecal shedding in cattle and have been well-established in peer-reviewed literature (Barkocy-Gallagher *et al.,* 2003; Edrington *et al.,* 2006; Callaway *et al.,* 2009; Ekiri *et al.,* 2014); therefore, future research is needed to address the effects of season, age, and diet in relation to non-O157 *E. coli* fecal shedding in

cattle. Due the limitations of the data retrieved from the publications, these factors could not be evaluated.

Our results indicated that most of the published literature reported data from cattle shedding non-O157 pathogens at low frequencies. Pooled fecal prevalence estimates in meta-analysis models significantly varied by continent in subgroup analyses. The lack of data prevented us from having a larger pool of relevant articles; however, based on the inclusion and exclusion criteria and the risk of bias assessment, the results of the present study are specific to the posed research question and can be generalized to scenarios describing cattle fecal prevalence of non-O157 organisms as detected by culture and molecular detection methods. While it is difficult to assess the validity of the magnitude and the precision of the summary prevalence estimates from the meta-analyses, the factors we identified as responsible for the between-study variability (e.g., specimen type, diagnostic methods, cattle type, time of harvest) may help refine research priorities for future studies. Minimal conclusions can be drawn from hide and carcass results reported due to the limited number of articles retrieved and the large variation between articles. Obtaining peri-harvest hide and carcass prevalence and concentration data are crucial as they are the closest indicators of the contamination burden of carcasses before being subjected to antimicrobial interventions at the harvest facility.

In conclusion, the results of this study illustrated the need for obtaining prevalence and concentration data for non-O157 STEC pathogens in different production systems, and in different matrices, in pre- and peri-harvest cattle. This study, to our knowledge, is the first systematic review and meta-analysis of studies reporting non-O157 serogroup and virulence gene fecal prevalence in cattle, in North America and worldwide. Our findings

demonstrate the importance of cattle as a reservoir of non-O157 serogroups and virulence genes. The prevalence estimates obtained using a systematic review and meta-analysis of peer-reviewed literature along with empirical data can be integrated into a quantitative microbial risk assessment model to assess the potential risks attributable to non-O157 STEC in the beef chain.

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Disclosure Statement

No competing financial interests exist.

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Tables and Figures

Table 2.1 Study inclusion and exclusion criteria for eligibility of articles for a systematic review of the literature

Criteria	Inclusion	Exclusion
Language	English	Languages other than English
Publication year	2000-2014	Prior to 2000
Population	Healthy, adult cattle (8 months and older) pre- and peri-harvest	Calves (< 8 months) Species other than cattle Diseased cattle
Sample type	Fecal: pen-floor, rectal swab, rectal grab/contents, cecal content (sampled pre- or post-harvest) Hide and carcass: samples (e.g., sponge, swab, etc) prior to any in-plant intervention	Hide and carcass: samples post in-plant interventions (e.g., hide wash, carcass wash).
Study type	Observational studies (cross-sectional, cohort, case-control) Laboratory trials (using field samples)	Experimental studies In vitro (laboratory) experiments Non-primary research (e.g., literature reviews)
Outcomes	*Escherichia coli* O26, O45, O103, O111, O121, and O145 Virulence genes: *stx*1, *stx*2, *eae*	Bacterial species other than *Escherichia coli* All other *Escherichia coli* O serogroups All other virulence genes
Outcome measures	Prevalence (or proportion positive), concentration	Outcomes other than prevalence and concentration
Location	North America (United States, Mexico, and Canada)	See below†

† Initially, the search was restricted to articles produced in North America; however, given the low number of articles, we expanded the search to include articles available in English from peer-reviewed literature and cattle populations worldwide.

Table 2.2 Risk of bias assessment criteria

Criteria	Outcome	No. Articles
1. Was the sample size justified?	No / unknown / not reported	41
	Yes	4
†2. Was the study population properly described?	No / unknown	0
	Yes (cattle; beef and/or dairy cattle)	45
†3. Were the animals housed or grouped in a way that is representative of field/ commercial conditions?	No / unknown / not reported	0
	In part - closed system; research farms	4
	Yes - typical of commercial operations	41
4. Study catchment area	Single-site (one operation / farm / processing plant)	15
	Multi-site (multiple operation / farms / processing plants / multiple states)	30
†5. Were the numerator and denominator for the prevalence provided?	No numerator and/or denominator (can't calculate prevalence)	0
	Provided both numerator and denominator (or prevalence and numerator/denominator; can calculate prevalence)	45
6. Was time/duration (month, season) of study reported?	No / unknown / multiple seasons but cumulative prevalence	33
	Less than 3 months	1
	Three months or more (full season)	11
†7. Can clearly identify at least one non-O157 STEC serogroup (O26, O45, O103, O111, O121, or O145)	No	0
	Yes	45

†Articles that did not meet quality assessment criteria 2, 3, 5 or 7 were excluded and were not considered for data extraction.

Table 2.3 List of the articles included in the meta-analysis of fecal prevalence by study variables.

Variable	No. articles	References
Continent		
Africa	1	Musa *et al.,* 2012
Asia	10	Das *et al.,* 2005; Islam *et al.,* 2008; Jeon *et al.,* 2006; Kang *et al.,* 2014; Khan *et al.,* 2002; Kijima-Tanaka *et al.,* 2005; Kobayashi *et al.,* 2001; Sasaki *et al.,* 2011, 2013a, 2013b
Australia	2	Cobbold and Desmarchelier, 2001; Midgley and Desmarchelier, 2001
Europe	14	Bonardi *et al.,* 2005, 2007; Cobeljić *et al.,* 2005; Jenkins *et al.,* 2002; Joris *et al.,* 2011; Lynch *et al.,* 2012; Monaghan *et al.,* 2011; Orden *et al.,* 2002; Pearce *et al.,* 2006; Pradel *et al.,* 2000; Shaw *et al.,* 2004; Thomas *et al.,* 2012; Zschöck *et al.,* 2000; Zweifel *et al.,* 2005
North America	9	Baltasar *et al.,* 2014; Cernicchiaro *et al.,* 2013; Dargatz *et al.,* 2013; Ekiri *et al.,* 2014; Karama *et al.,* 2008; Paddock *et al.,* 2012; Renter *et al.,* 2007; Schurman *et al.,* 2000; Thran *et al.,* 2001
South America	6	Farah *et al.,* 2007; Fernández *et al.,* 2010; Meichtri *et al.,* 2004; Padola *et al.,* 2004; Timm *et al.,* 2007; Vicente *et al.,* 2005
Time of Harvest		
Pre-harvest	30	Baltasar *et al.,* 2014; Cernicchiaro *et al.,* 2013; Cobbold and Desmarchelier, 2001; Cobeljić *et al.,* 2005; Dargatz *et al.,* 2013; Das *et al.,* 2005; Ekiri *et al.,* 2014; Fernández *et al.,* 2010; Jenkins *et al.,* 2002; Jeon *et al.,* 2006; Kang *et al.,* 2014; Khan *et al.,* 2002; Kijima-Tanaka *et al.,* 2005; Kobayashi *et al.,* 2001; Lynch *et al.,* 2012; Midgley and Desmarchelier, 2001; Monaghan *et al.,* 2011; Musa *et al.,* 2012; Orden *et al.,* 2002; Paddock *et al.,* 2012; Padola *et al.,* 2004; Pearce *et al.,* 2006; Renter *et al.,* 2007; Sasaki *et al.,* 2011, 2013a, 2013b; Shaw *et al.,* 2004; Thran *et al.,* 2001; Vicente *et al.,* 2005; Zschöck *et al.,* 2000;
Peri-harvest	12	Bonardi *et al.,* 2005, 2007; Farah *et al.,* 2007; Islam *et al.,* 2008; Joris *et al.,* 2011; Karama *et al.,* 2008; Meichtri *et al.,* 2004; Pradel *et al.,* 2000; Schurman *et al.,* 2000; Thomas *et al.,* 2012; Timm *et al.,* 2007; Zweifel *et al.,* 2005
Cattle Type†		
Beef	18	Baltasar *et al.,* 2014; Cernicchiaro *et al.,* 2013; Dargatz *et al.,* 2013; Ekiri *et al.,* 2014; Farah *et al.,* 2007; Kang *et al.,* 2014; Karama *et al.,* 2008; Kijima-Tanaka *et al.,* 2005; Meichtri *et al.,* 2004; Midgley and Desmarchelier, 2001; Paddock *et al.,* 2012; Padola *et al.,* 2004; Renter *et al.,* 2007;

		Sasaki *et al.,* 2011, 2013a; Schurman *et al.,* 2000; Thomas *et al.,* 2012; Timm *et al.,* 2007
Dairy	13	Bonardi *et al.,* 2005; Cobbold and Desmarchelier, 2001; Das *et al.,* 2005; Fernández *et al.,* 2010; Kang *et al.,* 2014; Kobayashi *et al.,* 2001; Lynch *et al.,* 2012; Paddock *et al.,* 2012; Sasaki *et al.,* 2013a, 2013b; Thran *et al.,* 2001; Vicente *et al.,* 2005; Zschöck *et al.,* 2000
Unknown	14	Bonardi *et al.,* 2007; Cobeljić *et al.,* 2005; Islam *et al.,* 2008; Jenkins *et al.,* 2002; Jeon *et al.,* 2006; Joris *et al.,* 2011; Khan *et al.,* 2002; Monaghan *et al.,* 2011; Musa *et al.,* 2012; Orden *et al.,* 2002; Pearce *et al.,* 2006; Pradel *et al.,* 2000; Shaw *et al.,* 2004; Zweifel *et al.,* 2005
IMS‡		
Yes	11	Bonardi *et al.,* 2005, 2007; Cernicchiaro *et al.,* 2013; Ekiri *et al.,* 2014; Jeon *et al.,* 2006; Joris *et al.,* 2011; Lynch *et al.,* 2012; Paddock *et al.,* 2012; Pearce *et al.,* 2006; Sasaki *et al.,* 2011; Thomas *et al.,* 2012
No	35	Baltasar *et al.,* 2014; Cernicchiaro *et al.,* 2013; Cobbold and Desmarchelier, 2001; Cobeljić *et al.,* 2005; Dargatz *et al.,* 2013; Das *et al.,* 2005; Ekiri *et al.,* 2014; Farah *et al.,* 2007; Fernández *et al.,* 2010; Islam *et al.,* 2008; Jenkins *et al.,* 2002; Kang *et al.,* 2014; Karama *et al.,* 2008; Khan *et al.,* 2002; Kijima-Tanaka *et al.,* 2005; Kobayashi *et al.,* 2001; Lynch *et al.,* 2012; Meichtri *et al.,* 2004; Midgley and Desmarchelier, 2001; Monaghan *et al.,* 2011; Musa *et al.,* 2012; Orden *et al.,* 2002; Paddock *et al.,* 2012; Padola *et al.,* 2004; Pradel *et al.,* 2000; Renter *et al.,* 2007; Sasaki *et al.,* 2013a, 2013b; Schurman *et al.,* 2000; Shaw *et al.,* 2004; Thran *et al.,* 2001; Timm *et al.,* 2007; Vicente *et al.,* 2005; Zschöck *et al.,* 2000; Zweifel *et al.,* 2005
Specimen Type		
Pen-floor	6	Jenkins *et al.,* 2002; Midgley and Desmarchelier, 2001; Monaghan *et al.,* 2011; Paddock *et al.,* 2012; Pearce *et al.,* 2006; Renter *et al.,* 2007
Rectal grab	15	Baltasar *et al.,* 2014; Cernicchiaro *et al.,* 2013; Ekiri *et al.,* 2014; Islam *et al.,* 2008; Joris *et al.,* 2011; Karama *et al.,* 2008; Kobayashi *et al.,* 2001; Musa *et al.,* 2012; Orden *et al.,* 2002; Sasaki *et al.,* 2011, 2013a, 2013b; Shaw *et al.,* 2004; Thomas *et al.,* 2012; Thran *et al.,* 2001
Rectal swab	10	Dargatz *et al.,* 2013; Farah *et al.,* 2007; Fernández *et al.,* 2010; Kang *et al.,* 2014; Lynch *et al.,* 2012; Padola *et al.,* 2004; Schurman *et al.,* 2000; Timm *et al.,* 2007; Vicente *et al.,* 2005; Zschöck *et al.,* 2000
Cecal	3	Bonardi *et al.,* 2005, 2007; Meichtri *et al.,* 2004
Unknown	8	Cobbold and Desmarchelier, 2001; Cobeljić *et al.,* 2005; Das *et al.,* 2005; Jeon *et al.,* 2006; Khan *et al.,* 2002; Kijima-Tanaka *et al.,* 2005; Pradel *et al.,* 2000; Zweifel *et al.,* 2005

† Three articles contain more than one cattle type (Kang *et al.,* 2014; Sasaki *et al.,* 2013a; Paddock *et al.,* 2012).

‡Four articles used two types of detection methodology (IMS and molecular test) (Cernicchiaro *et al.,* 2013; Ekiri *et al.,* 2014, Lynch *et al.,* 2012; Paddock *et al.,* 2012).

Table 2.4 Pooled serogroup, STEC, and EHEC fecal prevalence estimates by continent obtained from random-effects meta-analysis models*

Outcome	Continent	No. articles	No. datasets#	Prevalence, %	95% Confidence Interval (CI), %	Cochrane's chi-square statistic (*Q*)	I^2, %	*P*-value
Serogroup								
	Africa‡	1	1	-	-	-	-	-
	Asia	3	50	5.01	3.45-7.20	165.73	70.4	<0.01
	Australia	0	0	-	-	-	-	-
	Europe	6	22	2.34	1.19-4.52	1497.41	98.6	<0.01
	North America	3	39	5.68	3.54-8.97	3674.94	99.0	<0.01
	South America‡	1	1	-	-	-	-	-
	Worldwide	12	111	4.33	3.22-5.81	5640.56	98.0	<0.01
STEC								
	Africa	0	0	-	-	-	-	-
	Asia	10	28	0.82	0.50-1.33	107.43	74.9	<0.01
	Australia	0	0	-	-	-	-	-
	Europe	9	39	0.31	0.19-0.51	158.09	76.0	<0.01
	North America	6	60	3.27	2.46-4.35	578.22	89.8	<0.01
	South America	5	17	0.62	0.24-1.61	48.96	67.3	<0.01
	Worldwide	30	144	1.01	0.78-1.32	1920.23	92.6	<0.01
EHEC								
	Africa	0	0	-	-	-	-	-
	Asia	9	19	0.81	0.45-1.44	75.71	76.2	<0.01
	Australia†	2	3	0.09	0.02-0.45	0.29	0.0	0.86
	Europe	12	46	0.33	0.22-0.49	134.76	66.6	<0.01
	North America	4	18	2.17	1.32-3.54	374.8	95.5	<0.01
	South America	4	11	0.66	0.18-2.36	40.23	75.1	<0.01
	Worldwide	31	97	0.55	0.40-0.77	1338.54	92.8	<0.01

* This table depicts results from three meta-analysis models, one for each outcome classification (Serogroup, STEC and EHEC).

#Each dataset represents data pertaining to one outcome classification, in a specific study period, as classified by a specific diagnostic test, representing one cattle type, at different hierarchical levels. An article can include multiple datasets.

‡Africa and South America were omitted from the worldwide meta-analyses as only one study was identified in each of those continents.

† Articles retrieved from Australia (Cobbold and Desmarchelier, 2001; Midgley and Desmarchelier, 2001) did not detect EHEC. Estimates extracted as zero, were assigned a value equal to 0.5 during logit transformation.

Table 2.5 Univariable and multivariable meta-regression models for non-O157 serogroup fecal prevalence in cattle worldwide

Variables	Covariate	No. articles‡	No. datasets	Univariable		Multivariable	
				Prevalence (95%CI), %	***P*-value**	**Prevalence (95%CI), %**	P-value
Continent					**<0.01**		0.58
	Asia	3	50	4.26 (2.39-7.46)	<0.01	Ref.†	-
	Europe	6	22	2.10 (0.42-9.80)	0.16	2.91 (0.00-94.87)	0.34
	North America	3	39	5.39 (1.33-19.48)	0.56	3.11 (0.01-95.09)	0.31
Time of Harvest							0.14
	Peri-harvest	3	9	4.56 (0.34-40.00)	0.04	Ref.	-
	Pre-harvest	9	102	1.12 (0.31-3.95)	<0.01	3.83 (0.01-93.88)	0.31
Cattle Type					**0.02**		<0.01
	Beef	5	38	3.30 (1.84-5.86)	<0.01	Ref.	-
	Dairy	3	15	13.50 (2.68-46.93)	0.01	3.87 (0.02-89.75)	0.03
	Unknown	5	58	3.31 (0.84-12.18)	0.10	0.10 (0.00-27.97)	0.02
IMS							0.21
	No	8	39	2.76 (1.51-5.00)	<0.01	Ref.	-
	Yes	7	72	5.09 (1.31-17.82)	0.11	2.10 (0.01-81.40)	0.21
Specimen Type					**<0.01**		<0.01
	Pen-floor	2	16	18.85 (0.95-23.35)	<0.01	Ref.	-
	Rectal grab	5	19	3.72 (0.50-22.93)	<0.01	0.53 (0.00-57.87)	0.43
	Rectal swab	2	26	1.58 (0.22-10.33)	<0.01	0.08 (0.00-16.71)	<0.01
	Unknown	3	50	4.23 (0.66-22.35)	<0.01	4.48 (0.01-97.16)	0.22

‡Few articles present data for more than one covariate (e.g., both beef and dairy cattle-types).

†Ref = referent category; *P*-values highlighted in bold indicate overall significance of polychotomous variables based on Wald tests.

Table 2.6 Univariable and multivariable meta-regression models for non-O157 STEC fecal prevalence in cattle worldwide

Variables	Covariate	No. articles‡	No. datasets	Univariable		Multivariable	
				Prevalence (95%CI), %	*P*-value	Prevalence (95%CI), %	*P*-value
Continent					**<0.01**		**0.02**
	Asia	10	28	0.75 (0.43-1.32)	<0.01	Ref.†	-
	Europe	9	39	0.31 (0.08-1.15)	0.02	0.07 (0.00-1.58)	0.60
	North America	6	60	2.70 (0.79-8.78)	<0.01	0.17 (0.01-3.81)	0.10
	South America	5	17	0.67 (0.14-3.09)	0.81	0.50 (0.01-14.96)	<0.01
Time of Harvest					**<0.01**		**<0.01**
	Peri-harvest	9	43	1.41 (0.46-4.25)	<0.01	Ref.	-
	Pre-harvest	21	101	0.40 (0.24-0.68)	<0.01	0.39 (0.02-6.98)	<0.01
Cattle Type					**<0.01**		**0.18**
	Beef	14	88	1.75 (1.26-2.43)	<0.01	Ref.	-
	Dairy	9	22	0.34 (0.11-1.05)	<0.01	0.04 (0.00-0.96)	0.08
	Unknown	9	34	0.42 (0.16-1.12)	<0.01	0.09 (0.00-2.24)	0.94
IMS							**0.04**
	No	24	94	0.77 (0.54-1.10)	<0.01	Ref.	-
	Yes	8	50	1.43 (0.55-3.63)	0.04	0.15 (0.01-2.41)	0.04
Specimen Type					**<0.01**		**<0.01**
	Cecal	1	2	0.00 (0.06-4.36)	<0.01	Ref.	-
	Pen-floor	2	8	0.12 (0.00-11.61)	0.26	0.03 (0.00-3.59)	0.38
	Rectal grab	14	85	1.87 (0.02-61.65)	0.24	0.36 (0.00-25.97)	0.22
	Rectal swab	8	36	0.34 (0.00-23.36)	0.75	0.09 (0.00-6.74)	0.99
	Unknown	5	13	0.89 (0.01-46.08)	0.63	0.37 (0.00-29.35)	0.23

‡Few articles present data for more than one covariate (e.g., both beef and dairy cattle-types).

†Ref = referent category; *P*-values highlighted in bold indicate overall significance of polychotomous variables based on Wald tests.

Table 2.7 Univariable and multivariable meta-regression models for non-O157 EHEC fecal prevalence in cattle worldwide

Variables	Covariate	No. articles‡	No. datasets	Univariable		Multivariable	
				Prevalence (95%CI), %	*P*-value	Prevalence (95%CI), %	*P*-value
Continent					**<0.01**		**0.21**
	Asia	9	19	0.76 (0.39-1.47)	<0.01	Ref.†	-
	Australia*	2	3	0.09 (0.01-1.57)	0.06	0.09 (0.00-5.44)	0.55
	Europe	12	46	0.31 (0.07-1.34)	0.03	0.21 (0.01-3.67)	0.79
	North America	4	18	1.76 (0.36-8.14)	0.07	0.47 (0.02-8.84)	0.09
	South America	4	11	0.78 (0.12-4.84)	0.95	1.14 (0.02-38.40)	0.08
Time of Harvest							**<0.01**
	Peri-harvest	9	39	0.39 (0.22-0.67)	<0.01	Ref.	-
	Pre-harvest	22	58	0.67 (0.19-2.32)	0.12	0.81 (0.06-10.85)	<0.01
Cattle Type					**<0.01**		**0.29**
	Beef	12	39	1.07 (0.66-1.71)	<0.01	Ref.	-
	Dairy	9	18	0.26 (0.06-1.08)	<0.01	0.07 (0.00-1.74)	0.16
	Unknown	11	40	0.38 (0.12-1.21)	<0.01	0.19 (0.01-3.19)	0.95
IMS							**0.11**
	No	23	66	0.54 (0.35-0.82)	<0.01	Ref.	-
	Yes	9	31	0.55 (0.18-1.72)	0.93	0.31 (0.02-4.02)	0.11
Specimen Type					**<0.01**		**<0.01**
	Cecal	3	7	0.45 (0.13-1.60)	<0.01	Ref.	-
	Pen-floor	5	14	0.14 (0.01-2.36)	0.14	0.02 (0.00-0.94)	0.03
	Rectal grab	12	43	0.94 (0.07-11.73)	0.28	0.23 (0.01-6.47)	0.73
	Rectal swab	5	19	0.37 (0.02-5.66)	0.79	0.07 (0.00-2.13)	0.21
	Unknown	6	14	0.70 (0.04-10.38)	0.56	0.27 (0.01-9.17)	0.64

‡Few articles present data for more than one covariate (e.g., both beef and dairy cattle types).

†Ref = referent category; *P*-values highlighted in bold indicate overall significance of polychotomous variables based on Wald tests.

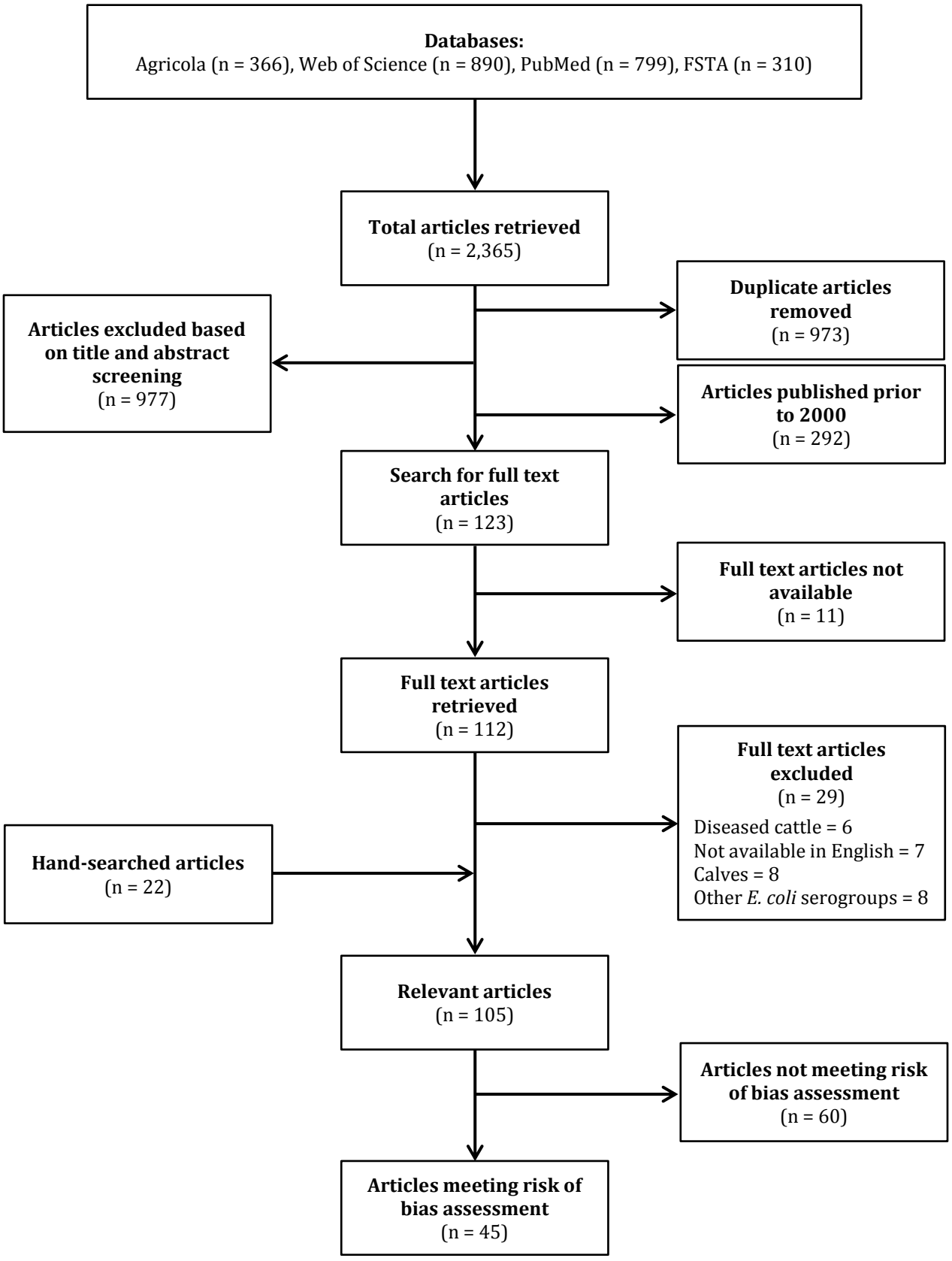
* Articles retrieved from Australia (Cobbold *et al.,* 2001; Midgley and Desmarchelier, 2001) did not detect EHEC. Estimates extracted were zero, however during logit transformation a non-zero prevalence estimate was generated for Australia.

Table 2.8 Random-effects meta-analysis pooled fecal non-O157 specific serogroup, STEC, and EHEC prevalence estimates in North America stratified by outcome

Outcome	O gene	No. articles	No. datasets	Prevalence, %	95% Confidence Interval (CI)	Cochrane's chi-square statistic (*Q*)	I^2, %	*P*-value
Serogroup[†]								
	O26	3	7	19.25	6.56-44.72	1010.50	99.4	<0.01
	O45	3	6	9.46	3.10-25.43	500.73	99.0	<0.01
	O103	3	7	11.85	3.69-32.08	881.12	99.3	<0.01
	O111	3	7	1.49	0.43-5.04	172.06	96.5	<0.01
	O121	3	6	6.31	1.44-23.69	568.74	99.1	<0.01
	O145	3	6	0.69	0.19-2.47	63.05	92.1	<0.01
	Overall non-O157	3	39	5.68	3.54-8.97	3674.94	99.0	<0.01
STEC								
	O26	5	11	5.84	3.36-9.96	71.25	86.0	<0.01
	O45	3	8	1.69	0.46-6.05	26.78	73.9	<0.01
	O103	5	12	4.20	2.42-7.18	86.49	87.3	<0.01
	O111	3	9	1.36	0.51-3.59	34.14	76.6	<0.01
	O121	5	12	3.10	1.41-6.67	86.74	87.3	<0.01
	O145	3	8	2.58	0.87-7.38	36.88	81.0	<0.01
	Overall non-O157	6	60	3.27	2.46-4.35	578.22	89.8	<0.01
EHEC								
	O26	2	3	3.75	0.79-15.95	88.44	97.7	<0.01
	O45	2	2	1.15	0.02-47.13	9.83	89.8	<0.01
	O103	3	4	4.46	2.32-8.42	21.62	86.1	<0.01
	O111	3	4	0.79	0.27-2.32	14.85	79.8	<0.01
	O121	2	2	1.50	0.01-68.00	12.70	92.1	<0.01
	O145	3	3	0.46	0.05-4.12	10.52	81.0	<0.01
	Overall non-O157	4	18	2.17	1.32-3.54	374.80	95.5	<0.01

[†]United States is the only country representing North America in this analysis; no articles were identified for serogroup data in Canada or Mexico.

Figure 2.1 Flow chart of study selection for meta-analysis eligibility



Chapter 3: Summer and Winter Prevalence of Shiga Toxin-producing *Escherichia coli* (STEC) O26, O45, O103, O111, O121, O145, and O157 in Feces of Feedlot Cattle

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Abstract

The United States Department of Agriculture Food Safety and Inspection Service have declared seven STEC serogroups (O26, O45, O103, O111, O121, O145, and O157) as adulterants in raw, non-intact beef products. The objective of this study was to determine the prevalence of these seven serogroups and the associated virulence genes (*stx*1, *stx*2, and *eae*) in cattle feces during summer (June to August 2013) and winter (January to March 2014) months. Twenty-four pen floor fecal samples were collected from each of 24 cattle pens, in both summer and winter months, at a commercial feedlot in the United States. Samples were subjected to culture-based detection methods that included enrichment, serogroup-specific immunomagnetic separation and plating on selective media, followed

by a multiplex PCR for serogroup confirmation and virulence gene detection. A sample was considered STEC positive if a recovered isolate harbored an O gene, a Shiga toxin gene (*stx*1 and/or *stx*2), and the intimin gene (*eae*). All serogroups of interest were detected in summer months, O26 (17.8%), O45 (14.6%), O103 (59.9%), O111 (0.2%), O121 (2.0%), O145 (2.7%), and O157 (41.6%); however most non-O157 isolates did not harbor virulence genes. The cumulative model-adjusted sample-level prevalence estimates of STEC O26, O103, O145, and O157 during summer (n=576) were 1.0, 1.6, 0.8, and 41.4%, respectively; STEC O45, O111, and O121 were not detected during summer months. In winter, serogroups O26 (0.9%), O45 (1.5%), O103 (40.2%), O121 (0.2%) were isolated; however, no virulence genes were detected in isolates from cattle feces collected during winter (n=576). Statistically significant seasonal differences were identified for STEC O103 and O157 ($P < 0.05$), but data on other STEC were sparse. The results of this study indicate that although non-O157 serogroups were present, non-O157 STEC were rarely detected in feces from the feedlot cattle populations tested in summer and winter months.

Key words: cattle; non-O157 STEC; prevalence; Shiga toxin-producing *Escherichia coli*; STEC

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens of public health importance. Cattle serve as a principal reservoir of STEC and shed these bacteria in their feces (Bettelheim, 2000). Human STEC infections are acquired through direct or indirect fecal-oral contact with human or animal feces (Evans and Evans, 1996), and can

lead to illnesses including mild to severe diarrhea, hemorrhagic colitis, and potentially life threatening complications such as hemolytic uremic syndrome or thrombotic thrombocytopenia purpura (Kuter *et al.,* 2014; Siegler *et al*., 2005). Several foods, including beef, have been implicated in STEC outbreaks in humans in North America (Painter *et al*., 2013; Scallan *et al*., 2011). As a result, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) has declared STEC of seven serogroups (O26, O45, O103, O111, O121, O145, and O157) as adulterants in raw, non-intact beef products.

Although there has been extensive research on the epidemiology and ecology of STEC O157 in cattle, information on the prevalence and distribution of non-O157 STEC in the cattle reservoir is limited. A strong seasonal pattern has been identified for *E. coli* O157:H7, where fecal prevalence in cattle peaks during summer months and greatly decreases in winter months (Barkocy-Gallagher *et al.,* 2003; Smith *et al.,* 2005; Edrington *et al.,* 2006). In a mathematical model, prevalence of *E. coli* O157:H7 in cattle was correlated with ground beef prevalence and human illnesses attributed to *E. coli* O157:H7 (Williams *et al.,* 2010). Frequency of human disease attributed to STEC O157 has declined since 2000; however, illnesses due to non-O157 STEC in the United States (US) have increased (Gould *et al*., 2013), with the majority (58%) of outbreaks occurring during summer months (Luna-Gierke *et al.,* 2014). The recent apparent increase in the incidence of non-O157 STEC human illness may be overstated due to a recent increase in the development and application of detection methods (Gould *et al*., 2013; Crim *et al*., 2014). However, accounting for the under-reporting of foodborne illness, it is estimated that more non-O157 STEC infections occur annually compared to STEC O157 (Scallan *et al*., 2011).

Recently, fecal prevalence of non-O157 serogroups and virulence genes in cattle and their environment has been studied in the US. Non-O157 serogroup and STEC fecal prevalence estimates reported in cattle range from 0.0 to 90.7% and 0.9 to 16.9%, respectively, in the US (Paddock *et al.,* 2012; Cernicchiaro *et al.,* 2013; Dargatz *et al.,* 2013; Baltasar *et al.,* 2014; Ekiri *et al.,* 2014). However, only a few studies have assessed seasonality of non-O157 STEC shedding in cattle (Barkocy-Gallagher *et al.,* 2003; Alexa *et al.,* 2011; Tanaro *et al.,* 2012). Often publications report combined prevalence estimates for all non-O157 STEC collectively, rather than reporting serogroup-specific prevalence estimates. Collective prevalence estimates may inaccurately represent the prevalence of the non-O157 STEC serogroups of regulatory or public health importance. Therefore, the objective of this study was to determine the prevalence of seven STEC O serogroups (O26, O45, O103, O111, O121, O145, and O157) and their virulence genes (*stx*1, *stx*2, and *eae*) in feces of pre-harvest commercial feedlot cattle during summer and winter months.

Materials and Methods

Study Population and Sample Collection

Pens of crossbred beef cattle from a large commercial feedlot in the central US were sampled weekly for 12 weeks in summer (June to August 2013) and 10 weeks in winter (January to March 2014). Cattle were managed following standard operating procedures of the feedlot. This study followed a repeated cross-sectional design. During summer months, two pens of finishing cattle within 24 hours (h) of harvest were sampled each week. Two to four pens of cattle, within two weeks of harvest, were sampled weekly during winter months. Sample sizes were determined using prevalence estimates from a previous study (Cernicchiaro *et al.,* 2013) to detect seasonal differences as low as 5%, with

a power of 80%, and Type I error of 5%. Twenty-four pen floor fecal samples were collected from each of 24 pens in both summer and winter months. Approximately 10 grams (g) of freshly voided feces were collected in individual plastic bags (WHIRL-PAK®, Nasco, Wisconsin, US) using plastic spoons, placed in a cooler on ice, and transported to the Pre-harvest Food Safety Laboratory at Kansas State University for processing within 36 h.

Culture-based Detection

Approximately 2 g of feces were added to 18 mL of *Escherichia coli* broth (EC; Difco, ThermoFisher), vortexed, and incubated for 6 h at 37°C. Post-enrichment, immunomagnetic separation (IMS) procedures were performed in a Kingfisher Flex Magnetic Particle Processor (Thermo Scientific, Waltham, MA). An aliquot of 980 µL of enriched fecal suspension was added to 20 µL serogroup-specific IMS beads (Abraxis®, Warminister, PA), for each of the *E. coli* serogroups of interest (O26, O45, O103, O111, O121, O145, and O157); a total of seven IMS runs were completed for each sample tested. The IMS method consisted of a 30-minute binding step, three two-minute washes with 1,000 µL of PBS Tween 20 and a one minute elution step using 100 µL PBS Tween 20. Following IMS, 50 µL of IMS bead suspension were spread-plated onto either Sorbitol MacConkey agar with cefixime and potassium tellurite (CT-SMAC) for O157, or Modified Possé (MP; Possé *et al*., 2008) agar for non-O157 beads. The modification included reduction of novobiocin and potassium tellurite concentrations to 5.0 mg L^{-1} and 0.5 mg L^{-1}, respectively. After the 20 to 24 h incubation, six chromogenic colonies were chosen from each of the six MP plates and six non-sorbitol fermenting colonies were chosen from the CT-SMAC plate. These colonies were streaked onto blood agar plates and incubated for 24 h at 37°C.

PCR Serogroup Confirmation and Virulence Gene Detection

Putative O157 colonies grown on blood agar were tested for the O157 antigen by latex agglutination and, if positive, then tested for indole production. Isolates positive by latex agglutination and indole assays were then confirmed by a multiplex PCR for detection of the six major genes (*rfbE*$_{O157}$, *fliC*$_{H7}$, *eae*, *stx*1, *stx*2, and *ehxA*) of *E. coli* O157 as outlined in Bai *et al.* (2010).

Putative non-O157 colonies grown on blood agar were pooled and tested by a multiplex PCR targeting serogroup-specific genes (O26, O45, O103, O111, O121, and O145; Paddock *et al*., 2012). If the colony pool tested positive for one of the six non-O157 serogroups, then each colony was individually tested by a multiplex PCR (Bai *et al*., 2012) targeting six non-O157 serogroups and three virulence genes (*stx*1, *stx*2, and *eae*).

Samples were considered *serogroup positive* if the individual isolate tested positive for an O gene of interest by the multiplex PCR for non-O157 serogroups, or for the *rfbE*$_{O157}$ gene by the 6-plex PCR. A sample was considered *serogroup negative* if colony pools tested negative for all O genes of interest by the 7-plex PCR; for serogroup O157, samples were considered negative if colonies tested negative to latex agglutination, indole production, or 6-plex PCR assays. Samples were considered *STEC positive* if a serogroup-positive (O157 or non-O157) isolate carried at least one Shiga toxin gene (*stx*1 and/or *stx*2), and the intimin (*eae*) gene. Samples were classified as positive if at least one isolate tested positive to the respective serogroup and STEC definitions.

Statistical Analyses

Crude cumulative pen-level prevalence estimates and 95% confidence intervals were computed for the serogroup case definitions for each season. Prevalence at the pen-

level was calculated as the number of positive samples per pen divided by the total number of samples tested per pen.

Model-adjusted cumulative pen-level prevalence estimates and their 95% confidence intervals (CI) were estimated from model intercepts using generalized linear mixed models. Monthly STEC prevalence estimates were also obtained by including the variable month (June, July, and August) as a fixed effect. Models were run using Proc Glimmix (SAS 9.3, SAS Institute Inc., Cary, NC) with a binomial distribution, logit link, residual pseudo-likelihood estimation technique, Kendward-Roger degrees of freedom approximation, and a random effect of pens within weeks to account for the clustering effect of pens nested within sampling week. Non-parametric permutation tests were used to compare the within-pen mean number of positive samples between seasons, using the exact option for hypothesis testing (Proc Npar1way command in SAS 9.3). *P*-values less than 0.05 were deemed significant.

Results

Study Population

Pens of healthy cattle closest to harvest, identified weekly by the feedlot manager, were sampled; consequently, study pens were sampled only once. A total of 48 pens housing 17,511 cattle were sampled. During summer months, 24 pens housing a total of 6,473 steers were sampled. Each pen sampled between June and August 2013 housed an average of 270 cattle (range=121 to 299 cattle, median=283 cattle). In winter months, 24 pens were sampled, housing a total of 11,038 cattle (22 pen of steers (n=10,036) and 2 pens of heifers (n=1,002)). In winter, each pen housed an average of 460 cattle (range=189

to 627 cattle, median=544 cattle). A total of 1,152 pen-floor fecal samples were collected from 48 pens (576 samples from 24 pens in both summer and winter months).

Summer Prevalence

Serogroup O103 was the most prevalent serogroup isolated from this cattle population. In summer months, serogroup O103 was detected in 340 (59.0%) fecal samples; cumulative crude prevalence for serogroups O26, O45, O111, O121, O145, and O157 was 22.0, 16.5, 0.2, 2.1, 3.1, and 43.1%, respectively (Table 3.1). All seven serogroups were identified during summer months; however, serogroup O111 was isolated only in a single sample. Although non-O157 serogroups were detected during summer, non-O157 isolates rarely harbored *stx* and *eae* genes in our study population; however, nearly all O157 isolates harbored *stx* and *eae* genes.

The most common STEC isolated in summer months was STEC O157 (247/576, 42.9%): 248 samples tested positive for O157 and *stx* genes, only one O157 isolate lacked the *eae* gene (Table 3.1). Shiga toxin-producing *E. coli* O157 samples predominately tested positive for *stx*2 (n=246, 42.7%) versus *stx*1 (n=95, 16.5%) genes; however, 93 samples (16.7%) harbored isolates that tested positive for both *stx*1 and *stx*2 genes. Shiga toxigenic *E. coli* O26, O103, and O145 were isolated in summer months; however, STEC O45, O111, and O121 were not detected during this period. Crude cumulative prevalence of STEC O26, O103, and O145 was 1.2, 1.7, and 1.0%, respectively, in summer (Table 3.1). In contrast to STEC O157, the Shiga toxin gene most commonly associated with non-O157 STEC was *stx*1 (n=20, 87%) while *stx*2 was present in only a few samples (n=3, 13%), and no isolates harbored both genes. Serogroup and STEC model-adjusted cumulative prevalence estimates are presented in Table 3.2.

Pen-level crude prevalence values varied greatly between pens of cattle; pen-level crude prevalence of serogroups O26, O45, O103, O111, O121, O145, and O157 ranged from 0 to 95.8, 0 to 79.2, 16.7 to 91.7, 0 to 4.2, 0 to 12.5, 0 to 16.7, and 0 to 79.2%, respectively (Table 3.3). Similarly, variability in pen-level prevalence was also observed for STEC shedding among pens during summer months; the crude pen-level prevalence of STEC O26, O103, O145, and O157 ranged from 0 to 8.3, 0 to 12.5, 0 to 12.5, and 0 to 79.2%, respectively (pen-level data not shown).

Model-adjusted fecal prevalence of STEC O157 significantly differed ($P < 0.01$) between study months: June (32.3%; 95% CI = 25.7-39.7%), July (56.3%; 95% CI = 48.7-63.5%), and August (40.1%; 95% CI = 33.0-47.6%). Significant differences in STEC O157 fecal prevalence were established between June and July ($P < 0.01$) and between July and August ($P < 0.01$); however, June and August were not significantly different. Sampling month was not statistically significant for STEC O26, O103, and O145. Monthly model-adjusted prevalence estimates (and 95% CI) for STEC O26, O103, and O145 in June were 3.1% (1.3-7.1%), 3.6% (1.7-7.8%), and 2.6% (1.0-6.4%), respectively; in July, monthly estimates for STEC O26, O103, and O145 were 0.5% (0.1-4.0%), 1.6% (0.5-5.1%), and 0.5% (0.1-4.0%), respectively. In August, STEC O26, O103, and O145 were not detected. The effect of month on fecal prevalence was not tested for STEC O45, O111, and O121 as these STEC were not detected in this group of samples during summer months.

Winter Prevalence

Serogroup O103 was the most prevalent serogroup isolated from this cattle population in winter. Cumulative crude prevalence for serogroups O26, O45, O103, and O121 was 1.2, 1.9, 41.0, and 0.5%, respectively (Table 3.1). Serogroups O111, O145, and

O157 were not isolated in this period. Model-adjusted cumulative serogroup prevalence estimates for winter are presented in Table 3.2. Crude prevalence estimates remained variable among pens; however, at lower proportions than observed in summer months (Table 3.4). In winter, serogroup O26, O45, O103, and O121 shedding ranged from 0 to 12.5, 0 to 12.5, 8.3 to 79.3, and 0 to 12.5%, respectively. Notably, serogroup O103 shedding was highly variable between study pens and was detected throughout all pens sampled during winter.

Although serogroups (O26, O45, O103, and O121) were isolated during winter months, no virulence genes were detected in those isolates. None of the samples collected during winter months tested positive for any of the seven STEC of interest.

Statistical Comparison between Summer and Winter Prevalence

Significant seasonal differences ($P < 0.05$) were identified for pen-level prevalence of serogroups O26, O45, O103, O145, and O157. Statistically significant differences ($P < 0.05$) were also observed for pen-level fecal prevalence of STEC O103 and O157 between study seasons; however, no significant differences were observed for the STEC less commonly identified.

Discussion

The prevalence of seven STEC serogroups (O26, O45, O103, O111, O121, O145, and O157) and their associated major virulence genes was determined in feces of commercial feedlot cattle prior to harvest for both summer and winter seasons. Although a few studies have addressed the detection of non-O157 STEC in cattle feces, data regarding the frequency of non-O157 STEC serogroups in pre-harvest cattle in the US are still limited

(Cernicchiaro *et al.,* 2013; Dargatz *et al.,* 2013; Baltasar *et al.,* 2014; Ekiri *et al.,* 2014). The prevalence of non-O157 STEC organisms obtained in our study was lower than what has been reported in previous studies (Barkocy-Gallagher *et al.,* 2003; Cernicchiaro *et al.,* 2013; Ekiri *et al.,* 2014) but comparable to others (Cobbold *et al.,* 2004). Different case definitions, study populations, and detection protocols may explain differences in prevalence estimates obtained across studies.

Diagnostic methods employed for detection and isolation of serogroups and virulence genes influence prevalence estimates (Cernicchiaro *et al.,* 2013). Methods for culturing and confirming STEC O157 have been well-established; however, various non-O157 diagnostic methods and their impact on detection specificity and sensitivity are still being explored. The current lack of standardized and well-established detection methods for non-O157 STEC in cattle feces poses a challenge when comparing prevalence estimates across studies. The use of IMS techniques has been shown to increase the sensitivity of detection of STEC O157 compared to direct plating culture methods (Chapman *et al.,* 1994). Recently, the use of IMS in culture-based detection methods has been shown to increase the apparent prevalence of non-O157 serogroups detected in feces, when compared to direct PCR (Cernicchiaro *et al.,* 2013). Despite the recent development of increasingly sensitive molecular methodologies, subjecting samples to culture methods may still be needed to obtain isolates for further characterization and accurate linkage of O serogroup and virulence genes from bacterial isolates.

Serogroup O103 was the predominant serogroup isolated from these cattle populations in both seasons. In winter months, serogroup O103 remained prevalent throughout all study pens unlike other serogroups of interest. We hypothesize that

serogroup O103 may have different microbial properties allowing the bacterium to endure harsher conditions and out-compete other microbiota, potentially allowing for longer survival in the cattle reservoir and subsequent environment. In another United States study, using similar detection methods, serogroup O103 also was the most frequently isolated non-O157 serogroup among cattle (Ekiri *et al.,* 2014).

Previous research has demonstrated that non-O157 IMS bead specificity is variable and non-specific binding of beads occurs (Cernicchiaro *et al.,* 2013). This issue needs to be further evaluated as diagnostic sensitivity and specificity directly impact prevalence estimates. Other potential reasons for the low or no detection of some serogroups and virulence genes in either summer or winter may be related to insufficient samples per pen or study design limitations. Although repeated sampling from pens provides a perspective of how prevalence varies within pens close to harvest, the intermittent nature of fecal shedding can impact sample-level detection. In the present study, different pens of cattle were tested each week, which provide useful data pertaining to the STEC prevalence in cattle at harvest. However, point in time estimates may not be representative of the prevalence that exists in pens of cattle over time due to intermittent fecal shedding (Sargeant *et al.,* 2000; Renter *et al.,* 2002; Chase-Topping *et al.,* 2008; Menrath *et al.,* 2010). Furthermore, because we measured prevalence of STEC, we cannot separate factors related to new animals shedding bacteria (i.e., incidence) and those associated with duration of shedding. Regardless, measures targeted at reducing STEC prevalence in pre-harvest cattle will be beneficial to potentially decrease microbial transmission to other pen-mates and the environment, as well as to reduce contamination of hides and subsequent transfer to carcass surfaces as those animals are harvested.

Seasonality of *E. coli* O157 has been well established in the bovine reservoir and in human illnesses (Barkocy-Gallagher *et al.,* 2003; Rangel *et al.,* 2005; Smith *et al.,* 2005; Williams *et al.,* 2010). An increase in non-O157 STEC human illness outbreaks has been observed in the United States for the warmer months (Luna-Gierke *et al.,* 2014); however, the seasonality of non-O157 STEC shedding in cattle has not yet been established. The increased recognition of the importance of non-O157 STEC human infections, and the seasonality associated with human illness outbreaks, emphasizes the need for non-O157 STEC data in the bovine reservoir, particularly prior to harvest. In the present study, significant seasonal differences between summer and winter months were identified for serogroups O26, O45, O103, O145, and O157, as well as STEC O103 and O157. Cobbold *et al.* reported that STEC were more prevalent in cattle feces in the fall than in winter (Cobbold *et al.,* 2004). Similarly, another study showed that cattle shed non-O157 STEC more frequently in spring and fall, than summer and winter (Barkocy-Gallagher *et al.,* 2003). In our study, we did not detect non-O157 STEC in winter months. Future research is needed to generate data on prevalence of non-O157 STEC in other seasons (i.e., fall and spring), as well as in other feedlot operations, geographic regions, and cattle types (e.g., dairy, pasture, culled cattle).

In conclusion, our results indicate that non-O157 STEC in feedlot cattle were rarely detected in summer and were undetected in winter months. The most commonly identified serogroups were O26, O103, and O157 during summer; and O26, O45, and O103 in winter. Conversely, the most common STEC detected in this cattle population in summer was STEC O157 followed by STEC O103, O26, and O145 in much lower frequency. These findings contribute to filling the data gaps regarding prevalence of non-O157 STEC in cattle

prior to entering the food supply, in two seasons. These estimates will be used to populate quantitative microbial risk assessment models that are crucial to understanding the risk of human illnesses due to STEC that are attributed to cattle and subsequent beef products.

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Tables

Table 3.1 Distribution of positive samples for *Escherichia coli* O serogroups and virulence genes isolated in summer and winter months from cattle feces

	Summer						Winter*
Serogroup	O gene n (%)	O gene + *stx*1 n (%)	O gene + *stx*2 n (%)	O gene + *stx*1 and *stx*2 n (%)	O gene + *stx*1 and/or *stx*2 n (%)	O gene + *stx*1 and/or *stx*2 + *eae* n (%)	O gene n (%)
O26	127 (22.0)	7 (1.2)	0 (0.0)	0 (0.0)	7 (1.2)	7 (1.2)	7 (1.2)
O45	95 (16.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	11 (1.9)
O103	340 (59.0)	9 (1.6)	1 (0.1)	0 (0.0)	10 (1.7)	10 (1.7)	236 (41.0)
O111	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
O121	12 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.5)
O145	18 (3.1)	4 (0.7)	2 (0.3)	0 (0.0)	6 (1.0)	6 (1.0)	0 (0.0)
O157	248 (43.1)	95 (16.5)	246 (42.7)	93 (16.7)	248 (43.1)	247 (42.9)	0 (0.0)

Proportions were calculated over the total number of fecal samples tested in winter and summer (n=576), respectively.

*No isolates with virulence genes were detected in winter months.

Table 3.2 Model-adjusted cumulative prevalence estimates* of O serogroups and Shiga toxin-producing *E. coli* (STEC) for summer and winter months

	Serogroup prevalence, % (95% CI)		STEC prevalence, % (95% CI)	
O gene	Summer	Winter	Summer	Winter
O26	17.8 (15.1, 20.4)	0.9 (0.0, 4.1)	1.0 (0.0, 4.2)	0.0
O45	14.6 (12.2, 17.0)	1.5 (0.0, 4.5)	0.0	0.0
O103	59.9 (57.5, 62.2)	40.2 (37.9, 42.5)	1.6 (0.0, 4.4)	0.0
O111	0.2 (0.0, 5.5)	0.0	0.0	0.0
O121	2.0 (0.0, 4.7)	0.2 (0.0, 5.6)	0.0	0.0
O145	2.7 (0.0, 5.4)	0.0	0.8 (0.0, 4.1)	0.0
O157	41.6 (39.2, 44.0)	0.0	41.4 (39.0, 43.8)	0.0

Denominator for summer and winter is 576 fecal samples.

*Estimates and 95% confidence intervals from generalized linear mixed models using binomial distribution, logit link, and a random intercept of pens within week to estimate prevalence by season.

Table 3.2 Crude pen-level and cumulative prevalence* of O serogroups by pen and sampling week during summer months (June to August 2013)

Pen	Date	No. of cattle	O26 % (n)	O45 % (n)	O103 % (n)	O111 % (n)	O121 % (n)	O145 % (n)	O157 % (n)
1	6/9/2013	284	25.0 (6)	16.7 (4)	45.8 (11)	0.0 (0)	0.0 (0)	4.2 (1)	50.0 (12)
2	6/9/2013	278	95.8 (23)	12.5 (3)	54.2 (13)	0.0 (0)	0.0 (0)	16.7 (4)	33.3 (8)
3	6/16/2013	281	25.0 (6)	12.5 (3)	41.7 (10)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
4	6/16/2013	283	25.0 (6)	12.5 (3)	25.0 (6)	0.0 (0)	0.0 (0)	12.5 (3)	0.0 (0)
5	6/23/2013	284	83.3 (20)	25.0 (6)	41.7 (10)	0.0 (0)	0.0 (0)	4.2 (1)	70.8 (17)
6	6/23/2013	279	50.0 (12)	37.5 (9)	54.2 (13)	0.0 (0)	4.2 (1)	0.0 (0)	12.5 (3)
7	6/30/2013	284	16.7 (4)	8.3 (2)	62.5 (15)	0.0 (0)	4.2 (1)	4.2 (1)	58.3 (14)
8	6/30/2013	281	33.3 (8)	16.7 (4)	16.7 (4)	0.0 (0)	4.2 (1)	0.0 (0)	33.3 (8)
9	7/7/2013	273	25.0 (6)	8.3 (2)	66.7 (16)	0.0 (0)	4.2 (1)	0.0 (0)	50.0 (12)
10	7/7/2013	283	12.5 (3)	12.5 (3)	66.7 (16)	0.0 (0)	8.3 (2)	0.0 (0)	54.2 (13)
11	7/14/2013	284	25.0 (6)	29.2 (7)	83.3 (20)	0.0 (0)	0.0 (0)	0.0 (0)	58.3 (14)
12	7/14/2013	291	20.8 (5)	16.7 (4)	83.3 (20)	0.0 (0)	0.0 (0)	8.3 (2)	45.8 (11)
13	7/21/2013	277	8.3 (2)	16.7(4)	83.3 (20)	0.0 (0)	0.0 (0)	0.0 (0)	58.3 (14)
14	7/21/2013	298	12.5 (3)	4.2 (1)	70.8 (17)	0.0 (0)	0.0 (0)	0.0 (0)	29.2 (7)
15	7/28/2013	292	4.2 (1)	4.2 (1)	50.0 (12)	0.0 (0)	0.0 (0)	12.5 (3)	75.0 (18)
16	7/28/2013	123	0.0 (0)	12.5 (3)	41.7 (10)	0.0 (0)	4.2 (1)	4.2 (1)	79.2 (19)
17	8/4/2013	299	20.8 (5)	79.2 (19)	91.7 (22)	0.0 (0)	12.5 (3)	0.0 (0)	33.3 (8)
18	8/4/2013	299	0.0 (0)	0.0 (0)	70.8 (17)	0.0 (0)	0.0 (0)	0.0 (0)	54.2 (13)
19	8/11/2013	279	4.2 (1)	4.2 (1)	62.5 (15)	4.2 (1)	0.0 (0)	0.0 (0)	37.5 (9)
20	8/11/2013	282	4.2 (1)	20.8 (5)	45.8 (11)	0.0 (0)	4.2 (1)	0.0 (0)	45.8 (11)
21	8/18/2013	294	4.2 (1)	20.8 (5)	66.7 (16)	0.0 (0)	0.0 (0)	4.2 (1)	41.7 (10)
22	8/18/2013	284	16.7 (4)	4.2 (1)	87.5 (21)	0.0 (0)	0.0 (0)	0.0 (0)	29.2 (7)
23	8/25/2013	240	8.3 (2)	16.7 (4)	66.7 (16)	0.0 (0)	0.0 (0)	4.2 (1)	33.3 (8)
24	8/25/2013	121	8.3 (2)	4.2 (1)	37.5 (9)	0.0 (0)	4.2 (1)	0.0 (0)	50.0 (12)
Pen prevalence range, %			0.0-95.8	0.0-79.2	16.7-91.7	0.0-4.2	0.0-12.5	0.0-16.7	0.0-79.2
Crude cumulative prevalence, %			22.0	16.5	59.0	0.2	2.1	3.1	43.1
95% confidence interval, %			18.7-25.7	13.6-19.8	54.9-63.1	0.0-1.0	1.1-3.6	1.9-4.9	39.0-47.2

*The number of positive samples divided by the total number of samples tested expressed as a percentage. The denominator (n=24) was the same for all pens.

Table 3.3 Crude pen-level and cumulative prevalence* of O serogroups by pen and sampling week during winter months (January to March 2014)

Pen†	Date	No. of cattle	O26 % (n)	O45 % (n)	O103 % (n)	O121 % (n)
25	1/6/2014	275	0.0 (0)	0.0 (0)	33.3 (8)	0.0 (0)
26†	1/6/2014	461	0.0 (0)	0.0 (0)	20.8 (5)	0.0 (0)
27	1/6/2014	280	0.0 (0)	0.0 (0)	25.0 (6)	0.0 (0)
28	1/13/2014	189	0.0 (0)	0.0 (0)	41.7 (10)	0.0 (0)
29	1/13/2014	565	0.0 (0)	0.0 (0)	58.3 (14)	0.0 (0)
30	1/13/2014	553	0.0 (0)	4.2 (1)	58.3 (14)	12.5 (3)
31	1/13/2014	623	0.0 (0)	4.2 (1)	75.0 (18)	0.0 (0)
32	1/20/2014	289	4.2 (1)	0.0 (0)	25.0 (6)	0.0 (0)
33	1/20/2014	560	0.0 (0)	0.0 (0)	54.2 (13)	0.0 (0)
34	1/20/2014	557	0.0 (0)	12.5 (3)	54.2 (13)	0.0 (0)
35†	1/27/2014	541	0.0 (0)	4.2 (1)	29.2 (7)	0.0 (0)
36	1/27/2014	560	0.0 (0)	0.0 (0)	50.0 (12)	0.0 (0)
37	2/3/2014	294	0.0 (0)	0.0 (0)	79.2 (19)	0.0 (0)
38	2/3/2014	624	4.2 (1)	0.0 (0)	33.3 (8)	0.0 (0)
39	2/10/2014	561	0.0 (0)	0.0 (0)	45.8 (11)	0.0 (0)
40	2/10/2014	627	0.0 (0)	0.0 (0)	12.5 (3)	0.0 (0)
41	2/17/2014	443	0.0 (0)	0.0 (0)	25.0 (6)	0.0 (0)
42	2/17/2014	546	0.0 (0)	0.0 (0)	45.8 (11)	0.0 (0)
43	2/24/2014	554	8.3 (2)	0.0 (0)	62.5 (15)	0.0 (0)
44	2/24/2014	530	0.0 (0)	8.3 (2)	37.5 (9)	0.0 (0)
45	3/3/2014	281	0.0 (0)	12.5 (3)	8.3 (2)	0.0 (0)
46	3/3/2014	564	12.5 (3)	0.0 (0)	20.8 (5)	0.0 (0)
47	3/10/2014	284	0.0 (0)	0.0 (0)	25.0 (6)	0.0 (0)
48	3/10/2014	277	0.0 (0)	0.0 (0)	62.5 (15)	0.0 (0)
Pen prevalence range, %			0.0-12.5	0.0-12.5	8.3-79.2	0.0-12.5
Crude cumulative prevalence, %			1.2	1.9	41.0	0.5
95% confidence interval, %			0.5-2.5	1.0-3.4	36.9-45.1	0.1-1.5

*The number of positive samples divided by the total number of samples tested expressed as a percentage. The denominator (n=24) was the same for all pens.

†Indicates the sex of the pen is heifers, the remaining pens are comprised of steers

Serogroups O111, O145, and O157 were not detected in winter months.

Chapter 4: Conclusion

Shiga toxin-producing *Escherichia coli* are foodborne pathogens of public health importance. Although the USDA-FSIS has declared the STEC-7 as adulterants in raw, non-intact beef products, there are many areas pertaining to the cattle reservoir and their environments that require additional research to understand the epidemiology of non-O157 STEC. The review of literature (Chapter 1) relating to the epidemiology of Shiga toxigenic *E. coli* and the bovine reservoir revealed a few inconsistencies and data gaps that exist in the published literature to date, in addition to demonstrating the lack of existing data regarding non-O157 STEC in cattle. This thesis has addressed major data gaps necessary to assess the risk of these non-O157 STEC pathogens in the beef supply by evaluating the presence of these pathogens in the cattle reservoir.

Firstly, a formal systematic review of the literature was conducted to identify all peer-reviewed literature regarding the six non-O157 serogroups (O26, O45, O103, O111, O121, and O145) and their associated virulence genes (*stx*1, *stx*2, and *eae*) in cattle. Following the initial retrieval of articles, the pre-set inclusion and exclusion criteria were expanded to include articles published worldwide, rather than articles published only in North America, as very few articles were retrieved from the United States (n = 7), Canada (n = 3), and Mexico (n = 0). After articles were screened, and underwent the risk of bias assessment and critical evaluation, data were extracted. Meta-analyses were conducted for non-O157 fecal prevalence for three different outcomes worldwide and in North America. Random-effects meta-analyses of data indicated that worldwide non-O157 serogroup and virulence gene fecal prevalence significantly differed ($P < 0.001$) between geographic regions (e.g., Africa, Asia, Australia, Europe, North America, and South America). Non-O157

serogroup and virulence gene pooled prevalence estimates were highest for North America; further evaluation indicated that the United States was the country of highest non-O157 serogroup and virulence gene prevalence in North America. Meta-regression analyses of worldwide studies were conducted to assess factors (continent, time of harvest, cattle type, IMS, and specimen type) contributing to between-study heterogeneity of non-O157 fecal prevalence estimates. To our knowledge, this is the first meta-analysis regarding non-O157 serogroups and virulence genes in cattle. This manuscript will greatly contribute to the limited body of knowledge regarding non-O157 STEC by evaluating the STEC burden in cattle by region and summarizing the prevalence along a major portion of the beef continuum (fecal-hide-carcass) prior to harvest.

Major data gaps were identified for fecal, hide, and carcass prevalence and concentration data regarding non-O157 serogroups and virulence genes in pre- and peri-harvest cattle; therefore, empirical data was obtained in a second study using a repeated cross-sectional study design. In my second study, we identified and assessed the frequency of the STEC-7 serogroups and virulence genes in feces of pre-harvest commercial feedlot cattle during summer and winter. This will be one of the first peer-reviewed studies to evaluate the seasonality of these specific non-O157 STEC serogroups and virulence genes in cattle in two seasons. In summer, all seven serogroups of interest were detected; however, most non-O157 isolates lacked the necessary virulence genes to cause human illness. Non-O157 STEC were detected in very low frequencies in cattle feces during summer months, especially compared to STEC O157. In winter months, serogroups of interest were detected but no STEC—of any serogroup of interest—were isolated from cattle feces. We observed seasonal differences in STEC shedding; STEC O103 and O157

shedding were significantly different between summer and winter. Potentially due to the low numbers of positive samples, significant seasonal differences were not observed for the less common STEC. The prevalence estimates obtained in this study will be used to populate quantitative microbial risk assessment models that are necessary to evaluate the risk of STEC human illnesses attributed to the cattle reservoir. Further research is needed to confirm these findings in other feedlots, regions, and production systems as this study included only 48 pens of cattle housed at a single feedlot in the central United States. However, these findings suggest that cattle commonly harbor and shed *E. coli* O157, yet cattle feces may not be a frequent source of non-O157 STEC.

Additional research is needed to assess the frequency of STEC pathogens throughout the beef chain, especially in peri-harvest cattle, in order to contribute toward initiatives for reducing STEC human illnesses resulting from cattle and beef products. The work herein indicates that cattle feces harbor non-O157 STEC pathogens but at very low frequencies. Overall, this thesis contributes to the limited body of data, summarizes current peer-reviewed literature, and furthers the knowledge regarding the epidemiology of non-O157 STEC in the bovine reservoir.