Data collection, analysis and development of a peri-harvest quantitative microbial risk assessment (QMRA) for Shiga toxin-producing *Escherichia coli* (STEC) in beef production

by

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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Abstract

Shiga-toxin-producing *Escherichia coli* (STEC), of which enterohemorrhagic *E. coli* (EHEC) are a pathogenic sub-group, are foodborne pathogens of significant public health importance in the United States. STEC belong to the family *Enterobacteriaceae* commonly found in the large intestine of humans and other warm-blooded animals. EHEC harbors shiga toxin (*stx*$_1$ and/or *stx*$_2$) and *eae* genes which confers the ability to cause human illnesses. The U.S. Department of Agriculture Food Safety and Inspection Service declared seven STEC (O26, O45, O103, O111, O121, O145, and O157) as adulterants in ground beef and non-intact beef products to reduce/eliminate the burden of the pathogens in the beef production chain. STEC control efforts in the U.S. include the development of quantitative microbial risk assessment (QMRA) to identify mitigation strategies that are effective and economical in reducing exposure and reduce occurrence and public health risk from STEC in the beef chain.

Collection of accurate and unbiased data is critical for the development of a QMRA that is valid for decision making. Determining the prevalence and concentration of the seven STEC in the different cattle types and seasons is valuable for the development a valid QMRA for STEC in beef production in the U.S. Our systematic review and meta-analysis study of the prevalence and concentration of *E. coli* O157 along the beef production chain indicated differences in the fecal prevalence of *E. coli* O157 among cattle types and seasons, revealed decreasing prevalence and concentration of *E. coli* O157 on cattle hides and carcass surfaces from pre-evisceration to the final chilled carcass stage, and identified study setting, detection method, hide or carcass swab area, and study design as significant sources of heterogeneity among studies reporting prevalence of *E. coli* O157 along the beef production chain.

Bayesian estimation of the diagnostic performance of three laboratory methods (culture, conventional PCR [cPCR], and multiplex quantitative PCR [mqPCR]) used for the detection of the seven STEC in the feces of cattle is necessary to estimate true prevalence of EHEC in cattle. The analysis revealed highest sensitivity of mqPCR, followed by cPCR, and culture for the detection of *E. coli* O157; the cPCR and mqPCR had comparable specificity, but specificity of mqPCR method was heavily dependent on prior specification. The mqPCR method was the most sensitive for the detection O26, O45, and O103 serogroups. The cPCR method was more sensitive than the culture method for serogroups O26, and O121, but comparable for serogroups O45, O103, O111, and O145. The cPCR method showed higher specificity than mqPCR within serogroups O45, O121, and O145 but no apparent differences within serogroups O26, O103, and O111.

A second order quantitative microbial risk assessment was developed to quantify the prevalence and concentration of the seven STEC on pre-evisceration beef carcasses and evaluate the impact of peri-harvest interventions. Simulation scenarios of current industry peri-harvest intervention practices showed variable effectiveness in reducing STEC contamination on pre-evisceration beef carcass, however, a scenario of increased adoption of peri-harvest interventions was more effective at reducing STEC contamination. Fecal-to-hide transfer and hide-to-carcass transfer had a large effect on prevalence and concentration of STEC on pre-evisceration carcasses.

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A second order quantitative microbial risk assessment was developed to quantify the prevalence and concentration of the seven STEC on pre-evisceration beef carcasses and evaluate the impact of peri-harvest interventions. Simulation scenarios of current industry peri-harvest intervention practices showed variable effectiveness in reducing STEC contamination on pre-evisceration beef carcass, however, a scenario of increased adoption of peri-harvest interventions was more effective at reducing STEC contamination. Fecal-to-hide transfer and hide-to-carcass transfer had a large effect on prevalence and concentration of STEC on pre-evisceration carcasses.

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Dedication

To my family and friends. Thank you for your support!

Preface

Shiga-toxin-producing *Escherichia coli* (STEC) are important foodborne pathogens of significant public health impact. In the U.S., they cause over 175,000 human illnesses annually. Cattle are the principal reservoir of STEC and they shed the bacteria in their feces, contaminating the environment and hides of cattle within herds. Bacterial contamination of beef carcasses may occur from transfer of fecal material from hides or rarely, gut rupture during processing, and consumption of contaminated and undercooked beef and beef products may result in significant human morbidity and mortality. The goal of this project is to develop a probabilistic second order quantitative microbial risk assessment (QMRA) for the seven major STEC–O26, O45, O103, O111, O121, O145, and O157– (collectively known as STEC-7) in the beef production chain with focus on the peri-harvest phase. Data were obtained from existing literature and new research studies to populate the QMRA. Chapter 1 begins with a review of the sources, reservoir, and transmission of STEC-7, methods for the detection and quantification, and control of STEC-7 in the beef production system, and a review of existing QMRA for STEC in beef in the United States and Canada. Chapter 2 uses a systematic and transparent approach, known as systematic review and meta-analysis, to collect and summarize exiting published data on the prevalence and concentration of *E. coli* O157 in different seasons and cattle types processed in North America. The results of this study were utilized in the QMRA. In Chapters 3 and 4, we use Bayesian latent class analysis to estimates the diagnostic performance (diagnostic sensitivity and specificity) of three laboratory methods use for the detection of STEC-7 in cattle feces. Briefly, the Bayesian estimation procedure combines prior knowledge about the unknown parameters of interest with data obtained from sample testing (i.e. likelihood function) to produce updated posterior distributions for parameters of interest. i.e.,

$$\text{posterior} \propto \text{prior} \times \text{likelihood}$$

The prior is a probability distribution that describe the current state of knowledge about the parameter of interest. The likelihood describes the additional information obtained through the collection of data. The posterior is a probability distribution that describe the new state of knowledge after combining the prior with the data. The posterior distribution is summarized as means or medians with estimates of variability (standard deviation, quantile, credibility interval). The estimated diagnostic sensitivity and specificity were used to adjust the apparent prevalence of *E. coli* O157 obtained from chapter 2 and the six non-O157 obtained from recent studies to derive the true prevalence of each of the STEC-7 serogroup in cattle feces. These estimates of true prevalence were utilized in the QMRA. Finally, Chapter 5, incorporates the true prevalence and concentration data for STEC-7, fecal-to-hide transfer, and the hide-to-carcass transfer data in a second order QMRA to derive an estimate of the prevalence and concentration of STEC-7 on cattle hides and on pre-wash, pre-evisceration beef carcass. Impact of pre-harvest intervention (*E. coli* O157 vaccination and inclusion of direct fed microbials in feed of fed cattle) and peri-harvest intervention (application of hide washes on cattle processed at large plants) on the prevalence and concentration of STEC-7 on pre-wash pre-evisceration beef carcass was assessed.

Chapter 1 - Literature Review – Epidemiology, control, and quantitative risk assessment of Shiga toxin-producing *Escherichia coli* (STEC) in beef production

Introduction

Escherichia coli is a Gram-negative, rod-shaped, facultative anaerobic bacterium of the family *Enterobacteriaceae* commonly found in the large intestine of humans and other warm-blooded animals in a mutually beneficial relationship (Farrokh et al., 2013). Most *E. coli* strains are commensal, providing resistance against pathogenic organisms and often used as indicator organisms for fecal contamination; however, some *E. coli* strains have virulence factors that have allowed them in some cases to cause serious illness in humans (Callaway et al., 2009), although commensal strains may also cause illness in immunocompromised hosts (Katouli, 2010). Pathogenic *E. coli* that affect the intestines of humans are classified into six categories: Shiga-toxin-producing *E. coli* (STEC), of which enterohemorrhagic *E. coli* (EHEC) are a pathogenic sub-group; enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); enteroaggregative *E. coli* (EAEC); enteroinvasive *E. coli* (EIEC); and diffusely adherent *E. coli* (DAEC) (Farrokh et al., 2013).

The disease usually associated with the pathogenic *E. coli* strains include mild bacillary dysentery for EIEC, infantile diarrhea for EPEC and EAEC, infantile and traveler's diarrhea for ETEC and DAEC, and bloody diarrhea, hemolytic uremic syndrome and death for STEC / EHEC (Nataro and Kaper, 1998; FDA, 2012). Among the pathogenic *E. coli* strains, the most significant is enterohemorrhagic *E. coli* O157:H7 (EHEC O157). This bacterium is highly virulent and capable of

producing large quantities of toxins, called Shiga toxin 1 (*stx*$_1$) and Shiga toxin 2 (*stx*$_2$), which cause severe damage to the intestinal mucosa and may lead to severe disease symptoms. Of the two toxins, the presence of *stx*$_2$ is linked with more severe human disease (Boerlin et al., 1999). In humans, *E. coli* O157:H7 colonize mostly the large intestine (Katouli, 2010). Intimin, an adhesin encoded by the *E. coli* attaching and effacing gene (*eae*) and present on the locus for enterocyte effacement (LEE) island of the bacteria chromosome, enables the attachment of the bacteria to the intestinal epithelial cells (Boerlin et al., 1999; Fairbrother and Nadeau, 2006). The adherent bacteria produce Shiga toxin (Stx) which is transported across the epithelial cells to the blood vessels resulting in abdominal symptoms (Schuller, 2011). *E. coli* serogroups are characterized by the presence of an O antigen. STEC possess an O antigen, along with *stx*$_1$ and/or *stx*$_2$. EHEC possess the intimin gene (*eae*) in addition to an O antigen and *stx*$_1$ and/or *stx*$_2$. *E. coli* O157:H7 is the most prevalent cause of STEC-induced human disease and outbreaks. A dose of 5-50 pathogenic *E. coli* O157:H7 viable cells can cause illness (Farrokh et al., 2013), with symptoms ranging from mild to bloody diarrhea, abdominal cramps, and may result in hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) characterized by acute renal failure, anemia, and lowered platelet counts. The presence of Stx in blood circulation results in renal failure in 5-15% of HUS patients. Stx circulating in blood binds to a glycosphingolipid receptors (Gb3) present in the kidney resulting in inflammatory responses or cell death (Obrig, 2010). In the United States, *E. coli* O157:H7 is estimated to cause 63,153 human foodborne illnesses, 2,138 hospitalizations, and 20 deaths annually (Scallan et al., 2011; Painter et al., 2013). Six other non-O157 STEC serogroups have emerged as leading causes of infection. They are *E. coli* O26, O45, O103, O111, O121, and O145. They cause the same type of disease as *E. coli* O157:H7. (Koohmaraie et al., 2005). The six non-O157 STEC and *E. coli* O157:H7 are collectively referred to as STEC-7 / EHEC-7 in this study. The non-O157 STEC cause a total of 112,752

illnesses and 271 hospitalizations annually in the US (Scallan et al., 2011; Painter et al., 2013). STEC foodborne illness imposes a significant health burden on the US population. The U.S. Department of Agriculture's Economic Research Service (USDA-ERS) estimates that illnesses due to STEC cost the United States more than $1 billion annually (Scharff, 2012; Hoffmann et al., 2015). Additionally, STEC management activities are a significant economic burden, including cost to cattle producers and the beef industry in terms of investment in beef safety research, food safety technology implementation and process validation. Also, significant costs of microbiological testing of products by the beef industry and the USDA-FSIS, and costs associated with frequent lost product sales, product diversion, and product recalls must be considered (BIFSCo, 2011; USDA-FSIS, 2013b).

STEC: Sources, Reservoir, and Transmission

Cattle are the major reservoir of STEC bacteria (Gyles, 2007; Hussein, 2007), and cattle-derived foods, particularly ground beef contaminated with pathogenic strains of STEC, are believed to be a principal source of STEC related human illnesses (Bell et al., 1994; Bettelheim, 2007). Based on outbreak data obtained from 1998 to 2008 by the U.S Centers for Disease Control and Prevention, 2,469 of 4,589 (53.8%) foodborne disease outbreaks were attributable to bacterial agents (Painter et al., 2013). Of the 2,469 bacterial associated outbreaks, 186 (7.5%) and 3 (0.12%) were further attributable to *E. coli* O157 and non-O157 STEC, respectively. Of the 186 outbreaks caused by O157 STEC, 103 (55.3%) were attributable to beef, and 59 (31.7%) were attributable to leafy vegetables. Other implicated food commodities include sprouts, fruit nuts, beans, dairy, and eggs. Of the 6 outbreaks caused by non-O157 STEC, 3 (50.0%) were attributable to beef, and 3 (50.0%) were attributable to fruits, nuts, and leafy vegetables.

Cattle and other ruminants are colonized by STEC in their intestinal tract and they may shed the organism in the feces. Young cattle are most likely to excrete the organism (Cray and Moon, 1995) but importantly, cattle shedding STEC in their feces show no clinical disease (Wray et al., 2000). STEC have also been isolated from other animals including sheep, goats, pigs, deer, and wildlife (Beutin et al., 1996), flies (Ahmad et al., 2007), and manure from cattle farms (Cernicchiaro et al., 2012). Observational and experimental studies showed that cattle intermittently shed STEC in their feces (Besser et al., 1997; Mechie et al., 1997; Sargeant et al., 2000), but may also shed persistently over several months (Widiasih et al., 2004; Hussein and Sakuma, 2005b; Gyles, 2007). Shedding of STEC among cattle varies by production system (LeJeune and Wetzel, 2007), geographic area (LeJeune et al., 2006; Islam et al., 2014), season (Berry and Wells, 2010), age (Wilson et al., 1998), diets (Jacob et al., 2009), stress (Rostagno, 2009), presence of pigs, dogs or wild geese on the farm (Synge et al., 2003; Schouten et al., 2004), and introduction of new animals into the herd (Schouten et al., 2004).

Apart from contaminated and undercooked beef products, other bovine-derived food such as unpasteurized milk (Centers for Disease Control and Prevention, 2007), raw milk cheeses and butter (Rangel et al., 2005), and dry-cured salami (Centers for Disease Control and Prevention, 1995) have been implicated as modes of transmission of STEC to humans. Plant produce such as spinach, lettuces, unpasteurized apple cider and juice, coleslaw, salad, melons, and sprouts have been linked with outbreaks (Rangel et al., 2005). Other STEC-linked human outbreaks have been traced to contaminated drinking water and recreational waters (Bruce et al., 2003). Human contacts with cattle or their manure on farms, at fairs, and at petting zoos, as well as person to person transmission have also resulted in outbreaks (Centers for Disease Control and Prevention, 2005).

STEC in the beef production system

The first association of STEC with human illnesses in the United States was made in 1982 among patients who consumed undercooked hamburgers at a fast-food restaurant chain in Oregon and Michigan (Riley et al., 1983). Since then, several STEC-caused outbreaks have been reported (Sparling, 1998; Brooks et al., 2005; Rangel et al., 2005; Heiman et al., 2015). These illnesses have been traced to both O157 and non-O157 STEC serogroups (Hussein and Bollinger, 2005). Following a large foodborne outbreak, in the fall of 1992 and spring of 1993, caused by the consumption of undercooked hamburgers (Centers for Disease Control and Prevention, 1993), the United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) in 1994 declared *E. coli* O157:H7 as an adulterant in raw ground beef, and in 1999 in all non-intact raw beef products (Federal Register, 1999), and established a zero-tolerance policy for this pathogen in these food products. Similarly, the six selected non-O157 strains were declared adulterants in September 2011 (Federal Register, 2011), and testing for pathogenic Top-7 STEC in beef trimmings of cattle slaughtered on-site was initiated by USDA-FSIS in June 2012.

Contamination of beef carcasses or products with STEC occurs during harvest at the slaughter facility and it is associated with both the prevalence and concentration of the pathogens in cattle feces and on hides (Arthur et al., 2008; Fox et al., 2008). STEC-colonized cattle shed the bacteria in feces and contaminate the production environment (Arthur et al., 2010b). Typically, cattle shed *E. coli* O157:H7 at levels $< 10^2$ CFU/g of feces, some cattle (referred to as high/super shedders) can shed the organism at levels $\geq 10^4$ CFU/g of feces at a single or multiple periods in time (Low et al., 2005; Chase-Topping et al., 2007). There is a large variation in shedding concentration from animal to animal (between-host variability), as well as within a single animal (within-host variability), and both are important drivers of transmission of *E. coli* O157 in cattle

farms (Chen et al., 2013). The high shedders have been estimated to constitute about 9% of cattle positive for *E. coli* O157, but they are estimated to be responsible for > 80% of the *E. coli* O157 shed by cattle (Matthews et al., 2006). Limited data suggest the same might be true for the six non-O157 STEC. In a recent study, Shridhar et al. (2017) reported that 15.5% of culture positive samples contain at least one or more of the six non-O157 serogroups at levels $\geq 10^4$ CFU/g of feces by spiral plating method. A high load of STEC in the environment can contaminate cattle hides which in turn can serve as the major source of carcass contamination during slaughter and dressing of cattle at abattoirs (McEvoy et al., 2000; Fox et al., 2008; Jacob et al., 2010). Arthur et al. (2007a) reported a marked increase in the prevalence of *E. coli* O157:H7 on cattle hides and the number of cattle having >0.4 CFU/cm^2 of pathogen on their hides from the time cattle were loaded at the feedlot to the time hides were removed in the processing plant. Arthur et al. (2008) highlighted the significance of bacteria present in the lairage environments of U.S beef processing plants to the overall contamination of hides and beef carcasses during processing. Their study indicated that the transfer of bacteria onto cattle hides that occur in the lairage environment accounts for a larger percentage of the hide and carcass contamination than does the initial bacterial population found on the cattle leaving the feedlot. Jacob et al. (2010) from a study evaluating association among fecal, hide, and pre-evisceration carcass prevalence of *E. coli* O157:H7 reported significant correlation between fecal shedding and hide prevalence ($R^2 = 0.55$), and between hide prevalence and carcass contamination prevalence ($R^2 = 0.70$). The presence of a high shedder within the cattle cohort, high within-cohort fecal and hides prevalence, and having a positive hide were significantly associated with the probability of detecting *E. coli* O157:H7 on a pre-evisceration beef carcass.

Prevalence of STEC in cattle feces

Based on available literature, there is high variability in the prevalence estimates of O157 and non-O157 STEC in cattle, ranging from 0 to > 90% of animals and from 0 to 100% of herds (Sargeant et al., 2003; Gyles, 2007). Some of the variations in prevalence estimates of *E. coli* O157:H7 have been attributed to factors such as world region and country where cattle are located, type of cattle, type of specimen, detection methods, and health status of animals (Islam et al., 2014). In the U.S, fecal prevalence of O157 and the six non-O157 STEC varies by season and cattle type (Barkocy-Gallagher et al., 2003; Hussein, 2007; Dewsbury et al., 2015). Fecal prevalence of *E. coli* O157:H7 is usually greater in the summer than in winter months (Barkocy-Gallagher et al., 2003; Omisakin et al., 2003); non-O157 STEC were found in more fecal samples in the fall and spring than in the summer and winter (Barkocy-Gallagher et al., 2003). Among dairy cows, fecal prevalence ranged from 0.2 to 48.8% for EHEC O157:H7 and 0.4 to 74.0% for non-O157 STEC (Hussein and Sakuma, 2005a). Fecal prevalence of *E. coli* O157:H7 within a pen or group of feedlot cattle has been observed to vary widely from 0% to > 90% among pens and over time (Sargeant et al., 2003; Renter et al., 2008). Dargatz et al. (2013) using a polymerase chain reaction assay (PCR) on 1145 fecal pools collected from July to September in 21 feedlots from four U.S states, reported O-serogroup prevalence of 19.7%, 13.8%, 9.9%, 9.3%, 5.5%, 1.1%, and 0.5% for O157, O45, O26, O103, O121, O145, and O111, respectively.

In a recent study conducted in a large commercial feedlot during summer and winter months in the U.S, Dewsbury et al. (2015) reported a 0 to 79.2%, 0 to 8.3%, 0 to 12.5%, and 0 to 12.5% pen-level fecal prevalence for STEC O157, O26, O103, and O145, respectively, but 0% prevalence for STEC O45, O111, and O121 during summer months, and likewise, 0% fecal prevalence for all seven STEC during the winter months. Cull et al. (2017) reported 0 to 12.5%, 0 to 6.5%, 0 to 68.7%, 0 to

33.3%, 0 to 41.7%, and 0 to 83.3% within pen-level fecal prevalence for EHEC O26, O45, O103, O111, O145, and O157, respectively, and 0% for O121, from a study conducted in eight commercial feedlots in two major U.S cattle feeding areas during the summer months.

Estimates of fecal prevalence vary by cattle types, season, detection method, and world region (Islam et al., 2014; Ekong et al., 2015). Likewise, it is important to note the case definition used in different prevalence estimation studies as the case definitions (O-serogroup vs. STEC/EHEC) have different implications for food safety risk assessment. Apart from the prevalence of STEC in cattle feces, the level or concentration of shedding of STEC by individual infected cattle is of much greater importance to food safety risk than the prevalence rate (Omisakin et al., 2003).

Concentration of STEC in cattle feces

The concentration at which *E. coli* O157 and non-O157 STEC are shed in cattle feces varies from animal to animal (Omisakin et al., 2003; Farrokh et al., 2013). Cattle can shed *E. coli* O157:H7 in their feces at concentrations ranging from 10^2 to 10^6 CFU/g, or occasionally at levels exceeding 10^6 CFU/g (Robinson et al., 2004a), however, the majority shed at a low level ($<10^2$ CFU/g), representing the minimum detection limit of the enumeration assay (Pearce et al., 2004). Some cattle transiently shed STEC at levels $>10^4$ CFU/g of feces and have been reported to have significant impact on the persistence of these pathogens in feedlot environments, transmission among cattle within the pen (Matthews et al., 2006), and subsequent hide and carcass contamination (Arthur et al., 2007a; Fox et al., 2008; Arthur et al., 2009).

Fecal enumeration studies of the concentration of *E. coli* O157:H7 in feedlot cattle in the United States during summer months revealed *E. coli* O157:H7 shedding levels of 10^2 to $<10^7$ CFU/g of feces (Arthur et al., 2007a; 2008; 2011), and $<10^2$ to $<10^{9.3}$ CFU/g of feces among Canadian cattle during summer and winter (Stephens et al., 2009). In a recent study, Shridhar et al.

(2016) using multiplex quantitative PCR (mqPCR) assays found the concentrations of *E. coli* serogroups O26, O45, O103, O111, O121, O145, and O157 to range from 10^4 to 10^7 CFU/g of feces. The six non-O157 serogroups had concentrations from 10^4 to $<10^6$ CFU/g, and the O157 serogroups had concentrations from 10^4 to $<10^7$ CFU/g of feces. In another study, Shridhar et al. (2017) recorded concentrations ranging from 10^3 to $<10^6$ CFU/g for O26, O45, and O145 serogroups and 10^3 to $<10^7$ CFU/g for O103 and O121 using a spiral plating method. However, quantification of the same samples using the mqPCR method revealed a concentration ranging from 10^4 to $<10^7$ CFU/g for all serogroups except O111 and O121 which had concentrations ranging from 10^4 to $<10^6$ CFU/g. Based on the spiral plating method, approximately 16% of culture positive samples had concentration values $\geq 10^4$ CFU/g (high shedders) for at least one of the six non-O157 serogroups. Conversely, based on the mqPCR method, approximately 32% of PCR positive samples were high shedders for at least one of the six non-O157 serogroups. The two quantification methods differ in sensitivity, consistent with the findings for *E coli* O157 (Jacob et al., 2014). The spiral plating method, however, can be used to quantify pathogenic EHEC, whereas quantification with the mqPCR method is at the serogroup level (Shridhar et al., 2017).

Prevalence of STEC on cattle hides

Cattle hides are the primary source of beef carcass contamination with STEC during cattle processing at slaughter (Van Donkersgoed et al., 2001; Barkocy-Gallagher et al., 2003; Arthur et al., 2004; Bosilevac et al., 2005; Arthur et al., 2008; Moxley and Acuff, 2014). The hide becomes contaminated through pathogen transmission at the feedlot, during transport, and in the lairage environment at the processing plant (Arthur et al., 2007c; 2011). Data suggest that the survival of *E. coli* O157:H7 on cattle hides ranges from 7-9 days (Arthur et al., 2011). As with fecal prevalence,

available evidence suggests that the prevalence of *E. coli* O157:H7 and non-O157 STEC on cattle hides is higher during summer than winter months (Barkocy-Gallagher et al., 2003).

The prevalence for *E. coli* O157:H7 in North American beef cattle hide samples was reported to range from 9 to 85% (Arthur et al., 2007c). STEC prevalence on cattle hides has been shown to increase on lots of cattle between load-out at the feedlot and the beginning of processing after lairage. Arthur et al. (2007a) recorded an increase in hide prevalence of *E. coli* O157:H7 from 50.3% to 94.4% between when cattle were loaded on transport trailers at the feedlot to hide removal in the processing plant. Similarly, using pulse field gel electrophoresis, Arthur et al. (2008) confirmed the transfer of *E. coli* O157:H7 from the lairage environment area onto cattle hides and concluded that the lairage-derived pathogens account for a larger proportion of hide and carcass contamination than the STEC population on the cattle hides when leaving the feedlot.

In a recent study conducted in a large commercial feedlot in the central U.S during summer months, Stromberg et al. (2015a) using the PCR/mass spectrometry-based NeoSEEK^{TM} STEC detection and identification test (NeoSEEK) reported 49.9%, 37.1%, 12.5%, 11.0%, 2.2%, 2.0%, and 0.2% prevalence for EHEC O145, O45, O103, O157, O111, O121, and O26, respectively on hide samples from fed cattle at harvest. Similarly, using NeoSEEK, Stromberg et al. (2016) reported 27.1%, 18.6%, 12.0%, 10.0%, 1.5%, 6.0%, and 1.7% prevalence for EHEC O45, O145, O111, O103, O26, O157, and O121, respectively on hide samples from culled dairy cows at harvest.

Concentration of STEC on cattle hides

Similar to the hide prevalence studies, several studies have reported the concentration of *E. coli* O157:H7 on cattle hide samples collected during cattle load-out at the feedlot and during harvest at the processing plant (Arthur et al., 2004; 2007a; 2007b; Brichta-Harhay et al., 2007; Arthur et al., 2008; Brichta-Harhay et al., 2008; Bosilevac et al., 2009; Arthur et al., 2011). From

these studies, concentration of *E. coli* O157:H7 on cattle hides ranges from 10^1 to 10^4 CFU/100cm^2. Brichta-Harhay et al. (2007) observed a median count of 8.0 x 10^1 CFU/100cm^2 and a maximum count of 9.8 x 10^3 CFU/100cm^2 for *E. coli* O157:H7 on hides of cattle at beef-processing plants.

In a study conducted in four cull cattle plants, Brichta-Harhay et al. (2008) observed a median count of 8.0 x10^1, minimum count of 4.0 x 10^1, and maximum of 9.8 x 10^3 CFU/100cm^2 for *E. coli* O157:H7 on hides during the summer, and median, minimum, and maximum of 4.0 x 10^1, 4.0 x 10^1, and 7.2 x 10^2 CFU/100cm^2, respectively during winter. The proportion of hide samples that carried *E. coli* O157:H7 at enumerable levels varied significantly between plants and was associated with the prevalence of *E. coli* O157:H7 on the hides of cattle sampled at the farm before load-out, hide contamination during transportation to the plants, and contamination in the lairage environment.

Comparable hide concentrations of *E. coli* O157:H7 were observed among large and small beef processing plants. In a study conducted at a U.S abattoirs processing fewer than 1,000 head of cattle per day, Bosilevac et al. (2009) observed median, minimum, and maximum counts of 8.4 x 10^1, 4.0 x 10^1, and 4.0 x 10^3 CFU/100cm^2, respectively. In a recent study conducted by Stromberg et al. (2016) to quantify the level of EHEC-7 on hides of cull cows at harvest, only one hide sample was enumerable at 3.7 log CFU/100cm^2; none of the non-O157 EHEC were enumerable by spiral plating.

Prevalence of STEC on beef carcasses

The primary source of contamination of beef carcasses during processing is the hides of animals (Elder et al., 2000; Barkocy-Gallagher et al., 2001; 2003; Arthur et al., 2007b). Direct contact between carcass and hide during the skinning process may result in the transfer of pathogens onto carcass. Pathogens may also be transferred by direct contact with contaminated equipment, surfaces and hands of personnel (Thomas et al., 2012). Although a rare event, rupturing of the

intestinal tract during the evisceration process may also result in carcass contamination (Bell, 1997). Significant plant-to-plant variations in levels and prevalence of pathogens on carcasses were reported from several studies (Arthur et al., 2004; 2008; Brichta-Harhay et al., 2008; Bosilevac et al., 2009), and the levels of contamination found to be associated with the incoming load of *E. coli* O157:H7, as well as *Salmonella*, on the hide. In-plant interventions, such as antimicrobial washes, are applied at different processing stages to reduce microbial contamination on carcass surfaces (Arthur et al., 2004).

The prevalence of *E. coli* O157:H7 on pre-evisceration carcasses from four cull cattle processing plants in the U.S was reported to range from 7 to 42% (Brichta-Harhay et al., 2008). Bosilevac et al. (2009) reported a 0 to 93% range for pre-evisceration carcass prevalence of *E. coli* O157:H7 recorded over multiple days and at seven different small processing plants in the U.S. Barkocy-Gallagher et al. (2003) from a study conducted at three large beef processing plants in the Midwestern U.S reported a significant drop in overall prevalence of *E. coli* O157:H7 from 26.7% on pre-evisceration carcasses to 1.2% on post-intervention carcasses (carcasses that had received complete antimicrobial interventions), suggesting the effectiveness of the interventions to reduce pathogens on carcass.

Based on the recent study by Stromberg et al. (2015a) in feedlot cattle, the prevalence of EHEC O157, O145, O103, O45, O26, O111, and O121 on pre-intervention carcass surface samples was estimated as 2.8%, 1.6%, 1.2%, 1.1%, 0.2%, 0.0%, and 0.0%, respectively using the NeoSEEK test method. In a similar study conducted in a plant processing culled dairy cows, the prevalence of EHEC O103, O145, O26, and O157 on pre-intervention carcass surface samples was estimated as 4.0%, 2.0%, 1.4%, and 1.0%, respectively. EHEC O45, O111, and O121 were not detected on pre-intervention carcass surfaces (Stromberg et al., 2016).

Concentration of STEC on beef carcasses

Several studies have reported on the concentration of *E. coli* O157:H7 on carcass samples collected during harvest at the processing plant (Arthur et al., 2004; 2007a; 2008; Brichta-Harhay et al., 2008; Bosilevac et al., 2009). From the studies, concentration of *E. coli* O157:H7 ranged from 0.5×10^0 to 10^2 CFU/100cm^2 on pre-evisceration carcasses. Brichta-Harhay et al. (2007) observed a median count of 1.6×10^0 CFU/100cm^2 and a maximum count of 4.6×10^1 CFU/100cm^2 on pre-evisceration carcass at beef-processing plants. In another study, Brichta-Harhay et al. (2008) reported a significantly lower concentration for *E. coli* O157:H7 on pre-evisceration carcasses during spring (median count = 0.8, minimum = 0.8, maximum = 5.6 CFU/100cm^2) compared to winter (median count = 1.6, minimum = 0.8, maximum = 45.6 CFU/100cm^2). Significant variation in pre-evisceration carcass concentration of *E. coli* O157:H7 across plants was also recorded. In a 2004 study, Arthur et al. (2004) observed that post-evisceration and post-intervention carcasses sampled during six visits at two commercial fed beef processing plants only harbored *E. coli* O157 at levels below the detection limit (<1.5 MPN/100cm^2) of the assay used. In the Stromberg et al. (2016) study, no EHEC-7 were quantifiable from the pre-intervention carcass samples. Available data suggested the presence of *E. coli* O157:H7 on pre-evisceration carcass during harvest is mainly due to contamination during hide removal (Koohmaraie et al., 2005; 2007; Brichta-Harhay et al., 2008). The prevalence and concentration varies by season and from plant to plant and drops significantly as interventions are applied (Brichta-Harhay et al., 2008; Bosilevac et al., 2009).

Methods for detection and quantification of STEC in the beef production system

STEC are genetically and biochemically diverse and their detection from different sample matrices can be challenging (Stromberg et al., 2015b). Many approaches have been developed to identify and quantify STEC in food and environmental samples. Traditional culture based methods

explore the specific biochemical characteristics of each serogroup. They involve an enrichment step, followed by immunomagnetic bead separation (IMS), and plating on a selective medium. Immunological detection methods use monoclonal or polyclonal antibodies to capture the target. Nucleic acid based detection methods detect DNA or RNA targets using complementary labeled nucleic acid probes.

Culture-based methods

Culture-based methods used to detect O157 and non-O157 STEC/EHEC in cattle feces, hides, and carcasses commonly include selective enrichment, IMS, and plating onto selective and differential media that contain antibiotics or chromogenic substrates. Modified Trypticase soy broth (mTSB) and *E. coli* (EC) broth are commonly used for enrichment in the detection of these STECs in all sample matrixes. Isolates obtained from the culture media are confirmed by biochemical tests, antigen agglutination, and/or PCR detection of virulence genes (Elder et al., 2000; Bai et al., 2010; Jacob et al., 2014). For the non-O157 STEC, the enriched suspensions are subjected to IMS procedures with serogroup-specific IMS beads, then spread-plated onto Possé differential agar for detection in fecal samples (Noll et al., 2015a; 2016), hides and carcass samples (Stromberg et al., 2015a). For the detection of O157 and non-O157 STEC in beef trim and other ground beef components, the FSIS (USDA-FSIS, 2013a) proposed a two-stage PCR screening test on post-enrichment samples, followed by IMS and inoculation onto Rainbow agar, with confirmation by latex agglutination and biochemical tests.

Culture methods used to quantify *E. coli* O157 in feces include the most-probable-number (MPN) dilution technique (Arthur et al., 2004; Fegan et al., 2004), spiral plating on selective or chromogenic media (Robinson et al., 2004b; Fox et al., 2007), and a technique of directly plating ground beef, cattle carcass, hide or fecal samples onto a selective medium (Brichta-Harhay et al.,

2007; Sanderson et al., 2007; Jacob et al., 2010). The culture methods are usually inexpensive, simple, and highly specific; however, they are low in sensitivity, time consuming and laborious.

Immunological-based methods

The detection of STEC by immunological-based methods is based on antibody-antigen reactions (Law et al., 2014). *E. coli* O157 antigen present in the test sample binds to specific antibody in the detection assay to form an antigen-antibody complex evident by color change in the conjugate or particle agglutination. Several immunoassays have been developed and used for the detection of foodborne pathogens including *E. coli* O157:H7 and non-O157 STEC in ground beef, cattle feces and hides. These assays include the enzyme-linked immunosorbent assay (ELISA) (Thompson et al., 2007; Hegde et al., 2012a; Shen et al., 2014), polymyxin-ELISA (Blais et al., 2005; Blais et al., 2006), lateral flow immunoassay (Ching et al., 2015; Wang et al., 2016), microplate enzyme immunoassay (EIA) (Hoefer et al., 2011), and flow cytometry (Flanagan and Martinez, 2010; Hegde et al., 2012b) for the detection of top six non-O157 STEC O groups in ground beef. Others include fluorescence polarization assay (Nielsen et al., 2007; Aydin et al., 2014), immunochromatography assay (Qi et al., 2011), latex agglutination (March and Ratnam, 1989; Medina et al., 2012), and optical immunoassay (Wang et al., 2013). These assays are generally less sensitive than traditional cultural techniques; however they offer advantages in terms of rapidity, reduced labor costs and high volume throughput (Chapman et al., 2001).

Nucleic acid-based methods

Several nucleic acid-based methods have been developed for the detection and characterization of STEC. The methods use polymerase chain reaction (PCR) to amplify specific gene targets in STEC (Hara-Kudo et al., 2000). The recent nucleic acid-based methods described are

conventional PCR (Arthur et al., 2005; Islam et al., 2006; Jacob et al., 2014), multiplex PCR (mPCR) (Stefan et al., 2007; Wang et al., 2007; Bai et al., 2012; Jacob et al., 2014), real-time / quantitative PCR (qPCR) (Fu et al., 2005; Jacob et al., 2012; Conrad et al., 2014; Jacob et al., 2014; Luedtke et al., 2014; Verstraete et al., 2014), loop-mediated isothermal amplification (LAMP) (Dong et al., 2014), and NeoSEEK^{TM} detection and identification test, a PCR/mass spectrometry-based method (Stromberg et al., 2015a; 2016). In two recent studies, mqPCR assays were developed and validated for quantification of STEC O157 (Noll et al., 2015b), and *E. coli* O26, O45, O103, O111, O121, and O145 serogroups and three virulence genes (*stx*$_1$, *stx*$_2$, *eae*) in preenriched cattle feces (Shridhar et al., 2016). The nucleic acid-based methods have high analytical sensitivity and specificity, detect multiple pathogens, have faster turnaround time, high throughput capacity, and produced reliable results. However, the method is affected by PCR inhibitors, requires DNA purification, and can be difficult to differentiate between viable and non-viable cells (Law et al., 2014).

Evidence from published literature revealed well established diagnostic methods for detection of STEC O157 and recently the non-O157 STEC in food matrices. Combinations of methods have also been used to improve the sensitivity of detection. However, none of these methods constitute a reference standard for the detection of STEC in feces, hide or beef products. Further research is needed on evaluating the diagnostic sensitivity and specificity of the detection methods in order to determine the accuracy of these methods in quantifying the true prevalence of STEC in food matrices and avoiding misclassification bias.

Control of STEC in animal food products

Successful control and /or reduction of human exposures and resultant illnesses due to STEC requires the implementation of pathogen-reduction interventions in live cattle (pre-harvest) to reduce

the level of STEC entering the food supply (Loneragan and Brashears, 2005; Callaway et al., 2013a), as well as in-plant (post-harvest) intervention strategies during cattle slaughter, dressing and processing to reduce carcass contamination and levels of STEC and other pathogens on beef products (Koohmaraie et al., 2005; Moxley and Acuff, 2014; Wheeler et al., 2014). Implementing effective pre-harvest controls would help reduce pathogen loads on hides of cattle entering processing plants and make in-plant interventions more effective, reduce animal-to-animal cross contamination that occurs during transport and lairage, and reduce potential environmental contamination and risk to persons in direct contact with animals such as through petting zoo and open farms (Callaway et al., 2013b; Wheeler et al., 2014). Implementation of good on-farm animal-health management practices, such as maintenance of clean cattle feed and water, a well maintained environment and appropriate biosecurity have been described as important prerequisites for successful pre-harvest interventions (Wheeler et al., 2014).

Pre-harvest interventions

Pre-harvest intervention is the first control step proposed for the reduction of food safety risks in the integrated beef production system (USDA-FSIS, 2014). Pre-harvest interventions used for the reduction of STEC shedding in cattle can be classified into three types: (i) exposure reduction strategies, (ii) exclusion reduction strategies, and (iii) direct anti-pathogen strategies. Exposure reduction strategies are targeted at limiting exposure of cattle to various sources of contamination in the environment, thereby lowering the prevalence of STEC in live animals (USDA-FSIS, 2014). This involves practices such as provision of clean water and feed, appropriately drained and cleaned environment, proper housing, adequate biosecurity (Loneragan and Brashears, 2005), and good transportation to reduce cross-contamination among animals (Stanford et al., 2011). Exclusion reduction strategies are targeted at modifying the gut microflora of cattle in favor of bacteria less

harmful to humans (USDA-FSIS, 2014). This involves provision of treated water (Callaway et al., 2002), fasting of cattle before and during transportation (Buchko et al., 2000), inclusion of feed additives and probiotics (Younts-Dahl et al., 2005; Stephens et al., 2007b), feed type and feeding strategies (Callaway et al., 2009) which may decrease STEC counts in cattle feces. The direct anti-pathogen strategies target and kill the STEC in and on cattle (USDA-FSIS, 2014). This involves practices such as cattle hide washing prior to slaughter (Arthur et al., 2011; Schmidt et al., 2012), use of bacteriophages (Sheng et al., 2006; Garcia et al., 2008), feeding of competitive exclusion products (Brashears et al., 2003b), and vaccination (Potter et al., 2004; Snedeker et al., 2012). These strategies are effective for reducing STEC shedding in feces and their levels on cattle hides and beef carcasses in the production environment.

Direct-fed microbials

Direct-fed microbials (DFM) are widely used in the livestock industry to improve ruminal fermentation and enhance production efficiency of meat and milk (Martin and Nisbet, 1992). In recent years, they have been used to reduce foodborne pathogens in live cattle. They include probiotics and competitive exclusion cultures, mainly made up of yeast, fungal or bacterial cultures or end-products of fermentation (Callaway et al., 2013a) with the potential to decrease pathogen carriage on the hides and in the feces of livestock. The mechanisms of action of the DFM products may include alterations to the intestinal microbiome, competition for colonization sites, enhancement of intestinal efficiency, and stimulation of the host innate immune response (Buntyn et al., 2016).

Numerous DFM intervention studies comprising different culture products, doses, duration and timing of the intervention are reported in the literature. These studies reported variable efficacy of DFM to reduce *E. coli* O157:H7 shedding in cattle. In a *Lactobacillus*-based DFM study, the fecal

prevalence of *E. coli* O157:H7 was reduced by more than 50% in individual cattle during the feeding period in addition to a significant decrease in number of *E. coli* O157:H7 positive hide samples at harvest (Brashears et al., 2003a). In a 2007 study, Stephens et al. (2007a) fed various strains and dosages of *Lactobacillus*-based DFM to feedlot cattle throughout the feeding period and observed a reduction in prevalence and concentration of *E. coli* O157 among specific strains (NP51 and NP28) or combination of strains (NP51 with NP35) compared with controls, and a 1 to 2-log reduction in concentration as determined using the MPN technique. In a similar study, Stephens et al. (2007b) evaluated the effectiveness of DFM comprising different doses of *Lactobacillus acidophilus* in combination with *Propionibacterium freudenreichii* and reported up to 74% reduction in *E. coli* O157:H7 fecal shedding among treated groups with no significant dosing effect.

A study by Arthur et al. (2010a) evaluated the efficacy of a *Bacillus*-based DFM fed to feedlot cattle and found no significant differences in *E. coli* O157:H7 fecal or hide prevalence and concentration between treatment and control groups. Cull et al. (2012) found no evidence of effect of a low dose *Lactobacillus*-based DFM (Bovamine®) on *E. coli* O157:H7 shedding in feedlot cattle. Additionally, the Bovamine®, though formulated for *E. coli* O157:H7, had no significant effects on fecal prevalence of the six non-O157 STEC serogroups in the feedlot (Cernicchiaro et al., 2014; Paddock et al., 2014).

In a recent systematic review and meta-analysis (SR-MA) study of the use of DFM to reduce fecal shedding of *E. coli* O157 in beef cattle, Wisener et al. (2015) observed that the combination of *L. acidophilus* (NP51) and *P. freudenreichii* (NP24) DFM was more efficacious at reducing the fecal prevalence of *E. coli* O157 in cattle at the time of harvest and throughout the trial period. The summary effect size of DFM (measured as fecal prevalence) based on 26 comparisons as measured at the end of the trial, and based on 23 comparisons as measured through-out the trials was

protective; odds ratio = 0.46 (95% CI: 0.36–0.60) and 0.55 (95% CI: 0.45–0.68), respectively. A recent study (Luedtke et al., 2016) compared the use of two different doses of a commercially available DFM (Bovamine®: 10^9 *L. acidophilus* + 10^6 *P. freudenreichii* CFUs/head/day and Bovamine Defend®: 10^9 *L. acidophilus* + 10^9 *P. freudenreichii* CFUs/head/day) on total EHEC load based on a PCR assay targeting *ecf1* gene in a commercial feedlot. No significant difference in the fecal concentration of total EHEC was observed between the two doses suggesting that the increased dosage provided no additional reduction. According to the USDA-National Animal Health Monitoring System's (NAHMS) Feedlot 2011 study, 28.5% of U.S. feedlots with a capacity of 1,000 or more head of cattle incorporate probiotics in cattle rations, overall, 53.8% of cattle receive probiotic in their diet (USDA-NAHMS, 2013b).

Vaccination

Cattle vaccination is a viable pre-harvest intervention for the reduction of public health risk associated with *E. coli* O157:H7. Vaccine confers immunity against *E. coli* O157:H7 by reducing colorectal colonization and duration of carriage in cattle, minimizes shedding of this pathogen into the farm environment, and reduces hide contamination (Smith, 2014). Two *E. coli* O157:H7 vaccines have been used in the livestock industry for reduction of fecal shedding. A Siderophore Receptor and Porin (SRP) protein vaccine (Epitopix SRP®), conditionally approved in the U.S, targets the bacterial cell membrane proteins (siderophore proteins) and disrupts iron transport into the bacterium leading to cell death (Callaway et al., 2013a; Wheeler et al., 2014). The second is *E. coli* O157 bacterial extract vaccine (type III secreted proteins [TTSP]) licensed in Canada as Econiche®. This vaccine confers protection by blocking the protein that allows *E. coli* to colonize the terminal rectum of the bovine intestine (Potter et al., 2004; Peterson et al., 2007; Martorelli et al., 2017). Two or three doses of the vaccine are administered to cattle at the feedlot. Results from

several studies indicated that these vaccines reduced *E. coli* O157:H7 shedding in feedlot cattle (Potter et al., 2004; Fox et al., 2009; Smith et al., 2009a; 2009b; Thomson et al., 2009; Cull et al., 2012).

In a feedlot study, vaccination of cattle with SPR vaccine reduced the fecal prevalence of *E. coli* O157 by nearly 50%, significantly reduced the number of days cattle tested culture positive for *E. coli* O157, and the number of days cattle were identified as high-shedders (Fox et al., 2009). Similarly, using the SPR vaccine, Cull et al. (2012) reported a significant reduction in fecal prevalence of *E. coli* O157:H7 and prevalence of high shedders in a commercial feedlot with observed vaccine efficacy of 53.0% and 77.3%, respectively. In a large-scale clinical vaccine trial conducted in 19 commercial feedlots across Nebraska using a two-dose regimen of the TTSP vaccine, Smith et al. (2009b) reported reduced rectal colonization by *E. coli* O157:H7, with vaccinated cattle being 92% less likely to be colonized with *E. coli* O157:H7 compared to non-vaccinated cattle. In a similar study, Smith et al. (2009a) reported a 52% to 63% vaccine efficacy for fecal shedding of *E. coli* O157:H7 and 55% efficacy for hide contamination among vaccinated fed cattle compared to non-vaccinated cattle.

In a recent SR-MA study of the use of vaccine treatment to reduce fecal shedding of *E. coli* O157 in beef cattle, Snedeker et al. (2012) concluded that both TTSP and SPR protein vaccines significantly reduce fecal prevalence of *E. coli* O157 in cattle. The summary effect size of TTSP vaccine based on 8 comparisons and the SPR protein vaccine based on 4 comparisons was protective; odds ratio = 0.38 (95% CI: 0.29–0.51) and 0.42 (95% CI: 0.20–0.61), respectively. In a different systematic review and meta-analysis study of vaccine treatment, Varela et al. (2013) demonstrated the efficacy (pre-harvest and at-harvest) of the two commercial vaccines combined (TTSP and SPR) for all dose regimens combined (OR = 0.43; 95% CI: 0.35–0.53) and two-dose

regimens (OR = 0.52; 95% CI: 0.44–0.61) to reduce the prevalence of *E. coli* O157:H7 under field conditions. Using simulation modeling, Vogstad et al. (2014) defined efficacy of a three dose regimen of TTSP vaccine with a log normal distribution (μ=0.58, SE=0.13) and predicted a decrease in average summer-fed pen level prevalence of STEC O157 from 30% (range: 0% - 80%) to 13% (range: 0% - 52%) indicating a decrease in mean pen prevalence, a reduced variability of STEC O157 fecal pen shedding, and a reduction of the highest prevalence pens due to vaccine intervention.

In a 2009 study that quantified the efficacy of the SRP protein-based vaccine on the prevalence and concentration of *E. coli* O157:H7 in two commercial feedlots, Thomson et al. (2009) reported a 49.7% reduction in prevalence of *E. coli* O157:H7 among vaccinated cattle compared to control cattle over the study period, and a 98.2% reduction in concentration of *E. coli* O157 in feces of SRP vaccinated cattle, equivalent to a 1.7 mean log reduction in concentration (0.80 ± 0.56 $\log_{10}$ MPN per gram in vaccinated group and 2.53 ± 0.24 $\log_{10}$ MPN per gram in the placebo group). Contrary to the efficacy of the *E. coli* SPR vaccine in reducing STEC O157 prevalence and concentration, the vaccine produced no significant effects on fecal prevalence of the six non-O157 STEC serogroups in feedlot cattle (Cernicchiaro et al., 2014; Paddock et al., 2014), suggesting the vaccine may not provide cross-protection against the non-O157 STEC.

It has been estimated that vaccination of cattle would reduce human illnesses due to O157 STEC in ground beef by 60 - 85% reduction (Hurd and Malladi, 2012; Matthews et al., 2013); however, despite this potential, adoption of *E.coli* vaccination by cattle producers is very limited (Callaway et al., 2013a; Matthews et al., 2013). According to the USDA-National Animal Health Monitoring System's (NAHMS) Feedlot 2011 study, only 2.4% of U.S. feedlots with a capacity of 1,000 or more head of cattle are using the *E. coli* O157 vaccine on at least some cattle; overall, only 0.1% of cattle receive *E. coli* O157 vaccination (USDA-NAHMS, 2013a). The low adoption rate of

E. coli vaccination by cattle producers has been attributed to risk of animal injury and impacts on production as a result of handling cattle for the recommended three-dose vaccine regimen. Additional factors include added cost for the vaccine and the labor to administer it (USDA-NAHMS, 2013a), given that cattle health and production efficiency are not adversely affected by presence of *E. coli* O157 and there is no economic compensation to the producer for vaccinating cattle against *E. coli* O157. Tonsor and Schroeder (2015) estimated 1-1.8 billion dollars in welfare losses over 10 years to cattle producers if they adopt *E. coli* O157 vaccination but proposed a 2-3% increase in domestic consumer demand for beef, 18-33% increase in export wholesale market, 2-4% reduction in retailer cost, or 1-2% reduction in processor cost as sufficient economic incentives for cattle producers to adopt *E. coli* O157 vaccination. Pre-harvest controls in cattle hold enormous potential to reduce spread of STEC on farms, in the environment, and entering the food supply. However, pre-harvest controls must be implemented along with in-plant interventions in a multiple-hurdle approach, in order to maximize reduction of pathogens in the food supply (Callaway et al., 2013b). Research into new intervention strategies with the potential to further reduce or eliminate food borne pathogens from the beef system will go a long way.

Quantitative microbial risk assessment of STEC in beef production

Quantitative microbial risk assessment (QMRA) has been described as a valuable tool and is increasingly used in the management of microbial food safety risks and establishment of standards for food in international trade (FAO/WHO, 2014). QMRA provides scientific evidence for the development of regulatory impact assessment of food safety risk to inform policy decisions. QMRA follows a structured and systematic process that includes hazard identification, exposure assessment, hazard characterization, and risk characterization, and considers the whole “production-to-consumption” food pathway.

Hazard identification identifies known or potential biological pathogens capable of causing adverse effects (illness or death) on the public health and which might be present in a particular food or group of foods. Exposure assessment is a quantitative estimation of the presence and number of pathogens at key points in the food chain – production, slaughter, food preparation and storage, and estimates of amount of contaminant in a single serving. Hazard characterization estimates the relationship between the exposure level (dose) and the frequency of adverse health effects (response). The dose-response relationship is used to estimate the number of pathogens required to cause illness and the susceptibility of different populations. Risk characterization combines the outputs of exposure assessment with outputs of hazard characterization to estimate the likelihood of adverse health effects on given population groups as a consequence of exposure to the pathogen, as well as estimates of the uncertainty associated with the predicted risk values. This may be calculated as an annual risk of illness or a prediction of illness per typical serving of food (USDA-FSIS, 2001; Duffy et al., 2006).

Quantitative risk assessment should distinguish between variability and uncertainty in input parameters since ecological systems are both highly variable and our knowledge of model input parameters is uncertain (Regan et al., 2003). Variability represents a true heterogeneity of the population. It can occur because a quantity fluctuates over time, location or within a group, and it is not reducible by further study. It is a quantitative measure of the range or spread of a given population parameter. In exposure risk assessment, variability is classified as temporal, spatial, intra-individual, and inter-individual (US-EPA, 2011). Temporal variability refers to differences over time, for example, seasonal differences in *E. coli* O157 fecal prevalence among fed cattle. Spatial variability refers to differences due to location, for example, environmental conditions such as soil temperature, rainfall, and humidity may directly affect *E. coli* O157 survival. Intra-individual

variability refers to differences in individual physiologic or behavioral characteristics over time or space, for example, differences in *E. coli* O157 fecal concentration within individual cattle over time. Inter-individual variability refers to differences across individuals or subpopulations, for example, variation in fecal prevalence of *E. coli* O157 among different cattle types or between production systems. Variability in exposure parameters may be defined as standard deviation, variance, percentile, range or probability distributions with defined parameter estimates and confidence intervals from which random samples are drawn to represent variability across the study population (US-EPA, 2011).

Uncertainty in exposure assessment represents a lack of perfect knowledge about the true value of one or more components of the system under assessment. It may be reduced by collecting additional data or conducting further study (Cummins et al., 2008). Uncertainty in exposure assessment is broadly categorized as parameter uncertainty, scenario uncertainty, and model uncertainty (Ozkaynak et al., 2008). Sources of parameter uncertainty include random error relating to imperfections in measurement techniques, systematic error relating to biases in measurement, use of surrogate data for a parameter due to lack of data for the parameter of interest, and non-representativeness with regards to specified criteria (US-EPA, 2011). Sources of scenario uncertainty include errors in information used in formulating exposure pathways and incomplete specification of exposure scenarios. Sources of model uncertainty includes oversimplified representation of the system of interest, exclusion of relevant variables, failure to account for correlation between variables, and incorrectly specified causal pathway (US-EPA, 2011). Guiding principles for reducing uncertainty in exposure assessment includes collecting new data using unbiased study design, identifying the appropriate target population or a more direct measurement

method, using models to estimate missing values, using default assumptions, using surrogate data, narrowing the scope of the assessment, and obtaining expert opinion (US-EPA, 2011).

Variability and uncertainty should be treated separately in risk assessment (Pouillot and Delignette-Muller, 2010). The separation allows for the estimation of uncertainty bounds on the risk estimate (Cassin et al., 1998), as well as provides greater accountability and transparency for key elements of the model, and clarifies communication of results to decision-makers (Cummins et al., 2008; Pouillot and Delignette-Muller, 2010). Risk assessors use a probabilistic second-order Monte Carlo modeling approach to separately classify variability and uncertainty in model inputs and parameters. The distributions representing variability and uncertainty are sampled separately in the Monte-Carlo simulation framework to separately estimate the variability and uncertainty in the model output.

Sensitivity analysis

Sensitivity analysis is a tool used by risk assessors to provide insight on the relative importance of the components of a risk assessment (FAO, 2009). It provides a clearer understanding of how model outputs respond to changes in model input values and assumptions. It can be used to assess the robustness of model results when making decisions and as an aid in identifying important sources of uncertainty, variability or both to aid additional data collection or model refinement (Ozkaynak et al., 2008; FAO, 2009).

Quantitative sensitivity analysis is based on four approaches, namely: scenario, graphical, mathematical, and statistical analysis (Yoe, 2012). Scenario analysis is used to compare different situations to identify differences in important model outputs. The different situations may include the best-case, worst-case, common practice, or a new policy case scenario where model inputs are entered as point estimates that represent the different scenarios. The different scenarios may lead to

different characterization of risk which may help to guide the risk managers in their decision-making process. In a recent risk assessment study to estimate the risk of illness due to *E. coli* O157:H7 contamination of ground beef and beef cuts in Canada, Smith et al. (2013) evaluated 20 intervention scenarios that included worst-case, no intervention, single or combined interventions, and current practices intervention scenarios in a sensitivity analysis.

Graphical methods for sensitivity analysis represent sensitivity in the form of graphs, such as scatter plots used to visually assess the influence of individual input on model output. Patterns (linear or non-linear) in the plot could depict potential relationship between an input and an output (Yang et al., 2012). Tornado graphs may be used to show relative sensitivity of model output to input variables. Sensitivity is measured by a correlation or normalized regression coefficient. The bar from the plot may extend to the right or left of the zero axis, indicating a positive or negative relationship with the length of the bar indicating the relative strength of the relationship. In a risk assessment study that evaluated the effects of pre-harvest and harvest interventions for *E. coli* O157 contamination on beef carcasses, Dodd et al. (2011) used tornado graphs and Spearman rank correlation coefficients and fixing input parameters at the 5^{th}, 50^{th}, and 95^{th} percentiles to compare the risk of carcass contamination in a sensitivity analysis.

The mathematical methods for sensitivity analysis are deterministic and rely on calculating outputs for a range of input values or different combinations of input values (Yang et al., 2012). The statistical methods rely on simulations in which inputs are represented by probability distributions. The statistical methods for sensitivity analysis use methods such as regression analysis, correlation, analysis of variance (ANOVA), response-surface methods (RSM), Fourier amplitude sensitivity test (FAST), and mutual information index (MII) (Yang et al., 2012).

Quantitative risk assessment for STEC in beef in the United States and Canada

A number of quantitative risk assessments have been developed for STEC, specifically *E. coli* O157:H7, in beef production in the United States and Canada (Cassin et al., 1998; USDA-FSIS, 2001; Ebel et al., 2004; Smith et al., 2013; Kiermeier et al., 2015).

Cassin et al. (1998): E. coli O157:H7 in ground beef hamburgers in Canada

This risk assessment was based on a Process Risk Model. The model applied quantitative risk assessment with scenario analysis and predictive microbiology to predict public health risk associated with *E. coli* O157:H7 in home-prepared ground beef hamburgers. The exposure assessment modeled the prevalence and concentration of *E. coli* O157:H7 in cattle feces, through production of beef trim at processing plants, grinding and storage at retail, cooking and consumption of hamburgers. The data used to estimate the distribution of *E. coli* O157:H7 prevalence in cattle feces was based on six studies conducted at dairy farms, feedlots and processing plants in Canada and the USA. The *E. coli* O157:H7 fecal prevalence data used in the model ranged from 0 to 3.1%. Data for concentration of *E. coli* O157:H7 in cattle feces was from a Zhao et al. (1995) study and ranged from < 2 to 5.0 $\log_{10}$ CFU/g. The dose-response assessment used a Beta-Binomial model derived from *Shigella dysenteriae* human outbreak data based on the assumption that the virulence of *E. coli* O157:H7 was similar to that of *Shigella*. Using Monte Carlo simulation with Latin Hypercube sampling in the @Risk software package, the exposure estimate was used as input in the dose-response model to estimate the health risk association with the consumption of contaminated hamburger.

The exposure model predicted a mean prevalence of 2.9% for *E. coli* O157:H7 in contaminated packages of fresh retail ground beef with concentration ranging from 0 to 8 $\log_{10}$ CFU/package with approximately 90% of contaminated packages having pathogen load of < 3 $\log_{10}$

CFU/package. The model predicted a probability of illness of 5.1×10^{-5} from a single meal for adults and 3.7×10^{-5} for children; a probability of HUS of 3.7×10^{-6} and a probability of mortality of 1.9×10^{-7} per meal for children.

Using the Spearman rank correlation coefficient, the model ranked the concentration of *E. coli* O157:H7 in cattle feces, host susceptibility, carcass contamination factor, cooking preference, retail storage time and temperature as the most important predictors of human health risk from *E. coli* O157:H7. Using scenario analysis, the model predicted retail storage temperature control (minimum 4ºC, mode 8ºC, and maximum 13ºC) as the most effective control strategy resulting in 80% reduction in *E. coli* O157:H7 illness. Pre-slaughter screening (reducing the maximum concentration of cattle fecal *E. coli* O157:H7 shedding to 4 $\log_{10}$ CFU/g) was predicted to reduce illness rates by 46%, while consumer education programs on cooking temperature was predicted to reduce *E. coli* O157:H7 illness by 16%.

The exposure assessment incorporated prevalence and concentration of *E. coli* O157:H7 in harvest ready cattle to predict probability of illness, mortality and HUS for the young and adult populations. However, important parameters such as seasonality, differences in shedding levels among cattle types, and effect of on-farm interventions were not considered. The dose-response model was based on human *Shigella* outbreak data which may have resulted in over-estimation of predicted risk.

USDA-FSIS (2001) and Ebel et al. (2004): E. coli O157:H7 in ground beef in USA

This risk assessment estimated the occurrence and number of *E. coli* O157:H7 in servings of ground beef from production of cattle, through slaughter, preparation, and consumption of ground beef. Exposure assessment estimated the prevalence of *E. coli* O157:H7 in culled breeding cattle and feedlot cattle in the U.S. during the low (October – May) and high (June – September) seasons. The

mean prevalence in breeding herds was 63% (95% CI: 55 – 72%), and within-herd prevalence was 3% (95% CI: 2 – 4%) during low season, and 4% (95% CI: 3 – 5%) during high season. For feedlot cattle, the mean prevalence was 88% (95% CI: 78 – 97%), within-feedlot prevalence was 9% (95% CI: 6 – 14%) during low season, and 22% (95% CI: 21 – 24%) during high season. Prevalence of infection within truckloads of cattle served as inputs to the slaughter phase. Slaughter plants that processed culled cattle and those that processed feedlot cattle were modeled separately. Following carcass contamination during the de-hiding process and decontamination as a result of in-plant interventions, on average 6% (range: 3 – 12%) of combo bins from slaughter of culled cattle contained *E. coli* O157:H7 during the low season, and an average 8% (range: 3 – 15%) were positive during the high season. For combo bins from slaughter of feedlot cattle, an average of 13% (range 3 – 45%) of bins contained *E. coli* O157:H7 during the low season, and an average of 43% (range: 17 – 58%) were contaminated during the high season. Final internal product temperature data from a commercial food temperature database was used to determine effect of product cooking.

The model predicted that 0.007% (range: 0.002 – 0.014%) of post-cooked servings consumed during the low-prevalence season and 0.018% (range: 0.004 – 0.042%) during the high-prevalence season were contaminated with at least one *E. coli* O157:H7 cell. Based on a Beta-Poisson dose-response relationship adapted from (Powell et al., 2000), the model predicted a probability of illness of 9.6×10^{-7} from a single meal for adults and 2.4×10^{-6} for children (about 2.5 times higher in children). The average population risk of developing HUS was 4.2×10^{-9}, and the average population risk of death from *E. coli* O157:H7 in ground beef was 5.9×10^{-10} per serving. The average population risk of illness was three times higher during the high-prevalence season (1.7×10^{-6}) compared to the low-prevalence season (6.0×10^{-7}).

Using sensitivity analysis, the model ranked feedlot and within-feedlot prevalence, probability of carcass contamination following de-hiding, and surface area of carcass contaminated, effectiveness of decontamination procedures, and carcass chilling as the most important predictors of *E. coli* O157:H7 contamination of beef trim; these predictors varied by season and type of cattle. The model ranked the occurrence and extent of *E. coli* O157:H7 contamination in beef trim and grinder loads, proportion of ground beef that is frozen, the maximum population density of *E. coli* O157:H7 in ground beef, home storage temperature, and cooking as the most important predictors of *E. coli* O157:H7 contamination of cooked ground beef servings.

The exposure assessment considered seasonality in prevalence of *E. coli* O157:H7 among feedlot and breeding cattle in predicting the probability of illness as a result of consumption of *E. coli* O157 contaminated ground beef among children and adults. However, the assessment did not include the concentration of *E. coli* O157:H7, which is a more important parameter for risk estimation. Effects of pre-harvest interventions on levels of *E. coli* O157:H7 in live cattle and carcasses were not considered. The dose-response model used in this assessment may have resulted in underestimation of predicted probability of illness reported (Duffy et al., 2006).

Smith et al. (2013): E. coli O157:H7 in ground beef and beef cuts in Canada

This assessment estimated the human health risk associated with consumption of ground beef and beef cuts contaminated with *E. coli* O157:H7 from cattle production, through processing at slaughter, storage at retail and home, and consumption; evaluating the effect of pre-harvest and processing interventions. The exposure assessment predicted *E. coli* O157:H7 prevalence and concentration throughout the farm-to-fork continuum. *E. coli* O157:H7 fecal prevalence values for adult cows, heifers, or steers in farms or feedlots obtained from peer-reviewed sources were adjusted

for diagnostic test sensitivity and represented as beta distributions for the high shedding (May–September) and low shedding (October–April) periods.

Hide prevalence was simulated by adjusting fecal prevalence with a fecal-to-hide transfer ratio derived from literature. The simulated hide *E. coli* O157:H7 prevalence was adjusted using the hide-to-carcass transfer ratio derived from literature to estimate the pre-intervention carcass prevalence. The data used to estimate the distributions for *E. coli* O157:H7 concentration in cattle feces were based on Stephens et al. (2009) study and were adjusted using a transfer factor to derive concentration on hides. Hide concentration distributions were equally adjusted using a hide-to-carcass transfer factor to derive concentrations on carcasses. Efficacy of interventions on prevalence and concentration of *E. coli* O157:H7 were obtained from systematic review and meta-analyses studies (Sargeant et al., 2007; Greig et al., 2012; Snedeker et al., 2012) and defined mostly as triangular distributions for effects on prevalence and mixture of uniform and triangular distributions for effects on concentration for pre-harvest and processing interventions.

Retail and consumer storage temperatures were modeled using ground beef storage temperature data from a USA survey (EcoSure, 2008). *E. coli* O157:H7 growth throughout retail and consumer storage at temperature $\geq 0^{o}$C were modeled using a model developed by Baranyi and Roberts (1994) and Tamplin et al. (2005). Consumer storage growth at temperature $< 0^{o}$C was modeled based on a cumulative distribution described by USDA-FSIS (2001). Thermal inactivation of *E. coli* O157:H7 in ground beef was modeled using the relationship determined by Juneja et al. (1997) for cooking hamburgers to internal temperatures between 56.1 and 74.4^{o}C. The amount of ground beef and beef cuts consumed in a single serving were assumed to follow a lognormal distribution with a mean of 64.8 g and standard deviation (SD) of 72.6 g for ground beef, and mean of 104 g and SD of 97.3 g for beef cuts. The Beta-binomial dose-response model described in Cassin

et al. (1998) was used to determine the probability of illness from exposure to *E. coli* O157:H7 in ground beef, non-intact, and intact beef cuts.

Effect of pre-harvest interventions (probiotic, SPR and TTSP vaccines) on *E. coli* O157:H7 fecal prevalence was modeled as a log logistic distribution for probiotic and triangular distribution for vaccines. Effect on fecal concentration was modeled as a uniform distribution for both probiotics and vaccines. Application of a single pre-harvest intervention resulted in 30.9%-61.9%, 37.1%-61.4%, and 35.3%-72.1% reduction in average probability of illness per serving of ground beef, non-intact beef cut, and intact beef cuts, respectively. Effect of processing interventions (carcass wash, hot water wash, steam pasteurization, acid spray chill, dry-aged chill, and water spray chill) on *E. coli* O157:H7 carcass prevalence and concentration was modeled as triangular distributions. Application of a single processing intervention post-evisceration resulted in 45.3%-92.4%, 44.3%-95.5%, and 44.0%-96.5% reduction in average probability of illness per serving of ground beef, non-intact beef cut, and intact beef cuts, respectively. For a combination of pre-harvest and processing interventions, the model predicted a reduction in average probability of illness of 95.1% - 99.6%, 95.3% - 99.9%, and 95.1% - 99.9% per serving of ground beef, non-intact beef cuts, and intact beef cuts, respectively. Based on intervention practices applied in Canada, the model predicted an average probability of illness per serving of 8.7×10^{-6}, 3.3×10^{-8}, and 2.9×10^{-9} for ground beef, non-intact beef cuts, and intact beef cuts, respectively. Average risks from consumption of ground beef were 263 and 2965 times greater than average risks associated with non-intact beef cuts and intact beef cuts, respectively.

In a sensitivity analysis based on Spearman's rank order correlation, the model ranked cooking temperature, host susceptibility, home storage method, contamination area, shedding period, serving size, fecal concentration, and retail storage method as the most important predictors of

probability of illness from *E. coli* O157:H7 in ground beef. For non-intact beef cuts and intact beef cuts, host susceptibility, cooking temperature, concentration in feces, hide-to-carcass transfer factor, consumer storage method, shedding period, contaminated area, and feces-to-hide transfer factor were the most important predictors of probability of illness, including tenderization method for the non-intact beef cuts.

This study is the first to evaluate the human health risk associated consumption of *E. coli* O157:H7 contaminated ground beef, intact beef cuts, and non-intact beef cuts. The exposure assessment modeled prevalence and concentration of *E. coli* O157:H7 in cattle while accounting for seasonality, cattle types (though not separated), sensitivity of the detection method, effect of probiotics and vaccines on level of pathogen on live cattle, and effect of multiple on-farm interventions. The dose-response model, however, was based on human *Shigella* outbreak data used in the Cassin et.al. (1998) model considered to result in overestimation of the risk of illnesses (Duffy et al., 2006).

Kiermeier et al. (2015): E. coli O157 in USA hamburgers made from Australian beef

This assessment estimated the public health risk associated with *E. coli* O157 contaminated hamburgers produced from manufacturing beef imported from Australia but consumed in the United States. The assessment was based on prevalence and concentration of *E. coli* O157 in lots of beef that were withdrawn from the export chain following detection of the pathogen. The model assumed no product was removed from the supply chain following testing; and modeled contamination from grinding and patty forming, retail storage, transport home, home storage, cooking and consumption. Australian manufacturing beef (trim) is imported frozen in 60 lb cartons arranged in container lots of up to 700 cartons. The proportion of *E. coli* O157 in contaminated cartons from contaminated lots was modeled with a beta distribution. The concentration of *E. coli* O157 in contaminated lots,

derived by an MPN enumeration technique, was modeled with a lognormal distribution. Growth during retail storage, transport to home, and home storage for ground beef was modeled using temperature data from the Ecosure survey (EcoSure, 2008). Thermal inactivation of *E. coli* O157:H7 in ground beef was modeled using the relationship determined by Juneja et al. (1997) for cooking hamburger patties. It was assumed that only one patty was consumed per serving. The Beta-binomial dose-response model described in Cassin et al. (1998) was used to determine the probability of illness from exposure to *E. coli* O157 in a hamburger patty. The model was implemented in the open-source R software utilizing functions from "mc2d" and "fitdistrplus" packages.

Two product consumption pathways were modeled, namely, home consumption and quick-service restaurant (QSR) consumption pathways. The home consumption pathway included chilled storage at retail, transport to home, chilled storage at home, and home cooking at variable temperature, while the QSR pathway included only frozen storage and cooking at fixed temperature (68^{o}C). For the home consumption pathway, the model predicted an average number of illnesses per contaminated lot of 13.5 (range: 12.3 – 14.9) for the 50 g patties, and an average of 10.8 illnesses (range: 9.8 – 11.8) for the 100 g patties per contaminated lot. The authors attributed the higher number of illnesses associated with the consumption of the 50 g patties to the doubling of the number of burgers obtained and consumed from each lot when the patty size is halved. The number of illnesses per 10^{7} burgers was 1.78 and 2.83 when 50 g and 100 g patties are consumed, respectively. For the QSRs consumption pathway, the number of illnesses per 10^{11} burgers from all Australian lots was 1.72 and 7.30 when 50 g and 100 g patties are consumed, respectively. A total of 49.59 illnesses were predicted to occur yearly from patties produced from only Australian manufacturing beef, all of which were due to patties cooked and consumed in the home.

In the sensitivity analysis, exclusion of the retail storage temperature outlier (19.4ºC), limiting retail and home storage durations to 10 days, and using spoilage-based maximum storage, produced little impact on the average number of illnesses per lot and the illness rate per 100,000 patties consumed. Spreading carton contamination over fewer patties resulted in 10% reduction in rate of illness, while spreading carton contamination over more patties produced a 10% increase in rate of illness. Cooking patties to temperature of 69ºC instead of 68ºC, reduced the rate of illness to 1.1 and 3.1 per 10^{11} burgers when 50 g and 100 g patties were consumed, respectively.

The risk assessment modeled the risk of *E. coli* O157:H7 illnesses in the U.S from consumption of burgers produced from beef trim imported from Australia. The exposure assessment monitored the prevalence and concentration of *E. coli* O157:H7 from trim in cartons through storage, retail and cooking at home and at restaurants. The dose-response used the Cassin et al. (1998) model considered to result in overestimation of the risk of illnesses (Duffy et al., 2006).

Overall, the available QMRA for the estimation of the public health risk associated with STEC contamination in beef in the U.S are based on *E. coli* O157:H7 contamination of ground beef, intact beef cuts, and non-intact beef cuts produced in the U.S, and beef trim imported from Australia. Some of the models have incorporated measures of prevalence and concentration of *E. coli* O157:H7 in the assessment of exposure while accounting for major confounding variables, such as seasonality of shedding, differences among cattle types, and impact of pre-harvest and processing interventions. Two dose-response models have been used; each suspected to have either over- or underestimated the risk of illness. Concentration of *E. coli* O157:H7 in feces, host susceptibility, probability of carcass contamination, effectiveness of decontamination procedure, storage temperature, and cooking temperature are some of the important predictors identified for *E. coli* O157:H7 contamination of beef products.

Conclusion

STEC are important foodborne pathogens of significant public health impact in the U.S. Cattle are the principal reservoir and they shed the bacteria in their feces, contaminating the environment and hides of cattle within the herd. Bacterial contamination of carcasses may occur from transfer of fecal material from hides or rarely, gut rupture during processing. STEC transmission to humans may occur through contaminated food, water, environment, direct contact with animals, or person-to-person contact, and may lead to significant morbidity and mortality in young, elderly, and immunocompromised individuals.

Prevalence and levels of STEC in cattle production systems vary by region of origin of the cattle, age, season, cattle type, type of specimen, and at various stages in the beef production continuum. There is high variability among studies on the estimated prevalence of the O157 and non-O157 STEC in cattle and along the beef production chain. It is crucial to systematically identify, evaluate, critically appraise, and summarize the data from the various and frequently small sample size studies evaluating pathogen burden in the same specimen, season, cattle types, and under similar conditions, to obtain more informative pooled effect estimates across studies.

Several diagnostic methods have been developed and evaluated for the detection and quantification of the seven STEC serogroups–O26, O45, O103, O111, O121, O145, and O157–already declared adulterants in ground beef and non-intact raw beef products. These methods, however, need to be validated for their diagnostic performance, to allow establishment of unbiased estimates of the true prevalence and levels of individual STEC serogroups. This is required for implementation of quantitative microbial risk assessment of the burden of the pathogens along the beef production chain.

A number of quantitative microbial risk assessments have been developed in the United States and Canada to estimate the public health risk associated with consumption of ground beef and beef cuts contaminated with *E. coli* O157:H7 from cattle production, processing, through consumption. The goal of this project is to create a probabilistic second order quantitative microbial risk assessment for the seven major STEC–O26, O45, O103, O111, O121, O145, and O157–in the beef production chain. The model will use current data to simulate and track prevalence and concentration of the seven STEC in different cattle types and season from primary production at the feedlot, through harvest and processing in the slaughter plant, retail to consumption in order to estimate their public health impact. In addition, these models will evaluate the efficacy of existing (pre-harvest and processing) and novel interventions for reducing beef carcass contamination and risk of illness in humans following consumption of contaminated beef products.

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Chapter 2 - Prevalence and concentration of *Escherichia coli* O157 in different seasons and cattle types processed in North America: A systematic review and meta-analysis of published research

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Abstract

Systematic review (SR) and meta-analyses (MA) methodologies were used to identify, critically evaluate and synthesize prevalence and concentration estimates for *Escherichia coli* O157 contamination along the beef production chain, and to illustrate differences based on cattle types and seasonality in North America from the scientific peer-reviewed literature. Four electronic databases were searched to identify relevant articles. Two independent reviewers performed all SR steps. Random effects MA models were used to estimate the pooled prevalence and concentration of *E. coli* O157 in feces, hides and carcasses of cattle processed in North America, including their

seasonal estimates. The potential sources of between studies heterogeneity were identified using meta-regression and sub-group analysis. Results indicated differences in the fecal prevalence of *E. coli* O157 among cattle types: 10.68% (95% CI: 9.17–12.28%) in fed beef, 4.65% (95% CI: 3.37–6.10%) in adult beef, and 1.79% (95% CI: 1.20–2.48%) in adult dairy. Fed beef fecal prevalence was 10.65% (95% CI: 8.93–12.49%) during summer and 9.17% (95% CI: 5.24–13.98%) during the winter months. For adult beef, the fecal prevalence was 7.86% (95% CI: 5.43–10.66%) during summer, and 4.21% (95% CI: 1.95–7.13%) during winter. Among adult dairy, the fecal prevalence was 2.27% (95% CI: 1.5–3.18%) during summer, and 0.36% (95% CI: 0.09–0.74%) during winter. There was a significantly higher percentage of hides with *E. coli* O157 concentration ≥ 40 CFU/100cm^2 on hides of fed beef sampled at the processing plant (23.81%; 95% CI: 14.79–34.15%) compared to those sampled at the feedlot (1.74%; 95% CI: 0.53–3.44%). Prevalence of *E. coli* O157 on carcass surfaces differed by season only at the post-evisceration stage, but decreased considerably through the subsequent processing stages. Country, study setting, detection method, hide or carcass swab area, and study design were identified as significant sources of heterogeneity among studies reporting prevalence of *E. coli* O157 along the beef production chain. The pooled prevalence and concentration estimates from this study provide a sound and reliable microbiological basis for risk assessment modeling of *E. coli* O157 and other pathogens in the food chain.

Keywords: *E. coli* O157; Prevalence; Concentration; Beef production, Food safety, Systematic review, Meta-analysis, North America

Introduction

Foods of bovine origin are frequently implicated in human outbreaks of Shiga toxin-producing *Escherichia coli* (STEC) O157 (Bell et al., 1994; Dodson and LeJeune, 2005) and non-O157 STEC (Mead et al., 1999; Scallan et al., 2011). *E. coli* O157 is estimated to be responsible for 63,153 episodes of domestically acquired foodborne illnesses, 2,138 hospitalizations, and 20 deaths among humans in the United States (US) annually (Scallan et al., 2011). Approximately 58% of foodborne disease outbreaks caused by *E. coli* O157 in the US during 1998 – 2008 were attributed to beef, about 17% to leafy vegetables, 10% to dairy products, 9% to fruits / nuts, and about 3% for each of poultry and game (CDC, 2013). Beef includes ground beef (Riley et al., 1983; Hussein, 2007; CDC, 2013), intact, and non-intact beef cuts (USDA-FSIS, 1999). Interestingly, the beef source (beef or dairy cattle) was not known in most cases.

Ground beef is produced mainly from culled dairy and beef cattle and low-value cuts from finished steers or heifers. In the US, an estimated 34% of domestic ground beef comes from culled cows; 17% from culled dairy cows and 17% from culled beef cows (Troutt and Osburn, 1997). The remaining percentage is from young beef cattle raised on pasture and/or feedlots. In New York State alone, culled dairy cows contribute 10.5 million pounds of hamburger annually (Segelken, 1996). In general, it is difficult to trace a specific ground beef outbreak to dairy or beef cattle. A statewide outbreak of *Escherichia coli* O157:H7 infections in Washington State in November 1997, which resulted in 37 cases, with 3 hospitalized for hemorrhagic colitis, was traced to consumption of contaminated ground beef originating from dairy cattle (Ostroff et al., 1990). Similar outbreaks, at other times, had been traced to beef cattle (Rodrigue et al., 1995), contact with animals at fairs or exposure to animal environments (CDC, 2001; Crump et al., 2003; CDC, 2005, 2009). The prevalence and concentration of STEC, especially *E. coli* O157:H7, vary in the environment, feces,

hides, and carcasses of cattle in different production systems—beef or dairy (Hancock et al., 1994; Hussein and Sakuma, 2005; Hussein, 2007), seasons (Renter and Sargeant, 2002; Barkocy-Gallagher et al., 2003; Williams et al., 2010), and at different stages of slaughter and meat processing at the harvesting plant (Arthur et al., 2008).

Understanding the epidemiology of *E. coli* O157 in the beef chain is important for risk assessments aiming to estimate the prevalence and concentration of the pathogen at different points along the beef chain, and ultimately help reduce its occurrence and public health risk. Obtaining data for the prevalence and concentration of *E. coli* O157 for different cattle types, seasons, and following control interventions at the pre-, post-harvest and post-processing stages of beef production is crucial for quantitative microbial risk assessments. Islam (Islam et al., 2014) in 2014 reported on the variation in the prevalence of *E. coli* O157 in cattle feces at the global level. Our review describes both the prevalence and concentration of *E. coli* O157 in cattle feces, hides and carcasses with a focus on the North American beef production chain, and further explores sources of variation in the prevalence estimates.

The objective of this study was to identify, critically evaluate and synthesize prevalence and concentration estimates for *E. coli* O157 along the beef production chain, and to illustrate differences based on cattle types and seasonality in North America, using a systematic review of the literature and meta-analyses.

Methods

Definitions

Escherichia coli O157, the outcome of interest in this study, include *E. coli* O157 or *E. coli* O157:H7 isolated from feces, hides or carcasses of adult cattle using the immunomagnetic separation (IMS) method for detection. Cattle types include fed cattle (young cattle being fed for

slaughter), adult beef (breeding cows on farms and cull cows) and adult dairy (lactating cows on farms and cull cows). Season was defined as summer (April, May, June, July, August and September) and winter (October, November, December, January, February and March). An identified peer-reviewed publication that described the prevalence and/or concentration of *E. coli* O157 in North American cattle was classified as an article. An article may contain one or more datasets, which could represent data obtained on different sampling dates, months, different sample types or different cattle types.

Literature search

A systematic search was conducted to identify all published studies reporting prevalence and/or concentration of *E. coli* O157 in cattle from North America. Four electronic databases – Agricola (EBSCO), CAB Abstracts, Food Science and Technology Abstracts (FSTA), and PubMed were searched using these terms: "(*Escherichia coli* OR *E. coli* OR STEC OR O157) AND (cattle OR dairy OR cow OR feedlot OR cull) AND (feces OR hide OR carcass) AND (prevalence OR concentration)". No language restrictions were applied. All searches were conducted from the 20th to 22nd of December 2013. We also included a hand search of authors' collections of relevant peer reviewed articles. All citations located in the searches were entered into EndNote X6 (Thomson ResearchSoft, Philadephia, PA). Duplicate references (where information about study setting / location, numerator, denominator, and the study period were exactly the same for different articles) were removed, and abstracts were obtained for all remaining citations.

Relevant screening, inclusion and exclusion criteria

Four inclusion criteria were applied to screen in abstracts considered relevant to the research question. The articles had to describe studies that: (1) measured animal level prevalence and/or concentration of *E. coli* O157 in natural field conditions (farm, feedlot or slaughter plant); (2)

measured prevalence or concentration in the feces, hides or carcasses of slaughter-ready fed cattle, or adult beef or dairy cows in North America (United States, Canada and Mexico); (3) included immunomagnetic separation (IMS) for detection of *E. coli* O157; and (4) had to be published from year 2000 through 2013. Descriptive studies (survey), observational studies (cross-sectional, case-control, and cohort), or field trials (control arms) were eligible for inclusion. Studies were excluded if they were a duplicate population group, i.e., separate articles reporting results from the same study, and for failing to meet the inclusion criteria above. Unpublished studies, conference abstracts, inoculation studies, and calf studies were not included in this meta-analysis. Full publications were obtained for all abstracts that passed the relevance screening. If there was not sufficient information in the abstract to assess whether all four inclusion criteria were met, the full publication was also obtained and screening was repeated using the full publication. Articles were identified as peer-reviewed research article by searching on Ulrichsweb (https://ulrichsweb.serialssolutions.com/), an electronic database that determines if a journal was indicated as being peer-reviewed. The abstracts were screened by two independent reviewers with all disagreements resolved by consensus. The relevance screening form, data extraction and assessment of risk of bias form were created in Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

Data extraction process

All article information (author's name, article title, and year of publication) were captured in the data extraction and assessment of risk of bias form. The form was pilot tested on seven articles by all three authors. Data were extracted on population (cattle type, production system, country, and study date), study design, type of specimens, specimen collection date, number of samples analyzed, number or proportion positive, percentage or number enumerable, and methods used for detection. Data were extracted for all articles at the farm/plant level based on sampling dates including studies

that were designated as longitudinal, surveys, and field trials, thus data represented outcome measurement at one specific point in time. Study design was therefore categorized as cross sectional (including longitudinal studies and surveys) studies, and field trials. Specimen collection dates were categorized into seasons. Data for the methods used for pathogen detection were extracted to include IMS, latex agglutination or ELISA, with or without PCR. This was re-categorized as methods that included IMS and PCR, and those that included IMS but no PCR. Data were extracted for three types of cattle; fed cattle (young cattle being fed for slaughter), adult beef (breeding cows on farm and cull cows) and adult dairy (lactating cows on farm and cull cows). For articles with insufficient or no information on the date when specimens were collected, the corresponding author was consulted by e-mail to request sampling dates and additional data where necessary. For articles that presented only the total sampled (denominator) and proportion of positives, the total positives (numerator) were calculated. Prevalence and / or percentage of enumerable samples were extracted or calculated for cattle feces, hides sampled at feedlots and at processing plants. Pre-evisceration carcass prevalence and percentage of enumerable samples were extracted for carcasses immediately after complete hide removal but before application of any antimicrobial interventions. Post-evisceration carcass prevalence was extracted for carcasses after evisceration, splitting, and trimming, but before further antimicrobial interventions. Post-intervention carcass prevalence was extracted for carcasses after final plant antimicrobial intervention on carcasses hanging in the cooler for no more than 2 hours after final intervention. Chilled carcass prevalence was extracted for carcasses hanging in the cooler for approximately 24 hours post- mortem. Data were extracted from the included studies by the first author and validated by a second author. All disagreements were discussed and resolved.

Statistical analysis

Descriptive statistics were calculated to determine the total number of cattle included at each level of analysis and the ranges of prevalence estimates. Random-effects meta-analyses were carried out (using the total sample size and number of positives) to estimate the prevalence of *E. coli* O157 in cattle feces, hides and carcasses. Between-study variations were assessed using: (1) the Cochran's Q (chi-square) test of heterogeneity, to evaluate whether the variation between studies exceeded that expected by chance, $P \leq 0.1$ indicated significant heterogeneity, and (2) the Higgins I^2 statistic, to estimate the percentage of total variation in effect estimates across the studies attributable to heterogeneity rather than chance, $I^2 > 50\%$ may indicate substantial heterogeneity (Higgins et al., 2003). Testing for subgroup differences was based on Cochran's Q (chi-square) test of heterogeneity, to test for heterogeneity across subgroups.

Separate meta-analyses (subgroup analysis) were conducted on data subsets to estimate the pooled prevalence and/or concentration (percentage enumerable) of *E. coli* O157 in different cattle types and seasons for fecal samples, hides sampled at farm or at slaughter plants, and beef carcasses (pre-evisceration and post-intervention) in North America and by country. For this analysis, our combinations of months to define season represented the most accurate definition of high and low prevalence months for *E. coli* O157:H7 in cattle, and was also supported by the USDA data (USDA-FSIS, 2002, 2013). Other months combinations to define seasons were assessed (data not shown). The point estimates (with 95% CIs) from separate datasets were pooled using the DerSimonian-Laird random effects (DerSimonian and Laird, 1986) method, with the variances of the raw proportions stabilized using the Freeman-Tukey double arcsine transformation (Freeman and Tukey, 1950; Viechtbauer, 2010).

Meta-regression was used to explore additional potential sources of heterogeneity below the initial grouping levels of type of sample (feces, hide or carcass), type of cattle (fed beef, adult beef or adult dairy), and season (summer or winter). The explanatory variables were: study setting (commercial vs. research facility), sampling setting (farm/feedlot vs. slaughter plant), study design (cross sectional studies vs. field trials), country of origin (USA, Canada, or Mexico), detection method (IMS with PCR vs. IMS no PCR), hide swab area (<1,000 vs. 1,000 cm^2), and carcass swab area (1,000 vs. >1,000 cm^2). Initially, univariable meta-regression models were built to explore the association between the variables and the prevalence of *E. coli* O157. Variables with $P < 0.2$ in the univariable analysis were included in the multivariable model. For models where a variable category contained fewer than 3 studies, the variable category was dropped (e.g. category "Mexico" within the variable "country". Subgroup analyses were conducted to obtain prevalence estimates by country given cattle types and seasons. All meta-analyses were carried out using "metaprop" in the "meta" package in open sources 64 bit *R* 3.0.2 (R Core Team, 2013).

Assessment of quality of reporting

This evaluation involved the assessment of information reported in the primary studies on factors related to internal validity (risk of bias) and external validity (generalizability). Ten criteria were extracted for further exploration. Factors related to internal validity were study design, study setting, sampling setting, and detection method. Factors related to external validity were cattle type, country, sample type, date/season of sampling, and coverage area for hide and carcass swabs.

Results

From the initial searches, 1,966 (1,961 from databases, 5 from hand-search) potentially relevant articles, all written in English, were identified. After primary screening of titles and

abstracts, 137 articles were selected for full text search. A total of 53 relevant articles (satisfied inclusion criteria) reporting prevalence and/or concentration of *E. coli* O157 in cattle feces, hide or carcasses were identified. Among these, we requested additional information (total number of samples tested and positive for *E. coli* O157 stratified by sampling date/month) through e-mail from the lead authors for 35 of the articles, of which 18 provided the requested data. Of the 17 articles for which additional data was not available from the authors, two were completely excluded (LeJeune et al., 2004a; Varela-Hernandez et al., 2007) in the MA, and 15 were included in the MA of *E. coli* O157 prevalence by cattle type based on the data reported in the articles (Sargeant et al., 2000; Barkocy-Gallagher et al., 2003; Riley et al., 2003; Rivera-Betancourt et al., 2004; Dewell et al., 2005; Dodson and LeJeune, 2005; Childs et al., 2006; LeJeune et al., 2006; Woerner et al., 2006; Brichta-Harhay et al., 2007; Stephens et al., 2007; Dewell et al., 2008; Fox et al., 2008b; Stephens et al., 2009; Fink et al., 2013). However, six of these 15 articles were excluded in the MA by season because sampling dates could not be separated into defined seasons. (Sargeant et al., 2000; Childs et al., 2006; Woerner et al., 2006; Brichta-Harhay et al., 2007; Stephens et al., 2007; Stephens et al., 2009).

Finally, a total of 51 articles (representing 631 datasets) were included in the MA for prevalence (575 datasets) and/or concentration (61 datasets) of *E. coli* O157 based on cattle types for all sample types. Out of the total 51 articles, 45 (representing 603 datasets) were included in the MA to assess the effect of season (Figure 2.1). Out of all articles, 44 represented studies conducted in the United States, five conducted in Canada and two in Mexico. Based on study design, included articles represented 43 cross-sectional-surveys, five longitudinal (data extracted as cross-sectional), and three field trial (control arm) studies. A total of 40 articles contained fecal prevalence and /or concentration data, 19 contained hide and 14 contained carcass data (Figure 2.1 and Table 2.1). Data

describing the characteristics of all studies included in the estimation of the prevalence and concentration of *E. coli* O157 in the beef production chain are presented in Table 2.2.

Prevalence of *E. coli* O157 in cattle feces

Cattle type and seasonality

The estimated fecal prevalence for *E. coli* O157 differed significantly ($P < 0.01$) among cattle types (fed beef, adult beef, and adult dairy). For fed beef, estimated fecal prevalence was 10.65 % (95% CI: 8.93–12.49%) during summer and 9.17 % (95% CI: 5.24–13.98%) during winter in North America ($P > 0.05$). For adult beef, the summer prevalence was 7.86% (95% CI: 5.43–10.66%), and 4.21% (95% CI: 1.95–7.13%) during winter ($P < 0.05$). Among adult dairy, the estimated fecal prevalence was 2.27% (95% CI: 1.5–3.18%) during summer, and 0.36% (95% CI: 0.09–0.74%) in winter ($P < 0.05$). The estimated percentage of enumerable (≥ 200 CFU/g of feces) fecal samples for fed beef sampled during summer was 5.78% (95% CI: 2.41–10.31%). For each of the prevalence estimates (fed beef, adult beef, adult dairy, and fed beef % enumerable), there was heterogeneity across studies [Cochran's Q (chi-square) $P < 0.1$ and the Higgins $I^2 > 50\%$], (Table 2.3). Univariable meta-regression analysis was conducted to assess study characteristics at each level of the explanatory variables. Detection method (IMS with PCR vs. IMS no PCR), and country (USA vs. Canada) were the only significant variables ($P < 0.2$) when exploring sources of heterogeneity among studies reporting fecal prevalence for fed beef during summer months. No variable was significant ($P > 0.2$) during winter. For adult beef, country was significant ($P < 0.2$) during summer, while detection method was significant ($P < 0.2$) during winter. Neither of these factors were significant for adult dairy in summer or winter. In a multivariable meta-regression model, country and detection method remained as the only significant sources of heterogeneity for fed beef fecal prevalence during summer months.

Prevalence of *E. coli* O157 on hides

There were reports of *E. coli* O157 measured on hides at the feedlot immediately prior to transport to processing plants and following the arrival of the cattle at the plant or post exsanguination (Table 2.4). Hide prevalence at the feedlot was reported for fed beef only. The estimated prevalence of *E. coli* O157 on hides measured at the feedlot was 52.01% (95% CI: 31.43–72.25%) during summer and 47.51 % (95% CI: 0.00–100.00%) during winter (based on 2 studies) in North America ($P > 0.05$). The percentage of enumerable hides (≥ 40 CFU/100 cm^2) at the feedlot was 1.74% (95% CI: 0.53–3.44%) for summer, but no data were available to estimate percentage of enumerable hide samples for winter (Table 2.4). For all hide estimates (summer and winter prevalence, % enumerable), there was heterogeneity across studies [Cochran's Q (chi-square) $P < 0.1$ and the Higgins $I^2 > 50\%$]. Univariable meta-regression was conducted to assess study characteristics at each level of the explanatory variables. Study setting (Commercial feedlot vs. Research farm), country (USA vs. Canada) and hide swab area (<1,000 vs. 1,000 cm^2) were the only significant variables ($P < 0.2$) when exploring sources of heterogeneity among studies reporting hide prevalence during summer months; there was not sufficient data to assess sources of heterogeneity for winter months. In a multivariable meta-regression model, only area of hide swabbed remained a significant source of between-study heterogeneity.

For hide prevalence measured at the processing plant (Table 2.5), the estimated prevalence of *E. coli* O157 was 54.63% (95% CI: 44.5–64.57%) during summer and 59.33% (95% CI: 46.76–71.33%) during winter in fed beef ($P > 0.05$). The percentage of enumerable hides (≥ 40 CFU/100 cm^2) was 23.81% (95% CI: 14.79–34.15%) for summer, and 14.5% (95% CI: 6.72–24.56%) during winter ($P > 0.05$). For all the hide in-plant prevalence and the percentage of enumerable hide estimates, there was heterogeneity across studies [Cochran's Q (chi-square) $P < 0.1$ and the Higgins

I^2 > 50%]. Univariable meta-regression was conducted to assess study characteristics at each level of the explanatory variables. Study design (cross sectional vs. field trial), country (USA vs. Canada) and hide swab area (<1,000 vs. ≥1,000 cm^2) were the only significant variables ($P < 0.2$) when exploring sources of heterogeneity among studies reporting hide prevalence during summer months. For winter months, country and hide swab area were significant ($P < 0.2$). In the multivariable meta-regression model, only country and hide swab area remained significant sources of heterogeneity for summer, and only country was significant during winter. In adult beef and dairy cattle, the estimated prevalence of *E. coli* O157 on hides at the plant was 45.34% (95% CI: 30.38–60.73%) during summer and 47.49% (95% CI: 20.57–75.22%) during winter ($P > 0.05$). There were significant differences in the overall, and estimated seasonal prevalence of *E. coli* O157 on hides of fed beef cattle when sampled at the slaughter plant compared to when sampled at the feedlot ($P < 0.05$).

Prevalence of *E. coli* O157 on carcasses

Pre-evisceration-pre-intervention carcass prevalence estimates were available for fed beef, adult beef and adult dairy cattle (Table 2.6). There was no difference ($P > 0.05$) in the estimated prevalence of *E. coli* O157 on pre-evisceration carcasses comparing fed beef with adult beef and adult dairy cattle. Prevalence was estimated as 14.06% (95% CI: 9.24–19.64%) during summer and 22.49% (95% CI: 13.45–33.00%) during winter for fed beef ($P > 0.05$). The estimate was 14.38% (95% CI: 8.34–21.70%) during summer and 13.79% (95% CI: 1.38–35.53%) during winter for adult beef and adult dairy cattle ($P > 0.05$). However, the percentage of pre-evisceration enumerable carcasses (≥ 0.5 CFU/100 cm^2) in fed beef was 0.0% (95% CI: 0.00–0.37%) during summer and 3.26% (95% CI: 1.01–6.62%) during winter ($P < 0.05$). For each of the prevalence estimates (fed beef, adult beef and dairy and % enumerable for fed beef), there was heterogeneity across studies

[Cochran's Q (chi-square) $P < 0.1$ and the Higgins $I^2 > 50\%$]. Univariable meta-regression was conducted to assess study characteristics at each level of the explanatory variables. Study design (cross-sectional vs. field trial) and country (USA vs. Mexico) were the only significant variables ($P < 0.2$) when exploring sources of heterogeneity among studies reporting summer prevalence for fed beef, whereas only country was significant ($P < 0.2$) for winter. In a multivariable meta-regression model, country and study design remained significant ($P = 0.01$) in the summer data.

Post-evisceration carcass prevalence estimates (Table 2.7) for fed beef were 8.90% (95% CI: 2.49–18.16%) during summer, and 1.25% (95% CI: 0.0–4.09) during winter ($P < 0.05$). There was a significant ($P < 0.2$) difference by country (USA vs. Mexico) in a univariable meta-regression. Post-intervention carcass prevalence estimates for fed beef were 1.02% (95% CI: 0.22–2.20%) during summer, and 0.04% (95% CI: 0.0–0.49%) during winter ($P < 0.05$). Chilled carcass prevalence estimates (Table 2.7) were 0.19% (95% CI: 0.0–1.83%) during summer, and 0.0% (95% CI: 0.0–0.70%) during winter ($P > 0.05$). The Cochran's Q (chi-square) tests of heterogeneity for the post-intervention estimates were non-significant ($P > 0.1$) and the Higgins $I^2 < 50\%$, indicating no between-study heterogeneity in the model.

Assessment of quality of reporting

For all sample types (feces, hide and carcass), the majority of the variables (study design, study setting, sampling setting, detection method, cattle type, country, and coverage area for hide) were reported in all studies. Among studies that reported the prevalence of *E. coli* O157 in cattle feces, hides, and carcasses, 5.8% (19/327), 3.6% (6/169), and 2.1% (2/97) of studies could not be clearly classified into our season variable, respectively. In addition, among studies that reported the prevalence of *E. coli* O157 on pre-, post-evisceration and chilled carcasses, 4.4% (4/92) of studies

did not report the coverage area of the carcass swab. No articles were excluded based on assessment of quality of reporting.

Discussion

Our systematic review identified a large number of articles reporting the prevalence of *E. coli* O157 in feces, hides, and carcasses of fed beef cattle; some articles reporting on the prevalence of *E. coli* O157 in feces of adult beef and dairy cattle; but no article reporting the prevalence of *E. coli* O157 in fed dairy cattle. Few articles reported on the concentration / levels of *E. coli* O157 in feces, hides, and carcasses of fed beef cattle. After the screening process was applied, 51 relevant articles reporting prevalence and six articles reporting concentration of *E. coli* O157 were available for quantitative analyses.

Results of MA for the fecal prevalence of *E. coli* O157 in North America indicated significant differences among cattle types. This finding supports the conclusion by Islam et al., (2014) who identified cattle type as one of the sources of heterogeneity when estimating the prevalence of *E. coli* O157 in cattle across the globe. The differences in the fecal prevalence of *E. coli* O157 in the different cattle types have been associated with animal (genetic / physiological) factors (Jeon et al., 2013), and diet (type of diet and diet practices) factors (Callaway et al., 2003; Callaway et al., 2009; Jacob et al., 2009). Potential environmental and management factors may further account for the differences in the estimates in the different cattle types. Adult beef are mostly raised on pasture and forage with occasional supplemental feeding. Their population structure is generally more dispersed, with seasonal perturbations in population density and size, mainly related to calving. Fed beef are fed a high-energy ration of grain, silage, hay, and/or protein supplement and housed in high population density with high population turnover (Nielsen et al., 2002; Callaway et al., 2013). Adult dairy cattle are also fed a high-energy ration of grain, silage, hay, and/or protein

supplement and housed in high population density but with much lower population turnover. These differences may alter infection and shedding dynamics of *E. coli* O157 and may at least partially account for the differences in prevalence. Further research on these factors may be useful to optimize implementation of pre-harvest control interventions.

Several studies have identified season as an important explanatory variable for the prevalence of *E. coli* O157 in cattle feces (Albihn et al., 2003; Barkocy-Gallagher et al., 2003; Ogden et al., 2004; Alam and Zurek, 2006; Khaitsa et al., 2006; Hussein, 2007; Arthur et al., 2009). In our study, we found the prevalence of *E. coli* O157 in feces of adult beef, and adult dairy to vary by season, with a higher prevalence during summer compared to the winter months. Possible influencing factors are day length and ambient temperature (Edrington et al., 2006). Increased temperatures have been hypothesized to enhance pathogen survival and proliferation (McClure and Hall, 2000; Kovats et al., 2004) leading to potentially increased exposure dose and prevalence in cattle (Lal et al., 2012). Human outbreaks of *E. coli* O157:H7 have been described by several authors to mirror the seasonal shedding patterns of cattle, occurring predominantly in the warm months (LeJeune et al., 2004b; Rangel et al., 2005; Hussein, 2007; Lal et al., 2012). In our study, we did not have sufficient data to assess the impact of season on the concentration of *E. coli* O157 shed in feces. A study conducted in Scotland found that high shedding beef cattle (excreting > 10^4 g^{-1}) shed a greater concentration of *E. coli* O157 in the warmer months (Ogden et al., 2004).

Hides have been established as a major source of carcass contamination during cattle processing (Koohmaraie et al., 2005; Arthur et al., 2007a; Arthur et al., 2008; Bosilevac et al., 2009). The overall estimated prevalence of *E. coli* O157 on hides of fed beef cattle, and estimated seasonal prevalence were comparable when sampled at the slaughter plants and when sampled at the feedlots prior to shipment to the plants. However, the percentage of enumerable hides (≥ 40

CFU/100 cm^2) increased from 1.74% when sampled at the feedlot to 23.81% when sampled at the plant during the summer months. These findings agree with those reported by Arthur et al. (Arthur et al., 2007a; Arthur et al., 2008), who also emphasized that pathogen enumeration rather than prevalence is a more precise indicator of the risk of food contamination (Arthur et al., 2007b) and of human exposure and infection with foodborne pathogens (Hussein, 2007). The difference in the level of *E. coli* O157 on cattle hides sampled on farm and at the plant could be attributed to the transfer of pathogens between cattle and from the surfaces of transport trailers and lairage environments at the processing plants to cattle hides (Arthur et al., 2007a; Arthur et al., 2008; Dewell et al., 2008), and to transportation stress (Barham et al., 2002; Dewell et al., 2008). The level of *E. coli* O157:H7 contamination on the hides of cattle entering the processing facility is of most concern (Nou et al., 2003; Bosilevac et al., 2004) as it plays a large role in carcass contamination rates (Barkocy-Gallagher et al., 2003; Arthur et al., 2004).

Our analysis showed no difference in the estimated prevalence of *E. coli* O157 on pre-evisceration carcasses from fed beef, adult beef or adult dairy cattle following hide removal during the summer or winter months. These estimates represent the level of hide-to-carcass contamination. However, the pooled estimate for percent enumerable carcasses ($\geq$ 0.5 CFU/100 cm^2) was significantly higher during winter in fed beef (Table 2.6). This finding is unusual. It may possibly be related to the variability in levels of pre-evisceration carcass contamination and different interventions applied at this processing point from plant to plant (Arthur et al., 2004; Bosilevac et al., 2009), however the data did not include any plant identification to separate plant and season variability. Additionally, our estimate during the summer months was based on a small sampling from four plants compared to nine plants during the winter months. Following evisceration and application of initial interventions, post-evisceration prevalence was reduced. The estimated post-

evisceration carcass prevalence was significantly higher during the summer than winter months. After the application of final antimicrobial interventions and cooling of the carcasses for between 2 to 29 hours, the pooled prevalence estimates markedly dropped and there was no significant summer to winter differences (Table 2.6). The low level of *E. coli* O157 contamination on the final carcass can be attributed to the effectiveness of the carcass intervention strategies implemented at the processing plant (Elder et al., 2000). Despite the success of multiple-sequential interventions during processing, intermittent outbreaks of *E. coli* O157 continue to occur perhaps due to the low infectious dose in humans (Nataro and Kaper, 1998; Kaper et al., 2004). Additionally, the phenomenon surrounding processing plant event days, where contamination levels are above normal, should be thoroughly researched to identify possible responsible factors. Other sources of human infection, besides consumption of undercooked contaminated ground beef and other meats, are consumption of unpasteurized dairy products, contaminated fresh fruits and vegetables, environment-mediated transmission (increased tendency for outdoor cooking with concomitant lapses in hygiene, recreational water use), and direct transmission (physical human–animal contact such as in petting zoos) (Ferens and Hovde, 2011).

The strengths of this study are that it provided a systematic and robust analysis of current primary research on the prevalence and concentration of *E. coli* O157 along the beef production chain in North America, and measured the degree of heterogeneity among studies reporting similar outcomes. There are some limitations of this study. First, there was no report of prevalence and/or concentration of *E. coli* O157 in fed dairy cattle, thus a comparison of these estimates with those of other cattle types could not be made. Secondly, there were limited studies available for some categories in our analysis, and limited studies to estimate the percentage of enumerable samples of *E. coli* O157 in feces and on hides at feedlots for the summer and winter months, thereby limiting

the precision of the reported estimates. In addition, studies were not available to estimate the prevalence and percent of enumerable carcasses for adult beef and adult dairy. The small number of studies used for some of the subgroup analysis and meta-regression call for caution in the interpretation of some of the estimates due to low statistical precision. Thirdly, imperfection of the sensitivity and specificity of the detection methods further limits the precision of the studies' and overall meta-analysis estimates. Finally, unpublished studies, conference abstracts, and government reports were not included in this systematic review-meta analysis. Though these forms of publications could bring more data into the analysis, these publications rarely contain sufficient information to allow for relevance screening, data extraction and analysis.

From this review, no estimate was found for the prevalence and/or concentration of *E. coli* O157 in fed dairy cattle. Limited data existed for concentration of *E. coli* O157 among other cattle and sample types. Future research should include the estimation of the prevalence and concentration of non-O157 STEC in beef production in order to generate complete and reliable data needed to inform quantitative assessment of the risk of *E. coli* O157 and non-O157 serogroups (O26, O45, O103, O111, O121, O145) in the beef production chain.

Conclusion

The prevalence and concentration of *E. coli* O157 varied along the beef production chain in North America. In feces, the prevalence varied by cattle type and season. The concentration on cattle hide differed at the feedlot and when sampled at the plant. The prevalence decreased substantially from the pre-evisceration carcass to the final chilled carcass demonstrating the effectiveness of post-harvest interventions. The prevalence and concentration estimates from this study, obtained using

systematic review and meta-analysis of similar studies, provide a sound and reliable microbiological basis for risk assessment modeling of *E. coli* O157 in the food chain.

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Figure 2.1 - Flow of information through the different stages of article selection process for the systematic review and meta-analysis

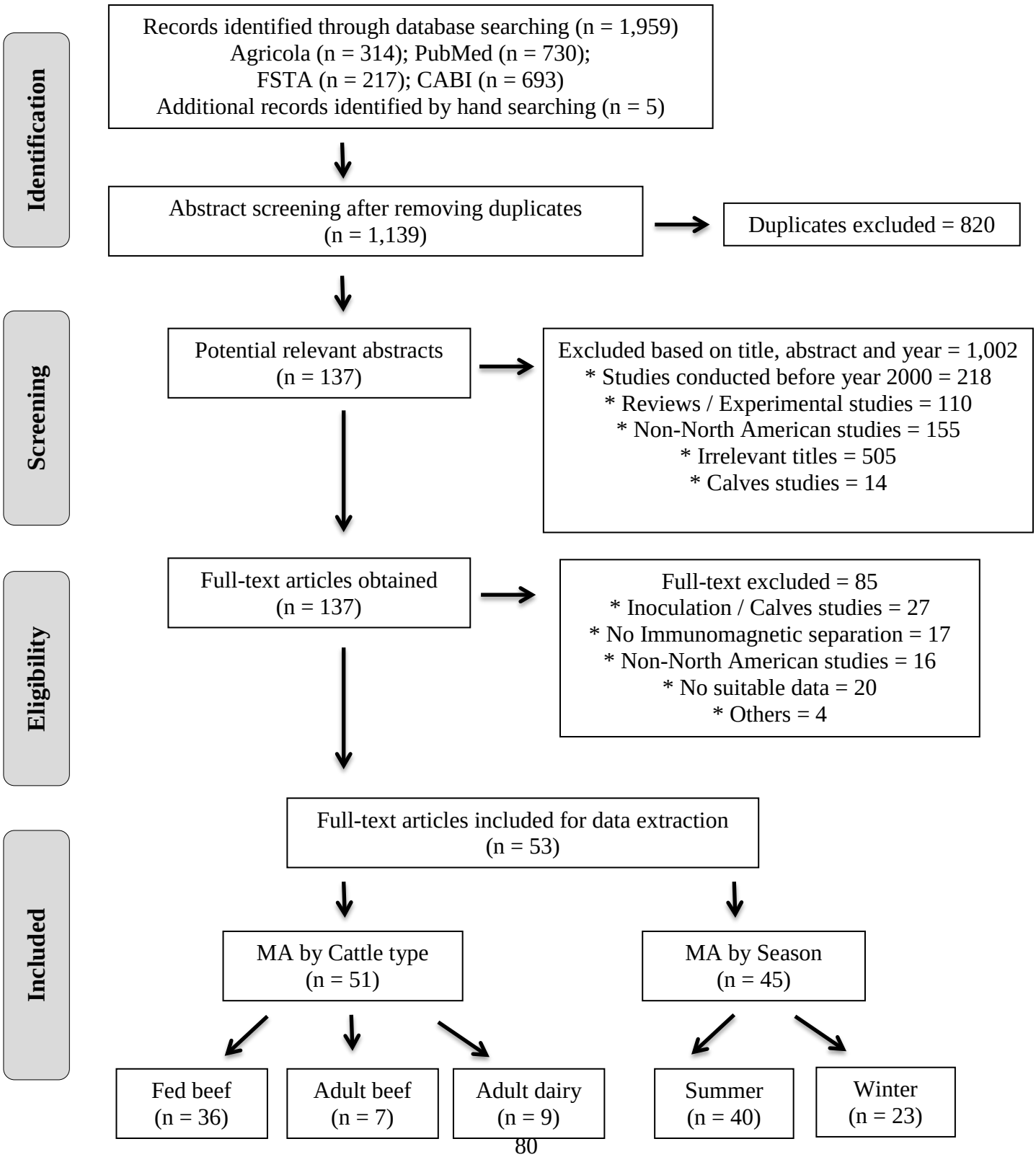

Table 2.1 - List of the articles included in the systematic review meta-analysis by the inclusion criteria

Variable	Articles in MA (#)	Reference
Cattle types	51	
Fed Beef	36	Elder et al., 2000; Smith et al., 2001; Barkocy-Gallagher et al., 2003; Nou et al., 2003; Sargeant et al., 2003; Arthur et al., 2004; Rivera-Betancourt et al., 2004; Bosilevac et al., 2005; Dewell et al., 2005; Greenquist et al., 2005; Alam and Zurek, 2006; Callaway et al., 2006; Childs et al., 2006; Khaitsa et al., 2006; Woerner et al., 2006; Arthur et al., 2007a; Arthur et al., 2007b; Arthur et al., 2007c; Brichta-Harhay et al., 2007; Stephens et al., 2007; Arthur et al., 2008; Brichta-Harhay et al., 2008; Dewell et al., 2008; Fox et al., 2008a; Fox et al., 2008b; Miller et al., 2008; Renter et al., 2008; Bosilevac et al., 2009; Kalchayanand et al., 2009; Stephens et al., 2009; Jacob et al., 2010; Walker et al., 2010; Cernicchiaro et al., 2013; Fink et al., 2013; Narvaez-Bravo et al., 2013; Stanford et al., 2013
Adult Beef	7	Sargeant et al., 2000; Gannon et al., 2002; Riley et al., 2003; Callaway et al., 2004; Branham et al., 2005; Cernicchiaro et al., 2009; Kondo et al., 2010
Adult Dairy	9	Byrne et al., 2003; Callaway et al., 2004; Dunn et al., 2004; Edrington et al., 2004a; Edrington et al., 2004b; Dodson and LeJeune, 2005; LeJeune and Kauffman, 2005; LeJeune et al., 2006; Cernicchiaro et al., 2012
Seasonality	45	
Summer	40	Elder et al., 2000; Smith et al., 2001; Gannon et al., 2002; Barkocy-Gallagher et al., 2003; Byrne et al., 2003; Nou et al., 2003; Sargeant et al., 2003; Arthur et al., 2004; Dunn et al., 2004; Edrington et al., 2004a; Edrington et al., 2004b; Rivera-Betancourt et al., 2004; Bosilevac et al., 2005; Branham et al., 2005; Dewell et al., 2005; Dodson and LeJeune, 2005; LeJeune and Kauffman, 2005; Alam and Zurek, 2006; Callaway et al., 2006; Khaitsa et al., 2006; LeJeune et al., 2006; Arthur et al., 2007a; Arthur et al., 2007b; Arthur et al., 2007c; Arthur et al., 2008; Brichta-Harhay et al., 2008; Dewell et al., 2008; Fox et al., 2008a; Fox et al., 2008b; Renter et al., 2008; Cernicchiaro et al., 2009; Kalchayanand et al., 2009; Jacob et al., 2010; Kondo et al., 2010; Walker et al., 2010; Cernicchiaro et al., 2012; Cernicchiaro et al., 2013; Fink et al., 2013; Narvaez-Bravo et al., 2013; Stanford et al., 2013

Winter	23	Gannon et al., 2002; Barkocy-Gallagher et al., 2003; Riley et al., 2003; Arthur et al., 2004; Callaway et al., 2004; Dunn et al., 2004; Edrington et al., 2004a; Rivera-Betancourt et al., 2004; Branham et al., 2005; Greenquist et al., 2005; Alam and Zurek, 2006; Arthur et al., 2007c; Brichta-Harhay et al., 2008; Renter et al., 2008; Bosilevac et al., 2009; Cernicchiaro et al., 2009; Kalchayanand et al., 2009; Kondo et al., 2010; Walker et al., 2010; Cernicchiaro et al., 2012; Fink et al., 2013; Narvaez-Bravo et al., 2013; Stanford et al., 2013
Country	51	
USA	44	Elder et al., 2000; Sargeant et al., 2000; Smith et al., 2001; Barkocy-Gallagher et al., 2003; Byrne et al., 2003; Nou et al., 2003; Riley et al., 2003; Sargeant et al., 2003; Arthur et al., 2004; Dunn et al., 2004; Edrington et al., 2004a; Edrington et al., 2004b; Rivera-Betancourt et al., 2004; Bosilevac et al., 2005; Branham et al., 2005; Dewell et al., 2005; Dodson and LeJeune, 2005; Greenquist et al., 2005; LeJeune and Kauffman, 2005; Alam and Zurek, 2006; Callaway et al., 2006; Childs et al., 2006; Khaitsa et al., 2006; LeJeune et al., 2006; Woerner et al., 2006; Arthur et al., 2007a; Arthur et al., 2007b; Arthur et al., 2007c; Brichta-Harhay et al., 2007; Stephens et al., 2007; Arthur et al., 2008; Brichta-Harhay et al., 2008; Dewell et al., 2008; Fox et al., 2008a; Fox et al., 2008b; Miller et al., 2008; Bosilevac et al., 2009; Kalchayanand et al., 2009; Jacob et al., 2010; Kondo et al., 2010; Walker et al., 2010; Cernicchiaro et al., 2012; Cernicchiaro et al., 2013; Fink et al., 2013
Canada	5	Gannon et al., 2002; Renter et al., 2008; Cernicchiaro et al., 2009; Stephens et al., 2009; Stanford et al., 2013
Mexico	2	Callaway et al., 2004; Narvaez-Bravo et al., 2013
Study type	51	
Cross sectional	43	Elder et al., 2000; Smith et al., 2001; Barkocy-Gallagher et al., 2003; Byrne et al., 2003; Riley et al., 2003; Sargeant et al., 2003; Arthur et al., 2004; Callaway et al., 2004; Dunn et al., 2004; Edrington et al., 2004a; Rivera-Betancourt et al., 2004; Bosilevac et al., 2005; Branham et al., 2005; Dewell et al., 2005; Dodson and LeJeune, 2005; Greenquist et al., 2005; Callaway et al., 2006; Childs et al., 2006; Khaitsa et al., 2006; LeJeune et al., 2006; Woerner et al., 2006; Arthur et al., 2007a; Arthur et al., 2007b; Arthur et al., 2007c; Brichta-Harhay et al., 2007; Stephens et al., 2007; Arthur et al., 2008; Brichta-Harhay et al., 2008; Dewell et al., 2008; Fox et al., 2008a; Fox et al., 2008b; Renter et al., 2008; Bosilevac et al., 2009; Cernicchiaro et al., 2009; Kalchayanand et al., 2009; Stephens et al., 2009; Jacob et al., 2010; Walker et al., 2010; Cernicchiaro et al., 2012; Cernicchiaro et al., 2013; Fink et al., 2013; Narvaez-Bravo et al., 2013; Stanford et al., 2013

Longitudinal	5	Sargeant et al., 2000; Gannon et al., 2002; LeJeune and Kauffman, 2005; Alam and Zurek, 2006; Kondo et al., 2010
Field trial	3	Nou et al., 2003; Edrington et al., 2004b; Miller et al., 2008
Sample type	51	
Fecal	40	Elder et al., 2000; Sargeant et al., 2000; Smith et al., 2001; Gannon et al., 2002; Barkocy-Gallagher et al., 2003; Byrne et al., 2003; Riley et al., 2003; Sargeant et al., 2003; Callaway et al., 2004; Dunn et al., 2004; Edrington et al., 2004a; Edrington et al., 2004b; Branham et al., 2005; Dewell et al., 2005; Dodson and LeJeune, 2005; Greenquist et al., 2005; LeJeune and Kauffman, 2005; Alam and Zurek, 2006; Callaway et al., 2006; Childs et al., 2006; Khaitsa et al., 2006; LeJeune et al., 2006; Woerner et al., 2006; Arthur et al., 2007a; Brichta-Harhay et al., 2007; Stephens et al., 2007; Arthur et al., 2008; Dewell et al., 2008; Fox et al., 2008a; Fox et al., 2008b; Renter et al., 2008; Cernicchiaro et al., 2009; Stephens et al., 2009; Jacob et al., 2010; Kondo et al., 2010; Walker et al., 2010; Cernicchiaro et al., 2012; Cernicchiaro et al., 2013; Fink et al., 2013; Narvaez-Bravo et al., 2013
Hide	19	Elder et al., 2000; Barkocy-Gallagher et al., 2003; Nou et al., 2003; Arthur et al., 2004; Rivera-Betancourt et al., 2004; Bosilevac et al., 2005; Arthur et al., 2007a; Arthur et al., 2007b; Arthur et al., 2007c; Arthur et al., 2008; Brichta-Harhay et al., 2008; Dewell et al., 2008; Miller et al., 2008; Renter et al., 2008; Bosilevac et al., 2009; Kalchayanand et al., 2009; Jacob et al., 2010; Narvaez-Bravo et al., 2013; Stanford et al., 2013
Carcass	14	Elder et al., 2000; Barkocy-Gallagher et al., 2003; Nou et al., 2003; Arthur et al., 2004; Rivera-Betancourt et al., 2004; Bosilevac et al., 2005; Woerner et al., 2006; Arthur et al., 2007a; Arthur et al., 2008; Brichta-Harhay et al., 2008; Fox et al., 2008a; Bosilevac et al., 2009; Jacob et al., 2010; Narvaez-Bravo et al., 2013

Table 2.2 - Descriptive statistics of the study characteristics assessed in the systematic review-meta analysis of the prevalence and concentration of *E. coli* O157 in the beef chain.

Characteristic	Fecal # Study (%)	Hide # Study (%)		Carcass # Study (%)			
		Feedlot	Plant	Pre-evisceration	Post-evisceration	Post-intervention	Chilled
Cattle type							
Fed beef	163 (57.8)	17 (100)	101(91.0)	82 (89.1)	19 (100)	18 (100)	11 (100)
Adult beef	46 (16.3)	-	-	-	-	-	-
Adult dairy	73 (25.9)	-	-	-	-	-	-
Adult beef and dairy	-	-	10 (9.0)	10 (10.9)	-	-	-
Season							
Winter	46 (16.1)	2 (11.8)	43 (38.7)	35 (38.0)	12 (63.2)	7 (38.9)	7 (63.6)
Summer	240 (83.9)	15 (88.2)	68 (61.3)	57 (62.0)	7 (36.8)	11 (61.1)	4 (35.4)
Study design							
Cross sectional survey	224 (79.4)	16 (94.1)	107 (96.4)	88 (95.7)	19 (100)	18 (100)	11 (100)
Longitudinal	54 (19.2)	-	-	-	-	-	-
Field trial	4 (1.4)	1 (5.9)	4 (3.6)	4 (4.3)	-	-	-
Study setting							
Commercial farm	263 (93.3)	13 (76.5)	111 (100)	92 (100)	19 (100)	18 (100)	11 (100)
Research facility	19 (6.7)	4 (23.5)	-	-	-	-	-
Sampling setting							
Farm	115 (40.8)	-	-	-	-	-	-
Feedlot	115 (40.8)	17 (100)	-	-	-	-	-
Plant	52 (18.4)	-	111 (100)	92 (100)	19 (100)	18 (100)	11 (100)
Country							
USA	253 (89.7)	15 (88.2)	101(91.0)	87 (94.6)	14 (73.7)	18 (100)	6 (54.6)
Canada	22 (7.8)	2 (11.8)	5 (4.5)	-	5 (26.3)	-	-
Mexico	7 (2.5)	-	5 (4.5)	5 (5.4)	-	-	5 (45.4)
Detection method							
IMS	99 (35.1)	-	4 (3.6)	-	-	-	-
IMS PCR	183 (64.9)	17 (100)	107 (96.4)	92 (100)	19 (100)	18 (100)	11 (100)
Area of hide swab (cm^2)							
< 1,000	-	3 (17.6)	44 (39.6)	-	-	-	-
1,000	-	14 (82.4)	67 (60.4)	-	-	-	-
Area of carcass swab (cm^2)							
1,000	-	-	-	29 (32.9)	8 (42.1)	8 (57.1)	-
>1,000	-	-	-	59 (67.1)	11 (57.9)	6 (42.9)	11 (100)

Table 2.3 - Pooled fecal prevalence and concentration of *E. coli* O157 in North American cattle stratified by cattle type, season and country.

Subgroup	# Articles (# studies)	N	Prevalence, %		I^2, % (*P*-value)
			Observed Range	Pooled Estimate (95% CI)	
Fed beef	25 (163)	32,264	0.00 – 55.00	10.68 (9.17–12.28)	94.8 (<0.01)
Meta-analysis by season in North America					
Summer (North America)	19 (140)	22,997	0.00 – 55.00	10.65 (8.93–12.49)	94.6 (<0.01)
Winter (North America)	7 (14)	2,626	0.30 – 27.78	9.17 (5.24–13.98)	92.4 (<0.01)
Meta-analysis of seasonal prevalence by country					
Summer (USA)	17 (136)	22,289	0.00 – 55.00	10.80 (9.11–12.61)	94.2 (<0.01)
Summer (Canada)	1 (1)	600	37.00*	–	–
Summer (Mexico)	1 (3)	108	0.00 – 0.00	0.00 (0.00–1.80)	–
Winter (USA)	5 (9)	2,155	0.30 – 27.78	8.37 (4.37–13.46)	92.2 (<0.01)
Winter (Canada)	1 (3)	390	8.33 – 26.67	13.86 (3.30–28.51)	0.0
Winter (Mexico)	1 (2)	81	3.33 – 7.84	–	–
Adult beef	7 (46)	11,111	0.00 – 26.00	4.65 (3.37–6.10)	86.5 (<0.01)
Meta-analysis by season in North America					
Summer (North America)	4 (17)	5,430	0.00 – 20.00	7.86 (5.43–10.66)	84.9 (<0.01)
Winter (North America)	6 (19)	3,623	0.00 – 26.00	4.21 (1.95–7.13)	87.3 (<0.01)
Meta-analysis of seasonal prevalence by country					
Summer (USA)	2 (7)	260	5.00 – 20.00	11.83 (7.98–16.25)	0.0 (0.65)
Summer (Canada)	2 (10)	5,170	0.00 – 18.00	6.46 (3.93–9.51)	89.7 (<0.01)
Winter (USA)	3 (11)	542	0.00 – 20.00	4.84 (1.50–9.49)	71.6 (<0.01)
Winter (Canada)	2 (7)	2,961	0.00 – 26.00	4.25 (1.04–9.10)	93.7 (<0.01)
Winter (Mexico)	1 (1)	120	0.83*	–	–
Adult dairy	8 (73)	17,168	0.00 – 35.00	1.79 (1.20–2.48)	86.4 (<0.01)
Meta-analysis by season					
Summer (USA)	7 (60)	13,966	0.00 – 35.00	2.27 (1.50–3.18)	88.0 (<0.01)
Winter (North America)	4 (13)	3,202	0.00 – 2.08	0.36 (0.09–0.74)	22.0 (0.22)
Winter (USA)	3 (12)	3,082	0.00 – 2.08	0.31 (0.06–0.67)	18.9 (0.31)
Winter (Mexico)	1 (1)	120	1.67*	–	–
Percentage Enumerable					
Fed beef					
Summer (USA)	2 (9)	818	1.34 – 18.56	5.78 (2.41–10.31)	81.59 (<0.01)

Estimates for subgroup categories with fewer than three studies were omitted. Summer (Apr, May, Jun, Jul, Aug, Sept); Winter (Oct, Nov, Dec, Jan, Feb, Mar); N = number of cattle included in the study; I^2 = between-study heterogeneity; *P*-value = Cochran's Q (chi-square) test of between study heterogeneity; North America = studies conducted in at least any two of Canada, Mexico and USA. *Point prevalence is presented because category contained only one study. Percentage enumerable = number of samples with *E. coli* O157 counts ≥ 200 CFU/g of feces / total number of samples analyzed

Table 2.4 - Pooled prevalence and concentration of *E. coli* O157 in hides of North American cattle, sampled at feedlots, stratified by cattle type and season.

Subgroup	# Articles (# studies)	N	Prevalence, %		I^2, % (*P*-value)
			Observed Range	Pooled Estimate (95% CI)	
Fed beef*: *Hide at feedlot	6 (17)	3,659	1.45–98.00	51.40 (31.97–70.62)	99.2 (<0.01)
Meta-analysis by season in North America					
Summer (North America)	6 (15)	3,057	4.21–98.00	52.01 (31.43–72.25)	99.2 (<0.01)
Winter (North America)	2 (2)	602	1.45–98.00	–	–
Meta-analysis of seasonal prevalence by country					
Summer (USA)	5 (14)	2,059	6.11–98.00	56.39 (34.14–77.40)	98.9 (<0.01)
Summer (Canada)	1 (1)	998	4.21*	–	–
Winter (USA)	1 (1)	50	98.00*	–	–
Winter (Canada)	1 (1)	552	1.45*	–	–
Percentage Enumerable: Hides at feedlot					
Fed beef					
Summer (USA)	2 (9)	181	0.00–7.41	1.74 (0.53–3.44)	49.5 (0.05)

Estimates for subgroup categories with fewer than three studies were omitted. Summer (Apr, May, Jun, Jul, Aug, Sept); Winter (Oct, Nov, Dec, Jan, Feb, Mar); N = number of cattle included in the study; I^2 = between-study heterogeneity; *P*-value = Cochran's Q (chi-square) test of between study heterogeneity; North America = studies conducted in at least any two of Canada, Mexico and USA; *Point prevalence is presented because category contained only one study. Percentage enumerable = number of hide samples with *E. coli* O157 counts ≥ 40 CFU/100 cm^2 / total number of samples analyzed

Table 2.5 - Pooled prevalence and concentration of *E. coli* O157 in hides of North American cattle, sampled at processing plants, stratified by cattle type and season.

Subgroup	# Articles (# studies)	N	Prevalence, %		I^2, % (*P*-value)
			Observed Range	Pooled Estimate (95% CI)	
Fed beef: Hide at plant	16 (101)	12,802	0.00–100.00	56.41 (48.56–64.11)	98.7 (<0.01)
Meta-analysis by season in North America					
Summer (North America)	15 (63)	8,264	0.00–100.00	54.63 (44.50–64.57)	98.8 (<0.01)
Winter (North America)	8 (38)	4,538	0.58–100.00	59.33 (46.76–71.33)	98.6 (<0.01)
Meta-analysis of seasonal prevalence by country					
Summer (USA)	13 (58)	7,523	0.00–100.00	59.04 (48.94–68.78)	98.7 (<0.01)
Summer (Canada)	1 (2)	591	3.69–8.75	–	–
Summer (Mexico)	1 (3)	150	0.00–42.00	12.38 (0.00–44.58)	95.2 (<0.01)
Winter (USA)	6 (33)	3,754	8.51–100.00	69.29 (59.43–78.35)	97.5 (<0.01)
Winter (Canada)	1 (3)	686	0.58–8.82	3.82 (0.57–9.45)	88.9 (<0.01)
Winter (Mexico)	1 (2)	98	2.00–4.17	–	–
Adult beef & dairy	1 (10)	3,040	6.84–90.00	46.39 (31.88–61.22)	98.5 (<0.01)
Meta-analysis by season					
Summer (USA)	1 (5)	1,520	20.53–66.84	45.34 (30.38–60.73)	96.9 (<0.01)
Winter (USA)	1 (5)	1,520	6.84–90.00	47.49 (20.57–75.22)	99.1 (<0.01)
Percentage Enumerable: Hides at processing plant					
Fed beef	5 (19)	3,263	0.00–50.00	19.06 (12.45–26.67)	96.3 (<0.01)
Summer (USA)	3 (10)	980	0.00–41.67	23.81 (14.79–34.15)	92.3 (<0.01)
Winter (USA)	2 (9)	2,283	3.16–50.00	14.50 (6.72–24.56)	97.4 (<0.01)

Estimates for subgroup categories with fewer than three studies were omitted. Summer (Apr, May, Jun, Jul, Aug, Sept); Winter (Oct, Nov, Dec, Jan, Feb, Mar); N = number of cattle included in the study; I^2 = between-study heterogeneity; *P*-value = Cochran's Q (chi-square) test of between study heterogeneity; North America = studies conducted in at least any two of Canada, Mexico and USA; Percentage enumerable = number of hide samples with *E. coli* O157 counts ≥ 40 CFU/100 cm^2 / total number of samples analyzed

Table 2.6 - Pooled pre-evisceration carcass prevalence and concentration of *E. coli* O157 in North American cattle stratified by cattle type and season.

Subgroup	# Articles (# studies)	N	Prevalence, %		I^2, % (*P*-value)
			Observed Range	Pooled Estimate (95% CI)	
Fed beef: Pre-evisceration	11 (82)	8,293	0.00–93.33	17.00 (12.39–22.15)	97.1 (<0.01)
Meta-analysis by season in North America					
Summer (North America)	10 (52)	5,299	0.00–93.33	14.06 (9.24–19.64)	96.6 (<0.01)
Winter (North America)	4 (30)	2,994	0.00–92.63	22.49 (13.45–33.00)	97.6 (<0.01)
Meta-analysis of seasonal prevalence by country					
Summer (USA)	9 (49)	5,149	0.00–93.33	15.25 (10.11–21.17)	96.7 (<0.01)
Summer (Mexico)	1 (3)	150	0.00–4.00	0.65 (0.0–3.91)	35.6 (<0.01)
Winter (USA)	3 (28)	2,894	0.00–92.63	25.10 (15.46–36.13)	97.6 (<0.01)
Winter (Mexico)	1 (2)	100	0.00–0.00	–	–
Adult beef & dairy	1 (10)	3,040	1.05–67.89	14.06 (6.56–23.76)	97.8 (<0.01)
Meta-analysis by season					
Summer (USA)	1 (5)	1,520	5.26–27.89	14.38 (8.34–21.70)	91.8 (<0.01)
Winter (USA)	1 (5)	1,520	1.05–67.89	13.79 (1.38–35.53)	98.9 (<0.01)
Percentage Enumerable: Pre-evisceration Carcass					
Fed beef	4 (18)	3,101	0.00–4.91	1.28 (0.25–2.87)	87.9 (<0.01)
Meta-analysis by season					
Summer (USA)	2 (9)	818	0.00–1.06	0.00 (0.00–0.37)	0.0 (0.98)
Winter (USA)	2 (9)	2,283	0.00–4.91	3.26 (1.01–6.62)	92.8 (<0.01)

Estimates for subgroup categories with fewer than three studies were omitted. Summer (Apr, May, Jun, Jul, Aug, Sept); Winter (Oct, Nov, Dec, Jan, Feb, Mar); N = number of cattle included in the study; I^2 = between-study heterogeneity; *P*-value = Cochran's Q (chi-square) test of between study heterogeneity; North America = studies conducted in at least any two of Canada, Mexico and USA; Percentage enumerable = number of carcass samples with *E. coli* O157 counts ≥ 0.5 CFU/100 cm^2 / total number of samples analyzed

Table 2.7 - Pooled post-evisceration, post-intervention and chilled carcass prevalence of *E. coli* O157 in North American cattle stratified by cattle type and season.

Subgroup	# Articles (# studies)	N	Prevalence, %		I^2, % (*P*-value)
			Observed Range	Pooled Estimate (95% CI)	
Fed beef: Post-evisceration	3 (19)	870	0.00–54.35	5.40 (1.66–10.72)	87.2 (<0.01)
Meta-analysis by season in North America					
Summer (North America)	3 (12)	530	0.00–54.35	8.90 (2.49–18.16)	89.5 (<0.01)
Winter (North America)	2 (7)	340	0.00–10.42	1.25 (0.00–4.09)	54.8 (0.04)
Meta-analysis of seasonal prevalence by country					
Summer (USA)	2 (9)	380	1.85–54.35	13.73 (4.92–25.61)	88.2 (<0.01)
Summer (Mexico)	1 (3)	150	0.00–2.00	0.32 (0.00–2.45)	0.0 (0.45)
Winter (USA)	1 (5)	240	0.00–10.42	2.20 (0.01–6.60)	60.1 (<0.01)
Winter (Mexico)	1 (2)	100	0.00–0.00	–	–
Fed beef: Post-intervention	3 (18)	1,852	0.00–5.41	0.49 (0.04–1.24)	36.3 (0.06)
Meta-analysis by season					
Summer (USA)	3 (11)	975	0.00–5.41	1.02 (0.22–2.20)	21.6 (0.24)
Winter (USA)	2 (7)	877	0.00–2.08	0.04 (0.00–0.49)	0.0 (0.43)
Fed beef: Chilled Carcass	2 (11)	538	0.00–2.00	0.03 (0.00–0.67)	0.0 (0.99)
Meta-analysis by season					
Summer (USA)	2 (4)	198	0.00–2.00	0.19 (0.00–1.83)	0.0 (0.69)
Winter (USA)	2 (7)	340	0.00–0.00	0.00 (0.00–0.70)	0.0 (0.99)

Estimates for subgroup categories with fewer than three studies were omitted. Summer (Apr, May, Jun, Jul, Aug, Sept); Winter (Oct, Nov, Dec, Jan, Feb, Mar); N = number of cattle included in the study; I^2 = between-study heterogeneity; *P*-value = Cochran's Q (chi-square) test of between study heterogeneity; North America = studies conducted in at least any two of Canada, Mexico and USA.

Chapter 3 - Bayesian estimation of true prevalence, sensitivity and specificity of three diagnostic tests for detection of *Escherichia coli* O157 in cattle feces

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Abstract

Cattle are a reservoir for *Escherichia coli* O157 and they shed the pathogen in their feces. Fecal contaminants on the hides can be transferred onto carcasses during processing at slaughter plants, thereby serving as a source of foodborne infection in humans. The detection of *E. coli* O157 in cattle feces is based on culture, immunological, and molecular methods. We evaluated the diagnostic sensitivity and specificity of one culture- and two PCR-based tests for the detection of *E. coli* O157 in cattle feces, and its true prevalence using a Bayesian implementation

of latent class models. A total of 576 fecal samples were collected from the floor of pens of finishing feedlot cattle in the central United States during summer 2013. Samples were enriched and subjected to detection of *E. coli* O157 by culture (immunomagnetic separation, plating on a selective medium, latex agglutination, and indole testing), conventional PCR (cPCR), and multiplex quantitative PCR (mqPCR). The statistical models assumed conditional dependence of the PCR tests and high specificity for culture (mode = 99%; 5^{th} percentile = 97%). Prior estimates of test parameters were elicited from three experts. Estimated posterior sensitivity (posterior median and 95% highest posterior density intervals) of culture, cPCR, and mqPCR was 49.1% (44.8–53.4%), 59.7% (55.3–63.9%), and 97.3% (95.1–99.0%), respectively. Estimated posterior specificity of culture, cPCR, and mqPCR were 98.7% (96.8–99.8%), 94.1% (87.4–99.1%), and 94.8% (84.1–99.9%), respectively. True prevalence was estimated at 91.3% (88.1–94.2%). There was evidence of a weak conditional dependence between cPCR and mqPCR amongst test positive samples, but no evidence of conditional dependence amongst test negative samples. Sensitivity analyses showed that overall our posterior inference was rather robust to the choice of priors, except for inference on specificity of mqPCR, which was estimated with considerable uncertainty. Our study evaluates performance of three diagnostic tests for detection of *E. coli* O157 in feces of feedlot cattle which is important for quantifying true fecal prevalence and adjusting for test error in risk modeling.

Keywords: *Escherichia coli* O157; true prevalence; sensitivity; specificity; diagnostic methods; culture; conventional PCR; quantitative PCR; Bayesian modeling; cattle feces

Introduction

Shiga toxin-producing *Escherichia coli* O157 is a foodborne pathogen of significant impact on beef industry and public health (Callaway et al., 2013). It can cause mild to bloody diarrhea in humans which may progress to hemolytic uremic syndrome (Centers for Disease Control and Prevention, 1993; Hussein, 2007) that can be fatal in children, the elderly and immuno-compromised individuals. In the United States, *E. coli* O157 is responsible for an estimated 63,153 illnesses, 2,138 hospitalizations and 20 deaths annually (Scallan et al., 2011). Cattle are a major reservoir of this pathogen (Gyles, 2007). *E. coli* O157 colonizes the hindgut of cattle and is shed in their feces, which can contaminate cattle hides and the environment (Jacob et al., 2010). Subsequently, hides may serve as main source of contamination of carcasses and beef products during processing (Arthur et al., 2008).

Laboratory methods used for the detection of *E. coli* O157 in cattle feces, on hides, and in beef products include traditional culture methods, immunological, and molecular based diagnostic methods (Barkocy-Gallagher et al., 2002; Deisingh and Thompson, 2004). The culture-based detection method exploits the specific biochemical characteristics of the pathogen for identification. It involves sample enrichment in a selective broth, followed by immunomagnetic bead separation, plating on selective agar medium, and confirmation of isolates by biochemical tests, agglutination, and/or PCR detection of serotype-specific and virulence genes (Elder et al., 2000; Bai et al., 2010; Jacob et al., 2014). Immunological-based detection methods use monoclonal or polyclonal antibodies to capture the pathogen specific antigen. Examples include ELISA-based assays (Thompson et al., 2007; Shen et al., 2014), microplate enzyme immunoassays (Hoefer et al., 2011), and fluorescence polarization assays (Nielsen et al., 2007). Molecular-based detection methods, which amplify serotype-specific DNA targets,

include conventional PCR (Arthur et al., 2005; Islam et al., 2006; Bai et al., 2012; Jacob et al., 2014), real-time / quantitative PCR (Stefan et al., 2007; Jacob et al., 2014; Luedtke et al., 2014; Verstraete et al., 2014; Noll et al., 2015), and loop mediated isothermal amplification (Dong et al., 2014). However, none of these methods constitute gold standards for the detection of *E. coli* O157 in cattle feces, hides, or beef products. Furthermore, quantitative assessment of the diagnostic performance (sensitivity and specificity) of these methods is lacking.

Estimating the sensitivity and specificity of the detection methods is crucial for the evaluation of the true *E. coli* O157 prevalence and the true public health risk. In the United States, estimated apparent prevalence of *E. coli* O157 depends on cattle type and season, and based on a recent systematic review and meta-analysis study, it ranges from 0.0% to 55.0% in cattle feces (Ekong et al., 2015). On hides, estimates of apparent prevalence ranged from 0.0% to 100.0%; on beef carcasses during slaughter, apparent prevalence estimates ranged from 0.0% to 93.3% in pre-eviscerated carcasses, and from 0.0% to 2.0% in chilled carcasses (Ekong et al., 2015). It is possible that due to imperfect sensitivity and specificity of the diagnostic tests currently available, apparent prevalence estimates reported may misrepresent the true prevalence of *E. coli* O157 due to misclassification of some of the tested samples. Therefore, there is need to assess the diagnostic performance of current detection methods using fecal samples from naturally shedding commercial cattle.

In the absence of a gold / reference standard, latent class models implemented through Bayesian estimation procedures have been successfully used to estimate true disease prevalence and diagnostic performance for two or more diagnostic tests applied to samples from test subjects of unknown underlying disease status (Berkvens et al., 2006; Fablet et al., 2010; Haley et al., 2011; Paradis et al., 2012; Paul et al., 2013; Kostoulas et al., 2017). The objective of this

study was to evaluate the diagnostic performance (diagnostic sensitivity and specificity) of culture-based, conventional PCR, and multiplex quantitative PCR tests used for the detection of *E. coli* O157 in cattle feces, and to estimate the true prevalence of *E. coli* O157 in feces of naturally shedding central US feedlot cattle, using latent class models in a Bayesian approach.

Materials and methods

Study population and sample collection

A total of 576 pen floor fecal samples were collected from crossbred finishing cattle, within 24 hours of harvest, from a large commercial finishing feedlot in the central United States from June to August 2013. The feedlot was visited weekly during a 12 week period (Dewsbury et al., 2015). Sample size was calculated to detect seasonal differences in prevalence and full complements of samples were collected at each visit. Two pens of cattle were sampled during each visit and 24 freshly excreted fecal samples were collected from each pen floor. In most cases, samples were collected from cattle observed to defecate. The presence of *E. coli* O157 in cattle is not associated with any clinical disease. The average number of finishing cattle housed per pen at this feedlot during the study period was 270 (range: 121- 299). About 10 grams of freshly excreted feces were collected in individual plastic bags (WHIRL-PAK®; Nasco, Fort Atkinson, WI) using plastic spoons, then shipped on ice to the Pre-harvest Food Safety Laboratory at Kansas State University for processing within 36 hours of sample collection. For each sample, approximately 2 grams of feces were added to 18 mL of *E. coli* enrichment broth, vortexed, and incubated for 6 hours at 40ºC. The enriched fecal suspension obtained from individual samples was used for the three diagnostic tests evaluated here which were run concurrently.

Culture-based testing

A culture-based test previously described by Dewsbury et al. (2015) was used. Briefly, the enriched fecal suspension was subjected to immunomagnetic separation (IMS) using O157-specific IMS beads (Abraxis, LLC, Warminster, PA). The IMS bead suspension was spread plated onto Sorbitol MacConkey agar with cefixime and potassium tellurite. Putative O157 colonies from the blood agar were tested for the O157 antigen by latex agglutination and if positive, tested for indole production. For this analysis, samples were considered positive for *E. coli* O157 serogroup by culture if recovered isolates tested positive for O157 antigen by latex agglutination and indole positive.

Conventional PCR testing

A multiplex conventional PCR (cPCR) assay targeting *rfb*E_{O157}, *stx*1, *stx*2, and *eae* genes (Bai et al., 2010; 2012) was used. DNA was extracted from the enriched fecal suspension using GeneClean DNA extraction kit (MP Biomedicals, Solon, OH), then subjected to the cPCR assay. The amplified DNA was separated on a capillary electrophoresis system in a QIAxcel Advanced System (Qiagen, Valencia, CA) and was analyzed using QIAxcel Screengel software. For this analysis, samples were considered positive for *E. coli* O157 serogroup if the DNA extracted from enriched fecal suspensions tested positive for the *rfb*E_{O157} gene.

Multiplex quantitative PCR testing

A multiplex quantitative PCR (mqPCR) assay targeting *rfb*E_{O157}, *stx*1, *stx*2, and *eae* genes (Noll et al., 2015) was used. DNA was extracted from the enriched fecal suspension as described above, and then subjected to the mqPCR assay. Samples with amplification of the

*rfb*E$_{O157}$ gene target below a cycle threshold (Ct) value of ≤ 38.1 were considered positive for *E. coli* O157. For this analysis, samples were considered positive for *E. coli* O157 serogroup if the DNA extracted from enriched fecal suspensions tested positive for *rfb*E$_{O157}$ gene. The detection methods have been previously validated for their analytical performance (Bai et al., 2012; Jacob et al., 2014; Noll et al., 2015)

Statistical analysis

Model description

The Bayesian analysis approach described by Branscum et al. (2005) was applied here to estimate the sensitivity and specificity of the three tests of interest, and also the true prevalence of *E. coli* O157 in cattle feces. Briefly, the Bayesian estimation procedure combines prior knowledge about the unknown parameters of interest with data obtained from sample testing (i.e. likelihood function) to produce updated posterior distributions for parameters of interest. For our analysis, cPCR and mqPCR tests were considered to be conditionally dependent because the two tests have a similar biologic basis; however, culture and the two PCR tests were considered conditionally independent because the two tests measure different biological attributes (biochemical activity vs DNA content). Conditional dependence between cPCR and mqPCR was explicitly modeled and estimated as a conditional correlation (Gardner et al., 2000; Branscum et al., 2005). The model required the estimation of nine parameters, namely sensitivity and specificity of the three tests, true prevalence, and the conditional correlation between cPCR and mqPCR amongst test positive (rhoD-Se) and amongst test negative (rhoD-Sp) samples. So specified, the likelihood for this model is non-identifiable because the data consists of eight (2^3, i.e., two possible outcomes [positive or negative] from each of three detection methods) ‘classes’

of test results, thereby allowing for a maximum of seven estimable parameters (Branscum et al., 2005). To ensure identifiability of all model parameters, proper informative priors were incorporated for apparent prevalence, sensitivity, and specificity of the three tests (Dendukuri and Joseph, 2001; Branscum et al., 2005; Berkvens et al., 2006).

Prior specifications

External prior information on sensitivity of culture, cPCR, and mqPCR, and on specificity of cPCR and mqPCR were elicited from three experts (a microbiologist, a molecular microbiologist, and an epidemiologist) individually through face-to-face interviews. In order to inform priors for test sensitivity, the experts were asked to give their best estimate of the most likely number of samples that each test would detect as positive given a hypothetical set of 100 known *E. coli* O157 positive fecal samples and the minimum number out of the 100 known positive samples that they were 95% sure the test could recognize as positive. For specifying priors on test specificity, experts were also asked to give their best estimate of the most likely number of samples that each test would detect as negative given a hypothetical set of 100 true negative samples and the minimum number out of the 100 known true negative samples that they were 95% sure the test could recognize as negative. Next, consensus prior information was generated from that elicited individually from the three experts. Each expert and consensus prior information was used to specify mode and 5th percentile of a beta distribution [Beta (a,b)] using the Beta Buster© software (http://betabuster.software.informer.com/1.0/), which in turn derived the corresponding a and b hyper parameters (Jones and Johnson, 2014). The prior apparent prevalence of *E. coli* O157 in feedlot cattle feces was specified as having a mode of 50% and a 5th percentile as low as 10%, corresponding to beta parameters a = 1.53 and b = 1.53. Such vague

prior specification was based on previous evidence that the fecal prevalence of *E. coli* O157:H7 within a pen or group of feedlot cattle can vary widely from 0% to > 80% among pens and over time (Sargeant et al., 2003; Renter et al., 2008). The data set represented 24 pens of cattle so we established our prior based on the expected average prevalence of 24 pens of cattle. The probability of obtaining extremely low or high prevalence for *E. coli* O157:H7 in samples of cattle feces from 24 pens is much lower compared to probability of obtaining values closer to the mode. In all cases, the culture method was considered to be highly specific (Cernicchiaro et al., 2011; Jacob et al., 2014) and modeled using a beta prior distribution with mode 99% and 5th percentile of 97% (a = 212.12 and b = 3.13). The prior information on sensitivity of the three tests and specificity of cPCR and mqPCR yielded by consensus of experts was used for the Bayesian analysis in this study and is summarized in Table 3.1. The models derived using the consensus prior information are here referred to as the consensus model (CM).

Sensitivity analysis

Sensitivity to prior specification using consensus information was assessed by comparing posterior inference with models that fitted widened priors reflecting greater uncertainty relative to the priors elicited by experts and their consensus (Kostoulas et al., 2006; Praud et al., 2012; Rahman et al., 2013). For parameters defining sensitivity of culture, cPCR and mqPCR, and specificity of cPCR and mqPCR, widened priors were generated by using the smallest values of prior mode elicited by the three experts and by halving the value of the 5th percentile of the consensus prior for each parameter. Our sensitivity analysis compared posterior inference for three models that considered widened priors and combinations of widened and consensus priors (Table 3.1). Specifically, sensitivity analysis model 1 (SM1) used widened priors for sensitivity

of culture, cPCR and mqPCR, and specificity of cPCR and mqPCR. Sensitivity analysis model 2 (SM2) used consensus priors for sensitivity of culture, cPCR and mqPCR, and widened priors for specificity of cPCR and mqPCR. Sensitivity analysis model 3 (SM3) used widened priors for sensitivity of culture, cPCR and mqPCR, and consensus priors for specificity of cPCR and mqPCR. Priors for the apparent prevalence of *E. coli* O157 and for specificity of culture were specified as previously described in all cases.

Software implementation and posterior inference

All models were fitted using OpenBUGS© version 3.2.3 rev 1012 (Thomas et al., 2006), with an R software (R Core Team, 2015) interphase through the "R2OpenBUGS" (Sturtz et al., 2005) package. For each model, three parallel Markov Chain Monte Carlo chains were run, each consisting of a burn-in period of 10,000 iterations followed by 100,000 saved iterations for posterior inference. Chain convergence was assessed by visual inspection of the Gelman-Rubin diagnostic plots and evaluation of the corresponding statistic (Gelman and Rubin, 1992). Convergence diagnostics were implemented using the "coda" (Plummer et al., 2006) package in R. Posterior inference on sensitivity and specificity for each of the three tests, on the conditional correlation between cPCR and mqPCR, and on the true prevalence was reported as posterior median and corresponding 95% highest posterior density intervals (HPDI), defined as the narrowest possible interval containing 95% of the posterior samples. In other words, HPDI is the collection of the most likely values of a parameter (Christensen et al., 2011). We can interpret a 95% HPDI as the interval within which we can be 95% sure that the parameter value of interest lies.

Model assessment

The relative fit of competing models to the data can be compared using the deviance information criterion (DIC) (Spiegelhalter et al., 2002) and the log pseudo marginal likelihood (LPML) (Christensen et al., 2011), both of which assess which of a subset of models fit the data better. DIC is a Bayesian measure of the model goodness-of-fit penalized by a complexity term *p*D interpreted as effective number of parameters (Spiegelhalter et al., 2002). Models with a lower DIC are considered better fitting to data (Spiegelhalter et al., 2003), with differences of at least 5 to 10 units indicating substantial improvement in model fit (Lunn et al., 2012). In turn, LPML is a posterior predictive statistic that is useful as an overall measure of model fit (Geisser and Eddy, 1979). It is derived from conditional predictive ordinate (CPO) statistics (Gelman et al., 2014), and expressed as $LPML = \sum_{i=1}^{n} \log(CPO_i)$, where *n* is the number of classes of test results as illustrated in table 3.2. The model with largest LPML is preferred (Christensen et al., 2011). Lack of fit of a proposed model to data can be assessed in absolute terms using the Bayesian *p*-value, which is a posterior predictive check statistic based on the difference between the deviance of the realized observations and the deviance of predictions generated from the fitted model (Berkvens et al., 2006). A model that adequately fits the data is expected to have a Bayesian *p*-value of around 0.50 (Rahman et al., 2013). As a rule of thumb, there is no evidence for lack of model fit when the Bayesian *p*-value is between 0.30 and 0.70 (Smit, 2013) and well away from the extremes at 0 and 1 (Gelman et al., 2014).

Results

Descriptive statistics on data

In total, 576 cattle pen floor fecal samples were analyzed by culture, cPCR, and mqPCR assays. The cross classified test results of the three detection methods for *E. coli* O157 are shown

in Table 3.2. Overall, 44.3% (255/576), 54.7% (315/576), and 89.8% (517/576) of enriched samples were positive by culture, cPCR, and mqPCR, respectively, though only 27.8% (160/576) of samples were positive by all three methods. In turn, 8.5% (49/576) of samples were negative by all three methods, whereas 91.5% (527/576) were positive by at least one detection method. For the culture method, a total of 98.0% (250/255) of samples that tested positive for O157 antigen by latex agglutination and indole tests were confirmed positive for *rfb*EO157 gene by PCR. A total of 97.3% (248/255) of samples that tested positive for O157 antigen (O157 serogroup) were also positive for *stx*1 and/or *stx*2 genes (O157 STEC). A total of 96.9% (247/255) of samples that tested positive for O157 antigen also tested positive for *eae*, *stx*1 and/or *stx*2 *genes* (O157 EHEC). Similarly, 96.9% (247/255) of samples that tested positive for O157 antigen also tested positive for the *eae*, *fli*C_{H7}, and at least one *stx* gene (O157:H7 EHEC). PCR based methods identified *E. coli* O157 based on the presence or absence of the *rfb*EO157 gene in the sample. Other genes (*stx*1, *stx*2, *eae*) may originate from non-O157 *E. coli* that may also be present in the sample, therefore they were not considered during analysis.

Bayesian posterior inference on diagnostic performance and true prevalence

Consensus priors based on information elicited independently from three experts were used in model fitting to obtain the posterior median and 95% HPDI used in assessing diagnostic performance of the three detection methods and true prevalence of *E. coli* O157 (Table 3.3). Based on these data, mqPCR yielded the greatest sensitivity (97.3%, HPDI: 95.1%–99.0%), followed by cPCR (59.7%, HPDI: 55.3%–63.9%); culture was the least sensitive of the detection methods (49.1%, HPDI: 44.8%–53.4%). All the three methods were relatively specific for *E. coli* O157, as indicated by the magnitude of their posterior medians (≥94.1%) and that of the lower

bound of the HPDI (≥84.1%). The true prevalence (sample level) of *E. coli* O157 in the study population was estimated to be 91.3% (HPDI: 88.1%–94.2%). There was evidence of conditional dependence between cPCR and mqPCR amongst test positive samples as the 95% HPDI of the correlation coefficient (i.e. rhoD-Se) did not include the null value of zero (Table 3.4). However, there was no evidence for conditional dependence between cPCR and mqPCR amongst test negative samples as the 95% HPDI of the correlation coefficient (i.e. rhoD-Sp) did include zero (Table 3.4). The posterior probability of rhoD-Sp was significantly different than zero.

Sensitivity analyses and model comparisons

Model fit statistics obtained from our sensitivity analyses indicated that competing models with either consensus or widened priors yielded comparable fit to the data (Table 3.3). Bayesian *p*-values for all models considered were in the range of 0.45–0.66, indicating no evidence for lack of fit of any of the models; in fact, all models seemed to fit the data reasonably well. Further, the differences in DIC between the consensus model and any of the sensitivity analysis models considered was 2.1 units, while their difference in LPML was less than 0.7 units, indicating no evidence for substantial differences in fit across models. Diagnostic sensitivity of all three diagnostic methods, specificity of cPCR, and true prevalence of *E. coli* O157 showed posterior medians of comparable numerical magnitude and overlapping 95% HPDI across the consensus prior model and the sensitivity analysis models considered (Table 3.3). However, the width of the 95% HPDI for specificity of cPCR and for true prevalence seemed to differ substantially between models, whereby SM1 and SM2 had the lowest posterior precision (i.e. wider intervals), consistent with the more diffuse priors specified on these models. Also,

posterior inference on specificity of the mqPCR method was sensitive to prior specification both in terms of point estimates and uncertainty estimation (Table 3.3). A lower posterior median and a wider 95% HPDI were obtained for specificity of mqPCR when wider, more diffuse priors were specified for this parameter. This result is consistent with the fact that only a few samples tested negative for *E. coli* O157 using mqPCR (Table 3.2), thereby indicating limited information available in the data for mqPCR specificity such that the influence of the prior was substantial.

Discussion

This is the first reported study using a Bayesian analysis framework to estimate the sensitivity and specificity of culture, cPCR, and mqPCR methods for detection of *E. coli* O157 in cattle feces. Posterior estimates of diagnostic sensitivity for the three detection methods seemed to differ, mqPCR being the most sensitive, followed by cPCR, and lastly culture. The posterior estimate of sensitivity of mqPCR was approximately twice as much as that of culture. This finding is in agreement with the study of Noll et al. (2015) which found a disagreement between the proportions of positive samples detected by these methods based on Cohen's K statistics and the McNemar's chi-square. Further, the optimum diagnostic sensitivity and specificity of cPCR was reported to be 85.7% and 96.9%, respectively at a Ct value of 31 based on receiver operating characteristic curve analysis (Noll et al., 2015). In a 2014 study conducted using fecal samples from cattle farms in Belgium (Verstraete et al., 2014), the diagnostic sensitivity and specificity of a quantitative PCR (qPCR) assay were estimated to be 82.8% (95% CI: 64.2–94.1%) and 76.7% (95% CI: 57.7–90.0%), respectively, using culture method as a reference standard. Also, based on culture as a reference standard, Jacob et al. (2014) compared multiplex real-time (mqPCR) and conventional PCR (cPCR) methods (both targeting the *rfb*E_{O157}, *stx*1, and *stx*2 genes) to a

culture-based method for detection and quantification *E. coli* O157 in cattle naturally shedding high ($\geq 10^4$ CFU/g of feces) and low ($\leq 10^2$ CFU/g of feces) concentrations of *E. coli* O157 in their feces. Their study estimates for diagnostic sensitivity of mqPCR and cPCR were 64% and 54%, respectively and diagnostic specificity were 60% and 71%, respectively. These estimates fall mostly within the ranges observed in our study for sensitivity of cPCR, specificity of cPCR and mqPCR, which assumed no gold standard among the three methods. Culture methods used for detection of *E. coli* O157 in cattle feces have very high specificity but limited sensitivity. Various estimates have been reported for the sensitivity of different culture-based methods. For example, Cernicchiaro et al. (2011) reported sensitivities of 72.9% (95% CI: 66.9–78.3%), 70.2% (95% CI: 64.1–75.9%), and 29.6% (95% CI: 23.9–35.7%) for rectoanal mucosal swab immunomagnetic separation (RAMS-IMS), fecal-IMS, and direct plating protocols, respectively for *E. coli* O157 at the sample level. These estimates were based on logistic regression modeling and assumed parallel interpretation of the three protocols (positive by at least one of RAMS-IMS, fecal-IMS, and direct plating) as the gold standard. Williams et al. (2014) reported diagnostic sensitivity of 67.0% (95% CI: 59.6–73.1%) for fecal-IMS relative to RAM-IMS. The estimates of sensitivity of the fecal-IMS protocol reported by Cernicchiaro et al. (2011) and Williams et al. (2014) were higher than the estimates obtained for the sensitivity of the culture method from our consensus and sensitivity analysis models. It is important to note that not all culture or PCR methods reported in the literature are equivalent, i.e., culture may include direct plating, fecal-IMS, RAMS-IMS, or spiral plating while PCR may differ in their targeted genes. Unlike previous studies (Cernicchiaro et al., 2011; Jacob et al., 2014; Verstraete et al., 2014; Williams et al., 2014), by implementing a latent class modeling approach, we were able to estimate the performance of the three methods without a gold standard. This method removed

naturally accounts for diagnostic uncertainty and removes the biases associated with using a single imperfect test as reference standard when estimating tests performance (Toft et al., 2007).

The estimated true prevalence of *E. coli* O157 in the study population was found to be high (91.3%). This estimate is consistent with previous reports of *E. coli* O157 shedding in pens of feedlot cattle, especially during the summer months (Smith et al., 2001; Keen and Elder, 2002; Khaitsa et al., 2003; Renter et al., 2008). The estimated true prevalence is also higher than the apparent prevalence detected by culture (44.3%) and cPCR (54.7%) methods, but similar to mqPCR (89.8%). This is not surprising since the median posterior estimates of specificities of both culture and cPCR methods are high (91.1 – 98.7%), implying few false positive results while the median posterior estimate for sensitivity of mqPCR is high implying few false negatives. The prevalence of *E. coli* O157 estimated in our study represents animal level true prevalence as the majority of the samples were collected from cattle observed to be defecating at the time of sample collection.

In this study, weak conditional dependence between cPCR and mqPCR among test positive samples and no evidence for dependence among test negative samples were observed. While both PCR tests are based on amplification of bacterial DNA, differences in cPCR and mqPCR reaction chemistry as well as differences in post-PCR analyses of these methods result in a substantial increase (~100-fold) in sensitivity for mqPCR compared to cPCR (Biassoni and Raso, 2014). This may explain the weak correlation observed between the two PCR among test positive samples. There were few samples tested as negative by the mqPCR method; this may help explain the wider, less precise, 95% HPDI for the coefficient of conditional dependence between mqPCR and cPCR for test negative samples (rhoD-Sp), which overlapped with zero for

all models. Given the limited information available for true negative parameters, the 5% level of significance may be too restrictive and a more liberal threshold might need to be considered.

In the consensus model, the computed Bayesian p-value (0.66) indicated adequate model fit to the data, thus supporting compatibility between prior and observed data. The effective number of parameters estimated by the model (*p*D) was positive (4.73), thereby supporting use of DIC to compare fit between competing models. As shown in the table 3.3, neither DIC nor LPML were particularly informative to discriminate between the models as DIC differences amongst the models were < 5 and LPML difference < 1 unit. Choosing to report only the model with the lowest DIC could be misleading especially when the competing models produces posterior estimates that are very different even if the DIC difference amongst models is < 5 units (Lunn et al., 2012). LPML does not suffer from the same limitations as DIC. In all cases considered here, model selection based on DIC was aligned with that of LPML, thus removing concerns about the validity of DIC for these models. A sensitivity analysis was used to determine the impact of consensus prior information on posterior inference of performance of the diagnostic tests. The posterior distributions of true prevalence, sensitivity of the three detection methods, and specificity of cPCR overlapped substantially across the competing models considered regardless of consensus or widened priors, thus suggesting robust inference to prior specification. Yet, inference on specificity of mqPCR showed considerable sensitivity to prior specification, as indicated by a markedly decreased posterior median and wider 95% HPDI when widened priors were used for specificity of cPCR and mqPCR. That specificity of mqPCR was sensitive to prior specification is not entirely unexpected as only few samples tested negative by the mqPCR method, thus indicating limited available information in the data to make inference on this parameter. Interestingly, this sensitivity to prior specification was not apparent from

indicators of model fit. The Bayesian *p*-value obtained for the consensus and the sensitivity analyses models ranged from 0.45 – 0.66 indicating no evidence of lack of fit for any of the models. Also, values of DIC and LMPL obtained for the consensus model and sensitivity analysis models (SM1-SM3) were comparable.

Conclusion

This study evaluated the diagnostic performance of culture, cPCR, and mqPCR methods for the detection of *E. coli* O157 in feces of feedlot cattle in central USA. These results showed that sensitivity estimates for the detection of *E. coli* O157 in cattle feces was highest for mqPCR, followed closely by cPCR, and last by culture. The estimated sensitivity for mqPCR was approximately twice as high as the estimated sensitivity for culture. The cPCR and mqPCR had comparable specificity in the consensus model, but a sensitivity analysis revealed that posterior inference on specificity of mqPCR method was heavily dependent on prior specification; this is likely associated with the few number of negative test results obtained by the mqPCR. There was evidence of weak conditional dependence between the two PCR methods amongst test positive samples. These data provide important estimates of test performance for calculating true prevalence of *E. coli* O157 in feedlot cattle and adjusting for test error in risk modeling.

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Table 3.1 - Elicitation of prior information on diagnostic performance of culture, conventional PCR (cPCR), and multiplex quantitative PCR (mqPCR) for detection of *Escherichia coli* O157 in feces of finishing feedlot cattle in central USA

Source	Variable	Culture		Conventional PCR		Multiplex quantitative PCR	
		Most likely	5th percentile	Most likely	5th percentile	Most likely	5th percentile
Expert 1	Sensitivity	0.80	0.70	0.70	0.50	0.95	0.90
	Specificity			0.90	0.80	1.00	0.95
Expert 2	Sensitivity	–	–	0.95	0.80	0.95	0.80
	Specificity			0.95	0.80	0.95	0.80
Expert 3	Sensitivity	0.70	0.40	0.70	0.30	0.90	0.50
	Specificity			0.99	0.88	0.99	0.88
Expert consensus	Sensitivity	0.75	0.55	0.78	0.53	0.93	0.73
	Specificity			0.95	0.83	0.98	0.88
Sensitivity model 1	Sensitivity	0.70	0.27	0.70	0.26	0.90	0.36
	Specificity			0.90	0.42	0.95	0.44
Sensitivity model 2	Sensitivity	0.75	0.55	0.78	0.53	0.93	0.73
	Specificity			0.90	0.42	0.95	0.44
Sensitivity model 3	Sensitivity	0.70	0.27	0.70	0.26	0.90	0.36
	Specificity			0.95	0.83	0.98	0.88

Sensitivity model 1 used the lowest most likely value of the three experts prior, and halved consensus prior 5th percentile for sensitivity of culture, cPCR, and mqPCR, and specificity of cPCR and mqPCR; Sensitivity model 2 used the lowest most likely value of the three experts prior, and halved consensus prior 5th percentile for specificity of cPCR and mqPCR but consensus priors for sensitivity of culture, cPCR, and mqPCR; Sensitivity model 3 used the lowest most likely value of the three experts prior, and halved consensus prior 5th percentile for sensitivity of culture, cPCR, and mqPCR but consensus priors for specificity of cPCR and mqPCR.

Table 3.2 - Tabulated detection results for *Escherichia coli* O157 in feces of finishing feedlot cattle in central USA based on culture, conventional PCR, and multiplex quantitative PCR diagnostic methods

Culture	Conventional PCR	Multiplex quantitative PCR	Number
1	1	1	160
1	1	0	1
1	0	1	88
1	0	0	6
0	1	1	151
0	1	0	3
0	0	1	118
0	0	0	49
Total			576

1: Positive; 0: Negative

Table 3.3 - Model fit statistics and posterior inference (i.e. posterior median and 95% HPDI) on true prevalence, sensitivity and specificity of culture, conventional PCR, and multiplex quantitative PCR obtained from consensus prior model and its sensitivity analysis for the detection of *Escherichia coli* O157 in feces of finishing feedlot cattle in central USA

Prior set	Models and Tests	Bayesp	*p*D	DIC	LPML	True prevalence (95% HPDI)	Sensitivity (95% HPDI)	Specificity (95% HPDI)
CM	Consensus prior (CP) for Se & Sp	0.66	4.73	46.57	–936.85	91.3 (88.1, 94.2)		
	Culture						49.1 (44.8, 53.4)	98.7 (96.8, 99.8)
	cPCR						59.7 (55.3, 63.9)	94.1 (87.4, 99.1)
	mqPCR						97.3 (95.1, 99.0)	94.8 (84.1, 99.9)
SM1	Widened prior for Se & Sp	0.47	5.12	44.78	–936.30	86.3 (77.3, 93.4)		
	Culture						51.2 (45.1, 58.2)	98.6 (96.8, 99.8)
	cPCR						61.9 (56.2, 67.8)	91.9 (74.6, 99.9)
	mqPCR						97.3 (95.0, 99.1)	60.9 (35.0, 94.7)
SM2	CP for Se; Widened for Sp	0.45	4.63	44.15	–936.22	84.5 (72.2, 93.2)		
	Culture						52.9 (45.8, 61.8)	98.6 (96.8, 99.8)
	cPCR						63.1 (57.2, 68.9)	91.1 (69.2, 99.9)
	mqPCR						97.3 (95.1, 99.0)	54.2 (26.7, 88.2)
SM3	CP for Sp; Widened for Se	0.65	4.87	46.59	–936.87	91.3 (88.1, 94.2)		
	Culture						48.3 (43.9, 52.7)	98.6 (96.8, 99.8)
	cPCR						59.3 (54.9, 63.6)	94.0 (87.3, 99.1)
	mqPCR						97.4 (95.1, 99.2)	95.0 (84.9, 99.9)

CM: Expert consensus model; SM: Sensitivity analysis model; Se: Sensitivity; Sp: Specificity; cPCR: Conventional PCR; mqPCR: Multiplex quantitative PCR; Bayesp: Bayesian *p*-value; *p*D: statistic for effective number of parameters estimated by the model, used for computation of DIC; DIC: Deviance Information Criterion; LPML: Log Pseudo Marginal Likelihood

Table 3.4 - Posterior median and 95% HPDI of conditional correlations between conventional PCR and multiplex quantitative PCR methods among test positive and test negative samples

Prior set model	Correlation coefficient	
	rhoD-Se	rhoD-Sp
Expert consensus model	0.13 (0.02, 0.23)	0.26 (–0.07, 0.74)
Sensitivity model 1	0.14 (0.02, 0.25)	0.16 (–0.19, 0.59)
Sensitivity model 2	0.14 (0.03, 0.25)	0.17 (–0.19, 0.52)
Sensitivity model 3	0.13 (0.01, 0.23)	0.27 (–0.07, 0.74)

rhoD-Se: Conditional correlation between cPCR and mqPCR among test positive samples;
rhoD-Sp: Conditional correlation between cPCR and mqPCR among test negative samples

Chapter 4 - Bayesian estimation of sensitivity and specificity of culture- and PCR-based methods for the detection of six major non-O157 *Escherichia coli* serogroups in cattle feces

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Abstract

Non-O157 Shiga toxin-producing *Escherichia coli* (non-O157 STEC) are foodborne pathogens of public health importance. Cattle serve as the principal reservoir, shedding these bacteria in their feces and contaminating the environment. In May 2012, the United States Department of Agriculture, Food Safety and Inspection Service declared six non-O157 STEC (O26, O45, O103, O111, O121, and O145) as adulterants in ground beef and non-intact beef products. Culture and PCR-based methods—conventional (cPCR) and multiplex quantitative PCR (mqPCR)—have since been developed for the detection of these serogroups in cattle feces. The objectives of this study were to evaluate the diagnostic sensitivity and specificity of PCR- and culture-based methods used for the detection of the six non-O157 serogroups, and to estimate their true prevalence in cattle feces, using a Bayesian latent class modeling approach that accounts for conditional dependence among the three methods. Fecal samples collected from 576 cattle in a commercial feedlot in the summer of 2013 were used. Fecal samples, suspended

in *E. coli* broth, were enriched and subjected to three detection methods: culture (involving immunomagnetic separation with serogroup specific beads and plating on a selective medium), cPCR, and mqPCR assays. Samples were considered serogroup positive if the sample or the recovered isolate tested positive by PCR for an O gene of interest; neither Shiga toxin (*stx*) nor intimin (*eae*) genes were assessed. External prior information on the performance of the three methods was elicited from three subject experts. Diagnostic sensitivity of culture method was estimated using posterior medians as 38.0%, 53.3%, 69.2%, 49.2%, 46.1%, and 71.9% for serogroups O26, O45, O103, O111, O121, and O145, respectively. For cPCR, the posterior median diagnostic sensitivity was 70.1%, 53.5%, 64.8%, 70.3%, 75.9%, and 71.9%; for mqPCR, it was 91.2%, 93.2%, 95.7%,80.9%, 87.8% and 87.2%, both in the same order as culture method. The diagnostic specificity of cPCR for serogroups O26, O45, O103, O111, O121, and O145 had posterior medians at 94.0%, 96.4%, 96.4%, 98.9%, 81.0%, and 99.2%, respectively, and at 89.7%, 62.2%, 92.3%, 98.8%, 46.5%, and 95.7%, respectively for mqPCR. Posterior medians for true prevalence were 58.5%, 28.9%, 85.8%, 0.2%, 4.5%, and 2.2% for serogroup O26, O45, O103, O111, O121, and O145, respectively. Sensitivity analysis indicated that posterior inferences on specificity of cPCR and mqPCR and on sensitivity of culture for O26 and O103, as well as on sensitivity of culture, cPCR and mqPCR for O111, O121, and O145, and sensitivity of culture for O45 were susceptible to prior specification due to few or no detections available in the data for selected combinations of diagnostic methods (i.e. extreme category problem). Our results characterize performance of detection methods and true prevalence of non-O157 serogroups, thus informing necessary adjustments for test bias in risk modeling.

Keywords: Non-O157 *Escherichia coli*; true prevalence; sensitivity; specificity; diagnostic methods; culture; conventional PCR; quantitative PCR; Bayesian modeling; cattle feces

Introduction

Shiga toxin–producing *Escherichia coli* (STEC) are major foodborne pathogens with significant negative impact on the beef industry, food safety, and public health. In the United States, STEC cause more than 175,000 human illnesses, over 2,000 hospitalizations, and 20 deaths each year (Scallan et al., 2011; Scharff, 2012). The annual total illness cost is estimated at more than $1 billion dollars (Scharff, 2010). For illustration, consider that in the 10-year-period following the *E. coli* O157 contamination of hamburger event of 1993, more than $2 billion dollars were spent by the cattle industry to combat STEC in processing plants (Kay, 2003). In May 2012, the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) declared six non-O157 STEC serogroups–O26, O45, O103, O111, O121, and O145–to be adulterants in ground beef and non-intact raw beef products (Federal Register, 2011). Since then, increasing efforts have been devoted to developing and validating laboratory methods for the detection and quantification of the non-O157 STEC in cattle and beef products.

Diagnostic methods recently developed for the detection and quantification of the six major non-O157 STEC serogroups in cattle feces include culture and PCR-based assays. The culture method uses suitable substrate to detect the presence of specific enzymes. It requires selective enrichment of samples, immunomagnetic separation (IMS) using serogroup specific beads, plating on selective and differential media that contain antibiotics and chromogenic substrates, and confirmation of serogroup specific genes by multiplex PCR (Kalchayanand et al., 2013; Dewsbury et al., 2015; Noll et al., 2015). The PCR based methods use conventional or real-time assays to amplify DNA targets in the samples (Bai et al., 2010; Anklam et al., 2012; Paddock et al., 2012). Several of these methods have been evaluated for the detection and quantification of non-O157 serogroups and associated virulence genes in feces of naturally

shedding cattle (Bai et al., 2012; Hofer et al., 2012; Baltasar et al., 2014; Verstraete et al., 2014; Noll et al., 2015; Shridhar et al., 2016). However, the diagnostic performance (i.e., diagnostic sensitivity and specificity) of these methods in populations of cattle naturally shedding non-O157 serogroups has not been evaluated. Therefore, there are no estimates of the true prevalence of the six non-O157 serogroups in cattle feces; these are crucial for quantitative microbial risk assessment of the potential contamination burden of the pathogens along the beef production chain. Classically, the performance of a diagnostic test is evaluated against an ideally-available gold standard. A gold standard test is a perfect test that has sensitivity and specificity of 100%. None of the tests used for the detection of non-O157 serogroups in cattle feces are perfect. When a gold standard test is unavailable, Bayesian latent class analysis can be used as a practical option for the evaluation of diagnostic performance of multiple tests and true prevalence (Branscum et al., 2005; Berkvens et al., 2006).

The objective of this study was to (i) evaluate the diagnostic performance (i.e., diagnostic sensitivity and specificity) of culture-based, conventional PCR, and multiplex quantitative PCR PCR tests used for the detection of the six major non-O157 serogroups: O26, O45, O103, O111, O121, and O145 in feces of naturally shedding commercial feedlot cattle, and to (ii) estimate the true prevalence of these serogroups using a Bayesian latent class modeling framework.

Materials and methods

Study population and sample collection

The study population consisted of cattle in finishing pens in a commercial feedlot in the central United States. During the summer (June-August) of 2013, a total of 576 pen floor fecal samples were collected from pens housing crossbred finishing cattle a day before they were

transported to a slaughter plant. The feedlot was visited weekly during a 12-week period (Dewsbury et al., 2015). Two pens of cattle were sampled during each visit and 24 freshly voided fecal samples were collected off the pen floor upon entering each pen. In most cases samples were collected from cattle observed defecating. The average number of finishing cattle housed per pen at this feedlot during the study period was 270 (range: 121- 299). Approximately 10 g of freshly voided feces were collected in individual plastic bags (WHIRL-PAK®; Nasco, Fort Atkinson, WI) using plastic spoons, and shipped on ice to the Pre-harvest Food Safety Laboratory at Kansas State University for processing within 36 h of sample collection. For each sample, approximately 2 g of feces were suspended in 18 mL of *E. coli* broth, vortexed, and incubated for 6 h at 40ºC. The enriched fecal suspension obtained from individual samples was used to conduct the three diagnostic methods.

Culture-based detection

A culture-based method described by Noll et al. (2015) was used. Briefly, the enriched fecal suspension was subjected to IMS using six serogroup-specific beads (Abraxis, LLC, Warminster, PA). Following IMS, beads were plated on chromogenic Posse´ (Posse´ et al., 2008) medium modified to include novobiocin (5 mg/l) and potassium tellurite (0.5 mg/l) and incubated for 20-24 h at 37ºC. Six chromogenic colonies were then picked and streaked onto blood agar plates and incubated for 18-24 h at 37ºC. Colonies from each of the six streaks on the blood agar plate were pooled in 50 mL distilled water, boiled for 10 min, and used as a template for multiplex PCR targeting the six serogroups (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*). For this analysis, samples were considered serogroup positive based on presence of the O gene regardless of the presence of virulence genes.

Conventional PCR method

A multiplex conventional PCR (cPCR) assay targeting O antigen genes, i.e. the *wzx* gene for the detection of serogroups O26, O45, O103, O111, and O145 and the *wbqE* and *wbqF* genes for the detection of serogroup O121 (Bai et al., 2012), was used. DNA was extracted from the enriched fecal suspension by boiling, centrifuging and purifying using a GeneClean DNA extraction kit (MP Biomedicals, Solon, OH), then subjected to the cPCR assay (Shridhar et al., 2016). Briefly, 2 µl of the extracted DNA were mixed with 18 µl of reaction mix and the amplification reaction was carried out as follows: 5 min denaturation at 94^{o}C, followed by 35 cycles of 94^{o}C for 30 s, and 67^{o}C for 80 s. The amplified DNA was separated on a capillary electrophoresis system in a QIAxcel Advanced System (Qiagen, Valencia, CA) and was analyzed using QIAxcel Screengel software. For this analysis, samples were considered positive for any of the six non-O157 serogroups if the DNA extracted from enriched fecal suspensions tested positive for that O gene.

Multiplex quantitative PCR method

Two sets of multiplex quantitative PCR (mqPCR) assays targeting the *wzx* gene for the detection of serogroups O26, O45, O103, O111, and O145, and the *wbqE* and *wbqF* genes for the detection of serogroup O121 in cattle feces (Shridhar et al., 2016) were used. The first assay targeted O26, O103, and O111 serogroups while the second assay targeted O45, O121, and O145 serogroups. DNA, extracted from the enriched fecal suspension as described above, was subjected to the two mqPCR assays. Briefly, 1 µl of the extracted DNA was mixed with 19 µl of

reaction mix and the reaction was carried out as follows: 10 minutes denaturation at 95^{o}C, followed by 45 cycles of 95^{o}C for 15 s, 56^{o}C for 20 s, and 72^{o}C for 40 s. An end-point cycle threshold (Ct) value of $\leq$ 37.6 and $\leq$ 37.9 were considered positive for the first and second assays, respectively. For this analysis, samples were considered positive for any of the six non-O157 serogroups if the DNA extracted from enriched fecal suspension tested positive for that O gene. The detection methods have been previously validated for their analytical performance (Bai et al., 2012; Paddock et al., 2012; Shridhar et al., 2016)

Statistical analysis

Model description

A Bayesian formulation of the latent class model described by Branscum et al. (2005) was used in this study to estimate the sensitivity and specificity of the three diagnostic tests, as well as the true prevalence of each of the six non-O157 serogroups in cattle feces. Briefly, the Bayesian estimation procedure can be described as a process of adjusting and updating knowledge of an event based on data (Sanogo et al., 2014). Bayesian inference combines prior knowledge (described using probability distributions on the unknown population parameters of interest, such as sensitivity, specificity, and true prevalence) with observed field data (likelihood) from sample testing to produce updated posterior distributions for parameters of interest. This approach is particularly useful in the absence of a gold standard (Enoe et al., 2000; Branscum et al., 2005; Kostoulas et al., 2017). For our analysis, diagnostic performance of all three tests (culture, cPCR and mqPCR) was considered to be conditionally dependent as all tests involve the detection of the nuclear DNA of specific non-O157 serogroups. Conditional dependence amongst the tests was explicitly modeled and estimated as a conditional correlation amongst test

positive samples and a conditional correlation amongst test negative samples (Gardner et al., 2000; Branscum et al., 2005). For each of the non-O157 serogroups, we fitted a separate model that required the estimation of 13 parameters, namely sensitivity and specificity of each of the three tests, true prevalence, conditional pairwise correlations between tests (culture and cPCR, culture and mqPCR, cPCR and mqPCR), for test positive (rhoDplus) and negative (rhoDmin) samples. So specified, the likelihood for each of the six non-O157 models was non-identifiable because the data for each serogroup consisted of eight 'classes' of test results (2^3, i.e., two possible outcomes [positive or negative] each from the three detection methods), thereby allowing for a maximum of seven estimable parameters for each model (Branscum et al., 2005). To ensure parameter identifiability, proper informative priors were incorporated for prevalence, sensitivity, and specificity of the three tests (Dendukuri and Joseph, 2001; Branscum et al., 2005; Berkvens et al., 2006).

For each of the non-O157 serogroups, we fitted competing models that accommodated 1) all three possible pairwise conditional dependences between culture, cPCR and mqPCR, 2) two pairwise conditional dependencies between culture, cPCR and mqPCR, or 3) only one conditional dependence between detection methods (Table 4.5). Model fit statistics, namely DIC and LPML, were compared to select the best fitting model for each non-O157 serogroup. Detailed description of model fit statistics is presented in the model assessment section below.

Prior specifications

External prior information on sensitivities of culture, cPCR, and mqPCR, and on specificities of cPCR and mqPCR for each of the six non-O157 serogroups were elicited from three experts, individually, through face-to-face interviews. To inform priors for test sensitivity,

the experts were asked to give their best estimate of the most likely and the minimum number of samples that each test would detect as positive given a hypothetical set of 100 known positive fecal samples that they were almost sure (i.e., 95% sure) the test could identify as positive. To inform priors for tests specificity, experts were also asked to give their best estimate of the most likely and the minimum number of samples that each test would detect as negative given a hypothetical set of 100 true negative samples that they were almost sure (i.e., 95% sure) the test could identify as negative. Prior information on test sensitivity and specificity was obtained in this way for each of the six non-O157 serogroups. Next, consensus prior information (simple arithmetic means) for the three experts was generated from that elicited individually. For each of the six non-O157 serogroups, information from each expert and from their consensus was used to specify the mode and 5th percentile of a beta distribution [Beta (a,b)] using Beta Buster© software (http://betabuster.software.informer.com/1.0/) and thus derive the a and b shape parameters of the corresponding beta prior distributions (Jones and Johnson, 2014). Consensus priors for sensitivity of the three methods, specificity of cPCR and mqPCR for serogroups O26, O45, and O103 are summarized in Table 4.1, and for serogroups O111, O121, and O145 in Table 4.2. In all cases, the culture method was considered to be highly specific (Cernicchiaro et al., 2011; Jacob et al., 2014), so that the prior distribution for culture specificity had mode 99% and 5th percentile of 97%, corresponding to a beta prior with shape parameters a = 212.12 and b = 3.13. External prior information on prevalence of each of the six non-O157 serogroups in cattle feces were obtained from a systematic review-meta analysis study (Dewsbury, 2015) and used to specify mode and 5th percentile of a beta distribution, whereby the corresponding a and b shape parameters were obtained from Beta Buster as explained above (Table 4.3). For each non-O157 serogroup, consensus priors based on information elicited independently from the three experts

were used in model fitting to obtain the posterior distributions for diagnostic performance of each of the three detection methods and for the true prevalence of each of the six non-O157 serogroup. The model specified using consensus prior is hereto referred to as the consensus model (CM).

Sensitivity Analysis

Sensitivity to consensus prior specification was assessed by comparing posterior inference of the model selected as best fitting for each serogroup to that of models that fitted widened priors reflecting greater uncertainty relative to the priors elicited by experts and their consensus (Kostoulas et al., 2006; Praud et al., 2012; Rahman et al., 2013). Widened priors were designed to represent more diffuse prior information compared to that of individual experts or their consensus. For parameters defining sensitivity of culture, cPCR and mqPCR, and specificity of cPCR and mqPCR, widened priors were generated by using the smallest values of the prior mode elicited by the three experts and by halving the value of the 5^{th} percentile of the consensus prior for each parameter. For each serogroup, sensitivity analysis compared posterior inference from three models using widened priors and combinations of widened and consensus priors (Tables 4.1–4.2). Specifically, sensitivity analysis model 1 (SM1) used widened priors for sensitivity of culture, cPCR and mqPCR, and for specificity of cPCR and mqPCR (i.e., increased prior uncertainty for all 5 parameters). Sensitivity analysis model 2 (SM2) used consensus priors for sensitivity of culture, cPCR and mqPCR but widened priors for specificity of cPCR and mqPCR (i.e., increased prior uncertainty for specificity of cPCR and mqPCR only). Sensitivity analysis model 3 (SM3) used widened priors for sensitivity of culture, cPCR, and mqPCR but consensus priors for specificity of cPCR and mqPCR (i.e., increased prior uncertainty for

sensitivity of culture, cPCR, and mqPCR only). For prevalence of each of the six non-O157 serogroups, the same priors (Table 4.3) specified in each initial consensus model were used for all sensitivity analysis models.

Software implementation and posterior inference

All models were fitted using OpenBUGS© version 3.2.3 rev 1012 (Thomas et al., 2006), with an R software (R Core Team, 2015) interphase through the "R2OpenBUGS" (Sturtz et al., 2005) package. For each model, three parallel Markov Chain Monte Carlo chains were run, each consisting of a burn-in period of 10,000 iterations followed by another 100,000 saved iterations for posterior inference. Chain convergence was assessed by visual inspection of the Gelman-Rubin diagnostic plots and evaluation of the corresponding statistics (Gelman and Rubin, 1992). Convergence diagnostics were implemented using the "coda" (Plummer et al., 2006) package in R. Sensitivity analyses were conducted using a similar implementation of MCMC as that used for the consensus model.

For each of the six non-O157 serogroups, posterior inference on sensitivity and specificity of each of the three tests, on the conditional correlations amongst the three methods, and on the true prevalence of the serogroups was reported as posterior median and corresponding 95% highest posterior density interval (HPDI). HPDI is defined as the shortest possible interval containing 95% of the posterior samples (Christensen et al., 2011). Using the best fitting model for each serogroup, we compare the sensitivity and specificity of detection methods by computing the posterior density of their pairwise differences in diagnostic sensitivity and the posterior density of their pairwise differences in diagnostic specificity, respectively. Two

detection methods were considered to differ in either sensitivity or specificity if the corresponding 95% HPDI on their pairwise differences did not include the null value zero.

Model assessment

The deviance information criterion (DIC) and the log pseudo marginal likelihood (LPML) were used to compare relative data fit amongst competing models (i.e., check which model fit the data better). The DIC is a Bayesian measure of relative model goodness-of-fit penalized by a complexity term *p*D (Spiegelhalter et al., 2002) that defines the effective number of parameters estimated by the model. A negative *p*D indicates substantial conflict between priors and data; when this happens the estimate for DIC is not reliable (Spiegelhalter et al., 2002; Berkvens et al., 2006). Given positive values of *p*D, models with smaller DIC values are considered better fitting (Spiegelhalter et al., 2003) and DIC differences of at least 5 to 10 units are indicative of substantial improvement in model fit between the competing models (Lunn et al., 2012). In turn, LPML is a posterior predictive statistic that is useful as an overall measure of relative model fit (Geisser and Eddy, 1979). It is derived from the conditional predictive ordinate (CPO) statistics (Gelman et al., 2014), and expressed as $LPML = \sum_{i=1}^{n} \log(CPO_i)$, where *n* is the number of classes of test results as illustrated in table 4.4. The model with largest LPML is preferred (Christensen et al., 2011).

In addition, we use the Bayesian *p*-value as a posterior predictive check statistic that expresses the absolute fit of a proposed model to selected features of the data. The Bayesian *p*-value is a probability calculated as a function of the difference between the deviance of the observations and the deviance of observations predicted from the fitted model (Berkvens et al., 2006). A model that provides adequate fit to the data is expected to yield a Bayesian *p*-value of

around 0.50 (Rahman et al., 2013). As a rule of thumb, Bayesian *p*-values between 0.30 and 0.70 (Smit, 2013) and well away from the extremes of 0 and 1 (Gelman et al., 2014) provide no evidence for lack of fit.

Results

Descriptive statistics on data

A total of 576 cattle pen floor fecal samples were analyzed by culture, cPCR, and mqPCR assays for the presence of the six non-O157 serogroups. The cross-classified test results of the three detection methods for each of the six non-O157 serogroups are shown in Table 4.4. All samples tested positive for at least one non-O157 serogroup and by at least one detection method.

Overall, 22.7% (131/576), 44.4% (256/576), and 59.0% (340/576) of enriched samples tested positive for serogroup O26 by culture, cPCR, and mqPCR respectively. Moreover, 61.8% (356/576) of samples tested positive for O26 by at least one detection method, and 14.9% (86/576) of samples tested positive for O26 by all three methods.

For the O45 serogroup, 16.7% (96/576), 17.9% (103/576), and 55.9% (322/576) of enriched samples tested positive by culture, cPCR, and mqPCR respectively. Moreover, 57.6% (332/576) of samples tested positive for O45 by at least one detection method, and 7.9% (46/576) of samples tested positive for O45 by all three methods.

For serogroup O103, 60.2% (347/576), 56.6% (326/576), and 84.7% (488/576) of enriched samples tested positive by culture, cPCR, and mqPCR respectively. Moreover, 87.3% (503/576) of samples tested positive for O103 by at least one detection method, and 42.2% (243/576) of samples tested positive by all three methods.

For serogroup O111, none of the enriched samples tested positive by culture, cPCR, and mqPCR. Only 0.9% (5/576) of enriched samples was positive by at least one detection method and 99.1% (571/576) were negative by all three methods.

For serogroup O121, 2.3% (13/576), 22.9% (132/576), and 57.8% (333/576) of enriched samples tested positive by culture, cPCR, and mqPCR, respectively. Moreover, 57.9% (334/576) of samples tested positive for O121 by at least one detection method, and 1.7% (10/576) of samples tested positive by all three methods.

Finally, for serogroup O145, 2.9% (17/576), 1.9% (11/576), and 5.9% (34/576) of enriched samples tested positive by culture, cPCR, and mqPCR respectively. Moreover, 7.3% (42/576) of samples tested positive for O145 by at least one detection method, and 1.2% (7/576) of samples tested positive by all three methods.

Model selection by serogroup

Fit statistics to compare competing models (DIC and LPML, *p*D) for each of the six non-O157 serogroup are presented in Table 4.5. Models were selected based on lowest DIC and largest LPML values indicating improved fit to data relative to other models. In all cases, models selected as best fitting explicitly accommodated conditional dependence between cPCR and mqPCR. In addition, best fitting models for *E. coli* O103 and O121 also included conditional dependence between culture and cPCR, and best fitting model for *E. coli* O111 also included conditional dependence between culture and mqPCR. For all selected best-fitting models, Bayesp indicated no evidence of lack of fit (Table 4.5).

Inference on diagnostic performance and true prevalence

Tables 4.6-4.11 show the posterior median and 95% HPDI for sensitivity and specificity of the three detection methods and for the true prevalence of serogroups O26, O45, O103, O111, O121, and O145 respectively, as well as model fit statistics based on the selected consensus and sensitivity analysis models. Further, Table 4.12 presents relevant posterior inference to compare diagnostic sensitivity and specificity between detection methods.

Sensitivity analyses of selected models

Serogroup O26

Sensitivity analyses indicated comparable data fit regardless of prior specification of the competing models (Table 4.6). Bayesian *p*-values for all models examined were in the range 0.52 – 0.55, indicating no evidence for lack of fit of any of the models. The DIC difference between CM and any of the sensitivity analysis models examined was smaller than 3 units, while their LPML difference was smaller than 0.3 units, indicating no evidence for substantial differences in fit across models. Diagnostic sensitivity of cPCR and mqPCR methods showed posterior medians and 95% HPDI of comparable ranges across the models considered (Table 4.6). In contrast, the posterior medians and corresponding 95% HPDI's for diagnostic sensitivity of culture, specificity of cPCR, specificity of mqPCR and true prevalence of O26 showed susceptibility to prior specification across models. In particular, SM1 and SM2 showed the greatest posterior medians and lowest estimation precision, as indicated by the broader 95% HPDI, for sensitivity of culture. SM1 and SM2 also showed lowest posterior medians and estimation precisions for specificity of cPCR, specificity of mqPCR and true prevalence of O26, consistent with the more diffused priors specified on these models. The posterior distribution of

specificity of cPCR and mqPCR methods appeared to be particularly sensitive to prior specification in SM1 and SM2 (Table 4.6).

Based on the consensus prior specification for the selected best-fitting model, diagnostic sensitivity to O26 differed significantly between the 3 methods, as indicated by a 95% HPDI on their pairwise differences that did not include the null value zero (Table 4.12). Specifically, diagnostic sensitivity was greatest for mqPCR, intermediate for cPCR and smallest for culture (Table 4.6). However, there was no evidence for differences between the detection methods in specificity to O26, as the corresponding 95% HPDI on their pairwise differences included zero.

Serogroup O45

Sensitivity analyses indicated comparable data fit across competing models regardless of prior specification (Table 4.7). Bayesian *p*-values for all models examined were in the range 0.52 – 0.59, indicating no evidence for lack of fit of any of the models. The DIC difference between CM and any of the sensitivity analysis models examined was smaller than 2 units, while their LPML difference was smaller than 0.4 units, indicating no evidence for substantial differences in fit across models. Diagnostic sensitivity of cPCR and mqPCR, specificity of cPCR and mqPCR methods, and true prevalence of O45 showed posterior medians and 95% HPDI of comparable ranges across the models considered (Table 4.7). The posterior medians for diagnostic sensitivity of culture showed susceptibility to prior specification across models, SM1 and SM2 showed greater posterior medians though the corresponding 95% HPDI considerably overlapped (Table 4.7).

Based on the consensus prior specification for the selected best-fitting model for O45, there was no evidence for any difference in diagnostic sensitivity or specificity of culture compared to that of cPCR (Table 4.12). However, diagnostic sensitivity of mqPCR was higher

than that of both culture and cPCR and conversely, specificity of mqPCR was significantly lower than that of culture and cPCR (Table 4.7 and 4.12).

Serogroup O103

Sensitivity analyses indicated comparable data fit regardless of prior specification of the competing models (Table 4.8). Bayesian *p*-values for all models examined were in the range 0.57 – 0.66, indicating no evidence for lack of fit of any of the models. The DIC difference between CM and any of the sensitivity analysis models examined was smaller than 4 units, while their LPML difference was smaller than 0.3 units, indicating no evidence for substantial differences in fit across models. Diagnostic sensitivity of cPCR and mqPCR methods showed posterior medians and 95% HPDI of comparable ranges across the models considered (Table 4.8). In contrast, the posterior medians and corresponding 95% HPDI's for diagnostic sensitivity of culture, specificity of cPCR, specificity of mqPCR and true prevalence of O103 showed susceptibility to prior specification across models. In particular, SM1 and SM2 showed the greatest posterior medians and lowest estimation precision, as indicated by the broader 95% HPDI, for sensitivity of culture. However, SM1 and SM2 also showed lowest posterior medians and estimation precisions for specificity of cPCR, specificity of mqPCR and true prevalence of O103, consistent with the more diffused priors specified on these models. The posterior distribution of specificity of mqPCR method appeared to be particularly sensitive to prior specification in SM1 and SM2 (Table 4.8).

Based on the consensus prior specification for the selected best-fitting model, diagnostic sensitivity of mqPCR was higher than that of culture and cPCR for O103 (Table 4.8 and 4.12).

However, there was no evidence for difference in sensitivity of culture compared to cPCR, neither in specificity amongst the three methods.

Serogroup O111

Sensitivity analyses indicated comparable data fit regardless of prior specification of the competing models (Table 4.9). Bayesian *p*-values for all models examined were in the range 0.65 – 0.72, indicating no evidence for lack of fit of any of the models. The DIC difference between CM and any of the sensitivity analysis models examined was smaller than 1 unit, while their LPML difference was smaller than 0.4 units, indicating no evidence for substantial differences in fit across models. The diagnostic specificity of cPCR and mqPCR methods, the true prevalence of O111 in the study population showed posterior medians and 95% HPDI of comparable numerical magnitude across all models, thereby indicating robust inference to prior specification. However, diagnostic sensitivity for all three methods appeared to be highly sensitive to prior specification (Table 4.9).

Based on the consensus prior specification for the selected best-fitting model, diagnostic sensitivity of mqPCR was greater than that of culture for O111 (Table 4.9 and 4.12). However, there was no evidence for difference in sensitivity between culture and cPCR, or between cPCR and mqPCR, neither in diagnostic specificity amongst any of the three methods.

Serogroup O121

Despite negative *p*D value and thus unreliable DIC for SM3, sensitivity analyses indicated comparable data fit of the remaining models regardless of prior specification (Table 4.10). The Bayesian *p*-value indicated no problems of lack of fit and the LPML did not suggest substantial relative advantages for any of the remaining models. The DIC difference between

CM, SM1, and SM2 was smaller than 2 units, while their LPML difference was smaller than 1 unit, indicating no evidence for substantial differences in fit across models. Diagnostic sensitivity of all three methods was highly susceptible to prior specification. Notably, posterior medians for sensitivity were smaller in magnitude and 95% HPDI were wider when models specified widened priors on diagnostic sensitivity. In turn, the diagnostic specificity of cPCR and mqPCR methods and the true prevalence of O121 in the study population showed robustness to prior specification, as their posterior medians and 95% HPDI were of comparable magnitude across CM, SM1 and SM2 (Table 4.10).

Based on the consensus prior specification for the selected best-fitting model, diagnostic sensitivity of mqPCR was significantly higher than that of culture for O121 (Table 4.10 and 4.12). However, there was no evidence for difference in sensitivity between culture and cPCR, or between cPCR and mqPCR. Specificity of culture was significantly higher than that of cPCR and mqPCR, and that of cPCR higher than mqPCR (Table 4.10 and 4.12).

Serogroup O145

Model fit statistics obtained from the sensitivity analyses indicated that the different sets of priors examined yielded alternative models with comparable fit to the data (Table 4.11). Bayesian *p*-values for all models examined were in the range 0.40 – 0.55, indicating no evidence of lack of fit of any of the models. The DIC difference between CM and any of the sensitivity analysis models examined was smaller than 2 units, while the LPML difference was smaller than 0.7 units, indicating no evidence for substantial differences in fit across models. The diagnostic specificity of cPCR and mqPCR methods, the true prevalence of O145 in the study population showed posterior medians and 95% HPDI of comparable numerical magnitude across the models

considered. However, diagnostic sensitivity of all three methods was susceptible to prior specification, particularly notable in the widened 95% HPDI between models that assumed widened priors on diagnostic sensitivity (SM1 and SM3) versus those that did not (CM and SM2) (Table 4.11).

Based on the consensus prior specification for the selected best-fitting model, diagnostic specificity of mqPCR was lower than that of culture and cPCR method for O145, though there was no evidence for method differences in sensitivity neither on specificity of culture and cPCR (Table 4.11 and 4.12).

Inference on dependence between diagnostic methods

For serogroups O26 and O45, results indicated evidence of moderate to high conditional correlation between cPCR and mqPCR both amongst test positive samples [i.e., posterior mean = 0.45 (95% HPDI: 0.30, 0.59) and 0.24 (95% HPDI: 0.06, 0.40), respectively] and amongst test negative samples [i.e., posterior mean = 0.71 (95% HPDI: 0.30, 0.99) and 0.23 (95% HPDI: 0.05, 0.36) respectively], as the 95% HPDI of the corresponding correlation coefficient did not include the null value of zero in either case. Similarly, for samples testing positive for serogroup O103, there was evidence of conditional correlation between cPCR and mqPCR [i.e., posterior mean = 0.27 (95% HPDI: 0.19, 0.34)] and between culture and cPCR [i.e., posterior mean = 0.18 (95% HPDI: 0.08, 0.28]. For serogroup O111, O121, and O145, there was also evidence of conditional correlation between cPCR and mqPCR amongst test negative samples [i.e., posterior mean = 0.85 (95% HPDI: 0.59, 0.99), 0.44 (95% HPDI: 0.39, 0.50), and 0.31 (95% HPDI: 0.04, 0.53), respectively]. No evidence of any additional pairwise conditional dependence was

apparent amongst test positive as well as test negative samples, as the 95% HPDI for the corresponding conditional correlations included zero.

Discussion

In this study, we report Bayesian estimates for diagnostic sensitivity and specificity of culture, cPCR, and mqPCR methods for detection of the six major non-O157 serogroups in feces of finishing feedlot cattle, and for true prevalence of each serogroup in the absence of a gold standard. Our modeling approach is based on latent class analysis implemented on a Bayesian framework (Branscum et al., 2005) and allows for assessment of diagnostic performance in the absence of a gold standard. Further, our Bayesian implementation strengthens inference by recognizing correlations in the diagnostic performance of the methods based on the partial overlap of their diagnostic mechanisms, as implemented in this study (Branscum et al., 2005). We explicitly acknowledge that this analysis focused on the detection of O serogroups and not specifically STEC. The frequency of STEC positive samples (i.e., isolates with at least one Shiga toxin gene) was too low (range from 0.0% - 1.7%) in this dataset to support reasonable inference on diagnostic performance of detection methods for the six non-O157 STEC.

In the absence of a gold standard, the Bayesian approach to estimating performance of diagnostic tests, as well as true prevalence of infection/disease using a combination of imperfect diagnostic tests, prevents biased estimations of disease burden and tests performance (Speybroeck et al., 2013). The inferential quality of the posterior estimates, however, depends on the availability and quality of prior knowledge specified in the model (Sanogo et al., 2014). Informative prior knowledge, when available, can be elicited from relevant historical data (published values from previous similar studies) or from subject-matter expert opinion. The

combination of prior knowledge and data likelihood in the Bayesian model provides the best possible parameter estimates of in realistic settings (Sanogo et al., 2014). Where conditional correlation existed among tests, such as when combined tests target a similar biological phenomenon, Bayesian estimation methods allow for the estimation of the test parameters while accounting for such conditional dependence amongst tests. In turn, Bayesian estimation requires sensitivity analyses to evaluate robustness of posterior inference to prior information by using alternative prior distributions. The Bayesian estimation method provides an alternative approach for generating unbiased accurate estimates for performance of tests, as well as generating estimates of true pathogen prevalence in animal populations. This information is a critical requirement for microbial risk assessment of non-O157 STEC in the beef production chain.

In this study, we targeted inference that relied heavily on prior elicitation from consensus of opinions elicited from three subject-matter experts. Elicitation of expert-opinion in this manner has been described to represent reasonable prior knowledge that reflects uncertainty about the parameters of interest (Garabed et al., 2009; Christensen et al., 2011). Based on procurable data, the results from this study represent the best estimates available at the moment for diagnostic performance for the three methods evaluated for the detection of the six non-O157 serogroups in cattle feces. Keeping with the idea of Bayesian learning, these estimates of diagnostic performance can be further improved as more data becomes available. In particular, our consensus-based posterior estimates for diagnostic sensitivity of mqPCR were greater than those of cPCR and culture for O26, O45, and O103 serogroups, whereas the sensitivity estimates of mqPCR were greater than those of culture but not distinguishable from those of cPCR for O111 and O121 serogroups. Finally, sensitivity estimates were of comparable magnitude for the three methods for O145 serogroup. Also, based on the consensus model, the posterior estimate of

sensitivity of cPCR was higher than that of culture for serogroup O26, but not for serogroups O45, O103, O111, O121, and O145. There was no evidence of difference in diagnostic specificity among the three methods for serogroups O26, O103, and O111, nor between culture and cPCR for O45 and O145. However, there was significant difference in specificity of the three methods for the O121 serogroup and between culture and mqPCR, cPCR and mqPCR for serogroups O45 and O145.

Previous studies have suggested differences in diagnostic performance among the detection methods as illustrated by disagreement in test results (Cernicchiaro et al., 2013; Noll et al., 2015; Shridhar et al., 2016). Shridhar et al. (2016) reported disagreement between the proportion of positive samples detected by mqPCR, cPCR, and the culture method applied to common enrichments based on a McNemar's test from a study that applied the three methods for the detection of the six non-O157 *E. coli* serogroups in cattle feces. They found cPCR to be less sensitive than mqPCR. Cernicchiaro et al. (2013) and Noll et al. (2015) in separate studies reported only fair to no agreement between cPCR and culture-based results for detection of O26, O45, O103, and O111 serogroups. Our study provides quantitative estimates (posterior medians and their 95% HPDIs) for the sensitivity and specificity of each detection method for O26, O45, O111, O103, O121, and O145 serogroups in cattle feces.

Based on the consensus model, the posterior median true prevalence was high for serogroups O26 (54.3%), O45 (26.3%), and O103 (85.5%), but low for serogroups O111 (0.3%), O121 (3.9%), and O145 (1.8%). This pattern is consistent with the estimates of apparent prevalence reported by previous studies. Cernicchiaro et al. (2013), using multiplex PCR and a culture procedure that involved IMS, found serogroups O26 (23.4%), O121 (16.4%), and O103 (11.8%) to be the most frequently detected non-O157 serogroups in feces of cattle from a

commercial feedlot operation in the central United States. Similarly, Dargatz et al. (2013) investigated the apparent prevalence of the six major non-O157 serogroups from bovine pooled fecal samples from 21 feedlots in four U.S. states using a multiplex PCR method and reported apparent prevalence of 13.8%, 9.9%, 9.3%, 5.5%, 1.1%, and 0.5% for O45, O26, O103, O121, O145, and O111 serogroups, respectively. Also, from studies conducted during summer and winter months at a large commercial feedlot in the central United States, Dewsbury et al. (2015) used the culture-based method and found serogroups O26 and O103 to be the most commonly identified non-O157 *E. coli* during the summer and serogroups O26, O45, and O103 in the winter.

Sensitivity analysis on prior specifications allowed us to assess the robustness of our posterior inference on diagnostic test performance to the priors elicited from expert consensus. For serogroups O26 and O45, the posterior distributions of true prevalence, sensitivity of the three detection methods, and specificity of cPCR and mqPCR overlapped substantially across the competing models considered regardless of consensus or widened priors, thus suggesting robust inference to prior specification, particularly for O26. For O103, the posterior distributions of true prevalence, sensitivity of the three detection methods, and specificity of cPCR also overlapped substantially across the competing models considered regardless of consensus or widened priors, thus suggesting relatively robust inference to prior specification. However, inference on specificity of mqPCR showed considerable sensitivity to prior specification, as indicated by a markedly decreased posterior median and wider 95% HPDI when widened priors were used for specificity of cPCR and mqPCR. This result is consistent with the fact that few samples tested negative for O103 using mqPCR, thereby indicating limited information available in the data for mqPCR specificity such that the influence of the prior was substantial.

For O111, O121, and O145 serogroups, the posterior distributions of true prevalence, and the specificity of cPCR and mqPCR overlapped substantially across the competing models considered, thus suggesting robust inference to prior specification. Yet, inference on sensitivity of the three methods showed considerable sensitivity to prior specification, as indicated by decreased posterior median and wider 95% HPDI when widened priors were used for sensitivity of culture, cPCR and mqPCR. For these serogroups, susceptibility to prior specification is to be expected as only few samples tested positive by one or more methods, thereby indicating limited available information in the data to make precise inference for sensitivity of the methods. In turn, for these serogroups, posterior estimates were largely dependent on expert-derived priors. Regarding model comparison, the fit statistics considered in this study (i.e. DIC, LPML or Bayesian p-value) showed little, if any, power to discriminate fit across competing models. This was despite the evident differences in posterior inference due to sensitivity to prior specification.

The three diagnostic methods considered here involve amplification of specific gene targets in individual non-O157 serogroups, though they differ in sample preparation, reaction chemistry and post-PCR analyses. As a result, a substantial increase in sensitivity of mqPCR over cPCR (Biassoni and Raso, 2014), and cPCR and mqPCR over culture, was expected, as only samples with viable colonies on the Posse´ medium were tested by the PCR method. Altogether, it is sensible to consider potential correlations in the diagnostic performance of these methods. Indeed, we found evidence for conditional dependence between cPCR and mqPCR amongst test positive as well as test negative samples for O26 and O45 serogroups. For samples testing positive for serogroup O103, there was evidence of conditional dependence between cPCR and mqPCR and between culture and cPCR, but no evidence for any dependence amongst test negative samples. Fewer samples tested negative by the mqPCR methods compared to the

cPCR for O103 serogroup and this may be responsible for the lack of significant conditional dependence between the methods amongst test negative samples. Conversely, for samples testing negative to serogroups O111, O121, and O145, there was evidence of conditional dependence between cPCR and mqPCR. Generally, there were fewer test positive samples by both cPCR and mqPCR for serogroup O145 and almost no positives for O111. For O45, there were fewer tests positive samples by the cPCR compared to the mqPCR methods. The limited number of test positive samples in addition to differences in the number of test positive samples by the methods may be responsible for the lack of significant conditional dependence between the methods amongst test positive samples. The finding of conditional correlation between pairs of methods amongst the test positive and/or test negative samples support the *a priori* assumption of conditional dependence among the three methods. It is recommended that a conditional dependent model be considered when there is a moderate to high correlation between two or more tests (Georgiadis et al., 2003) as it may be possible to take advantage of the shared information between methods that is indicated by their correlation. Failure to account for correlation between tests can not only impairs precision of inference but it can also lead to a biased assessment on the diagnostic performance of the tests (Vacek, 1985).

The diagnostic performance of the methods analyzed in this study represents the diagnostic sensitivity, specificity, as well as the true prevalence of each of the six O serogroups evaluated in the absence of the virulence genes. The presence of the virulence genes describes the pathogenicity of the isolate and its ability to cause human illnesses following exposure. Based on available data, the assessment of diagnostic sensitivity and specificity obtained from this study represent the best estimates of performance of these methods for the detection of six non-O157 serogroups in feces of naturally shedding commercial feedlot cattle.

Conclusion

This study evaluated the diagnostic performance of culture, cPCR, and mqPCR methods for the detection of the six major non-O157 *E. coli* serogroups in feces of feedlot cattle. Our analysis on data from test samples draws heavily from consensus knowledge carefully elicited from subject-matter experts. Evidence showed that mqPCR was the most sensitive detection method for the O26, O45, and O103 serogroups. The cPCR method was more sensitive than the culture method for serogroups O26, and O121, though their sensitivity was not distinguishable for serogroups O45, O103, O111, and O145. The cPCR method showed higher specificity than mqPCR within serogroups O45, O121, and O145 but no apparent differences within serogroups O26, O103, and O111. The posterior median true prevalence of the different non-O157 evaluated in the study varied by serogroup. These results provide important estimates of test performance and also of true prevalence of the six major non-O157 *E. coli* in feces of feedlot cattle, which will be useful to make necessary adjustment for test error in risk modeling.

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Table 4.1 - Elicitation of prior information on diagnostic performance of culture, conventional PCR (cPCR), and multiplex quantitative PCR (mqPCR) for detection of *Escherichia coli* O26, O45, and O103 in bovine feces

Serogroup	Source	Variable	Culture		Conventional PCR		Multiplex quantitative PCR	
			Most likely	5th percentile	Most likely	5th percentile	Most likely	5th percentile
O26; O45	Expert consensus	Sensitivity	0.60	0.40	0.75	0.52	0.87	0.67
		Specificity			0.94	0.80	0.97	0.85
	Sensitivity model 1	Sensitivity	0.60	0.20	0.60	0.26	0.70	0.34
		Specificity			0.90	0.40	0.95	0.43
	Sensitivity model 2	Sensitivity	0.60	0.40	0.75	0.52	0.87	0.67
		Specificity			0.90	0.40	0.95	0.43
	Sensitivity model 3	Sensitivity	0.60	0.20	0.60	0.26	0.70	0.34
		Specificity			0.94	0.80	0.97	0.85
O103	Expert consensus	Sensitivity	0.65	0.40	0.78	0.53	0.93	0.73
		Specificity			0.95	0.83	0.98	0.88
	Sensitivity model 1	Sensitivity	0.60	0.20	0.70	0.27	0.90	0.37
		Specificity			0.90	0.42	0.95	0.44
	Sensitivity model 2	Sensitivity	0.65	0.40	0.78	0.53	0.93	0.73
		Specificity			0.90	0.42	0.95	0.44
	Sensitivity model 3	Sensitivity	0.60	0.20	0.70	0.27	0.90	0.37
		Specificity			0.95	0.83	0.98	0.88

Sensitivity model 1 used the lowest most likely value of the three experts prior, and halved consensus prior 5th percentile for sensitivity of culture, cPCR, and mqPCR, and specificity of cPCR and mqPCR; Sensitivity model 2 used the lowest most likely value of the three experts prior, and halved consensus prior 5th percentile for specificity of cPCR and mqPCR but consensus priors for sensitivity of culture, cPCR, and mqPCR; Sensitivity model 3 used the lowest most likely value of the three experts prior, and halved consensus prior 5th percentile for sensitivity of culture, cPCR, and mqPCR but consensus priors for specificity of cPCR and mqPCR.

Table 4.2 - Elicitation of prior information on diagnostic performance of culture, conventional PCR (cPCR), and multiplex quantitative PCR (mqPCR) for detection of *Escherichia coli* O111, O121, and O145 in bovine feces

Serogroup	Source	Variable	Culture		Conventional PCR		Multiplex quantitative PCR	
			Most likely	5th percentile	Most likely	5th percentile	Most likely	5th percentile
O111; O121	Expert consensus	Sensitivity	0.50	0.35	0.72	0.47	0.83	0.65
		Specificity			0.92	0.77	0.95	0.82
	Sensitivity model 1	Sensitivity	0.40	0.18	0.50	0.24	0.60	0.33
		Specificity			0.90	0.38	0.90	0.41
	Sensitivity model 2	Sensitivity	0.50	0.35	0.72	0.47	0.83	0.65
		Specificity			0.90	0.38	0.90	0.41
	Sensitivity model 3	Sensitivity	0.40	0.18	0.50	0.24	0.60	0.33
		Specificity			0.92	0.77	0.95	0.82
O145	Expert consensus	Sensitivity	0.70	0.50	0.75	0.52	0.87	0.67
		Specificity			0.93	0.80	0.97	0.85
	Sensitivity model 1	Sensitivity	0.60	0.25	0.60	0.26	0.70	0.34
		Specificity			0.90	0.40	0.95	0.43
	Sensitivity model 2	Sensitivity	0.70	0.50	0.75	0.52	0.87	0.67
		Specificity			0.90	0.40	0.95	0.43
	Sensitivity model 3	Sensitivity	0.60	0.25	0.60	0.26	0.70	0.34
		Specificity			0.93	0.80	0.97	0.85

Sensitivity model 1 used the lowest most likely value of the three experts prior, and halved consensus prior 5th percentile for sensitivity of culture, cPCR, and mqPCR, and specificity of cPCR and mqPCR; Sensitivity model 2 used the lowest most likely value of the three experts prior, and halved consensus prior 5th percentile for specificity of cPCR and mqPCR but consensus priors for sensitivity of culture, cPCR, and mqPCR; Sensitivity model 3 used the lowest most likely value of the three experts prior, and halved consensus prior 5th percentile for sensitivity of culture, cPCR, and mqPCR but consensus priors for specificity of cPCR and mqPCR.

Table 4.3 - Prior information elicited, and corresponding beta distributions, for prevalence of each of the six non-O157 *Escherichia coli* serogroups in bovine feces

Serogroup	Prior prevalence		Prior beta distribution	
	Most likely	95th percentile	*a*	*b*
O26	0.19	0.40	3.99	13.78
O45	0.10	0.30	2.56	15.03
O103	0.12	0.40	2.11	9.10
O111	0.02	0.20	1.30	15.80
O121	0.07	0.30	1.84	12.17
O145	0.02	0.20	1.30	15.80

Table 4.4 - Frequency table of detection results* based on culture, conventional PCR (cPCR), and multiplex quantitative PCR (mqPCR) for the six non-O157 *Escherichia coli* serogroups in bovine feces

Diagnostic test result			Serogroup					
Culture	**Conventional PCR**	**Multiplex quantitative PCR**	**O26**	**O45**	**O103**	**O111**	**O121**	**O145**
+	+	+	86	46	243	0	10	7
+	+	–	0	0	0	0	0	0
+	–	+	29	40	89	0	2	2
+	–	–	16	10	15	1	1	8
–	+	+	170	57	83	4	122	4
–	+	–	0	0	0	0	0	0
–	–	+	55	179	73	0	199	21
–	–	–	220	244	73	571	242	534

+: Positive; –: Negative
*Out of a total of 576 samples

Table 4.5 - Model fit statistics for competing Bayesian models to estimate true prevalence, sensitivity and specificity of culture, conventional PCR, and multiplex quantitative PCR for the detection of *E. coli* O26, O45, O103, O111, O121, and O145 in bovine feces

Competing models accommodate	Serogroup O26				Serogroup O45			
	DIC	LPML	*p*D	Bayesp	DIC	LPML	*p*D	Bayesp
Pairwise conditional dependence (CD) amongst all methods	40.96	-863.06	3.58	0.87	40.79	-821.41	3.54	0.74
CD between CUL and cPCR, and between CUL and mqPCR	51.79	-867.96	4.56	0.89	47.50	-824.93	3.27	0.81
CD between CUL and cPCR, and between cPCR and mqPCR	40.78	-863.07	3.31	0.65	40.92	-821.43	3.63	0.67
CD between CUL and mqPCR, and between cPCR and mqPCR	39.54	-862.66	2.46	0.84	40.88	-821.33	3.66	0.68
CD between CUL and cPCR	51.56	-867.82	4.55	0.89	47.73	-824.94	3.34	0.82
CD between cPCR and mqPCR	**39.73**	**-862.56**	**2.62**	**0.55**	**40.72**	**-821.21**	**3.61**	**0.58**
CD between CUL and mqPCR	52.18	-868.07	4.13	0.90	49.13	-825.10	4.39	0.82
	Serogroup O103				Serogroup O111			
	DIC	LPML	*p*D	Bayesp	DIC	LPML	*p*D	Bayesp
Pairwise conditional dependence (CD) amongst all methods	42.78	-900.3	4.56	0.78	15.86	-36.75	1.08	0.76
CD between CUL and cPCR, and between CUL and mqPCR	60.90	-909.59	4.22	0.99	26.39	-42.01	1.49	0.86
CD between CUL and cPCR, and between cPCR and mqPCR	**42.66**	**-900.25**	**4.52**	**0.65**	16.13	-36.80	1.00	0.72
CD between CUL and mqPCR, and between cPCR and mqPCR	47.33	-902.81	4.31	0.91	**15.81**	**-36.64**	**0.97**	**0.71**
CD between CUL and cPCR	60.98	-909.38	4.56	0.99	27.15	-42.32	1.43	0.88
CD between cPCR and mqPCR	47.30	-902.81	4.23	0.81	16.48	-36.92	0.78	0.59
CD between CUL and mqPCR	59.39	-908.71	4.39	0.98	27.69	-42.57	1.43	0.89
	Serogroup O121				Serogroup O145			
	DIC	LPML	*p*D	Bayesp	DIC	LPML	*p*D	Bayesp
Pairwise conditional dependence (CD) amongst all methods	34.82	-676.12	3.04	0.67	30.37	-212.48	3.87	0.60
CD between CUL and cPCR, and between CUL and mqPCR	51.16	-684.30	2.92	0.95	34.36	-214.40	3.84	0.66
CD between CUL and cPCR, and between cPCR and mqPCR	**34.81**	**-675.99**	**2.94**	**0.67**	30.17	-212.36	3.79	0.61
CD between CUL and mqPCR, and between cPCR and mqPCR	35.68	-676.44	2.79	0.70	30.62	-212.54	3.65	0.56
CD between CUL and cPCR	50.41	-683.82	2.81	0.95	33.80	-214.10	3.77	0.63
CD between cPCR and mqPCR	35.45	-676.23	2.66	0.65	**29.96**	**-212.17**	**3.49**	**0.48**
CD between CUL and mqPCR	50.75	-683.60	3.08	0.95	34.34	-214.32	3.56	0.66

CUL; Culture; cPCR: Conventional PCR; mqPCR: multiplex quantitative PCR; DIC: Deviance Information Criterion; LPML: Log Pseudo Marginal Likelihood; *p*D: the number of parameters effectively estimated by a model; Bayesp: Bayesian *p*-value

Table 4.6 - Model fit statistics and posterior inference (i.e. posterior median and 95% HPDI) on prevalence, sensitivity and specificity of culture, conventional PCR, and multiplex quantitative PCR based on consensus prior model and its sensitivity analysis for the detection of *E. coli* O26 in bovine feces

Prior set	Models and Tests	Bayesp	*p*D	DIC	LPML	Prevalence (95% HPDI)	Sensitivity (95% HPDI)	Specificity (95% HPDI)
CM	Consensus prior (CP) for Se & Sp	0.55	2.62	39.73	–862.56	58.5 (50.1, 65.8)		
	Culture						38.0 (31.8, 44.7)	98.4 (96.4, 99.7)
	cPCR						70.1 (63.3, 76.9)	94.0 (85.1, 99.7)
	mqPCR						91.2 (85.5, 96.5)	89.7 (78.8, 98.0)
SM1	Widened prior for Se & Sp	0.53	0.18	37.01	–862.79	33.2 (20.8, 51.9)		
	Culture						64.6 (38.8, 91.8)	98.6 (96.7, 99.8)
	cPCR						66.7 (58.2, 75.0)	67.6 (58.9, 81.9)
	mqPCR						89.1 (82.7, 95.3)	57.2 (47.1, 75.3)
SM2	CP for Se; Widened for Sp	0.52	1.76	38.45	–862.66	35.4 (24.4, 50.0)		
	Culture						60.4 (41.7, 79.1)	98.5 (96.6, 99.8)
	cPCR						68.2 (60.0, 76.0)	68.9 (60.9, 80.7)
	mqPCR						90.1 (84.1, 95.6)	59.0 (49.4, 73.4)
SM3	CP for Sp; Widened for Se	0.53	2.82	39.61	–862.43	59.7 (51.6, 66.8)		
	Culture						36.5 (30.5, 42.9)	98.5 (96.6, 99.8)
	cPCR						68.9 (61.9, 75.8)	94.7 (86.4, 99.7)
	mqPCR						90.2 (83.9, 96.2)	90.9 (80.7, 98.4)

CM: Consensus model; SM: Sensitivity analysis model; Se: Sensitivity; Sp: Specificity; cPCR: Conventional PCR; mqPCR: multiplex quantitative PCR; Bayesp: Bayesian *p*-value; *p*D: the number of parameters effectively estimated by a model; DIC: Deviance Information Criterion; LPML: Log Pseudo Marginal Likelihood

Table 4.7 - Model fit statistics and posterior inference (i.e. posterior median and 95% HPDI) on prevalence, sensitivity and specificity of culture, conventional PCR, and multiplex quantitative PCR based on consensus prior model and its sensitivity analysis for the detection of *E. coli* O45 in bovine feces

Prior set	Models and Tests	Bayesp	*p*D	DIC	LPML	Prevalence (95% HPDI)	Sensitivity (95% HPDI)	Specificity (95% HPDI)
CM	Consensus prior (CP) for Se & Sp	0.58	3.61	40.72	–821.21	28.9 (20.2, 37.2)		
	Culture						53.3 (40.3, 69.5)	98.5 (96.8, 99.7)
	cPCR						53.5 (43.2, 63.8)	96.4 (91.9, 99.7)
	mqPCR						93.2 (87.3, 98.5)	62.2 (54.9, 69.3)
SM1	Widened prior for Se & Sp	0.52	2.89	39.03	–820.87	24.8 (14.9, 36.2)		
	Culture						61.6 (40.3, 90.8)	98.6 (96.9, 99.8)
	cPCR						51.5 (40.9, 62.2)	93.3 (81.1, 99.9)
	mqPCR						91.8 (84.8, 98.4)	56.9 (49.2, 66.4)
SM2	CP for Se; Widened for Sp	0.53	3.41	39.71	–820.85	26.0 (17.5, 35.1)		
	Culture						58.3 (42.7, 77.6)	98.4 (96.7, 99.7)
	cPCR						54.7 (44.6, 65.1)	94.7 (89.7, 99.9)
	mqPCR						92.7 (86.6, 98.4)	57.9 (50.7, 65.6)
SM3	CP for Sp; Widened for Se	0.59	3.60	40.91	–821.44	30.8 (19.7, 40.8)		
	Culture						49.9 (35.2, 71.5)	98.6 (96.9, 99.7)
	cPCR						49.8 (38.9, 60.7)	96.7 (91.5, 99.8)
	mqPCR						92.5 (85.9, 98.8)	63.4 (54.6, 72.0)

CM: Consensus model; SM: Sensitivity analysis model; Se: Sensitivity; Sp: Specificity; cPCR: Conventional PCR; mqPCR: multiplex quantitative PCR; Bayesp: Bayesian *p*-value; *p*D: the number of parameters effectively estimated by a model; DIC: Deviance Information Criterion; LPML: Log Pseudo Marginal Likelihood

Table 4.8 - Model fit statistics and posterior inference (i.e. posterior median and 95% HPDI) on prevalence, sensitivity and specificity of culture, conventional PCR, and multiplex quantitative PCR based on consensus prior model and its sensitivity analysis for the detection of *E. coli* O103 in bovine feces

Prior set	Models and Tests	Bayesp	*p*D	DIC	LPML	Prevalence (95% HPDI)	Sensitivity (95% HPDI)	Specificity (95% HPDI)
CM	Consensus prior (CP) for Se & Sp	0.65	4.52	42.66	–900.25	85.8 (81.7, 89.4)		
	Culture						69.2 (64.7, 73.6)	98.7 (96.8, 99.8)
	cPCR						64.8 (60.2, 69.3)	96.4 (90.6, 99.7)
	mqPCR						95.7 (93.5, 97.7)	92.3 (80.4, 99.7)
SM1	Widened prior for Se & Sp	0.58	1.39	39.04	–900.08	72.0 (59.8, 82.4)		
	Culture						81.8 (71.2, 96.4)	98.6 (96.8, 99.8)
	cPCR						70.5 (61.9, 77.4)	82.9 (60.9, 99.9)
	mqPCR						95.7 (93.3, 97.8)	47.0 (30.2, 68.6)
SM2	CP for Se; Widened for Sp	0.57	2.92	40.48	–899.99	73.9 (64.2, 83.4)		
	Culture						79.5 (70.2, 90.0)	98.6 (96.8, 99.8)
	cPCR						71.3 (62.6, 78.1)	89.7 (64.0, 99.9)
	mqPCR						95.8 (93.5, 97.7)	50.2 (34.0, 71.2)
SM3	CP for Sp; Widened for Se	0.66	4.58	42.75	–900.29	85.8 (81.8, 89.4)		
	Culture						69.2 (64.7, 73.6)	98.6 (96.7, 99.8)
	cPCR						64.5 (59.9, 69.0)	96.3 (90.6, 99.7)
	mqPCR						95.8 (93.4, 97.7)	92.4 (80.8, 99.7)

CM: Consensus model; SM: Sensitivity analysis model; Se: Sensitivity; Sp: Specificity; cPCR: Conventional PCR; mqPCR: multiplex quantitative PCR; Bayesp: Bayesian *p*-value; *p*D: the number of parameters effectively estimated by a model; DIC: Deviance Information Criterion; LPML: Log Pseudo Marginal Likelihood

Table 4.9 - Model fit statistics and posterior inference (i.e. posterior median and 95% HPDI) on prevalence, sensitivity and specificity of culture, conventional PCR, and multiplex quantitative PCR based on consensus prior model and its sensitivity analysis for the detection of *E. coli* O111 in bovine feces

Prior set	Models and Tests	Bayesp	*p*D	DIC	LPML	Prevalence (95% HPDI)	Sensitivity (95% HPDI)	Specificity (95% HPDI)
CM	Consensus prior (CP) for Se & Sp	0.71	0.97	15.81	–36.64	0.24 (0.00, 0.84)		
	Culture						49.3 (31.2, 66.6)	99.2 (98.4, 99.8)
	cPCR						70.3 (46.1, 90.9)	98.8 (97.8, 99.6)
	mqPCR						80.8 (63.8, 94.5)	98.8 (97.9, 99.6)
SM1	Widened prior for Se & Sp	0.65	0.99	14.91	–36.25	0.29 (0.00, 1.12)		
	Culture						38.8 (11.8, 68.8)	99.3 (98.6, 99.8)
	cPCR						50.9 (19.7, 79.4)	99.2 (98.2, 99.8)
	mqPCR						57.9 (29.1, 85.1)	99.1 (98.2, 99.8)
SM2	CP for Se; Widened for Sp	0.66	1.19	15.22	–36.44	0.28 (0.00, 0.99)		
	Culture						48.7 (31.0, 66.3)	99.3 (98.5, 99.8)
	cPCR						70.7 (46.4, 91.5)	99.1 (98.2, 99.9)
	mqPCR						81.0 (64.3, 95.1)	99.1 (98.2, 99.9)
SM3	CP for Sp; Widened for Se	0.72	0.84	15.90	–36.68	0.26 (0.00, 0.97)		
	Culture						39.9 (12.3, 69.9)	99.3 (98.4, 99.8)
	cPCR						49.6 (19.1, 79.1)	98.8 (97.8, 99.5)
	mqPCR						57.2 (27.9, 84.0)	98.8 (97.8, 99.5)

CM: Consensus model; SM: Sensitivity analysis model; Se: Sensitivity; Sp: Specificity; cPCR: Conventional PCR; mqPCR: multiplex quantitative PCR; Bayesp: Bayesian *p*-value; *p*D: the number of parameters effectively estimated by a model; DIC: Deviance Information Criterion; LPML: Log Pseudo Marginal Likelihood

Table 4.10 - Model fit statistics and posterior inference (i.e. posterior median and 95% HPDI) on prevalence, sensitivity and specificity of culture, conventional PCR, and multiplex quantitative PCR based on consensus prior model and its sensitivity analysis for the detection of *E. coli* O121 in bovine feces

Prior set	Models and Tests	Bayesp	*p*D	DIC	LPML	Prevalence (95% HPDI)	Sensitivity (95% HPDI)	Specificity (95% HPDI)
CM	Consensus prior (CP) for Se & Sp	0.67	2.94	34.81	–675.99	3.87 (0.81, 8.09)		
	Culture						47.2 (28.6, 65.8)	99.1 (97.9, 99.8)
	cPCR						71.8 (48.3, 90.6)	80.4 (76.5, 84.3)
	mqPCR						87.7 (75.4, 97.0)	46.2 (41.9, 50.6)
SM1	Widened prior for Se & Sp	0.70	1.59	33.26	–675.97	3.72 (0.05, 11.1)		
	Culture						36.9 (10.5, 66.7)	98.7 (97.4, 99.8)
	cPCR						48.6 (19.5, 77.3)	78.4 (74.1, 82.6)
	mqPCR						75.8 (51.6, 94.2)	43.7 (39.2, 48.5)
SM2	CP for Se; Widened for Sp	0.58	3.16	33.34	–675.16	3.33 (0.64, 6.68)		
	Culture						49.2 (31.7, 67.2)	98.9 (97.7, 99.8)
	cPCR						70.6 (46.9, 90.0)	78.8 (74.9, 82.5)
	mqPCR						87.0 (74.2, 96.7)	43.8 (39.5, 48.0)
SM3	CP for Sp; Widened for Se	0.69	–28.35	.	–676.32	–		
	Culture						–	–
	cPCR						–	–
	mqPCR						–	–

CM: Consensus model; SM: Sensitivity analysis model; Se: Sensitivity; Sp: Specificity; cPCR: Conventional PCR; mqPCR: multiplex quantitative PCR; Bayesp: Bayesian *p*-value; *p*D: the number of parameters effectively estimated by a model; DIC: Deviance Information Criterion; LPML: Log Pseudo Marginal Likelihood

Table 4.11 - Model fit statistics and posterior inference (i.e. posterior median and 95% HPDI) on prevalence, sensitivity and specificity of culture, conventional PCR, and multiplex quantitative PCR based on consensus prior model and its sensitivity analysis for the detection of *E. coli* O145 in bovine feces

Prior set	Models and Tests	Bayesp	*p*D	DIC	LPML	Prevalence (95% HPDI)	Sensitivity (95% HPDI)	Specificity (95% HPDI)
CM	Consensus prior (CP) for Se & Sp	0.48	3.49	29.96	–212.17	2.24 (0.95, 3.83)		
	Culture						71.9 (53.9, 88.2)	98.7 (97.7, 99.4)
	cPCR						71.9 (53.4, 88.5)	99.2 (98.3, 99.9)
	mqPCR						87.2 (73.0, 98.0)	95.7 (93.8, 97.3)
SM1	Widened prior for Se & Sp	0.47	3.60	29.69	–212.18	2.69 (1.10, 4.71)		
	Culture						66.5 (40.8, 90.9)	98.8 (97.9, 99.6)
	cPCR						61.7 (37.1, 85.1)	99.6 (98.8, 99.9)
	mqPCR						78.4 (54.8, 97.4)	95.9 (94.1, 97.6)
SM2	CP for Se; Widened for Sp	0.40	3.52	28.67	–211.53	2.39 (1.04, 4.02)		
	Culture						70.1 (52.1, 86.6)	98.7 (97.7, 99.4)
	cPCR						72.3 (54.0, 88.6)	99.6 (98.8, 99.9)
	mqPCR						87.4 (73.2, 97.8)	95.9 (94.0, 97.5)
SM3	CP for Sp; Widened for Se	0.55	3.57	31.02	–212.83	2.51 (0.99, 4.46)		
	Culture						70.3 (44.6, 93.9)	98.8 (97.8, 99.6)
	cPCR						61.2 (35.9, 84.6)	99.2 (98.3, 99.9)
	mqPCR						77.9 (54.1, 97.4)	95.7 (93.8, 97.4)

CM: Consensus model; SM: Sensitivity analysis model; Se: Sensitivity; Sp: Specificity; cPCR: Conventional PCR; mqPCR: multiplex quantitative PCR; Bayesp: Bayesian *p*-value; *p*D: the number of parameters effectively estimated by the model; DIC: Deviance Information Criterion; LPML: Log Pseudo Marginal Likelihood

Table 4.12 - Posterior inference on the difference between detection methods on diagnostic sensitivity and specificity of *E. coli* O26, O45, O103, O111, O121, and O145 in bovine feces

Serogroup	Method comparison	95% HPDI of Difference	
		Sensitivity	Specificity
O26	Culture vs. cPCR	–0.41, –0.23	–0.02, 0.14
	Culture vs. mqPCR	–0.61, –0.45	–0.00, 0.20
	cPCR vs mqPCR	–0.27, –0.15	–0.01, 0.10
O45	Culture vs. cPCR	–0.15, 0.17	–0.02, 0.07
	Culture vs. mqPCR	–0.54, –0.23	0.29, 0.44
	cPCR vs mqPCR	–0.50, –0.30	0.28, 0.40
O103	Culture vs. cPCR	–0.01, 0.10	–0.02, 0.09
	Culture vs. mqPCR	–0.31, –0.22	–0.02, 0.19
	cPCR vs mqPCR	–0.35, –0.27	–0.04, 0.16
O111	Culture vs. cPCR	–0.50, 0.09	–0.01, 0.02
	Culture vs. mqPCR	–0.54, –0.06	–0.01, 0.02
	cPCR vs mqPCR	–0.39, 0.17	–0.01, 0.01
O121	Culture vs. cPCR	–0.52, 0.07	0.15, 0.22
	Culture vs. mqPCR	–0.62, –0.17	0.48, 0.57
	cPCR vs mqPCR	–0.41, 0.05	0.30, 0.38
O145	Culture vs. cPCR	–0.24, 0.25	–0.02, 0.01
	Culture vs. mqPCR	–0.37, 0.07	0.01, 0.05
	cPCR vs mqPCR	–0.36, 0.05	0.02, 0.05

* Two detection methods were considered to differ in either sensitivity or specificity if the corresponding 95% HPDI on their pairwise differences did not include the null value zero

Chapter 5 - Quantitative risk assessment of *Escherichia coli* O26, O45, O103, O111, O121, O145, and O157 in beef production in the United States: The impact of peri-harvest interventions

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Abstract

A quantitative microbial risk assessment (QMRA) model was developed to estimate the prevalence and concentration of enterohemorrhagic *Escherichia coli* strains O26, O45, O103, O111, O121, O145, and O157, collectively known as "EHEC-7", on pre-evisceration beef carcasses processed in the United States. We derived probability distributions for the true prevalence and concentration of EHEC-7 in feces of harvest-ready cattle in the U.S, and model the impact of peri-harvest interventions on the prevalence and concentration of EHEC-7 on pre-evisceration beef carcass. The model incorporated data from targeted research, simulation, and from systematic review and meta-analysis of published literature. A second order Monte Carlo simulation model, programmed in R 3.2.2, accounting for variability and uncertainty in fecal concentrations of EHEC-7, was used to generate prevalence and concentration of EHEC-7 on

cattle hides and carcasses at harvest. Impact of on-farm control measures (vaccination and direct-fed microbials), in-plant animal/hide wash interventions, fecal-to-hide transfer, and hide-to-carcass transfer on the prevalence and concentration of EHEC-7 on pre-evisceration beef carcasses were evaluated. Each model run was based on 5,000,000 variability iterations and 100 uncertainty iterations for a total of 500,000,000 simulated results for each scenario. The model-estimated median prevalence of EHEC O26, O45, O103, O111, O121, O145, and O157 on pre-evisceration carcasses from fed beef during summer was 0.16% (95% PI: 0.02–0.50%), 0.04% (0.01, 0.14%), 0.21% (0.03, 0.59%), 0.09% (0.02, 0.24%), 0.03% (0.01, 0.06%), 0.10% (0.05, 0.16%), and 5.05% (0.27, 23.87%), respectively. The estimated median concentration (for positive and negative samples) of EHEC O26, O45, O103, O111, O121, O145, and O157 was –11.16 $\log_{10}$ CFU/100cm^2 (95% PI: –17.87, –4.41), –12.53 $\log_{10}$ CFU/100cm^2 (95% PI: –19.24, –5.81), –10.85 $\log_{10}$ CFU/100cm^2 (95% PI: –17.73, –4.01), –14.13 $\log_{10}$ CFU/100cm^2 (95% PI: –22.57, –5.82), –17.15 $\log_{10}$ CFU/100cm^2 (95% PI: –26.59, –7.82), –14.49 $\log_{10}$ CFU/100cm^2 (95% PI: –23.07, –5.72), and –7.80 $\log_{10}$ CFU/100cm^2 (95% PI: –14.75, –0.64), respectively. Implementation of a full peri-harvest intervention scenario (46% of fed beef vaccinated, 54% fed diet containing direct-fed microbials, and 100% of cattle processed at the large plants had hide wash prior to hide removal) resulted in 51% to 72% reduction in estimated median prevalence of EHEC-7 on beef carcasses and 1.4 to 2.8 $\log_{10}$ CFU/100cm^2 reduction in estimated median concentration of EHEC-7 compared to the no intervention scenario. Sensitivity analysis indicated that fecal-to-hide transfer and hide-to-carcass transfer factors significantly affected prevalence and concentration of EHEC-7 on beef carcasses. The outputs from the study serve as input variables for the post-harvest, retail, and consumer phases of the quantitative risk assessment of the probability of human illness from consumption of beef products contaminated with EHEC-7 in the United States.

Keyword: Quantitative microbial risk assessment, QMRA, *E. coli* O157, non-O157, beef production, vaccination, direct fed microbial, hide wash

Introduction

Enterohemorrhagic *Escherichia coli* strains O26, O45, O103, O111, O121, O145, and O157, collectively known as “top seven EHEC”, are important foodborne pathogens associated with severe illnesses such as bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and death in the United States (Centers for Disease Control and Prevention, 1993), especially in children, the elderly, and immunocompromised individuals. Cattle are a major reservoir of Shiga toxin-producing *Escherichia coli* (STEC) strains, of which EHEC are a pathogenic subset. Transmission to humans may occur through contaminated food, water, environment, or person-to-person contact. In the United States, 73,890 bacterial associated foodborne disease outbreaks were reported from 1998 to 2008 with 4,844 (6.65%) outbreaks attributable to *E. coli* O157 STEC and 37 (0.05%) to non-O157 STEC (Painter et al., 2013). Of the estimated 3,645,773 bacterial associated foodborne illnesses recorded during the period, 63,153 (1.73%) were due to O157 STEC and 112,752 (3.09%) were due to non-O157 STEC. Of the estimated 35,797 hospitalizations, 2,138 (5.97%) were due to O157 STEC and 271 (0.76%) were due to non-O157 STEC; while 20 (2.32%) of the estimated 862 deaths were due to O157 STEC and 1 (0.12%) was due to non-O157 STEC. In the same study, 103 of 186 (55.3%) foodborne disease outbreaks caused by O157 STEC were attributable to beef and 59 (31.7%) to leafy vegetables; of 6 outbreaks caused by non-O157 STEC, an equal proportion of outbreaks (50.0%) were attributable to beef and plant-based produce.

The beef industry in the United States is significantly impacted by STEC with billions of dollars spent by the industry to combat STEC in the beef production chain (Kay, 2003). Cattle shed STEC in their feces, which contaminates the environment and cattle hides (Jacob et al., 2010). Contamination of carcass surfaces may occur from transfer of fecal material from the hide during the process of hide removal at slaughter (Arthur et al., 2010; Greig et al., 2012; Moxley and Acuff, 2014), leading to contamination of beef products further down the production line. Evidence of significant positive correlation exists for the prevalence and levels of *E. coli* O157 in feces, on hides, and carcasses within lots of cattle slaughtered in the U.S. (Elder et al., 2000; Barkocy-Gallagher et al., 2001; Arthur et al., 2004). Since the devastating *E. coli* O157:H7 outbreak associated with the consumption of contaminated undercooked hamburgers in the U.S. in 1993, efforts have been made to ensure better meat safety and the protection of public health. These efforts include the application of pre-harvest interventions focused on reducing EHEC fecal shedding and concentration on hides of live cattle (LeJeune and Wetzel, 2007; Callaway et al., 2013), application of multi-hurdle intervention strategies focused at reducing carcass contamination and the decontamination of carcasses during the slaughtering process (Koohmaraie et al., 2005; Moxley and Acuff, 2014; Wheeler et al., 2014), and the testing of final beef products for EHEC presence to prevent contaminated products from entering the market (Koohmaraie et al., 2007). Other efforts included the development of quantitative microbial risk assessments (QMRA) to estimate the probability of illness from consumption of ground beef contaminated with *E. coli* O157 (Ebel et al., 2004; Kiermeier et al., 2015). QMRA is a tool increasingly used to provide scientific support for the assessment of food safety risks and development of regulatory policies. It has been used to estimate the burden of illness associated with exposure to a food hazard, to evaluate potential risk reduction strategies, and to inform risk management policy decisions (Smith et al., 2013; Pouillot et al., 2015). Our study objectives

were i) to evaluate the available scientific evidence to derive probability distributions for the true prevalence and concentration of EHEC O26, O45, O103, O111, O121, O145, and O157 (EHEC-7) in feces of harvest-ready cattle, on cattle hides and on carcasses after slaughter in the United States; ii) derive probability distributions for bacterial transfer factors and efficacy of peri-harvest interventions; and iii) generate a second order QMRA to evaluate the impact of peri-harvest interventions on the levels of EHEC-7 on cattle at primary production to the pre-evisceration carcass processing stage during slaughter. The outputs from the study serve as input variables for the quantitative microbial risk assessment of the probability of human illness from consumption of beef products contaminated with EHEC-7 in the United States.

Materials and methods

Model development

This model was designed to generate prevalence and concentration distributions for EHEC-7 on beef carcasses at United States slaughter plants. The model begins with distributions of the prevalence and concentration of EHEC-7 in feces of harvest-ready feedlot cattle, adult beef cattle, and adult dairy cattle during the high (April-September) and low (October-March) shedding periods (seasons) in the U.S. Fecal-to-hide and hide-to-carcass microbial transfer coefficients were generated to quantify the transfer of pathogens from cattle feces to hides prior to slaughter, and from cattle hides to carcasses during slaughter at processing plants. Effect of interventions (pre-harvest use of *E. coli* vaccines and direct-fed microbials in live cattle at the feedlot, and peri-harvest application of animal/hide washes at the plant during processing) on prevalence and concentration of EHEC-7 on cattle hides and pre-evisceration carcasses were estimated from published data. The conceptual model for the generation of EHEC-7 prevalence and concentration on cattle carcasses at slaughter is illustrated in Fig. 5.1. The second order

Monte Carlo simulation approach was used to account for variability and uncertainty inherent in the starting concentration of EHEC-7 in feces at production and in the model outcomes. The outputs of the model are (i) the prevalence and concentration of EHEC-7 on cattle hides at harvest, (ii) the prevalence and concentration of EHEC-7 on carcasses post-dehiding and before carcass interventions, and (iii) the effectiveness of peri-harvest interventions at reducing the prevalence and levels of EHEC-7 on beef carcasses at harvest.

Input data

Fecal prevalence

Data describing the apparent prevalence of *E. coli* O157 in feces of fed beef, adult beef, and adult dairy cattle in the U.S. classified by seasons were obtained from a systematic review and meta-analyses study (Ekong et al., 2015). Data describing the apparent prevalence of *E. coli* O26, O45, O103, O111, O121, and O145 in feces of fed beef and adult beef cattle were obtained from published field studies (Dewsbury et al., 2015; Cull et al., 2017), and data describing the apparent prevalence of *E. coli* O26, O45, O103, O111, O121, and O145 in feces of adult dairy cattle were obtained from a published field study (Stromberg et al., 2016). All data (Table 5.1) were based on culture detection methods.

The true fecal prevalence of *E. coli* O157, and its estimate of variability, in the different cattle types and season were computed by applying the Rogan-Gladen estimator (Rogan and Gladen, 1978) in a simulation model.

$$TP = AP + Sp - 1 / Se + Sp - 1$$

where TP = true prevalence, AP = apparent prevalence, Se = diagnostic sensitivity, and Sp = diagnostic specificity of the detection method obtained from a Bayesian estimation of diagnostic

test performance (Ekong et al., 2017a in review). Estimates of true prevalence of *E. coli* O26, O45, O103, O111, O121, and O145 were computed using the diagnostic sensitivity and specificity reported for detection of each O-group from a Bayesian estimation of diagnostic test performance (Ekong et al., 2017b in preparation). For the simulation, the apparent prevalence was fitted as a pert distribution using the minimum, most likely, and maximum values of the observed prevalence for each serogroup. Diagnostic sensitivity and specificity were defined as beta distributions based on the Bayesian analysis of test performance (Ekong et al., 2017a; 2017b in review). The true estimates of fecal prevalence were estimated using Latin Hypercube sampling in a Monte Carlo simulation model with 10,000 iterations. The model was constructed in Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, USA) with the add-on package @Risk (version 7, Palisade Corporation, New York, USA). The distributions of true fecal prevalence of *E. coli* O26, O45, O103, O111, O121, O145, and O157 used in the model are shown in Table 5.2.

Fecal concentration

Fecal concentration data for *E. coli* O157 during the high season were obtained from the literature (Arthur et al., 2007a; 2008; 2011) and from unpublished data from a 2013 field project (Cernicchiaro, unpublished). *E. coli* O157 fecal concentration during the low season was obtained from data reported by Stephens et al. (2009). These data represent fecal concentration of *E. coli* O157 among fed beef during summer and winter months. The high season fecal concentration dataset for *E. coli* O157 consisted of 1,752 samples. Each sample was first tested to confirm the presence/absence of *E. coli* O157 using immunomagnetic separation (IMS)–based culture method (after selective enrichment) with an assumed level of detection of 100 colony forming units (CFU) per gram of feces (2.0 $\log_{10}$ CFU/g). Samples that tested positive were

subjected to quantitative enumeration using a spiral plating method (Robinson et al., 2004) with a detection limit of 200 CFU/g (2.3 $\log_{10}$ CFU/g). Results from the culture and spiral plating assays were integrated to derive concentration data for *E. coli* O157 in cattle feces. Based on the 1,752 samples, the observed prevalence of *E. coli* O157 in the fecal samples was 33.9% (596 positives). A total of 152 samples had quantifiable concentrations above the detection limit with the highest observed concentration of 7.0 $\log_{10}$ CFU/g. The low season fecal concentration dataset for *E. coli* O157 consisted of 2,049 samples; the observed prevalence was 5.1% (105 positives). Fifteen samples had quantifiable concentrations above the detection limit, with the highest observed concentration > 6.0 $\log_{10}$ CFU/g. To derive a fecal concentration distribution for *E. coli* O157 for use in the QMRA, a maximum likelihood estimation algorithm (Shorten et al., 2006) was used to fit a lognormal curve to the observed concentration data. The $\log_{10}$ mean and standard deviation of the lognormal distribution, uncertainty about the lognormal parameters, and the correlation coefficient between the mean and standard deviation were computed. The lognormal distribution has been described as an appropriate fit for pathogen concentration in food microbiology (Kilsby and Pugh, 1981; Legan et al., 2001; Limpert et al., 2001; Crépet et al., 2007; Busschaert et al., 2010).

Fecal concentration data for the non-O157 *E. coli* were only available at the serogroup level (O-group), which includes *E. coli* without Shiga toxin or intimin virulence factors, because prevalence of non-O157 EHEC was too low to give reliable estimates of the concentration distribution. A total of 1,152 samples were tested to detect each of the 6 non-O157 serogroups. First, each sample was tested to confirm the presence/absence of each of the non-O157 serogroups using IMS–based culture from selective EC broth enrichments, with an assumed level of detection of 2.0 $\log_{10}$ CFU/g of feces. Samples that tested positive were subjected to

quantitative enumeration using a spiral plating method (Shridhar et al., 2017) with a detection limit of 3.0 $\log_{10}$ CFU/g of feces. Results from the culture and spiral plating assays were integrated to derive concentration data for *E. coli* serogroups O26, O45, O103, O111, O121, and O145 in cattle feces. These serogroup concentration distributions were inferred to apply to the non-O157 EHEC concentration distributions. For O26 serogroup, the observed prevalence was 23.9%, and 19 samples had quantifiable concentrations above the detection limit with the highest observed concentration being < 6.0 $\log_{10}$ CFU/g. The observed prevalence for serogroup O45 was 16.9%, and 12 samples had quantifiable concentrations above the detection limit, with the highest observed concentration being < 6.0 $\log_{10}$ CFU/g. For the O103 serogroup, the observed prevalence was 49.8%, and 99 samples had quantifiable concentrations above the detection limit, while the highest observed concentration was < 7.0 $\log_{10}$ CFU/g. The observed prevalence for serogroup O111 was 0.8%, and none of the samples were quantifiable. For the O121 serogroup, the observed prevalence was 6.3%, and 7 samples had quantifiable concentrations above the detection limit, with the highest observed concentration being < 7.0 $\log_{10}$ CFU/g. Observed prevalence for the O145 serogroup was 3.1%, and 2 samples had quantifiable concentrations above the detection limit, with the highest observed concentration being < 6.0 $\log_{10}$ CFU/g. As with *E. coli* O157, fecal concentration distributions were derived for *E. coli* serogroups O26, O45, O103, O111, O121, and O145 using the maximum likelihood estimation algorithm (Shorten et al., 2006; Busschaert et al., 2010).

Fecal concentration distributions were then generated for *E. coli* O157 and the six non-O157 at the EHEC level (not serogroup) for the different cattle types by season by integrating the standard deviation estimates of the fitted concentration distributions at the serogroup level with the true EHEC prevalence of *E. coli* O157 and the six non-O157 in the different cattle types and

season. This was accomplished by running a 100,000 iteration simulation in the open-source R software version 3.4.0 (R Core Team, 2015) using the "uniroot" function (Brent, 1973) for each of the seven EHEC groups. In the simulation, pairs of values were randomly selected from the distribution of concentration standard deviation for each serogroup and the distribution of prevalence for each EHEC group. These values were passed to the function to find the geometric mean that matched the prevalence of each EHEC group based on the detection limit of the assay and the selected standard deviation. This approach assumed that the standard deviation fit from the concentration distribution was constant, and only the mean concentration changes as the prevalence changes. The average frequency from the simulation was determined to give the variability of the mean estimate and the standard deviation to estimate the uncertainty about the mean concentration. The derived fecal concentration distributions for each of the seven EHEC serogroups are presented in Table 5.3. It includes estimate of variability ($\log_{10}$ mean and standard deviation), uncertainty (standard deviation of the mean and standard deviation of the standard deviation) about the lognormal parameters, and the correlation coefficient between the mean and standard deviation. The distributions at this stage represent concentration of the seven *E. coli* at the EHEC level.

Fecal-to-hide transfer coefficient

Field study data reported by Arthur et al. (2007a; 2008) were used to model transfer of *E. coli* O157 from cattle feces to hides. The study sampled feces and hides of tagged cattle at United States feedlots immediately prior to cattle loadout and again sampled hides at the processing plants immediately after stunning and bleeding. Concentrations of *E. coli* O157 on fecal samples (expressed as $\log_{10}$ CFU/g) and on hides samples (expressed as $\log_{10}$ CFU/100cm^2) were determined by spiral plating. Fecal-to-hide transfer factor (TF_{fh})was computed for use in

the model, $TF_{fh} = log_{10}(C_h) - log_{10}(C_f)$. C_h is the concentration on hides at the plants and C_f is the concentration in feces, each defined as a pert distribution having minimum, most likely, and maximum values of 0.003, 1.6, 3.6 $\log_{10}$ CFU/100cm^2 and 0.1, 2.3, and 5.4 $\log_{10}$ CFU/g, respectively. Minimum values were derived as described in (Smith et al., 2013). Concentration on hides and feces were correlated using a coefficient of 0.24 derived from Arthur et al. (2007a; 2008) data. The distribution of TF_{fh} was determined using Latin Hypercube sampling in a Monte Carlo simulation model constructed in Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, USA) with the add-on package @Risk (version 7, Palisade Corporation, New York, USA). The model was run with 25,000 iterations to generate TF_{fh}. The resulting normal distribution (normal [–0.783, 1.065]) of TF_{fh} (Table 5.4) was truncated at the minimum and maximum values determined in the simulation: –4.28 and 2.53 $\log_{10}$100cm^2/g, respectively. The same fecal-to-hide transfer coefficient was assumed for the six non-O157 serogroups. Concentrations of individual EHEC on hides were obtained by adding the log transfer factor to the concentration of individual EHEC in feces.

Hide-to-carcass transfer coefficient

Hide-to-carcass transfer was modeled using data on transfer of generic *E. coli* from cattle hides to carcasses following the de-hiding process at slaughter plants in the United States. Concentration of generic *E. coli* on cattle hides and pre-wash pre-eviscerated carcasses were used as a surrogate indicator for the transfer of *E. coli* O157 and the other six non-O157 serogroups. McKiearnan (KSU unpublished data) sampled cattle hides and pre-wash pre-eviscerated carcasses during the commercial slaughter process in four plants in the central United States during summer, winter and spring months. Hide samples were collected post-exsanguination, and pre-wash carcass samples were collected immediately after hide removal but

before any in-plant carcass interventions were applied. Approximately, 2,700 cm^2 area from the brisket to umbilicus and elbow to mid line region was sampled. The concentration of *E. coli* on samples were determined by sponge sampling, followed by plating serial dilutions on 3M™ *E. coli* / coliform (ECC) Petrifilm™ plates. Enumeration data, expressed as $\log_{10}$ CFU/100cm^2, from each sampling period were combined to represent the high and low season. Hide-to-carcass transfer factor, $TF_{hc} = log_{10}(C_{pwc}) - log_{10}(C_h)$, was computed for use in the model. C_{pwc} is the concentration on pre-wash carcasses and C_h is the concentration on hides. Distribution fitting to TF_{hc} was implemented in the open-source software R version 3.4.0 (R Core Team, 2015) utilizing functions from "MASS" (Venables and Ripley, 2002) and "fitdistrplus" (Delignette-Muller and Dutang, 2015) packages. The normal distribution provided the best fit for TF_{hc} during the high and low seasons. The high season distribution mean and standard deviation were –3.64 and 0.81 $\log_{10}$ CFU/100cm^2, respectively. For the low season, the distribution mean was –3.26 and standard deviation was 0.96 $\log_{10}$ CFU/100cm^2 (Table 5.4). Concentrations of individual EHEC on carcasses were obtained by adding the log transfer factor to the concentration of individual EHEC in hides.

Vaccination efficacy

Data from a Systematic Review and Meta-Analysis (SR-MA) on the effect of vaccine on *E. coli* O157 fecal prevalence in cattle (Varela et al., 2013) was used to estimate vaccine effect in the model. From the study, a significant reduction in fecal prevalence of *E. coli* O157 due to vaccine treatment was reported (odds ratio = 0.46; 95% CI: 0.39-0.54). From this meta-analysis, the lower and upper estimates from the 95% confidence interval (CI) of individual studies were each ranked. From these ranked upper and lower estimates, the values corresponding to the 5th percentile of the lower 95% bound and 95th percentile of the upper bound were obtained to

represent the variability of vaccine effect across the population (i.e. 90% CI: 0.13-1.52 to represent the population standard deviation rather than standard error of the mean effect). The odds ratio (OR) was converted to relative risk (RR), the RR was used to compute the vaccine efficacy (expressed as 1 - RR) on fecal prevalence of *E. coli* O157 using the method described by (Vogstad et al., 2014). Vaccine efficacy was described as a lognormal distribution (lognormal [–0.694, 0.531]) based on data extracted from the SR-MA (Varela et al., 2013). Vaccine efficacy was implemented on the *E. coli* fecal prevalence estimate for vaccinated cattle and converted to fecal concentrations using the "uniroot" function (Brent, 1973) in the open-source R software version 3.4.0 (R Core Team, 2015). The estimated mean log reduction (0.153) and standard deviation (1.0), representing vaccine efficacy (Table 5.4) on *E. coli* O157 fecal concentration, were derived by simulating the difference in fecal concentration between vaccinated and non-vaccinated cattle.

Direct fed microbial (DFM) efficacy

Data from an SR-MA on the effect of DFM on *E. coli* O157 fecal prevalence in fed cattle (Wisener et al., 2015) was used to estimate DFM effect in the model. From the study, a significant reduction in fecal prevalence of *E. coli* O157 due to DFM treatment was reported (odds ratio = 0.55; 95% CI: 0.45-0.68). A total of 3810 fed cattle constituted the study units for the estimation of DFM effect. The standard deviation of DFM effect across the population was computed using the total number of study units and the standard error of the mean effect from the SR-MA. This resulted in a 95% confidence interval that captures the variability of DFM effect across the study population (OR = 0.55; 95% CI: 0.13-0.97). The OR was converted to RR, and the RR was used to compute DFM efficacy (expressed as 1 – RR), using the method described by Vogstad et al. (2014). DFM efficacy was described as a lognormal distribution

(lognormal [–0.89, 0.48]) based on data extracted from the SR-MA. DFM efficacy was implemented on the *E. coli* fecal prevalence estimate for cattle fed DFM and converted to fecal concentrations using the "uniroot" function (Brent, 1973) in the open-source R software version 3.4.0 (R Core Team, 2015). The estimated mean log reduction (0.161) and standard deviation (1.0), representing DFM efficacy (Table 5.4) on *E. coli* O157 fecal concentration, were derived by simulating the difference in fecal concentration between DFM fed and non-DFM fed cattle.

Animal and hide wash efficacy

Data on the efficacy of hide washing was obtained from a recent meta-analysis study on the effect of in-plant interventions at reducing concentration of *E. coli* O157 and non-O157 on cattle hides and carcasses during processing (Zhilyaev et al., 2017). The study reported 3.66, 3.02, 2.21, and 0.08 log CFU/cm^2 reductions in pathogen concentration following hide wash with sodium hydroxide, lactic acid, acetic acid, and water, respectively. We defined hide wash efficacy as a normal distribution with a mean log reduction of 3.66 and standard deviation of 0.54 to represent hide wash using sodium hydroxide antimicrobial compounds (Table 5.4).

Model implementation

The model was written in the open-source R software version 3.4.0 (R Core Team, 2015) and used the "snowfall" package (Knaus, 2010) for parallelization. A second-order Monte Carlo modeling approach was used. Each model run was based on 5,000,000 variability iterations and 100 uncertainty iterations for a total of 500,000,000 simulated results for each scenario. To ensure reproducibility in model outcomes, the simulation seed was set at 12345. The fecal concentration of each of the seven EHEC pathogens was assumed to be uncertain and variable, other inputs were assumed to be certain but variable.

Scenario analysis

For each of the EHEC strains, cattle type and season combinations, three separate scenarios were explored to test the effect of peri-harvest interventions on the estimated prevalence and concentration of each EHEC strain on pre-wash pre-evisceration beef carcasses at slaughter. Scenario 1 represents the no intervention (reference scenario) model. In this scenario, no pre-harvest or peri-harvest interventions are applied. Scenario 2 represents the industry current practices (current practices) where selected STEC-targeted interventions were applied based on the proportion of cattle in U.S. establishments that receive the intervention. Based on data from the United States Department of Agriculture, National Animal Health Monitoring System (USDA-NAHMS), 2.4% of U.S. feedlots with a capacity of 1,000 or more head of cattle use the *E. coli* O157 vaccine; overall, only 0.1% of U.S. fed cattle receive the *E. coli* O157 vaccination (USDA-NAHMS, 2013a). Based on the same USDA-NAHMS data, only 28.5% of U.S. feedlots with a capacity of 1,000 or more head of cattle incorporate probiotics in cattle rations; overall, 53.8% of U.S. fed cattle receive probiotic in their diet (USDA-NAHMS, 2013b). Data from the USDA-Food Safety Inspection Service showed that 9% of slaughter operations apply pre-slaughter animal wash and 9% apply a pre-dehiding carcass wash as decontamination procedures to live or slaughtered cattle prior to hide removal (USDA-FSIS, 2008). Scenario 3 represents a hypothetical situation (full intervention) where 46% of cattle received *E. coli* O157 vaccination, 54% are fed probiotic incorporated in the diet, and all (100%) cattle processed in the large slaughter plants received an animal/hide wash intervention prior to de-hiding. Scenario 3 upgrades pre-harvest intervention coverage to 100%, already approximately 54% of fed cattle receive a probiotic diet, and we assume *E. coli* O157 vaccination of the remaining 46%.

Sensitivity analysis and assessment of model performance

Sensitivity analyses were performed to assess the independent influence of fecal-to-hide transfer and the hide-to-carcass transfer parameters on the estimated prevalence and concentration of EHEC-7 on pre-evisceration beef carcasses by fixing each of these input parameters at the 10^{th}, 50^{th}, and 90^{th} percentile values and comparing the estimated prevalence and concentrations. The influence of the application of a vaccination only intervention (i.e., all fed cattle received *E. coli* O157 vaccination but no DFM) and DFM only intervention (i.e., all fed cattle received DFM in their diet but no *E. coli* O157 vaccination) on the prevalence and concentration of *E. coli* O157 on fed beef carcasses was assessed. Similarly, the influence of the application of only a hide intervention on the prevalence and concentration of EHEC O157 and non-O157 EHEC on pre-evisceration carcasses was assessed. Our model-estimated prevalences of EHEC O157 and non-O157 EHEC on hides and pre-wash pre-evisceration carcasses were compared with data from two independent field studies (Stromberg et al., 2015; 2016) not used in building our model. Similarly, our model-estimated concentrations for EHEC O26, O103, O145, and O157 on hides were compared with independent hide data from a field study (Renter et al., unpublished), as there is no suitable data currently available for concentration of EHEC on pre-evisceration beef carcasses.

Results

Prevalence and concentration in feces, hides and carcass

Model-estimated prevalence and concentration for *E. coli* O26, O45, O103, O111, O121, O145, and O157 in feces of fed beef, adult beef, and adult dairy cattle during summer and winter are presented in Table 5.5, while estimated prevalence and concentration on hides are presented

in Table 5.6. Model-estimated median fecal prevalence (proportion detectable by culture with limit of detection set at ≥ 2.0 $\log_{10}$ CFU/g or ≥ 100 CFU/g of feces) of EHEC O157 was higher for fed beef and adult beef compared to adult dairy cattle during summer and winter. The model-estimated median fecal prevalences (based on detection limit of ≥ 2.0 $\log_{10}$ CFU/g of feces) were lower for the non-O157 EHEC (ranged from 0.04% to 0.44% [95% PI: 0.01% to 1.80%]) compared to EHEC O157; however, among the non-O157 EHEC, O26, O103, and O111 were more prevalent.

Model-estimated median fecal concentration (for all samples including positives and negatives) for EHEC O157 in fed beef, adult beef, and adult dairy cattle during summer ranged from –5.70 to –3.72 $\log_{10}$ CFU/g (95% PI: –12.18, 2.95), and ranged from –13.20 to –6.45 $\log_{10}$ CFU/g (95% PI: –23.03, 3.55) during winter (Table 5.5). The model-estimated median concentrations for the non-O157 EHEC in feces ranged from –12.87 to –6.81 $\log_{10}$ CFU/g (95% PI: –21.62, –0.57).

Model-estimated median prevalence of EHEC O157 (proportion detectable by culture with limit of detection set at ≥ 0.7 $\log_{10}$ CFU/100cm^2 or ≥ 5 CFU/100cm^2) on hides of fed beef, adult beef, and adult dairy cattle followed a similar trend as in fecal prevalence; higher in the summer than winter for adult beef and adult dairy, but generally higher than the fecal prevalence estimates (Table 5.6). The model-estimated median prevalence of non-O157 EHEC (based on detection limit of ≥ 0.7 $\log_{10}$ CFU/100cm^2) on cattle hides ranged from 0.09% to 0.97% (95% PI ranged from 0.03% to 3.52%); among the non-O157 EHEC, O26, O103, and O111 were more prevalent (Table 5.6).

Model-estimated median concentration of EHEC O157 on hides of fed beef, adult beef, and adult dairy cattle during summer ranged from –6.48 to –4.50 $\log_{10}$ CFU/100cm^2 (95% PI: –13.28, 2.49), and ranged from –13.98 to –7.23 $\log_{10}$ CFU/100cm^2 (95% PI: –24.03, 2.99) during winter (Table 5.6). The model estimated median concentration for the non-O157 EHEC on cattle hides ranged from –13.67 to –7.55 $\log_{10}$ CFU/100cm^2 (95% PI: –22.69, –0.99).

Model-estimated median prevalence of EHEC O157 (proportion detectable by culture with limit of detection set at ≥ -0.7 $\log_{10}$ CFU/100cm^2 or ≥ 0.2 CFU/100cm^2) on pre-wash pre-evisceration carcasses from fed beef, adult beef, and adult dairy cattle followed a similar trend as for hide and fecal prevalence (Table 5.7), but lower than the estimated median prevalence on hides. Model-estimated median prevalence of non-O157 EHEC (based on a detection limit of ≥ -0.7 $\log_{10}$ CFU/100cm^2) on pre-wash pre-evisceration beef carcasses ranged from 0.03% to 0.28% (95% PI ranged from 0.01% to 1.07%). EHEC O26, O103, and O111 were the most prevalent serogroups (Table 5.7).

Model-estimated median concentration of EHEC O157 on pre-wash pre-evisceration carcasses of fed beef, adult beef, and adult dairy cattle during summer ranged from –9.78 to –7.71 $\log_{10}$ CFU/100cm^2 (95% PI: –16.82, –0.52), and ranged from –17.19 to –10.44 $\log_{10}$ CFU/100cm^2 (95% PI: –27.40, –0.04) during winter (Table 5.7). The model-estimated median concentrations of the non-O157 EHEC on pre-evisceration carcasses ranged from –16.92 to –10.85 $\log_{10}$ CFU/100cm^2 (95% PI: –26.11, –4.01).

Scenario analysis

The simulated current practices (scenario 2) and full intervention (scenario 3) models resulted in a relative reduction in estimated median prevalence and concentration of EHEC O157

and non-O157 EHEC on beef carcasses (Table 5.8). Scenario 2 resulted in variable reductions in estimated median prevalence of EHEC O157 on beef carcasses ranging from 0.85% to 53.5% (95% PI: 0.12%, 62.4%) and reduction in estimated median prevalence of non-O157 EHEC ranging from 2.1% to 61.2% (95% PI: 0.14%, 68.4%) compared to the no intervention scenario (scenario 1), Table 5.8. Scenario 2 produced higher reductions in estimated median prevalence for EHEC O26 and O111 in adult dairy, and EHEC O157 among all cattle types during summer compared to the no intervention scenario. Similarly, scenario 3 resulted in reductions in estimated median prevalence of EHEC O157 ranging 53.1% to 71.6% (95% PI: 41.8%, 83.4%) and reductions in non-O157 EHEC prevalence ranging from 51.4% to 64.8% (95% PI: 32.9%, 71.4%) on beef carcasses compared to scenario 1 (Table 5.8). Scenario 3 produced higher reductions in estimated median prevalence for all EHEC groups and cattle types during summer and winter compared to scenarios 1 and 2.

For impact on estimated concentration, scenario 2 resulted in a $\log_{10}$ reductions in estimated median concentration ranging from 0.04 to 1.99 $\log_{10}$ CFU/100cm^2 (95% PI: 0.01 to 2.83 $\log_{10}$) for EHEC O157 on beef carcasses and reduction ranging from 0.09 to 1.95 $\log_{10}$ (95% PI: 0.07 to 2.77 $\log_{10}$ CFU/100cm^2) for non-O157 EHEC compared to scenario 1, (Table 5.9). Higher log reductions were observed for EHEC O26 and O111 in adult dairy, and for O157 in adult beef, and adult dairy cattle during summer. Scenario 3 resulted in a higher log reduction in estimated median concentration for all EHEC groups by cattle type and season combinations compared to scenarios 1 and 2. The log reductions in estimated median concentration due to scenario 3 range from 2.13 to 2.78 $\log_{10}$ CFU/100cm^2 (95% PI: 1.20 to 3.73 $\log_{10}$ CFU/100cm^2) for EHEC O157 and range from 1.37 to 2.50 $\log_{10}$ CFU/100cm^2 (95% PI: 1.04 to 3.28 $\log_{10}$ CFU/100cm^2) for non-O157 EHEC on beef carcasses compared to scenario 1 (Table 5.9).

Sensitivity Analysis

Sensitivity analysis indicated that fecal-to-hide transfer and hide-to-carcass transfer had a large effect on the prevalence and concentration of EHEC-7 on pre-wash pre-evisceration beef carcasses. Results are presented for EHEC O103 and O157 to represent the effect of transfer factors on O157 and non-O157 EHEC (Table 5.10). Reductions of 82.6% and 92.7% in estimated median prevalence of EHEC O157 and O103, respectively, on pre-evisceration beef carcasses were observed when fecal-to-hide transfer value was fixed at the 10^{th} percentile compared to when it was fixed at the 90^{th} percentile value; the difference in estimated median concentration was 2.7 $\log_{10}$ CFU/100cm^2 for both EHEC O157 and EHEC O103. Similarly, 81.2% and 89.5% differences in estimated median prevalence of EHEC O157 and O103, respectively, were observed on pre-wash pre-evisceration beef carcasses when the hide-to-carcass transfer value was fixed at the 10^{th} percentile compared to when it was fixed at the 90^{th} percentile value; the differences in estimated median concentration were 2.5 $\log_{10}$ CFU/100cm^2 and 2.4 $\log_{10}$ CFU/100cm^2, respectively.

The vaccination only intervention, applicable only to fed beef cattle in this model, resulted in an approximate 39% (95% PI: 27%, 45%) reduction in estimated median prevalence of EHEC O157 on pre-evisceration beef carcasses, and a 0.88 $\log_{10}$ CFU/100cm^2 (95% PI: 0.71, 0.97) reduction in estimated median concentration. Similarly, the DFM only intervention scenario resulted in an approximate 36% (95% PI: 27%, 44%) reduction in estimated median prevalence of EHEC O157 on pre-evisceration beef carcasses, and a 0.84 $\log_{10}$ CFU/100cm^2 (95% PI: 0.69, 0.91) reduction in estimated median concentration. The hide wash only intervention scenario resulted in approximately 47% to 67% reductions in estimated median

prevalence of EHEC O157 and non-O157 EHEC on pre-wash pre-evisceration beef carcasses, and a 1.6 to 2.8 $\log_{10}$ CFU/100cm^2 reduction in estimated median concentration for all EHEC.

Assessment of model performance

The industry current practice scenario (scenario 2) model outputs for the prevalence of EHEC O157 and non-O157 EHEC on cattle hides and pre-wash pre-evisceration carcasses were generally within the range of data obtained from two independent field studies (Stromberg et al., 2015; 2016) that were not included in generating input values in our model building. The data are presented in Table 5.11. On hides, our model-estimated prevalence for EHEC O26 in adult dairy cattle was within the lower end of observed prevalence in the field study, Conversely, for EHEC O157 in fed beef cattle, our model-estimated median hide prevalence and 95% prediction interval were higher than observed in the field study. For pre-wash pre-evisceration carcasses, our model-estimated median prevalence was within the range observed in the field study (Stromberg et al., 2015; 2016) as shown in Table 5.10. For EHEC concentration on cattle hides, our model output prediction intervals for the concentration of EHEC O26, O103, O145, and O157, for which suitable field concentration data were available, were within the range observed in the field study (Renter et al., unpublished). For EHEC O26 and O103, our model estimated ≈ 0 – 2% hide contamination at ≥ 2.0 $\log_{10}$ CFU/100cm^2 (detection limit of the culture-based enumeration assay) among fed beef cattle during summer, while observed field data indicated ≈ 1% hide contamination at this concentration (Figure 5.2). For EHEC O145, our model estimated ≈ 0 – 4% hide contamination at ≥ 2.0 $\log_{10}$ CFU/100cm^2 among fed beef cattle during summer, while observed field data indicated ≈ 2% hide contamination at this concentration. For EHEC O157, our model estimated ≈ 0 – 8% hide contamination at ≥ 2.0 $\log_{10}$ CFU/100cm^2 among adult

beef cattle during summer, while the observed field data indicated ≈ 2% hide contamination at this concentration (Figure 5.2).

Discussion

In this study, we conducted a quantitative microbial risk assessment using a second-order Monte Carlo simulation model to generate prevalence and concentration distributions for EHEC O26, O45, O103, O111, O121, O145, and O157 on cattle hides and pre-wash pre-evisceration beef carcasses. We generated starting concentrations for the seven EHEC strains in feces of harvest-ready fed beef, adult beef, and adult dairy cattle during summer and winter seasons, and derived an expected distribution for the transfer of the EHEC strains from cattle feces onto cattle hides to simulate the level of pathogens on cattle as they arrive the slaughter facility for processing. We also generated a distribution for the transfer of EHEC from cattle hides to the carcass surface (inferred from in-plant generic *E. coli* data) during the de-hiding process to simulate the level of pathogens that gets presented to the post-harvest phase of beef production. We evaluated the impact of *E. coli* O157 vaccination and inclusion of direct-fed microbials in feedlot cattle as a pre-harvest intervention for control of EHEC O157 in live cattle, and the impact of hide/animal washing as a peri-harvest intervention strategy to reduce the frequency and levels of the seven EHEC strains on beef carcasses.

The model-estimated prevalence and concentration of EHEC O157 on cattle hides and pre-evisceration carcasses were generally higher than those of non-O157 EHEC. This pattern is consistent with the observed prevalence of the seven EHEC strains in cattle feces, hides, and carcasses where EHEC O157 was the most frequently observed EHEC strain (Dewsbury et al., 2015; Stromberg et al., 2015; Stromberg et al., 2016; Cull et al., 2017).

The application of the industry current practices (scenario 2) as an intervention scenario resulted in variable reductions in median prevalence of EHEC strains on pre-wash pre-evisceration carcasses compared to the no intervention scenario. Scenario 2 interventions resulted in high reductions (> 20% reduction in prevalence) in pre-evisceration carcass prevalence of EHEC O26 and EHEC O111 on adult dairy, and EHEC O157 on fed beef, adult beef, and adult dairy during summer compared to scenario 1. Conversely, scenario 2 resulted in minimal reduction (< 20% reduction in prevalence) in median prevalence of EHEC O26, O45, O103, O111, O121, and O145 on fed beef, and EHEC O157 on fed beef, adult beef, and adult dairy pre-wash pre-evisceration carcasses during winter compared to scenario 1. The varied effectiveness of scenario 2 in reducing prevalence of EHEC on pre-evisceration carcasses may be related to the proportion of establishments/cattle that received the treatment. Given that vaccination and DFM interventions were only applied for fed beef cattle and only 18% of large processing plants (who harvest about 60% of beef cattle) applied animal/hide wash, appreciable numbers of cattle/carcasses did not receive the interventions, hence the minimal effect recorded with some strains and cattle types. We observed greater log reductions (median log concentration > 1.0 $\log_{10}$) for EHEC O26 and O111 in adult dairy and EHEC O157 in adult beef and adult dairy pre-wash pre-evisceration carcasses during summer comparing scenario 2 to scenario 1, but minimal reduction (median log concentration < 1.0 $\log_{10}$) for other EHEC, cattle types, season combinations. These findings indicate varied effectiveness of the current industry peri-harvest intervention approach at reducing EHEC contamination on pre-evisceration beef carcass, possibly due to differential application of intervention to different cattle types (i.e., higher prevalence and log reductions amongst adult beef and adult dairy) and / or due to limited opportunity for interventions to impact median concentration in low prevalence EHEC groups.

Implementation of an alternative scenario (scenario 3) where 46% of fed cattle are vaccinated, 54% are fed probiotic containing diets, and large slaughter plants perform a hide wash on all (100%) beef carcasses prior to hide removal resulted in a higher percent reduction in estimated median prevalence (51% to 72% reduction in median prevalence) and higher log reduction (1.4 to 2.8 $\log_{10}$ CFU/100cm^2 reduction) in estimated median concentration for all seven EHEC compared to the no intervention scenario. Cattle *E. coli* O157 vaccination at the feedlot and the feeding of direct-fed microbials at the feedlot have been shown in research trials to decrease *E. coli* O157 prevalence and concentrations in cattle feces (Stephens et al., 2007; Thomson et al., 2009; Cull et al., 2012). Use of animal/hide wash interventions at processing plants prior to hide removal has been demonstrated to be effective at reducing the presence and level of *E. coli* O157 and *Salmonella* on beef carcass (Bosilevac et al., 2005; Arthur et al., 2007b; 2008; Bosilevac et al., 2009), suggesting the possible effectiveness of hide wash interventions to reduce the presence and concentration of non-O157 EHEC on cattle hides. Our model showed a 47% to 67% reduction in median prevalence of the non-O157 EHEC on pre-wash pre-eviscerated carcasses, and a 1.6 $\log_{10}$ CFU/100cm^2 to 2.6 $\log_{10}$ CFU/100cm^2 reduction in estimated median concentration as a result of a hide wash only intervention approach. Importance of vaccination and use of direct-fed microbials in live cattle in supplementing in-plant interventions for reducing fecal, hide and carcass contamination have been emphasized (Dodd et al., 2011). Despite the limited adoption of *E. coli* O157 vaccination, our analysis suggests that moderate adoption of a probiotic in cattle diets, combined with a hide wash intervention at the plant, resulted in substantial reduction in EHEC O157 load on pre-evisceration beef carcass during harvest compared to a baseline no intervention scenario. Increasing the proportion of vaccinated cattle, however, lowered *E. coli* O157 prevalence and median concentration further in our model.

Sensitivity analysis regarding the fecal-to-hide and hide-to-carcass transfer parameters showed that the prevalence and concentration of EHEC on pre-wash pre-evisceration beef carcasses were sensitive to the values of the transfer parameters. As expected, separately fixing the transfer parameters to their 90^{th} percentile values while other model parameters were allowed to vary resulted in a considerable increase in estimated median prevalence and concentration of EHEC O157 and non-O157 EHEC serogroups on pre-evisceration beef carcasses. Likewise, fixing the parameter values to their 10^{th} percentile resulted in lower estimated median prevalence and concentration. Carcass contamination that occurs during harvest at the slaughter facility is associated with both the prevalence and concentration of the pathogens in cattle feces and on hides (Arthur et al., 2008; Fox et al., 2008). Evidence suggests that reduction in the prevalence and concentration of pathogens on hides can lower the prevalence and concentration of pathogens on carcasses (Arthur et al., 2004; Bosilevac et al., 2004). High fecal-to-hide transfer may indicate a peak in fecal prevalence, which may lead to increased pathogen load on hides of cattle entering the processing facility. Implementation of pathogen-reduction interventions in live cattle would help reduce pathogen loads on hides of cattle entering the processing plants. Similarly, high hide-to-carcass transfer may be an indication for high pathogen load on hides or significant hide-to-carcass cross-contamination during the de-hiding process, suggestive of poor dressing practices. Several hide and carcass interventions are implemented at the slaughter plants and they have been found to be effective at reducing EHEC contamination on beef carcasses. Combinations of pre-harvest and harvest interventions have been described as the most effective strategy for reducing pathogen contamination in the beef production chain (Loneragan et al., 2005; Callaway et al., 2013; Wheeler et al., 2014).

Conclusion

Our risk assessment model estimated the prevalence and concentration of EHEC-7 on pre-wash pre-evisceration carcasses. The simulation scenario analysis showed the variable effectiveness of current industry intervention practices at reducing carcass contamination, however, a scenario of increased adoption of pre-harvest and hide wash interventions was more effective at reducing risk of carcass contamination. Sensitivity of our model output to fecal-to-hide and hide-to-carcass transfer highlights the importance of good process management as a pathogen reduction strategy. In addition, this study provides the critical initial data required for the farm-to-fork quantitative risk assessment of the public health threat of EHEC O26, O45, O103, O111, O121, O145, and O157 in the beef production chain.

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Figure 5.1 - Flow diagram for the risk assessment of EHEC O26, O45, O103, O111, O121, O145, and O157 on pre-evisceration beef carcass. Dashed boxes indicate intervention point, bold boxes indicate model outputs.

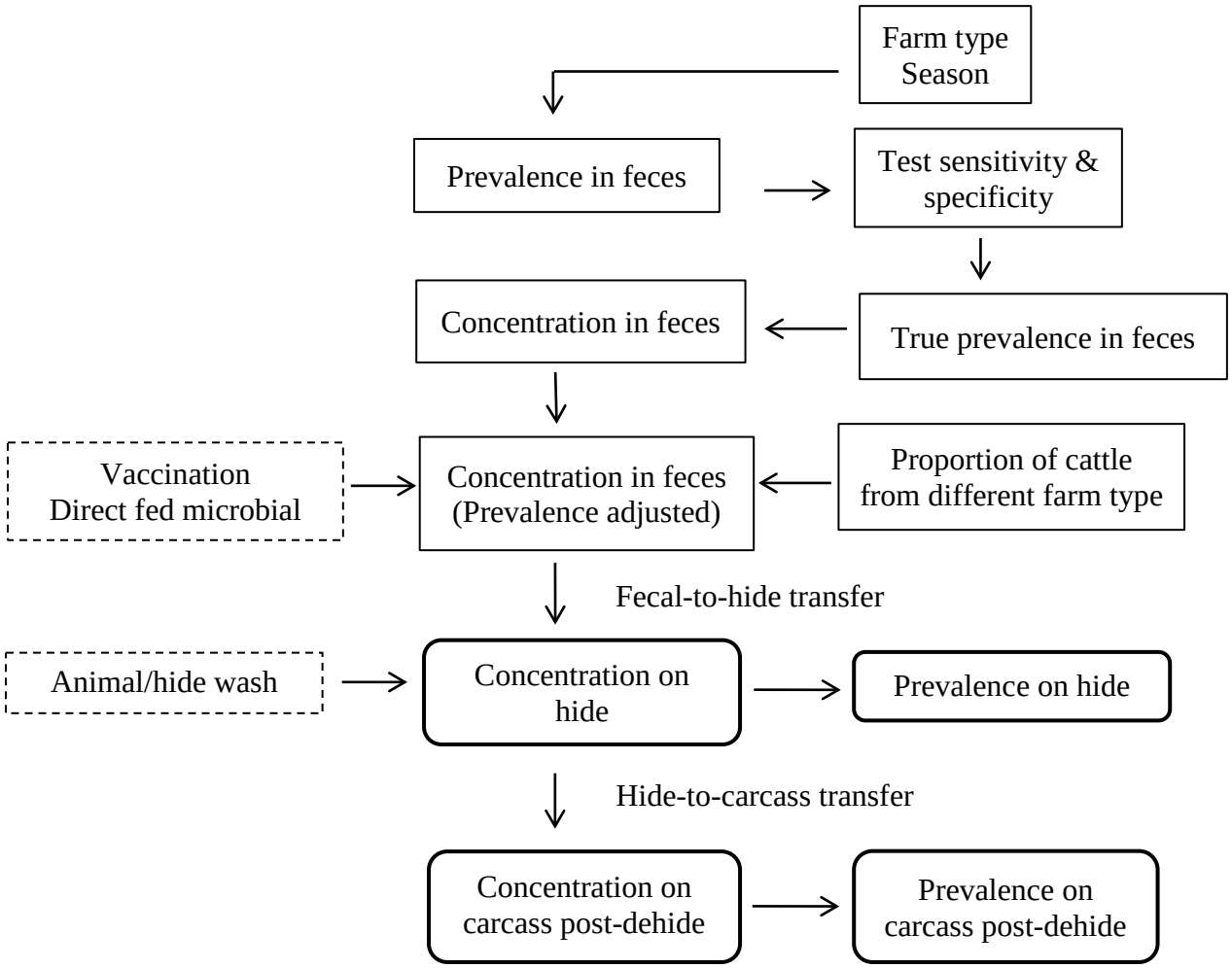


Table 5.1 - Apparent fecal prevalence of EHEC O26, O45, O103, O111, O121, O145, and O157 in fed beef, adult beef, and adult dairy cattle classified by seasons

Strain	Season	Fed beef Mean (Range)	Adult beef Mean (Range)	Adult dairy Mean (Range)
O26	Summer	1.0 (0.0–5.00)	–	1.01 (0.1–9.90)*
	Winter	0.0 (0.0–0.00)	–	
O45	Summer	0.2 (0.0–4.10)	–	0.0 (0.0–0.00)*
	Winter	0.0 (0.0–0.00)	–	
O103	Summer	2.4 (0.0–7.40)	–	0.0 (0.0–0.00)*
	Winter	0.0 (0.0–0.00)	–	
O111	Summer	0.3 (0.0–4.20)	–	1.0 (0.12–7.94)*
	Winter	0.0 (0.0–0.00)	–	
O121	Summer	0.0 (0.0–1.00)	–	0.0 (0.0–0.00)*
	Winter	0.0 (0.0–0.00)	–	
O145	Summer	0.8 (0.0–4.50)	–	0.0 (0.0–0.00)*
	Winter	0.0 (0.0–0.00)	–	
O157	Summer	10.8 (0.0–55.00)	11.83 (5.0–20.0)	2.27 (0.0–35.0)
	Winter	8.37 (0.3–27.78)	4.25 (0.0–20.0)	0.31 (0.06–0.67)

*Interval represents 95% confidence interval;

Source: Ekong et al., 2015; Dewsbury et al., 2015; Stromberg et al., 2016; Cull et al., 2017

Table 5.2 - Distribution for true fecal prevalence of *E. coli* O26, O45, O103, O111, O121, O145, and O157 after adjusting for diagnostic sensitivity and specificity of the detection method. The derived distributions based on best fit were truncated to represent the minimum and maximum values of the estimated true prevalence.

Strain	Season	Fed beef	Adult beef	Adult dairy
O26	Summer	Beta (1.91, 5.90, 0, 0.14)	–	Beta (1.45, 5.99, 0, 0.25)
O45	Summer	Beta (1.23, 7.06, 0, 0.09)	–	–
O103	Summer	Beta (2.33, 3.91, 0, 0.11)	–	–
O111	Summer	Gamma (1.48, 0.013, 0, 0.09)	–	Gamma (1.83, 0.024, 0, 0.17)
O121	Summer	Gamma (1.08, 0.004, 0, 0.03)	–	–
O145	Summer	Beta (1.77, 6.15, 0, 0.07)	–	–
O157	Summer	Beta (1.81, 4.59, 0, 1.18)	Beta (4.47, 4.07, 0, 0.46)	Beta (1.26, 4.83, 0, 0.73)
O157	Winter	Beta (2.26, 4.15, 0, 0.59)	Beta (1.87,4.54, 0, 0.43)	Beta (1.72, 4.74, 0, 0.05)

Table 5.3 - Input parameters for distribution of fecal concentration of *E. coli* O26, O45, O103, O111, O121, O145, and O157 by season and cattle type

			Variability estimate (log_{10} CFU/g)		Uncertainty estimate (log_{10} CFU/g)		
EHEC	Season	Cattle type	Mean	SD	SD of Mean	SD of SD	Correlation
O26	summer	Fed beef	–7.062	3.100	1.168	0.175	–0.782
O26	summer	Adult dairy	–6.614	3.100	1.343	0.175	–0.782
O45	summer	Fed beef	–8.609	3.164	1.269	0.224	–0.871
O103	summer	Fed beef	–6.928	3.198	0.939	0.107	–0.405
O111	summer	Fed beef	–10.500	4.128	2.310	0.743	–0.959
O111	summer	Adult dairy	–9.028	4.128	2.206	0.743	–0.959
O121	summer	Fed beef	–12.820	4.453	1.888	0.511	–0.961
O145	summer	Fed beef	–10.310	4.128	2.179	0.743	–0.979
O157	summer	Fed beef	–3.454	3.355	1.997	0.115	–0.641
O157	summer	Adult beef	–3.554	3.355	0.956	0.115	–0.641
O157	summer	Adult dairy	–5.612	3.355	1.715	0.115	–0.641
O157	winter	Fed beef	–5.955	4.975	2.250	0.451	–0.959
O157	winter	Adult beef	–7.851	4.975	2.242	0.451	–0.959
O157	winter	Adult dairy	–13.220	4.975	1.923	0.451	–0.959

SD: Standard deviation

Table 5.4 - Input parameters and references for transfer factors and peri-harvest intervention efficacies for modeling the concentration of *E. coli* O26, O45, O103, O111, O121, O145, and O157 on cattle hides and carcass

Parameter	Distribution	Unit	Reference(s)
Fecal-to-hide transfer factor	Normal (–0.78, 1.07)	$log_{10}CFU100cm^2/g$	Arthur et al., 2007; 2008
Hide-to-carcass transfer factor	Normal (–3.26, 0.96)	$log_{10}CFU$	McKiearnan (KSU data, unpublished)
Vaccine efficacy	Normal (0.15, 1.00)	$log_{10}CFU$	Varella et al., 2013
Direct fed microbial efficacy	Normal (0.16, 1.00)	$log_{10}CFU$	Wisener et al., 2014
Animal/Hide wash efficacy	Normal (3.66, 0.54)	$log_{10}CFU$	Zhilyaev et al., 2017

Table 5.5 - Model predicted prevalence and concentration of *E. coli* O26, O45, O103, O111, O121, O145, and O157 in cattle feces in the no intervention scenario. Prevalence was computed using culture method detection limit of ≥ 2.0 log_{10} colony forming unit (CFU) per gram of feces or ≥ 100 CFU/g of feces.

Season	Cattle type	Pathogen	Prevalence (%)			Concentration (log_{10} CFU/g)		
		EHEC	Median	2.5^{th} percentile	97.5^{th} percentile	Median	2.5^{th} percentile	97.5^{th} percentile
Summer	Fed beef	O26	0.247	0.034	0.807	–7.095	–13.140	–0.942
	Adult dairy	O26	0.443	0.044	1.795	–6.816	–12.930	–0.645
	Fed beef	O45	0.058	0.011	0.197	–8.490	–14.580	–2.359
	Fed beef	O103	0.337	0.045	0.962	–6.806	–13.110	–0.567
	Fed beef	O111	0.151	0.032	0.357	–10.083	–18.030	–2.279
	Adult dairy	O111	0.441	0.181	1.075	–9.122	–17.550	–0.840
	Fed beef	O121	0.044	0.017	0.084	–12.873	–21.620	–4.126
	Fed beef	O145	0.165	0.079	0.255	–10.440	–18.550	–2.176
	Fed beef	O157	8.120	0.434	35.822	–3.761	–10.110	2.827
	Adult beef	O157	5.111	1.865	11.289	–3.718	–10.260	2.952
	Adult dairy	O157	2.122	0.061	8.875	–5.695	–12.180	0.827
Winter	Fed beef	O157	6.301	1.959	17.837	–6.446	–16.330	3.552
	Adult beef	O157	2.750	0.704	7.248	–8.098	–17.870	1.810
	Adult dairy	O157	0.129	0.047	0.285	–13.200	–23.030	–3.429

Table 5.6 - Model predicted prevalence and concentration of *E. coli* O26, O45, O103, O111, O121, O145, and O157 on cattle hides in the no intervention scenario. Prevalence was computed using culture method detection limit of ≥ 0.7 log_{10} CFU/100cm^2 or ≥ 5 CFU/100cm^2.

Season	Cattle type	Pathogen	Prevalence (%)			Concentration (log_{10} CFU/100cm^2)		
		EHEC	Median	2.5th percentile	97.5th percentile	Median	2.5th percentile	97.5th percentile
Summer	Fed beef	O26	0.610	0.101	1.805	–7.879	–14.270	–1.393
	Adult dairy	O26	0.971	0.126	3.520	–7.597	–14.060	–1.089
	Fed beef	O45	0.178	0.034	0.539	–9.162	–15.690	–2.767
	Fed beef	O103	0.794	0.142	2.121	–7.547	–14.220	–0.985
	Fed beef	O111	0.323	0.099	1.035	–10.896	–19.100	–2.765
	Adult dairy	O111	0.835	0.351	2.222	–9.941	–18.620	–1.359
	Fed beef	O121	0.093	0.038	0.186	–13.668	–22.690	–4.695
	Fed beef	O145	0.326	0.175	0.541	–11.190	–19.580	–2.724
	Fed beef	O157	11.083	0.881	42.373	–4.542	–11.230	2.366
	Adult beef	O157	7.823	3.211	15.764	–4.500	–11.350	2.490
	Adult dairy	O157	3.478	0.173	13.129	–6.479	–13.280	0.364
Winter	Fed beef	O157	8.070	2.606	22.134	–7.231	–17.330	2.998
	Adult beef	O157	3.799	1.015	9.969	–8.876	–18.870	1.255
	Adult dairy	O157	0.223	0.079	0.520	–13.982	–24.030	–3.997

Table 5.7 - Model predicted prevalence and concentration of *E. coli* O26, O45, O103, O111, O121, O145, and O157 on beef carcass by simulated intervention scenarios. Prevalence was computed using culture method detection limit of ≥ –0.7 log_{10} CFU/100cm^2 or ≥ 0.2 CFU/100cm^2.

Scenario	Season	Cattle type	Pathogen	Prevalence (%)			Concentration (log_{10} CFU/100cm^2)		
			EHEC	Median	2.5th percentile	97.5th percentile	Median	2.5th percentile	97.5th percentile
No Intervention	Summer	Fed beef	O26	0.158	0.031	0.502	–11.157	–17.870	–4.405
		Adult dairy	O26	0.276	0.030	1.072	–10.849	–17.560	–4.068
		Fed beef	O45	0.041	0.008	0.135	–12.530	–19.240	–5.807
		Fed beef	O103	0.213	0.032	0.589	–10.850	–17.730	–4.007
		Fed beef	O111	0.096	0.029	0.244	–14.130	–22.570	–5.820
		Adult dairy	O111	0.274	0.109	0.667	–13.160	–22.070	–4.408
		Fed beef	O121	0.029	0.011	0.061	–16.920	–26.110	–7.751
		Fed beef	O145	0.104	0.050	0.160	–14.490	–23.070	–5.724
		Fed beef	O157	5.053	0.308	23.871	–7.800	–14.750	–0.641
		Adult beef	O157	3.091	1.144	6.292	–7.713	–14.840	–0.518
		Adult dairy	O157	1.248	0.041	5.386	–9.780	–16.820	–2.684
	Winter	Fed beef	O157	4.237	1.294	12.530	–10.366	–20.640	–0.051
		Adult beef	O157	1.852	0.527	5.026	–11.965	–22.050	–1.795
		Adult dairy	O157	0.081	0.029	0.174	–17.153	–27.240	–6.939
Current practice	Summer	Fed beef	O26	0.135	0.023	0.425	–11.246	–18.360	–4.483
		Adult dairy	O26	0.102	0.009	0.339	–12.795	–20.330	–5.163
		Fed beef	O45	0.036	0.007	0.126	–12.790	–19.990	–5.880
		Fed beef	O103	0.209	0.028	0.586	–11.110	–18.510	–4.121
		Fed beef	O111	0.078	0.024	0.157	–14.610	–23.350	–6.071
		Adult dairy	O111	0.118	0.055	0.235	–15.070	–24.460	–5.836
		Fed beef	O121	0.027	0.012	0.052	–17.150	–26.590	–7.816
		Fed beef	O145	0.089	0.039	0.189	–15.050	–24.100	–6.198
		Fed beef	O157	3.877	0.268	13.658	–8.164	–15.930	–0.743
		Adult beef	O157	1.498	0.458	4.144	–9.710	–17.660	–1.660
		Adult dairy	O157	0.560	0.024	2.254	–11.686	–19.650	–3.701

	Winter	Fed beef	O157	4.035	1.162	11.841	–10.442	–20.730	–0.037
		Adult beef	O157	1.817	0.462	4.883	–12.063	–22.220	–1.792
		Adult dairy	O157	0.080	0.029	0.171	–17.188	–27.400	–7.023
Full Intervention	Summer	Fed beef	O26	0.056	0.009	0.177	–13.392	–20.930	–5.807
		Adult dairy	O26	0.100	0.007	0.307	–12.872	–20.340	–5.265
		Fed beef	O45	0.015	0.003	0.044	–15.030	–22.520	–7.212
		Fed beef	O103	0.092	0.021	0.258	–13.220	–20.890	–5.430
		Fed beef	O111	0.036	0.011	0.092	–16.580	–25.680	–7.451
		Adult dairy	O111	0.111	0.039	0.216	–15.370	–24.530	–5.926
		Fed beef	O121	0.014	0.007	0.035	–18.290	–27.800	–8.791
		Fed beef	O145	0.041	0.021	0.063	–16.480	–25.770	–7.109
		Fed beef	O157	1.435	0.045	7.264	–10.456	–18.330	–2.315
		Adult beef	O157	1.450	0.387	3.206	–9.841	–17.660	–1.798
		Adult dairy	O157	0.456	0.022	2.022	–11.995	–19.880	–3.884
	Winter	Fed beef	O157	1.547	0.415	3.559	–12.798	–23.570	–1.994
		Adult beef	O157	0.824	0.199	2.615	–14.841	–25.950	–3.820
		Adult dairy	O157	0.031	0.013	0.063	–19.839	–30.790	–8.908

Table 5.8 - Model estimated percentage difference in prevalence of EHEC O26, O45, O103, O111, O121, O145, and O157 between the reference, industry standard, and the full intervention scenarios on beef carcass. Prevalence was computed using culture method detection limit of ≥ –0.7 log_{10} CFU/100cm^2 or ≥ 0.2 CFU/100cm^2.

Season	Cattle type	Pathogen	Percent difference in estimated prevalence					
			Scenario 1 vs 2			Scenario 1 vs 3		
		EHEC	Median	2.5th percentile	97.5th percentile	Median	2.5th percentile	97.5th percentile
Summer	Fed beef	O26	14.31	10.14	25.27	64.76	63.45	64.99
	Adult dairy	O26	61.24	42.60	68.38	63.80	48.41	71.40
	Fed beef	O45	12.31	6.95	19.50	62.81	49.09	67.31
	Fed beef	O103	2.08	0.59	14.50	55.70	43.53	56.24
	Fed beef	O111	19.28	11.41	35.86	60.03	53.56	62.55
	Adult dairy	O111	56.93	29.25	63.90	59.55	49.65	64.82
	Fed beef	O121	3.07	0.14	13.97	51.40	32.91	55.40
	Fed beef	O145	14.17	7.55	21.64	60.26	58.64	60.65
	Fed beef	O157	23.27	12.93	42.78	71.59	55.27	83.41
	Adult beef	O157	51.52	34.13	59.97	53.09	49.04	62.26
	Adult dairy	O157	53.46	45.80	62.43	55.15	41.80	66.16
Winter	Fed beef	O157	4.77	1.39	10.21	63.50	54.69	67.92
	Adult beef	O157	1.91	0.29	3.84	54.66	46.45	56.72
	Adult dairy	O157	0.85	0.12	3.20	62.26	53.85	63.89

Table 5.9 - Model estimated relative difference in concentration of EHEC O26, O45, O103, O111, O121, O145, and O157 between the reference, industry standard, and the full intervention scenarios on beef carcass

Season	Cattle type	Pathogen	Relative difference in estimated concentration (log_{10} CFU/100cm^2)					
			Scenario 1 vs 2			Scenario 1 vs 3		
		EHEC	Median	2.5th percentile	97.5th percentile	Median	2.5th percentile	97.5th percentile
Summer	Fed beef	O26	0.089	0.078	0.490	2.235	1.402	3.060
	Adult dairy	O26	1.946	1.094	2.770	2.023	1.197	2.780
	Fed beef	O45	0.260	0.073	0.750	2.500	1.405	3.280
	Fed beef	O103	0.260	0.114	0.780	2.370	1.423	3.160
	Fed beef	O111	0.480	0.251	0.780	2.450	1.631	3.110
	Adult dairy	O111	1.910	1.518	2.460	2.210	1.428	2.390
	Fed beef	O121	0.230	0.065	0.480	1.370	1.040	1.690
	Fed beef	O145	0.560	0.474	1.030	1.990	1.385	2.700
	Fed beef	O157	0.364	0.102	1.180	2.656	1.674	3.580
	Adult beef	O157	1.997	1.142	2.820	2.128	1.280	2.820
	Adult dairy	O157	1.906	1.017	2.830	2.175	1.200	3.060
Winter	Fed beef	O157	0.076	0.015	0.090	2.356	1.957	2.840
	Adult beef	O157	0.098	0.003	0.170	2.778	2.028	3.730
	Adult dairy	O157	0.035	0.008	0.160	2.705	1.885	3.390

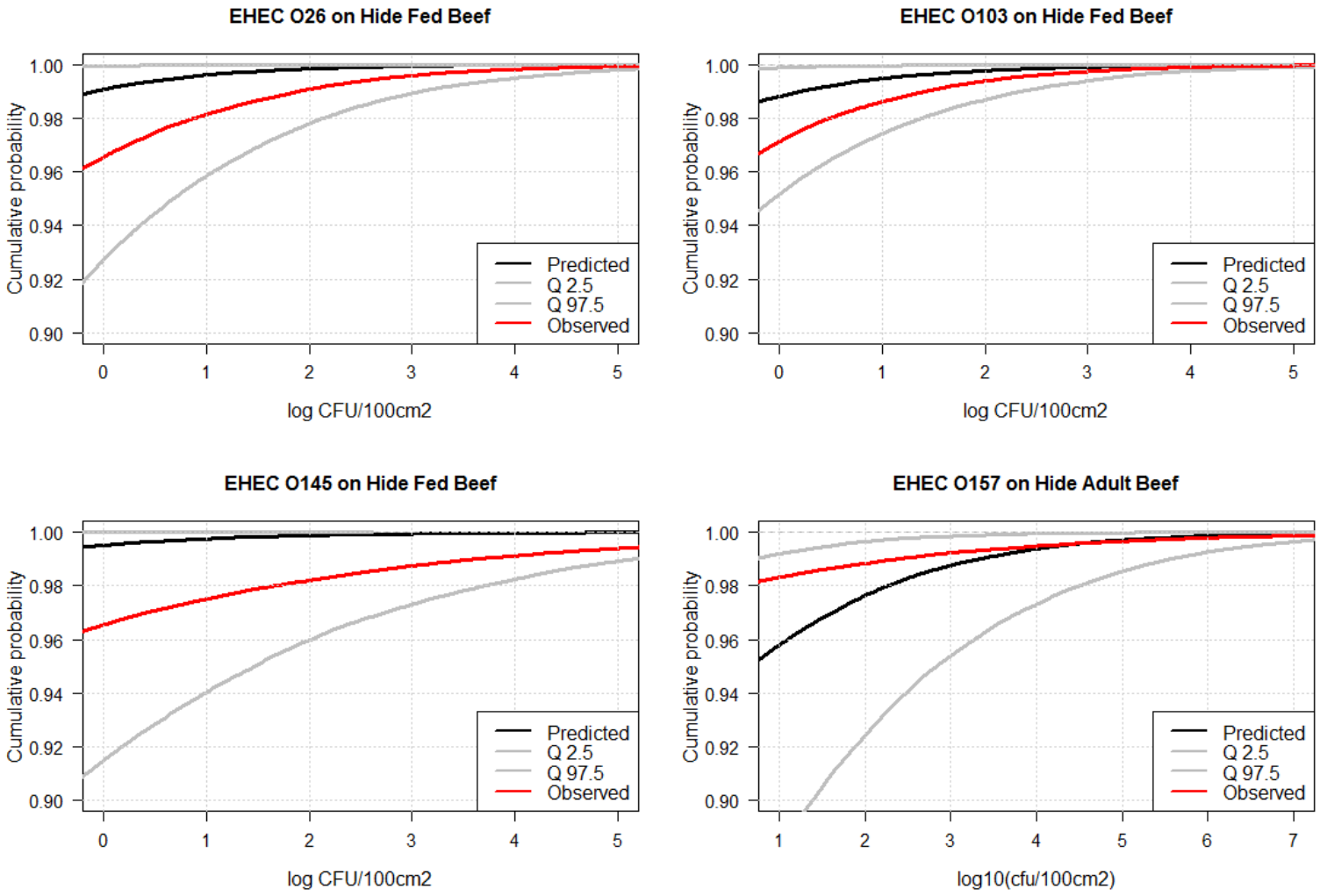
Table 5.10 - Sensitivity analysis showing the impact of fecal-to-hide transfer and hide-to-carcass transfer factors (10^{th}, 50^{th}, and 90^{th} percentiles) on the prevalence and concentration of EHEC O157 and O103 on beef carcass. Prevalence was computed using culture method detection limit of ≥ –0.7 log_{10} CFU/100cm^2 or ≥ 0.2 CFU/100cm^2.

Input parameter	Parameter distribution	Value	Prevalence (%)			Concentration (log_{10} CFU/100cm^2)		
			Median	2.5^{th} percentile	97.5^{th} percentile	Median	2.5^{th} percentile	97.5^{th} percentile
EHEC O157 FBS								
Fecal-to-hide transfer factor	10th	–2.118	1.14	0.08	8.00	–8.78	–15.63	–1.93
	50th	–0.829	2.97	0.24	15.30	–7.39	–14.23	–0.55
	90th	0.545	6.54	0.71	26.80	–6.08	–12.92	0.77
Hide-to-carcass transfer factor	10th	–4.504	1.08	0.10	10.95	–8.74	–15.64	–1.85
	50th	–3.321	2.53	0.29	18.83	–7.53	–14.42	–0.65
	90th	–2.022	5.73	0.85	30.62	–6.24	–13.14	0.64
EHEC O103 FBS								
Fecal-to-hide transfer factor	10th	–2.118	0.03	0.00	0.16	–12.20	–18.72	–5.67
	50th	–0.829	0.11	0.02	0.57	–10.92	–17.45	–4.39
	90th	0.545	0.41	0.09	1.82	–9.55	–16.08	–3.02
Hide-to-carcass transfer factor	10th	–4.504	0.04	0.00	0.15	–12.14	–18.73	–5.56
	50th	–3.321	0.12	0.02	0.50	–10.94	–17.51	–4.37
	90th	–2.022	0.38	0.08	1.22	–9.70	–16.28	–3.12

Table 5.11 - Model predicted median prevalence of EHEC O26, O45, O103, O111, O121, O145, and O157 on hides and pre-evisceration carcass and empirical data from published studies used in assessing model simulation accuracy. Prevalence in our model was computed using culture method detection limit of ≥ –0.7 log_{10} CFU/100cm^2 or ≥ 0.2 CFU/100cm^2.

		Median prevalence [(95% prediction interval)]			
		Hide		Pre-evisceration carcass	
EHEC	Cattle type	Model estimate	Stromberg et al., 2015; 2016	Model estimate	Stromberg et al., 2015; 2016
O26	Fed beef	0.54 (0.14, 1.71)	0.42 (0.11, 1.66)	0.14 (0.02, 0.43)	0.21 (0.03, 1.40)
O26	Adult dairy	0.35 (0.04, 1.04)	3.19 (0.50, 17.59)	0.10 (0.01, 0.34)	1.39 (0.09, 17.35)
O45	Fed beef	0.15 (0.03, 0.51)	0.00 (0.00, 0.00)	0.04 (0.01, 0.13)	1.13 (0.47, 2.71)
O103	Fed beef	0.07 (0.11, 1.92)	0.00 (0.00, 0.00)	0.21 (0.03, 0.59)	1.23 (0.50, 2.99)
O111	Fed beef	0.26 (0.09, 0.62)	0.00 (0.00, 0.00)	0.08 (0.02, 0.16)	0.00 (0.00, 0.00)
O111	Adult dairy	0.34 (0.12, 0.66)	1.00 (0.14, 6.75)	0.12 (0.06, 0.24)	
O121	Fed beef	0.09 (0.04, 0.20)	1.01 (0.09, 9.90)	0.03 (0.01, 0.05)	0.00 (0.00, 0.00)
O145	Fed beef	0.28 (0.15, 0.62)	0.21 (0.03, 1.48)	0.09 (0.04, 0.19)	1.58 (0.74, 3.36)
O157	Fed beef	8.63 (0.99, 26.84)	0.63 (0.20, 1.94)	3.88 (0.27, 13.66)	2.78 (1.71, 4.49)
O157	Adult beef	3.89 (1.38, 9.54)		1.50 (0.46, 4.14)	
O157	Adult dairy	1.33 (0.08, 5.59)	6.65 (1.85, 21.22)	0.56 (0.02, 2.25)	1.00 (0.14, 6.75)

Figure 5.2 - Comparison of model predicted cumulative probability of hide contamination from EHEC O26, O103, O145, O157 and data from published field studies.



Chapter 6 - Dissertation conclusion

The purpose of this research was to develop a quantitative microbial risk assessment (QMRA) model for the seven major Shiga toxin-producing *Escherichia coli* – O26, O45, O103, O111, O121, O145, and O157 – in beef production with a focus on the peri-harvest phase, identify intervention strategies that are effective at reducing risk of exposure, and generate data for the farm-to-fork quantitative risk assessment of the public health threat of the seven EHEC in the beef production chain. Integrating the results of this risk assessment with economic analysis will provide decision makers with the needed tool to determine the effectiveness (public health impact) and efficiency (economic cost) of risk reduction strategies.

Collection of accurate and unbiased data is critical for the development of a QMRA that is valid for decision making. This QMRA incorporated data on the true prevalence and concentration of the seven STEC at the EHEC level in cattle feces with data of fecal-to-hide transfer and hide-to-carcass transfer to estimate the prevalence and concentration of the seven EHEC on cattle hides and pre-evisceration carcasses. Data of the efficacy of pre-harvest (*E. coli* O157 vaccination and use of direct fed microbial in cattle feed at production stage) and peri-harvest (animal/hide wash during slaughter) intervention were incorporated into the QMRA to measure the impact of control strategies.

Estimates of the prevalence of *E. coli* O157 in cattle varies by region (country), cattle type, type of sample, and season. Our systematic review and meta-analysis study provide estimates of the prevalence and concentration of *E. coli* O157 along the beef production chain in the U.S. It revealed differences in the fecal prevalence of *E. coli* O157 among cattle types and seasons, as well as decreasing prevalence and concentration of *E. coli* O157 on cattle hides and carcass surfaces from pre-evisceration to the final chilled carcass stage. Additional sources of

heterogeneity among studies reporting prevalence of *E. coli* O157 along the beef production chain were identified.

Given the imperfection (possibility of misclassification of samples – false positives and false negatives) in the laboratory methods used in detection of the seven STEC in cattle feces, which produce bias estimates of true prevalence, we applied Bayesian formulation of the latent class analysis to estimate the diagnostic performance of three laboratory methods (culture, conventional PCR [cPCR], and multiplex quantitative PCR [mqPCR]) used for the detection of the seven STEC in the feces of cattle. This is the first time this approach was used to estimate the diagnostic sensitivity and specificity of culture, cPCR, and mqPCR methods for the detection of *E.coli* O157 in cattle feces which as enable us to estimate the true prevalence of the seven EHEC in cattle feces.

A second order quantitative microbial risk assessment was developed, based on the data generated studies reported in this research, to quantify the prevalence and concentration of the seven EHEC on pre-evisceration beef carcasses and to evaluate the impact of peri-harvest interventions on the risk of carcass contamination. Simulation scenarios of current industry peri-harvest intervention practices showed variable effectiveness in reducing STEC contamination on pre-evisceration beef carcass. A scenario of increased adoption of peri-harvest interventions was more effective at reducing EHEC contamination and the fecal-to-hide transfer and the hide-to-carcass transfers had a large effect on prevalence and concentration of EHEC on pre-evisceration carcasses. The studies provide the critical initial data required for the farm-to-fork quantitative risk assessment of the public health threat of EHEC O26, O45, O103, O111, O121, O145, and O157 in the beef production chain.