

**Exploring different components of disease transmission for
Verocytotoxigenic *Escherichia coli* (VTEC) infections in Ontario,
Canada**

by

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A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Master of Science
in
Population Medicine

Guelph, Ontario, Canada

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ABSTRACT

Exploring different components of disease transmission for Verocytotoxigenic *Escherichia coli* (VTEC) infections in Ontario, Canada

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The transmission dynamics of human verocytotoxigenic *Escherichia coli* (VTEC) infections are not well understood. This thesis examines the transmission of VTEC, exploring the role of environmental conditions on the risk of primary infections, and the impact of person-to-person transmission during outbreaks. The first study explored the effect of environmental factors on VTEC infections in Ontario using case-crossover analyses. While the magnitude and direction of the effect was variable between health units, the findings demonstrate that infections were associated with hydrological conditions. The second study focused on the potential impact of person-to-person transmission using a mathematical model to describe an outbreak in Ontario. Based on the model results, including public health interventions targeting person-to-person transmission reduced the outbreak size by ~16%. The results from these studies demonstrate that watershed hydrology may be associated with VTEC infections, and that person-to-person transmission is an important transmission route to target for interventions during outbreaks.

ACKNOWLEDGEMENTS

I am so lucky to have had tremendous support throughout my academic journey. First and foremost, I want to thank my advisor Dr. Amy Greer for giving me this opportunity, and for providing me with endless guidance, mentorship, and support. She pushed me to persist through the many challenges that come with doing research, improving my confidence and skills as a researcher. I am also grateful to have had Drs. Jan Sargeant and David Fisman a part of my advisory committee. I am thankful for their support in my research, and for their fascinating discussions during our meetings.

I would also like to thank everyone at the Department of Population Medicine that not only taught me the skills to complete my degree, but also helped me discover my love for epidemiology as a student. Special thanks to Dr. Pearl and Alex Swirski for taking me on as an undergraduate student, helping me get where I am today. I want to also thank my fellow members of the math epi lab for their assistance on my research.

I want to thank my family and friends (back home and in Guelph) for their limitless love and support these last few years. I am thankful that they kept me motivated to succeed, but also reminded me to not let life pass me by. Despite not always understanding what I do, my family has always been supportive of my goals. My friends have kept me sane through the long nights, brought me constant joy and laughter with their stories, and took me on incredible adventures. Lastly, I want to thank Emma, Gina, Muffin, Ryot, Dusty, and Kody for providing me with endless emotional support and attention, even when I did not think I needed it.

STATEMENT OF WORK

Chapter 2: In this chapter, Roksolana Hovdey identified environmental and hydrological factors which were associated with increased risk of verotoxigenic *Escherichia coli* (VTEC) infections in five Public Health Units in Ontario, Canada. The case data of VTEC infections were provided by Public Health Ontario. The environment data were obtained from Environment Canada. All data analysis was conducted by Roksolana Hovdey, with input from Drs. Amy Greer, Jan Sargeant, and David Fisman. The manuscript was written by Roksolana Hovdey, and reviewed and edited by Drs. Amy Greer, Jan Sargeant, and David Fisman.

Chapter 3: Roksolana Hovdey developed a deterministic, compartmental infectious disease model of a VTEC outbreak in Ontario, Canada. The model was parameterized using values from the peer-reviewed literature and calibrated to a documented VTEC outbreak in Ontario. The model was built by Roksolana Hovdey, with input by Drs. Amy Greer, Jan Sargeant, and David Fisman. All mathematical analyses were performed by Roksolana Hovdey, with input by Dr. Amy Greer. The manuscript was written by Roksolana Hovdey, and reviewed and edited by Drs. Amy Greer, Jan Sargeant, and David Fisman.

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CHAPTER 1

INTRODUCTION, LITERATURE REVIEW, STUDY RATIONALE

1.0 INTRODUCTION

Verocytotoxigenic *Escherichia coli* (VTEC) were first identified in Canada as a bacterial pathogen that played a role in causing diarrhea in the 1970s [1]. Compared to other enteric pathogens, VTEC infections are associated with more severe illnesses, including hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) [2,3]. These disorders, which have a higher risk of occurrence in children infected with VTEC (compared to adults), can lead to lifelong disability or death [4–6]. Exposure to VTEC can arise from contact with contaminated food and water, as well as from other people and animals, commonly cattle [4,5,7–13]. The bacteria have been associated with both sporadic infections and larger outbreaks [4,5,7]. In the last 20 years, VTEC have been associated with several major food and waterborne outbreaks across North America [5,13–18]. For example, the Walkerton, Ontario VTEC outbreak in 2000 led to over 2000 human infections [16]. In Canada, it is estimated that approximately 39 O157 VTEC and 63 non-O157 VTEC cases per 100,000 have occurred since 2006 [19]. While not as high compared to other enteric pathogens, management of both acute and long-term illness (due to the severe complications) represents an economic burden, estimated at \$404 million annually in Canada [20].

There are knowledge gaps related to the disease dynamics of VTEC. Despite VTEC illness being a reportable disease in Canada, incidence based on surveillance data on VTEC infections is known to be an underestimate, primarily due to the high rate

of underreporting of all clinical enteric illnesses including VTEC [21–23]. In addition, there are significant challenges associated with determining the specific sources of infection when a case is identified [24,25]. This has led to difficulties in determining the relative contribution of the different routes of transmission on the overall incidence of VTEC [26,27]. Furthermore, animals and the environment are known reservoirs for VTEC, but there are still uncertainties related to the factors that influence pathogen transmission between the reservoirs and between the reservoirs and humans [28]. Improving our understanding of the dynamics of VTEC, and factors that affect the risk of transmission of the pathogen to humans, will be useful contributions to enhance future public health prevention and control strategies.

2.0 CHARACTERISTICS OF *ESCHERICHIA COLI*

E. coli are a diverse species of facultative anaerobic bacteria with many strains residing as symbionts in the gastrointestinal tracts of humans and animals [29,30]. There are however, several pathogenic *E. coli* strains that can cause disease in both humans and animals [6,29–31]. There are multiple classification systems for *E. coli* based on the physical characteristics or clinical manifestations of the infection in humans [6,8,29]. Multiple changes to the definitions of *E. coli* subtypes based on either physical characteristics or clinical manifestations, and the interchangeable use of strain names has led to difficulties defining specific subgroups of *E. coli*. Verocytotoxigenic *E. coli* (VTEC), also known as shiga-like toxin producing *E. coli* (STEC) are a serotype of *E. coli* broadly defined by their ability to produce a vero-like (or shiga-like) cytotoxin [6]. However, not all VTEC strains are pathogenic in humans [8,32]. Broadly, all human

pathogenic *E. coli* can be classified as diarrhoeagenic or extraintestinal *E. coli* [6]. There are six pathovars of diarrhoeagenic *E. coli*: enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) [5,6,32,33]. Generally, VTEC strains that cause clinical illness in humans fall under the EHEC pathovar, and the terms have been used interchangeably in the literature [32]. Strains of EHEC are commonly classified as either VTEC O157 or non-O157 strains [6,8,29]. Non-O157 strains of VTEC that are commonly associated with human illness include serotypes O26, O45, O103, O111, O121, O145 [4,8,34–37].

E. coli are able to survive under a wide range of environmental conditions [30]. The bacteria are tolerant of acidic environments [30]. This characteristic contributes to the human pathogenicity as it allows *E. coli* to survive stomach acidity [30]. Furthermore, *E. coli* are able to form viable but non-culturable which permits survival in stressful environmental conditions for longer periods of time [7,8,30,38].

2.1 PATHOGENESIS AND HUMAN INFECTION

The pathogenesis of VTEC is well described in the literature [5,6,8,34,39]. The infectious dose of VTEC is very low and it is estimated that a dose of less than 100 organisms can cause clinical illness in humans [7,8,40]. After ingestion, the bacteria enter the gastrointestinal tract, where they can withstand the low pH of the stomach due to their acid tolerance [4,8,28,30]. Colonization involves attaching and effacing the epithelial cells within the gut, destroying the microvilli and forming lesions [5,29,33,39,41]. VTEC then produce a shiga-like toxin (Stx) which bind to specific

glycolipid receptors in the intestine [5,34,39]. As the same glycolipid receptors found in intestinal cells are also located on endothelial cells in the kidney and brain, circulating Stx that have been absorbed through the epithelium can bind to those organs as well [5,34,39]. Receptor binding triggers a complex chain of events including cell apoptosis and proinflammatory responses in the body [5,34,39]. The proinflammatory responses further potentiate the action of Stx on endothelial cells [42]. Damage to the endothelial cells eventually leads to thrombotic microangiopathy in the renal glomeruli and other organs [5,34,43].

Symptoms of VTEC infection typically appear 3-4 days after exposure but can range from 1–10 days [5,43,44]. Most commonly, damage from the infection causes watery diarrhea and abdominal cramping. Asymptomatic infection may also occur [8,33,45]. Severe infections can lead to hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) [5,6,8]. Hemorrhagic colitis can occur shortly after initial clinical symptoms and is categorized by the sudden onset of severe abdominal cramping, followed by watery diarrhoea progressing into bloody diarrhoea [5,34]. Haemolytic uremic syndrome is a consequence of severe damage to the colon and the renal endothelial cells, occurring approximately 5 – 13 days after the initial onset of diarrheal symptoms [5,8,46]. The disorder is generally defined by three features: acute renal failure, thrombocytopenia, and microangiopathic haemolytic anaemia [5,47–49]. Renal effects include vascular lesions leading to thrombotic occlusion of small renal arteries, and endothelial damage in the glomeruli. [5,34,43,46]. Haemolytic uremic syndrome also causes damage to the central nervous system, causing seizures or coma [43,49].

There is no specific treatment available for VTEC illnesses therefore, therapies are primarily supportive and targeted at symptoms [5,33,34]. Unlike other bacterial infections, antibiotic use to treat VTEC is not recommended, as it can lead to the release of Stx from the bacteria, increasing the risk of severe complications, including HUS [4,5,8]. Adults will typically recover from illness in under 1 week, however some children shed VTEC up to 3 weeks after the onset of symptoms [50]. Haemolytic uremic syndrome can lead to long-term disorders including chronic kidney disease, cardiac and gastral issues, as well as neurological disorders and behavioural and cognitive changes [4,8]. The risk of mortality with HUS is 2 – 10% in developed countries [4].

2.2 SOURCES OF INFECTION

Exposure to VTEC can occur as a result of contact with contaminated food or water, as well as through contact with infected humans or animals [4,5,7–13]. Determining the proportion of all documented infections attributable to each VTEC transmission route is challenging, as it is not always possible to identify sources of sporadic cases or outbreaks [26,27]. The level of underreporting of VTEC infections poses an additional challenge to determining the source attribution [23]. Expert elicitation has become a popular method to estimate the overall source attribution of enteric illnesses [26]. In these studies, an expert panel is recruited and used to estimate the proportion of enteric illness caused by each source of infection [26,27]. Overall, these studies have estimated similar source attributions for both O157 and non-O157 strains of VTEC [27,51–54]. In Canada, foodborne sources have been reported as the major route of transmission, with one expert elicitation study suggesting that approximately 60% of VTEC infections are due to foodborne sources [26]. A follow-up

study from the same researchers specifically identified beef products and produce as the most prominent foodborne sources [55]. Another study from Canada reported highly variable estimates for the proportion of cases attributable to each transmission route, where experts reported that foodborne sources contributed to either a high or low proportion of VTEC infections in Canada [52]. Outside of Canada, foodborne transmission is also considered to be a major source of VTEC infections [51,53,54]. Studies from Australia and the Netherlands have reported that greater than 40% of infections are attributable to foodborne sources [51,53,54]. Improvements in meat safety have caused a decrease in meat-sourced VTEC and an increase in the consumption of produce (produced in very intensive agricultural settings) has been associated with an increase in human VTEC cases acquired from non-bovine sources [13]. While not as prominent, other sources of transmission are also considered of significant importance [12]. Butler et al. (2015) estimated that approximately 12% of VTEC infections in Canada can be attributed to waterborne transmission, and approximately 12% due to person-to-person transmission. In the studies conducted outside of Canada, it was estimated that 17 – 25% of all cases are caused by direct zoonotic transmission, and approximately 8 – 17% of cases each arise from waterborne, environmental, and person-to-person transmission [51,53,54]. A systematic review examining transmission pathways of sporadic infections reported that consumption of undercooked meat, contact with the animals, and person-to-person transmission were the most common transmission routes [56]. Population attributable fractions (PAFs) were estimated from pooled meta-analysis: a PAF of 19% was reported for undercooked/raw meat, 15% for person-to-person transmission, and 14% for contact with animals [56]. Snedecker et al.

(2009) examined outbreak data from countries in North America, Europe, and Japan, to estimate the proportion of VTEC cases in an outbreak that arose from primary and secondary transmission. The study reported that approximately 20% of cases in an outbreak of VTEC arose from secondary (primarily person-to-person or water) transmission [57]. Furthermore, the proportion of secondary transmission in an outbreak differed based on the age of the cases; there were higher rates of secondary transmission if the cases in the outbreak were primarily children [57]. It is important to understand the sources of VTEC transmission to develop effective intervention programs to prevent VTEC illness.

Common food sources associated with human infections have included uncooked/undercooked meats, unpasteurized milk and apple cider, and produce including lettuce, spinach, alfalfa and radish sprouts [9,10,58–61]. Undercooked beef is a significant route of transmission for VTEC O157 [7,62]. Other meat products from ruminant animals such as deer or goats have also been identified as sources of VTEC [9,63,64]. Food can become contaminated with VTEC through cross contamination with faecal matter during the process of slaughtering colonized animals, or through contaminated irrigation of produce fields and the use of manure as fertilizer [7,8,15,38,59,63,65–67].

In addition to drinking water, recreational use of water has also been implicated in outbreaks [7,68,69]. Both unchlorinated pool water and contaminated fresh water have been associated with outbreaks of VTEC [7,11,68–71]. Contaminated water

supplies have also been associated with major waterborne outbreaks, such as the outbreak in Walkerton, Ontario in May, 2000 [7,14,16,68,69,72–74].

Direct zoonotic infection can occur as a result of contact with colonized animals and/or their faecal matter [5,7,8,13,75,76]. Direct contact can occur in farm settings, such as visits to cattle farms, agricultural fairs, or petting zoos [15,37,77–80].

Like animals, infected humans also shed VTEC during and after clinical illness and can infect others through the faecal-oral route [5,7,8,81]. Adults remain infectious for approximately 7 days after the onset of clinical symptoms [8,44]. Prolonged shedding and communicability have been reported in children [50,81,82]. Those with asymptomatic infections also shed and transmit the bacteria in their feces which can then cause secondary infections in other individuals [8]. However, given the lack of symptoms, it is difficult to estimate the proportion of asymptomatic infections of VTEC [8]. Person-to-person transmission commonly occurs as a secondary route of transmission following a point source (e.g. foodborne) outbreak [7]. This transmission route also occurs as clusters within households and institutional settings such as daycares or long-term care homes [7,83–87].

In the literature, VTEC O157 strains are usually assumed as the primary EHEC serotype associated with human illness [4,5,13,31,87]. Due to lack of systematic laboratory testing for non-O157 VTEC serotypes, estimates of non-O157 VTEC incidence are likely underestimated [21,23,34,35,37,87]. Research has indicated that non-O157 VTEC serotypes also contribute to a significant proportion of sporadic infections and outbreaks – estimated between 30-50% of infections [21,87,88].

2.3 EPIDEMIOLOGY OF VTEC

A 2014 systematic review estimated that the global incidence of VTEC infection was 2,801,000 infections annually, with 126,200 illnesses occurring in the North American World Health Organization sub-region [89]. In Canada, VTEC infection is a notifiable disease and cases are reported to provincial and federal departments of health [21,22]. However, as with other enteric pathogens, VTEC cases are commonly underreported [23]. The likelihood of reporting may be affected by the type and severity of symptoms experienced, as individuals experiencing mild symptoms may not seek medical attention [18,25,90]. In the United States, the degree of under-reporting for VTEC infections is estimated to be 20-fold [4]. Estimates in Ontario from the early 2000s reported that between 78 – 88% of symptomatic VTEC infections are missed [91]. A 2006 study estimated that for every reported VTEC case in Canada, there were between 10 – 47 additional undocumented VTEC cases occurring in the community [92]. Another study estimated that approximately 39 O157 VTEC and 63 non-O157 VTEC foodborne cases (per 100,000) have occurred annually in Canada since 2006 [19]. A telephone survey in Ontario conducted from May 2005 to April 2006 estimated the monthly prevalence of people experiencing symptoms of acute gastrointestinal illness including vomiting and diarrhea (which is caused by various enteric pathogens including VTEC) to be around 8.6% in Ontario [90]. Extrapolating to 2006 population demographics in Ontario translated to more than 1 million cases each month. [90]. While VTEC infection rates may be less than other enteric diseases, the severe medical sequelae experienced by some patients make it clinically important [38]. In the United States, it is estimated that 15% of children under the age of 10 develop HUS after a

VTEC infection [8,34,93]. A systematic review in 2014 estimated that the proportion of VTEC O157 cases that developed HUS ranged from 4.2 – 17.2% [94].

Younger children are most susceptible to VTEC infections [5,13,37,86,89,95–99]. Furthermore, children, the elderly, and immunocompromised people are most susceptible to developing HUS [13,81,100,101]. Several studies have reported that non-O157 VTEC had a higher incidence of hospitalization and HUS rates in England compared to VTEC O157 strains [37,87]. Females are more likely to experience diarrheal illness and acute gastrointestinal illness compared to men [13,37,90,102]. Studies have also reported higher proportions of VTEC infections and subsequent hospitalization in females compared to males [13,37,103].

Human illness due to VTEC appears to be more common in the summer months, between May – September [5,7,37,103–105]. This seasonality could be due to meteorological factors, such as increased temperatures which may promote growth and survival of VTEC in the environment increasing the chance of human exposure [38,104,105]. An alternative hypothesis is that this seasonality arises due to different human social behaviours in the summer months which lead to increased exposure to VTEC and other enteric pathogens [5,38,104]. Differences in social activities may include increased participation in outdoor activities such as swimming, or increased consumption of barbecued beef [5]. Additionally, studies have found a higher incidence of VTEC infections in rural areas compared to urban areas in Ontario [37,90,106,107]. As reported by Byrne et al. (2015), an increase in the incidence of VTEC in rural areas

may be due to the increased environmental exposure and contact with animals and/or their faecal matter.

3.0 RESERVOIRS OF VTEC

Ruminant animals, primarily cattle, are the primary reservoirs of VTEC. [5,7,8,13,28,76,87]. Both O157 and non-O157 strains of VTEC have been isolated from cattle and other animals including swine, sheep, goats, horses, deer, dogs, and rodents [4,5,8,28,76,87,88,108]. Colonization of animals by VTEC is asymptomatic, which may be due to a lack of Stx-binding receptors in animals [6,7,21,109]. Colonization in the absence of symptoms makes it difficult to estimate the prevalence of VTEC in animals, and estimates vary depending on region and sampling and diagnostic methods used [5,7,21,110,111].

VTEC can persist in animal faecal matter for several weeks [7,8,21,28,68,112]. VTEC has also been isolated from feed samples and water sources on farms and can persist in the agricultural environment for long periods of time [7,8,13,28,43,68,69,113]. Outside of farm environments, VTEC strains survive in soil, water, and even on metal surfaces [38,68,69,114]. VTEC have the ability to survive in soil as viable but non-culturable cells for several months and even up to a year [8,30,69,115].

3.1 ANIMAL-ENVIRONMENTAL TRANSMISSION

Cattle shed VTEC into the surrounding environment at varying concentrations, with some cattle shedding significantly higher amounts of VTEC, known as “super shedders” [5,7,13,21,28,38,43,76,116]. There is evidence of seasonal shedding of VTEC in cattle, where cattle more commonly shed VTEC in the summer months

[7,28,38,43,58,105,117,118]. Seasonality in animals appears to coincide with peak human incidence of VTEC, which also occurs more often in the summer [7,38].

VTEC can be transmitted from agricultural farms into surrounding environmental and water sources through runoff of manure [28,107]. Transport of bacteria in agricultural runoff has been demonstrated experimentally [119–121]. As such, hydrological factors that impact runoff may be associated with the transfer of VTEC into the broader environment. Heavy rain and flooding may lead to bacterial contamination in localized areas [38]. Runoff from high precipitation events (such as storms) is associated with pathogen erosion from soil surfaces and increased overland flow, transporting microorganisms (including bacterial pathogens) into nearby bodies of water [122,123].

The long term survival capabilities of *E. coli* in freshwater allow the bacteria to be transported through water sources such as streams and rivers [30,68,69,124–127]. Furthermore, simulation models have theorized that high waterflow rates can resuspend *E. coli* that have sedimented in streams, allowing the pathogen to be transported via water to other areas [122,128,129]. Increasing water levels and waterflow rates can lead to greater areas of runoff around water sources, resulting in a greater potential for bacterial contamination in the surrounding areas [122,130]. As high impact weather events such as storms impact runoff and waterflow rates in streams, these events can facilitate the movement of pathogens like VTEC into different areas, providing greater exposure of the population to the pathogen [122,130].

4.0 EPIDEMIOLOGICAL MODELS

There have been multiple studies examining the transmission dynamics of enteric pathogens including VTEC, using both traditional and non-traditional epidemiological methods [104,131–139]. Several systematic reviews and meta-analyses have been published to compare the results among studies of environmental effects on enteric pathogens [105,140,141]. One review on the seasonality of enteric bacterial disease in humans reported distinct peaks in incidence during the summer months for all examined pathogens including VTEC, which had a peak in the month of July [105]. Another systematic review and meta-analysis examined the results of 26 published studies on the relationship between ambient temperature and various bacterial and viral diarrheal diseases [141]. Pooled estimates from the meta-analysis found a significant positive association between ambient temperature and both all-cause and bacterial diarrhea in humans [141]. Phillipsborn et al. (2016) compared results from studies examining climatic drivers of diarrheagenic *E. coli* in humans and reported mixed findings, where studies reported both positive and negative associations between precipitation and incidence of infections. From the pooled analysis, researchers reported a positive association between diarrheal incidence and both mean monthly temperature and prior-month precipitation [140]. However, the association between mean precipitation of the month prior and diarrheal illness was not statistically significant when controlling for temperature [140].

4.1 TRADITIONAL EPIDEMIOLOGICAL MODELS

Poisson-regression is a method that has been used to examine the relationship between weather effects and rates of enteric illness and outbreaks [142]. As illnesses

caused by infections from pathogens such as *E. coli* have distinct seasonal patterns in North America, seasonal variation needs to be controlled in analyses [104,105,143]. Controlling for seasonality could be done by transforming the data itself, or by incorporating sinusoidal waves or smoothing functions into the models [143]. An assumption of Poisson regression analysis is that the mean and variance of the observations are equal [142]. However, overdispersion, where the variance of the observations is larger than the mean, occurs commonly [142]. To account for the overdispersion in the data, negative binomial and zero-inflated models (in cases of an excess of zero counts) can be used [142].

Poisson, negative-binomial, and zero-inflated Poisson regression models have previously been used to analyze environmental drivers of other enteric illnesses similar to VTEC [131–137]. In these examples, environmental variables were lagged up to 8 weeks to account for the time it would take a pathogen to move from the environment into the human population and then cause clinical illness. Six of these studies reported statistically significant seasonality of enteric illness case occurrence [132–137]. These studies controlled for seasonality by incorporating seasonal smoothers or categorical variables into the models [133–137], as well as by using deseasonalised data [132]. Two studies used negative-binomial models to account for the overdispersion found in the associated enteric datasets [135,136].

Several of these studies reported statistically significant associations between environmental and/or hydrological factors and enteric illnesses [131–136]. However, after controlling for seasonality, Brankston et al. (2018) found no statistically significant

associations between human cryptosporidiosis cases and environmental, hydrological, or farm level predictors in the Poisson regression analysis. Three studies reported positive associations between human case occurrences and ambient temperature [132,134,135]. Bentham and Langford (2001) reported an association with temperature (1-5 weeks prior) and food poisoning occurrences, and D'Souza et al. (2004) reported an association with temperature (in the month prior) and salmonellosis. Fleury et al. (2006) reported a non-linear association between ambient temperature and enteric infections (including *Salmonella*, *Campylobacter*, and enteropathogenic *E. coli*) pathogens; where a linear association was observed only above a temperature threshold between -10 – 0 °C (depending on the pathogen).

Associations between enteric illnesses and hydrological factors have been reported in other enteric studies [131,133,136]. Brunn et al. (2018) reported that increased water levels were positively associated with giardiasis (lagged 1 month), while Ng et al. (2008) reported a negative association between water level and legionellosis risk. Furthermore, both studies found positive associations between precipitation and disease occurrence [133,136]. Carlton et al. (2014) also examined the effects of rainfall on enteric illness. However, the study reported that the association between heavy rainfall (2 weeks prior) and diarrheal disease depended on the precipitation pattern; during dry periods there was a positive association between heavy rainfall and incidence of diarrheal illness, but during a high rainfall period there was a negative association with heavy rainfall and incidence of diarrheal illness [131].

4.2 CASE CROSSOVER ANALYSIS

Case-crossover studies are well suited for analysing the effects of acute exposures on rare outcomes [143]. This type of approach addresses some of the limitations described in section 4.1 that are associated with Poisson regression techniques. Analysis involves comparing differences in exposures that preceded days in which a case occurred to control days [143]. Control periods are self-matched to case days within a stratum period of nearby case days, thus controlling for any fixed or long term effects (such as seasonality) within the design [143]. Conditional logistic regression is then used to estimate the odds of case occurrence based on a defined hazard period that preceded the case.

Several of the studies cited in section 4.1 used case crossover analysis to analyze environmental impacts on the same enteric data set as a second, complementary analysis [133,136,137]. These studies stratified their study periods into either 3 or 4 week stratum, matching cases to controls within stratum. Other studies used case crossover methodology to examine the impact of weather changes on waterborne disease outbreaks in general, which include (but are not limited to) VTEC infections [138,139]. Similar to the Poisson regression analysis, all of the studies examined the weather impacts in the period up to 90 days prior to case occurrences [133,136–139]. Studies reported varying associations with temperature, water levels, and precipitation. Some studies reported that increased temperature (within approximately 90 days) was associated with disease outbreaks or enteric infections [133,137,139]. In examining hydrological effects, increases in water level and flow rate in the month prior have been reported to be positively associated with disease

occurrence [133,136,137]. Brunn et al. (2018) also reported significant associations with rainfall; precipitation (4 weeks lagged) was negatively associated with giardiasis [133]. Conversely, Thomas et al. (2016) reported that high precipitation (in the 6 week period prior) was positively associated outbreak occurrence [139]. Nichols et al. (2009) reported that outbreak occurrences were positively associated with both high (greater than 40 mm, in the week prior) and low (below 20 mm, 2-4 weeks prior) cumulative precipitation levels.

Case-crossover analysis has not been used to evaluate how environmental and hydrological factors may influence VTEC infections specifically. This method can provide additional understanding regarding the dynamics of VTEC and how environmental conditions may impact the survival and transmission of VTEC at the human-environment interface.

5.0 INFECTIOUS DISEASE MODELS

Infectious disease modelling is an epidemiological method that uses a system of mathematical equations to describe the dynamics of an infectious disease in a population [144]. Mathematical models are an important tool that can provide insight into the patterns and determinants of disease spread [144,145]. Mathematical models can also help researchers estimate key parameters of transmission and examine hypotheses about the transmission dynamics and biology of the host-pathogen system [144,145]. One such key parameter is the basic reproductive number (R_0) [144]. The R_0 is the average number of secondary cases generated by an infected person before they recover from their infection [144,146]. The value is a theoretical measure of infectivity,

as it relates to person-to-person transmission of an infection in populations [144,146]. An R_0 value greater than 1 indicates that each infected individual generates more than 1 new infection before they recover which leads to exponential growth of the epidemic within a population [144]. Mathematical models can also be used to test and evaluate the expected effectiveness of interventions for controlling disease spread, to inform development of control strategies [144,145].

As described in section 2.2, enteric pathogens such as VTEC have several different routes of transmission including food, water, and person-to-person transmission [5,33]. Infectious disease modelling has been used to examine the dynamics of enteric pathogens specifically, to estimate the relative contribution of the different transmission routes to the overall disease dynamics [17,146,147]. A study published in 2007 used data from a foodborne VTEC outbreak in the United States to examine interventions intended to reduce person-to-person transmission in VTEC outbreaks [17]. The researchers estimated person-to-person and person-environment transmission parameters using background baseline incidence, under various assumptions regarding the proportion of person-to-person transmission (12, 25, 50 and 75%). The best fit parameters were used to examine how an intervention targeted at reducing person-to-person transmission in the population may reduce outbreak size. The study reported that the model was unable to fit the observed outbreak data if person-to-person transmission was assumed to cause more than 50% of the infections, suggesting person-to-person transmission accounted for less than half of the infections in that US outbreak [17]. Examining the effect of interventions that reduce person-to-person transmission, it was reported that even with an inefficient intervention,

the total outbreak size could have been reduced by 5 – 11% by targeting person-to-person transmission during the course of the outbreak [17].

A study by Brookhart et al. (2002) developed a mathematical compartment model simulating the 1993 *Cryptosporidium* outbreak in Milwaukee. The model was used to examine how failure of water treatment increased primary transmission, and what the rate of secondary (person-to-person) transmission was in the outbreak. To estimate the transmission parameters, the study used a constrained profile-likelihood approach in order to control for collinearity issues in estimation [146]. Various assumptions of R_0 were used to estimate person-to-person transmission and fit all other parameters. It was reported that the parameter estimates were dependent on the value used for the proportion of asymptomatic infections in the population [146]. The researchers reported that person-to-person transmission was possible in the outbreak, estimating the highest possible R_0 of 0.35 in the outbreak [146]. Eisenberg et al. (2005) also developed a mathematical model based on the same *Cryptosporidium* outbreak in the United States. In this study, the model examined the attributable risk of person-to-person transmission in the outbreak and examined the proportion of infections that were prevented by the water plant closure. Similar to Brookhart et al. (2002), the study used profile likelihood estimation to estimate the transmission parameters for the model. An attributable risk of 0.1 (95% CI: 0.06 – 0.21) due to person-to-person transmission was estimated [147]. The percentage of infections that were prevented by closure of the water plant depended on the proportion of asymptomatic infections in the population. If 10% of those exposed to the pathogen were asymptotically infected, the preventable fraction

was estimated at 0.36 [147]. However, if there was a high proportion of asymptomatic infections, around 70%, then the preventable fraction decreased to 0.12 [147].

Using these types of disease models can help us understand the relative contribution of different transmission sources, and where intervention efforts should be targeted to prevent disease spread. While infectious disease modelling could be used as an effective tool in public health planning, it is limited by the structure and assumptions of the model [144,148,149]. Simple models may have underlying assumptions that are unrealistic [144,148]. Conversely, models that are more complicated can lead to uncertainty in model projections [144,148]. Moreover, quantitative results are not well suited for predicting specific outcomes, but rather for comparing hypothetical scenarios [149]. Results from these types of infectious disease models can be used to better understand the relative contribution of different transmission routes, and inform decision making related to public health response measures.

6.0 THESIS OVERVIEW, PURPOSE, AND RESEARCH

The dynamics of VTEC transmission are complex and occur at the human-animal-environment interface. Knowledge gaps exist that relate to 1) understanding the environmental factors that may play a role in the risk of human VTEC infections, and 2) understanding the relative importance of person-to-person transmission during human outbreaks of VTEC. Improving our understanding in these two areas will contribute to more nuanced disease prevention and control strategies for VTEC in Canada.

The overall goals of this thesis were to, 1) explore the role of environmental and hydrological conditions on the risk of primary VTEC infections, and 2) examine the potential impact of public health interventions for reducing secondary (person-to-person) transmission during VTEC outbreaks. These objectives were addressed using epidemiological case-crossover and infectious disease modelling methods. The first chapter of this thesis provides background on VTEC bacteria and some of the relevant methodologies used to examine VTEC dynamics in human populations. The following research objectives are addressed in Chapters 2 and 3:

1. To use a case crossover approach to identify associations between environmental and hydrological conditions and the occurrence of primary human VTEC cases in five different public health units in Ontario, Canada (Chapter 2).
2. To examine the impact of person-to-person transmission in VTEC outbreaks in Ontario, Canada (Chapter 3).
3. To examine the effectiveness of public health interventions to reduce person-to-person transmission during a large VTEC outbreak in Ontario, Canada (Chapter 3).

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CHAPTER 2

ENVIRONMENTAL DETERMINANTS OF PRIMARY HUMAN VEROCYTOTOXIGENIC *ESCHERICHIA COLI* INFECTIONS IN ONTARIO, CANADA

ABSTRACT

Background: Environmental conditions such as temperature and precipitation, and hydrological conditions such as watershed level and waterflow rate are hypothesized to be associated with human verocytotoxigenic *Escherichia coli* (VTEC) infections. This is because water sources can become contaminated with animal or human fecal matter which may contain VTEC, resulting in human exposures. The objective of this research was to evaluate the impact of environmental factors on the incidence of human VTEC cases in the province of Ontario, Canada.

Methods: Data were obtained from Public Health Ontario and Environment Canada. The data represent primary confirmed cases of VTEC from the Toronto, Waterloo, Peel, York, and Ottawa public health units. Secondary outbreak cases and travel associated cases were excluded. A case-crossover study design was used to examine the acute effect of environmental conditions (including temperature, precipitation, and watershed level/flow) 1 – 4 weeks prior to human case occurrence in Ontario. Effects were examined by estimating univariable conditional logistic regression analysis for all environmental variables.

Results: Across the five public health units, there were 648 reported primary cases of VTEC, from January 1st, 2005 to December 31st, 2013. In the Ottawa region, a positive

association was found between case occurrence and weekly average precipitation after a 2-week lag (OR = 1.10, 95% CI = 1.003 – 1.20, p value = 0.04). In Peel region, the odds ratio of case occurrence was 2.91 (95% CI = 1.13 – 4.27, p value = 0.02) when the cumulative weekly precipitation exceeded the 90th percentile after a 3-week lag. However, high precipitation occurring 4 weeks prior was negatively associated with VTEC case occurrences with an odds ratio of 0.42 (95% CI = 0.18 – 0.99, p value = 0.05) in Peel region, and by 0.41 (95% CI = 0.190 – 0.88, p value = 0.02) in Waterloo region.

Conclusion: Statistically significant and biologically plausible associations were identified between weekly lagged precipitation data and VTEC case occurrence within 4 of the 5 health units. However, the results were not consistent across the regions in terms of the relevant time lag or the other environmental factors examined. This suggests that cases of VTEC may not be strongly influenced by acute environmental conditions.

INTRODUCTION

Verocytotoxigenic *Escherichia coli* (VTEC) is a pathogenic form of *E. coli* implicated in numerous food and waterborne outbreaks worldwide. Infection results in a variety of clinical symptoms including diarrhea and haemorrhagic colitis [1,2]. Severe infections can further develop into haemolytic uremic syndrome (HUS), which can lead to long term renal problems and death [3]. Children under the age of 5 are most likely to develop HUS [1,4–6]. Management of both acute infection and long-term sequelae is an economic burden, costing Canadians \$404 million annually [7].

Ruminant animals, including cattle, are known reservoirs for VTEC [1,2,8,9]. They carry the pathogen asymptotically, and subsequently may shed the pathogen into the environment [4,9–11]. Bacterial shedding by livestock may contaminate water systems, exposing human populations to the bacteria [4,9,11,12]. There is speculation that environmental factors may affect the survival and transport of VTEC within the environment, thereby influencing human infections [4,9,12–20]. Human VTEC infections more commonly occur during the summer months which may be due to social or climatic changes in that period [10,12–15]. Increasing ambient temperature is positively associated with the incidence of human enteric illness including VTEC [14,16–20]. Precipitation has also been associated with the occurrence of human enteric infections, including VTEC [14,18,20–23]. More specifically, extreme precipitation events such as heavy periods of rainfalls have preceded enteric outbreaks [14,21,24–26]. While there were many factors that led to the Walkerton outbreak of *E. coli* O157:H7 in 2001, where 2000 people became ill, the outbreak occurred following unusually heavy rainfall in the area [24]. A study conducted to examine the association between weather events and waterborne outbreaks found that warmer temperatures and extreme rainfall events increased the relative odds of waterborne outbreak occurrence [26]. The effect of environmental conditions on VTEC dynamics specifically is still uncertain. Examining the possible drivers of VTEC infection could improve our understanding of VTEC disease risk in Canada, especially in the context of climate change. Climate change is altering the current environmental conditions, which may potentially alter the risk of human VTEC infections. This study used a time-stratified case-crossover methodology to examine how environmental factors influence the human incidence of VTEC infection

in Ontario. This approach is used to examine the association between acute environmental exposures and rare outcomes, by comparing differences in acute exposures prior to case days and prior to days in which a case did not occur [27,28]. As control periods are self-matched with case days within a defined time, fixed characteristics and long-term variation of exposures (e.g. seasonality) are controlled for by design [27]. Similar studies examining environmental exposures on enteric infections and waterborne outbreaks have used this methodology [20,21,26,29]. The objective of this study was to use a case-crossover methodology to identify associations between environmental and hydrological conditions and the occurrence of sporadic human VTEC cases in five different public health units in Ontario, Canada.

METHODS

This project was approved by The University of Guelph Research Ethics Board (#15NV011).

Public Health Units:

The province of Ontario is divided into 36 Public Health Regions. Based on data availability during the period of interest, we examined data from the Toronto, Waterloo, York, Peel, and Ottawa Public Health Regions (Figure 2.1). The five regions vary in size, population and agricultural density, and primary watershed characteristics (Table 2.1). The Toronto region is the most populous yet contains the smallest watershed [30–32]. The Waterloo region, containing the smallest population, has the largest watershed of the five health regions [30,33].

Human Case data:

Human case count data from Jan 31, 2005 to December 31st, 2013 were obtained from Public Health Ontario (PHO). The data represent confirmed cases of VTEC from Ontario's Integrated Public Health Information System (iPHIS), as VTEC illnesses are reportable in Canada. An individual was defined as a confirmed case if they exhibited clinically compatible signs and symptoms and either: laboratory identification of verocytotoxin in stool, laboratory isolation of VTEC from stool or blood, or the case had an epidemiological link to a laboratory confirmed case. Additionally, cases that were diagnosed with HUS by a physician were defined as confirmed cases of VTEC. Cases were excluded if the individual travelled outside of Ontario during the maximum incubation period of VTEC (10 days) [34], as it was assumed that these cases did not represent locally acquired cases. Furthermore, since the focus was on environmental drivers of disease occurrence, only sporadic and primary outbreak cases were used in the study (secondary outbreak cases were removed from the dataset as they were assumed to not be the result of an environmental exposure). Cases that did not have a documented date of onset of symptoms were also removed from the dataset (n = 28, 4.1%).

Environmental Exposure Data:

Daily meteorological data, including temperature (°C) and precipitation (mm), as well as hydrological data including water level (m) and waterflow rate (m³/s) were obtained from Environment Canada from January 1st, 2005 – December 31st, 2013 for each of the health units. Weather stations for both environmental and hydrological data were selected based on health unit centrality and data availability (Table 2.1). If one

location did not contain data for the entire study period, an average of two or more weather/hydrology stations was used. To obtain a good representation of the hydrological characteristics within the Toronto region, hydrology data were averaged across both primary watersheds, the Don and Humber Rivers. Environmental data were aggregated into weekly average exposures (from Sunday to Saturday). Precipitation data were also aggregated into cumulative weekly precipitation variables.

To investigate the possible association between extreme precipitation and VTEC cases, daily precipitation was also dichotomized based on the 95th percentile. A day was classified as an 'extreme precipitation day' if the daily precipitation exceeded the 95th percentile of the study period in the region. Extreme precipitation was then aggregated by week into three different weekly variables. A week was categorized as an extreme precipitation week if at least one extreme precipitation event occurred within the week. Additionally, extreme precipitation was aggregated into a cumulative weekly extreme precipitation event variable, to examine if increasing extreme precipitation events within a week was associated with VTEC case occurrence. Cumulative weekly precipitation was also dichotomized based on the 90th percentile, to examine the association between extreme weekly precipitation and case occurrence.

Statistical Analyses:

A merged dataset was created which combined the human and environmental data for the study period (January 1st, 2005 – December 31st, 2013) for each of the five public health units. The study period was stratified into equal, mutually exclusive strata with a length of 28 days. Cases and controls were only compared within the same

stratum period. Three control periods were selected for every case, matched by day of week. Control periods could be prior to, after, or straddling a case day in a stratum. Environmental variables were lagged 1– 4 weeks to examine the effect of different time delays on the occurrence of human VTEC cases. The 4-week time period was chosen to account for a biologically plausible time period to allow for adequate environmental transport of the bacteria, human exposure, and subsequent human infection. Univariable conditional regression analysis was used to obtain odds ratios (comparing the odds of being a case versus not being a case with environmental and hydrological variables lagged 1 – 4 weeks prior to the onset of symptoms). The season package [35] in R version 3.5.1 [36] and STATA 14.0 [37] were used for statistical analyses.

RESULTS

Descriptive statistics:

There were a total of 648 cases of primary VTEC infections in the five public health units between January 1st, 2005 and December 31st, 2013 (Table 2.2). Most of the cases were female (58.0%; Table 2.2). Toronto had the highest case count with 228 cases over the study period. There were 85 cases reported in Ottawa, the lowest number of cases out of all the health units. The largest proportion of cases were within the 5 – 19 years age group (Table 2.2). Based on population density, the Waterloo public health unit had the highest incidence of cases throughout the study period (Figure 2.2). On average, disease incidence was highest in the summer months (Figure 2.3). The highest number of cases occurred in the month of June in York, July in both Toronto and Waterloo, August in Ottawa, and September in Peel (Figure 2.3).

Case-crossover analyses:

In Toronto, there were no statistically significant univariable associations between any of the environmental and hydrological variables and human VTEC case occurrence (see Table A2.1 for full univariable results from Toronto). Also, temperature did not have a statistically significant association with human VTEC occurrence during any lag period, within any of the public health units considered. Figure 2.4 depicts the statistically significant univariable associations between precipitation variables and the odds of VTEC occurrence in the Waterloo, Ottawa, Peel, and York Public Health Units (Toronto excluded due to no statistically significant results for any variables). Complete results for all environmental variables examined in each public health unit can be found in Table A2.2.

In the Ottawa health region, there was a positive association between both the average and cumulative weekly precipitation values 2 weeks prior to case occurrence (Figure 2.4). For increases in average daily and cumulative weekly precipitation 2 weeks prior, the odds ratios of VTEC cases were 1.10 (95% CI: 1.00 – 1.20, p value = 0.04) and 1.02 (95% CI: 1.00 – 1.03, p value = 0.03) (Figure 2.4). There were no statistically significant associations between VTEC occurrence and waterflow rate, water level, or the extreme precipitation variables.

In Waterloo region, hydrological variables were not significantly associated with VTEC case occurrence. Average and cumulative weekly precipitation were also not significantly associated with VTEC occurrence. However, when the cumulative weekly

precipitation exceeded the 90th percentile 1 week prior, the odds ratio of case occurrence was 0.41 (95% CI: 0.19 – 0.88, p value = 0.02; Figure 2.4).

Average weekly precipitation was not significantly associated with case occurrence for any of the lag periods examined in York region. However, case occurrence was positively associated with both waterflow rate and water level from the local watershed 2 weeks prior, with odds ratios of 1.30 (95% CI = 1.06 – 1.61, p value = 0.01) and 80.05 (95% CI = 3.83 – 1673, p value = 0.01), respectively. Additionally, the odds of a case occurrence significantly increased when the cumulatively weekly precipitation exceeded the 90th percentile 2 weeks prior to a case occurrence (Figure 2.4).

In Peel, there were no statistically significant univariable associations between water level and flow rate from the local watershed, and VTEC occurrence. Increases in both average and cumulative weekly precipitation 4 weeks prior were associated with case occurrences (Figure 2.4). The odds ratios for average daily and cumulative weekly precipitation (lagged 4 weeks) were 0.89 (95% CI: 0.80 – 0.99, p value = 0.03) and 0.98 (95% CI: 0.97 – 1.0, p value = 0.02; Figure 2.4).

Furthermore, extreme precipitation events were also statistically associated with case occurrence of VTEC in Peel. The odds ratio of case occurrence was 0.53 (95% CI: 0.31 – 0.92, p value = 0.02) if daily precipitation exceeded the 95th percentile 4 weeks prior (Figure 2.4). The number of extreme precipitation events within the week (where the daily precipitation exceeded the 95th percentile) occurring 4 weeks prior, was negatively associated with case occurrence, with an odds ratio of 0.55 (95% CI: 0.35 –

0.86, p value = 0.01; Figure 2.4). When cumulative weekly precipitation 3 weeks prior exceeded the 90th percentile, the odds ratio was 2.91 (95% CI: 1.13 – 4.27, p value = 0.02; Figure 2.4). However, the odds ratio of a case occurrence was 0.42 (95% CI: 0.18 – 0.99, p value = 0.05) when the cumulative precipitation exceeded the 90th percentile 4 weeks prior to case occurrence (Figure 2.4).

DISCUSSION

The purpose of this research was to improve our understanding of how acute changes in environmental and hydrological conditions may influence primary VTEC infections in five public health units in Southern Ontario (Toronto, Waterloo, Ottawa, York, and Peel). This study found no statistically significant association between temperature and the odds of VTEC case occurrences in any of the health units. Positive associations between hydrological variables (increasing water level and waterflow rate) and case occurrence were found in one health unit (York). Both positive and negative associations were found with average daily and cumulative weekly precipitation in two public health units. When examining extreme precipitation events, the occurrence of extreme precipitation events 1 or 4 weeks prior was negatively associated with case occurrence. Conversely, there was a positive association with VTEC case occurrence and extreme precipitation 2 or 3 weeks prior. Despite these statistically significant and biologically plausible associations found in 4/5 public health units in the province of Ontario, the results were not consistent between the health units.

While our study found no significant association between temperature and the occurrence of VTEC infections, there are numerous studies that have reported this

association. Overall, human VTEC infections occur more often in the summer months, suggesting that temperature may play a role in infections [13,19,38]. Positive associations have been reported between increases in ambient temperature and enteric illness caused by pathogens including *E. coli* [14,16–18,20]. One systematic review found a significant positive association between ambient temperature and both all-cause and bacterial diarrhea [39]. However, only one of the studies included in that review came from Canada. One study on human cases of giardiasis found that low ambient temperatures had a protective effect on the risk of giardiasis [29]. It is possible that, in our study, other factors need to be controlled in multivariable analysis to see an effect. A systematic review done in 2016 found a positive association between average monthly temperature and diarrheal infections when controlling for precipitation levels [14]. Furthermore, it is possible that the lack of findings could be due our inability to distinguish between the source attribution of case data. Within the literature, a large proportion of VTEC infections are foodborne, which would not be influenced directly by acute changes in local temperature [40–46]. Since we were not able to specifically identify and exclude these cases, any true association between temperature and case occurrence may have been difficult to capture.

The occurrence of human cases of VTEC in the York health region was positively associated with both watershed level and flow rate 3 weeks prior to case occurrence. These findings appear to be contradictory to other results for enteric pathogens. In a study on *Cryptosporidium* infections in Ontario, it was reported that increasing water level 3 weeks prior and flow rate 4 weeks prior in the Waterloo region decreased the odds of case occurrence [20]. Another study found similar results, where the risk of

legionellosis in the greater Toronto area (GTA) was increased with decreases in water level and flow 4 weeks prior [47]. However, one recently published study reported results similar to those found in our study. A case-crossover analysis on the risk of giardiasis in Ontario also found positive associations with case occurrence and increasing water levels 1 week prior [29]. Our findings may be different due to differences in watershed structure in the regions examined, as some regions within the province have more heavily regulated water levels and flow rates. Increasing water level and flow rate can indicate greater overland flow, which can transport higher concentrations of bacteria [48]. In a model simulating transport of *E. coli* (and other enteric, waterborne pathogens) in watersheds, measurable concentrations of *E. coli* were observed with increasing waterflow rates [48]. Experimental studies have shown that strains of *E. coli* are able to be transported in bodies of water and can survive in freshwater for long periods of time, greater than 12 weeks [49–54]. High rates of stream flow can contribute to the resuspension of *E. coli* from sediments in the water, increasing bacterial spread [48,55,56]. Furthermore, increasing water level and flow rate can indicate greater areas of runoff in the watershed, leading to greater potential for sources of bacterial contamination [57].

This study demonstrated that the odds of case occurrences were both positively (2 weeks prior in Ottawa) and negatively (4 weeks prior in York) associated with average daily and cumulative weekly precipitation. Mixed results are documented in the literature, with studies reporting both positive and negative associations with *E. coli* incidence and rainfall/precipitation [14]. In a systematic review examining climatic drivers of diarrheagenic *E. coli* infections, pooled analyses found a positive association

between rainfall lagged 1 month and incidence of *E. coli* infections [14]. However, these results were not statistically significant when controlling for monthly temperature [14]. While there are other studies that have documented that precipitation was associated with increased risk of diarrheal illness and enteric disease outbreaks, these studies examined only precipitation levels above the 90th percentile as predictors [21,25,26,58]. Experimental studies have demonstrated that rainfall could lead to the erosion and transport of *E. coli* growing on cow manure into runoff [59], depositing into the soil. Periods of increased precipitation and runoff have been associated with increased counts of fecal bacteria, as high soil saturation could lead to increased transport of microorganisms [60]. Runoff from slurries have also led to contamination of *E. coli* in nearby soil and grasslands, where the bacteria can survive for up to 4 weeks [61]. Therefore, it is plausible that rainfall increases the transportation of VTEC through water, leading to contaminations of the pathogen into water systems and watershed.

Similar studies have also reported that precipitation is associated with decreased risk of enteric illness and outbreak occurrence [21,29,58] as our study demonstrated in York region. A previously published case-crossover study reported that increasing levels of precipitation 1 month prior decreased the risk of giardiasis in the Waterloo region [29]. Nichols et al. (2009), found that both low and high levels of precipitation preceded drinking water related outbreaks. Low levels of rainfall could increase contamination of source waters as a result of an increased percentage of sewage effluent in rivers, contamination of ground water with surface water, and low soil water content increasing the likelihood of run-off [21]. However, this study specifically focused on all drinking water related outbreaks, a fraction of which were caused by *E. coli*

pathogens. Negative associations with increasing levels of precipitation could be indicative of low precipitation being an important factor leading to outbreaks. In a study examining how levels of cumulative rainfall affect the association between heavy rainfall and the incidence of diarrheal disease, when the cumulative precipitation 8 weeks prior was low, there was a positive association between heavy rainfall lagged 2 weeks and the incidence of diarrheal disease [58]. When there was a high amount of precipitation in the preceding period, there was a significant negative association with heavy rainfall 2 weeks prior and diarrheal incidence [58]. While this study examined diarrheal illness in Ecuador, which has significant climatic, social, and sanitation differences from Ontario, the similar findings provide insight into how interactions between precipitation levels may lead to increased exposure and subsequent infection of humans by enteric pathogens such as VTEC [58].

When examining extreme precipitation, our study found different associations at different time lags. When weekly precipitation exceeded the 90th percentile, there was a positive association at the 2-week (York) and 3-week (Peel) lag, and a negative association at the 1-week (Waterloo) and 4-week (Peel) lags. Similar research into enteric outbreaks and diarrheal incidence has also shown positive associations with high precipitation prior to infection, but at different periods preceding onset of illness [21,25,26,58]. In a similar case-crossover study examining waterborne outbreaks in Canada, extreme rainfall events increased the risk of outbreak occurrence in the 6 week period prior [26]. Drinking water related outbreaks in England and Wales were also positively associated with high periods of rainfall 1 – 4 weeks prior [21]. Extremely high precipitation levels, for example during storm events, have been associated with

pathogen erosion from soil surface into streams, as they can generate a large amount of overland flow [48]. Increasing overland flow can lead to greater contamination of pathogens into surrounding areas, increasing exposure to the pathogen [48,57]. In contrast, the results of our study found significant negative associations with precipitation 4 weeks prior to VTEC case occurrences. The difference in findings may be due to the different measurement methods or outcomes of interest. Waterborne outbreaks are caused by numerous pathogens in addition to VTEC, which may have different dynamics within the environment [14].

Overall, there was a lack of consistency in statistically significant results between the five public health units. As these regions are in the same geographical region with similar climate patterns, we would expect some similarities between units. There is no reason to expect that the way in which environmental factors influence the pathogens persistence in the environment and subsequent human exposure would be different between the health regions being studied and yet, we found significant inconsistencies. This variability may be due to differences between the public health regions that we did not explicitly consider. The population densities vary, where Toronto has a significantly higher population density compared to the other four regions [30]. The agricultural density between regions is also highly variable. Waterloo Region is more than twice as agriculturally dense as the other four health units, while only a few farms are in the Toronto Region [62]. The primary watersheds of the five health regions (Don, Humber, Grand, Holland, Credit, and Rideau Rivers) all vary in size. All of these differences could affect the level of pathogen exposure and transmission capability in a region, leading to different results. Furthermore, the source of infection in the cases

used in the study could have affected the reproducibility between each health region. As we were unable to exclude foodborne cases from our study, which may not be driven by local environmental conditions such as temperature and/or precipitation. It is possible that foodborne cases are a significant proportion of our data, based on the literature [40–45]. Therefore, if an association truly did exist between environmental variables and case occurrences, it would be difficult to identify the effect.

Limitations:

As with other enteric illnesses, infections of VTEC are commonly underreported [63]. Therefore, the cases included within the study may not be representative of the true disease occurrence within each public health unit. Our inability to distinguish foodborne sources of infection within the dataset may have negatively impacted our ability to detect environmental drivers of VTEC. Case data do not contain confirmed information on the source of infection, therefore we were unable to remove foodborne cases, which would not be driven by changes of local environmental conditions. Additionally, out of the 32 univariable analyses conducted, 12 (37.5%) were found to be statistically significant. Given the number of analyses conducted, it is possible that some of the findings were found to be statistically significant by chance.

CONCLUSION

In conclusion, our results found statistical associations between VTEC infections in Ontario and watershed level and flow, and precipitation but the direction of the effects and time lags identified were highly variable across the regions studied. Future research should examine the interaction between both high and low precipitation, and how it may

affect the risk of enteric illness. This study highlights the importance of better understanding the complexity that exists in the relationship between environmental pathogen reservoirs and human illnesses caused by enteric pathogens such as VTEC. Further studies can enable us to better understand the biological mechanisms that transport the pathogen from animal sources into the environment and cause subsequent human infection. Improving our understanding of VTEC dynamics between the human-animal-environmental interfaces will allow us to better prepare for and prevent outbreaks in the future.

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TABLES

Table 2.1: Description of the five public health units examined. Data include human population in each of the health units, reference locations for environmental and hydrology data, and the size of the primary watershed.

Public Health Unit	Population (2016) ¹	Environment Data Reference Location	Hydrology Reference Location	Size of Primary Watershed (km ²)	Agricultural Density (count /km ²) ¹
Toronto	2,731,571	Toronto City Centre	Don River (at Todmorden) Humber river (at Weston)	Don river: 360 ² Humber river: 911 ²	0.12
Waterloo	535,154	Waterloo International Airport Kitchener/Waterloo Roseville	Grand River (at Galt)	6,800 ³	1.00
York	1,109,909	Buttonville airport	Holland River (at Holland landing)	600 ⁴	0.40
Peel	1,381,739	Lester B Pearson International Airport	Credit River (near Cataract)	860 ⁵	0.33
Ottawa	934,243	Ottawa Macdonald-Cartier International Airport	Rideau River (at Ottawa)	4000 ⁶	0.37

¹ (Statistics Canada, 2018)

² (Toronto and Region Conservation Authority, 2018)

³ (Grand River Conservation Authority, 2018)

⁴ (Lake Simcoe Region Conservation Authority, 2010)

⁵ (Credit Valley Conservation, 2011)

⁶ (Rideau Valley Conservation Authority, 2018)

Table 2.2: Characteristics of verocytotoxigenic *Escherichia coli* (VTEC) cases from each study health unit. Data include gender and age distribution.

Public Health Unit	Number of cases	Gender (%)		Age Group (years)				
		Male	Female	< 1–4	5–19	20–44	45+	Not known
Toronto	228	101 (44.3)	127 (55.7)	44	72	56	56	0
Waterloo	129	50 (38.8)	79 (61.2)	30	38	31	26	4
York	105	42 (40.0)	63 (60.0)	21	44	19	21	0
Peel	101	50 (49.5)	51 (50.5)	22	41	18	20	0
Ottawa	85	29 (34.1)	56 (65.9)	15	23	28	19	0
Total	648	272 (42.0)	376 (58.0)	132	218	152	142	4

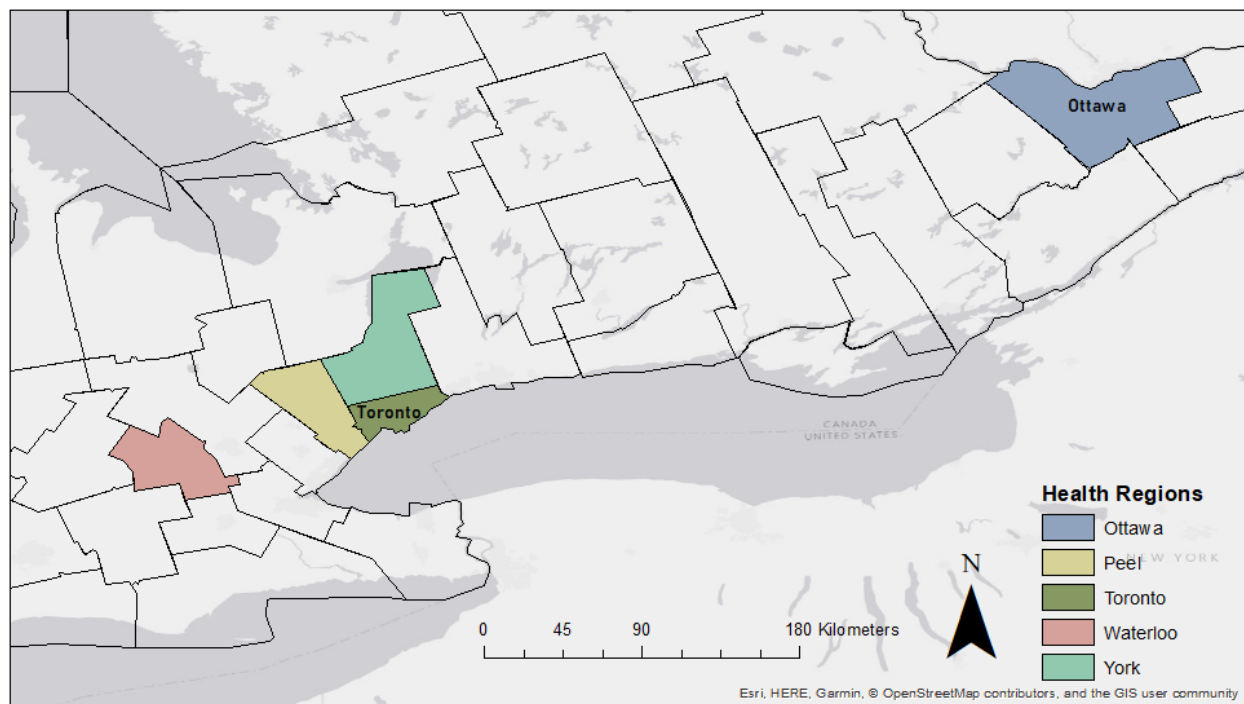
FIGURES

Figure 2.1: Map of the five public health units (coloured) in Southern Ontario, Canada that were included in this study.

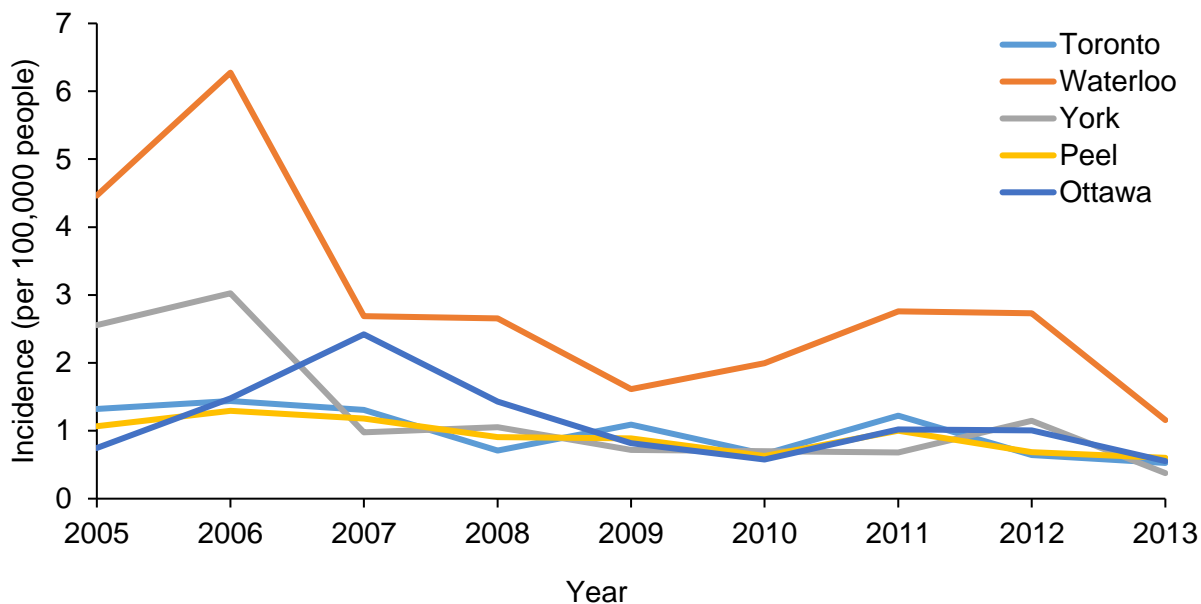


Figure 2.2: Yearly incidence per 100,000 individuals of verocytotoxigenic *Escherichia coli* (VTEC) cases in the five public health units of interest, from January 1st, 2005 – December 31st, 2013.

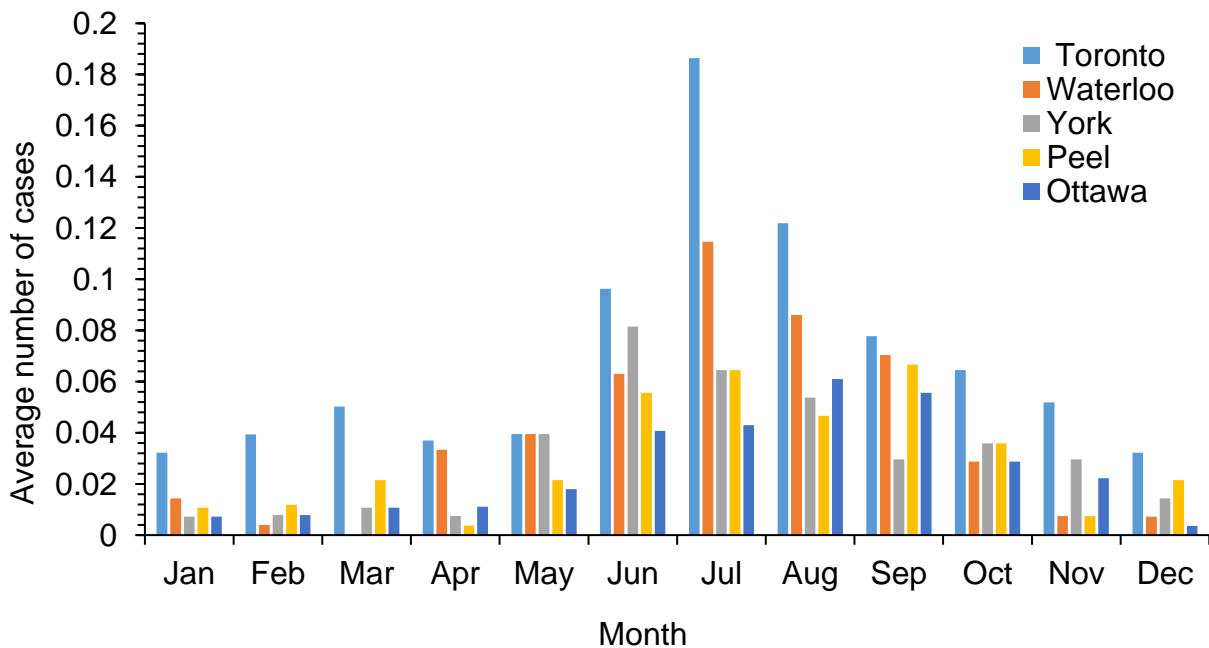


Figure 2.3: Average number of verocytotoxigenic *Escherichia coli* (VTEC) cases per month in the five public health units examined, from January 1st, 2005 – December 31st, 2013.

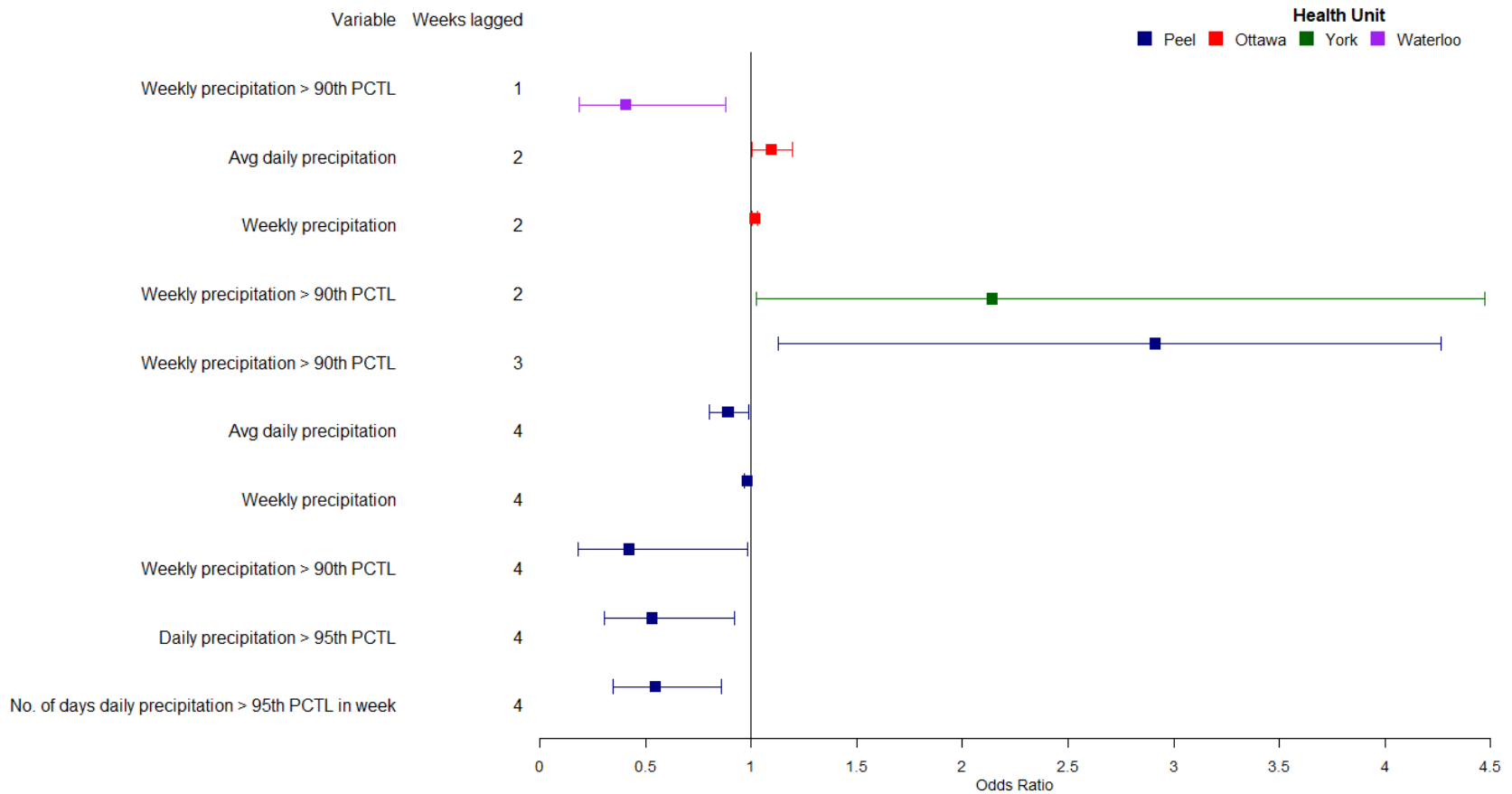


Figure 2.4: Forest plot of significant univariable precipitation results from the case-crossover analyses in the Ottawa, Waterloo, Peel, and York from January 1st, 2005 – December 31st, 2013. Midpoint and error bars represent odds ratio and 95% confidence interval, respectively.

CHAPTER 3

EXAMINING THE ROLE OF PERSON-TO-PERSON TRANSMISSION DURING A VEROCYTOTOXIGENIC *ESCHERICHIA COLI* OUTBREAK IN ONTARIO, CANADA

ABSTRACT

Background: During human outbreaks of verotoxigenic *Escherichia coli* (VTEC), cases arise from a combination of a primary source (e.g. contaminated food or water), and secondary person-to-person transmission. While person-to-person transmission likely contributes less to the overall final outbreak size, it is not always possible to ascertain the source of each case. It is important to understand the relative contribution of the different transmission routes. The objectives of this study were to examine the potential role of person-to-person transmission during a VTEC outbreak in Ontario, Canada, and to estimate the reduction in outbreak size due to public health interventions targeted at reducing person-to-person transmission.

Methods: A deterministic SEIR compartmental model describing a VTEC outbreak was constructed. The model was fit to data from a large VTEC outbreak in Ontario. Two outbreak scenarios were investigated; 1) assuming person-to-person transmission was constant during the entire outbreak, and 2) assuming the person-to-person transmission rate changed when interventions targeting this transmission route were implemented. Maximum likelihood estimation was used to determine the most parsimonious explanation for the observed outbreak data. The best fit person-to-person transmission rate was compared pre- and post-intervention to determine the reduction in the

transmission rate when the public health intervention was implemented. To investigate the potential role of person-to-person transmission, the proportion of cases that were infected from person-to-person transmission was calculated from the best fit model. We then simulated the “counterfactual” to better understand the possible outbreak trajectory in the absence of interventions targeting person-to-person transmission. The final outbreak size of the best fit outbreak model and the “no intervention” simulation were compared to describe the estimated reduction in outbreak size due to the public health interventions. A Latin hypercube sensitivity analysis was conducted to examine the sensitivity of the final outbreak size to the model parameters.

Results: Based on the best fit model, approximately 14.25% of the outbreak cases likely arose due to person-to-person transmission. After the time of the intervention, the person-to-person transmission rate was reduced by approximately 73%. In the absence of an intervention, the model projects that an additional 47 cases (compared to the best-fit model) would have likely arisen due to person-to-person transmission. Therefore, the observed final outbreak size was approximately 16.47% less than if there has been no intervention. The sensitivity analysis showed that the model was highly sensitive to changes in all parameters of the model. The model was most sensitive to changes in the foodborne transmission (β_f), as well as the duration of infection (γ) parameters.

Conclusion: The model demonstrates that person-to-person transmission is an important transmission route during VTEC outbreaks. Targeting this route of transmission, using a combination of approaches that include improved hand hygiene, and work exclusions for high risk work environments (e.g. food service workers, daycare

workers etc.), is important for reducing the final outbreak size. In the future, this model could be used to examine other intervention strategies, including examining how improved syndromic surveillance could impact outbreak size.

INTRODUCTION

Verotoxigenic *Escherichia coli* (VTEC) are bacterial pathogens that can cause enteric illness in humans, particularly children and immunocompromised individuals. Severe infections can lead to haemolytic uremic syndrome (HUS), characterized by renal failure, thrombocytopenia, and hemolytic anemia, which can cause lifelong disability and death [1–3]. Exposure can occur from a variety of sources, including: ingesting contaminated food or water, as well as contact with infected humans or colonized animals such as cattle (the primary reservoir of VTEC) [2,4,5]. Infected humans shed the bacteria in their faeces, and can spread the bacteria to others through the faecal-oral route [2,4,5]. During outbreaks of VTEC, cases arise from a combination of point source (e.g. contaminated food or water) and secondary (typically person-to-person) transmission [1,2,5–12]. In recent years, VTEC has been associated with multiple large, multi-state and multi-province outbreaks, such as the spinach-linked outbreak in 2006 that affected over 200 people in the United States and Canada [10,13]. However, there are still knowledge gaps that exist in relation to the relative contribution of the different transmission routes, specifically the role of person-to-person transmission in VTEC outbreaks [14,15]. This may be due to the challenges associated with ascertaining the specific source of infection for each individual case, as well as the high rate of under-reporting of enteric infections [14,15]. Current source attribution

literature (based on expert elicitation studies) has reported that food (primarily of bovine origin, or leafy greens) is a major source of transmission of VTEC infections overall [11,16–19]. Person-to-person transmission has been estimated to cause between 10-13% of VTEC illnesses [11,18,19]. Within outbreaks, one study estimated that approximately 20% of outbreak cases occurred due to secondary transmission [20]. It is important to better understand the contribution of the different transmission routes to strengthen and target disease prevention and control strategies.

Infectious disease modelling has been used to investigate different routes of transmission during outbreaks. Disease modelling uses a system of mathematical equations to describe the dynamics of an infectious disease in a population [21]. Models can provide insight into the observed patterns of disease spread and can be used to examine hypotheses about disease transmission and estimate key parameters of transmission [21,22]. A number of models have been published in the peer-reviewed literature that simulate enteric disease outbreaks with the objective being to investigate the impact of different transmission routes and interventions on the observed disease dynamics [7–9]. Seto et al. (2007) developed a mathematical model of a foodborne outbreak of VTEC and estimated that reducing person-to-person transmission could have reduced the outbreak size by 5 – 11%. Two other compartmental models describing a *Cryptosporidium* outbreak in the United States have been used to estimate the potential role of the various transmission routes, and how interventions may have reduced the outbreak size [8,9].

The objective of this study was to: 1) examine the potential role of person-to-person transmission in a large VTEC outbreak in Ontario, Canada, and 2) estimate the proportion of cases that were likely prevented due to the public health interventions that were targeted at person-to-person transmission during the outbreak period.

METHODS

Outbreak Data:

Outbreak data for this project were extracted from a publicly available report on a foodborne outbreak of VTEC O157 that was traced to a restaurant in Ontario, Canada in 2008 [23]. The outbreak was comprised of 235 cases (47 confirmed, 59 probable, 118 suspect, and 11 secondary). While there was no confirmed primary source of the outbreak, it was suggested that there was a risk of exposure to the point source contamination approximately one week prior to the restaurant closure (which was 1 day after the initial detection of the outbreak). The suspected source food item was shipped to the restaurant approximately one week prior to restaurant closure. Therefore, only cases with a date of symptom onset after the shipment of the suspected point source food were included in the dataset ($n = 225, 95.7\%$). Day 1 of the outbreak was considered the first case occurrence after this shipment. Additionally, cases with no documented date of symptom onset were excluded from the dataset ($n = 3, 1.3\%$).

The local public health unit was first notified on day 9 of the outbreak as cases began being identified within the community upon seeking medical attention (Figure 3.1). The restaurant that was the suspected source of the outbreak was closed on the

evening of day 10 (Figure 3.1). Following the closure of the restaurant, media releases, and print and radio advertisements were used within the region to communicate with the public, providing updates on the outbreak and to disseminate information focused on preventing further disease spread via person-to-person transmission. The public health unit also provided information (either written or verbal) to all identified symptomatic cases describing steps for preventing secondary transmission, and also recommended exclusion from workplaces until the case was no longer symptomatic and/or had a laboratory-confirmed negative faecal sample. Cases that worked in food service settings or daycares were required to have two laboratory confirmed negative faecal samples prior to returning to work.

Model Structure and Assumptions:

A deterministic compartment model, informed by the model structure of Seto et al. (2007), was developed to describe the outbreak of VTEC within the public health region (Figure 3.2). The model was comprised of five epidemiological states, Susceptible (S), exposed through food but not infectious (E_f), exposed through an infected person but not yet infectious (E_p), clinically ill and infectious to others (I), and recovered (R). In the model, susceptible individuals could become exposed to VTEC through contact with contaminated food (β_f), or through person-to-person transmission (β_p). Once exposed, individuals became clinically ill at a rate inversely proportional to the incubation period (δ). Infected individuals remained infectious for the duration of their clinical symptoms, after which they recovered from infection (at a rate of γ , inversely proportional to the duration of infection) and became immune to reinfection

(enter the R class). The disease transmission process is represented by the following differential equations:

$$\frac{dS}{dt} = -\beta_f S - \beta_p SI$$

$$\frac{dE_f}{dt} = \beta_f S - \delta E_f$$

$$\frac{dE_p}{dt} = \beta_p SI - \delta E_p$$

$$\frac{dI}{dt} = \delta(E_f + E_p) - I\gamma$$

$$\frac{dR}{dt} = I\gamma$$

Model parameter values can be found in Table 3.1. It was assumed that all individuals residing within the health unit at the time of the outbreak were susceptible to infection. Homogeneous mixing was assumed within the population of the health unit. Due to the short outbreak duration, population demographics (such as births and deaths) were not included in the model. While there is evidence of asymptomatic infection and transmission of VTEC, information regarding the proportion of asymptomatic infections that occur is still not well understood [2,12,24,25]. Therefore, our model structure assumes that all cases become symptomatic. This is similar to the model structure of Seto et al. (2007), which assumed that both asymptomatic and symptomatic VTEC infections transmit the bacteria at the same rate.

As previously described, there were multiple interventions implemented during the outbreak response, targeting both the primary source (at the restaurant), and secondary person-to-person transmission. The restaurant closure was modelled by assuming that transmission of VTEC through food (β_f) decreased to zero after day 10. The public health interventions targeted at reducing person-to-person transmission (e.g. work exclusion and public health messaging) were modelled as one intervention that reduced the person-to-person transmission rate. It was assumed that the interventions targeted at reducing person-to-person transmission began after day 10 and were continued until the outbreak was declared over.

Model Fitting and Public Health Intervention:

The model was fit to incident cases of the outbreak data by considering two different outbreak scenarios: where person-to-person transmission rate remained constant for the duration of the outbreak (scenario 1), and where the transmission rates differed prior-to and after the implementation of the intervention (scenario 2; Table 3.2). The parameter values estimated for Scenario 1 were used in Scenario 2 (with the person-to-person transmission rate from Scenario 1 used as the post-intervention transmission rate in Scenario 2) to estimate the person-to-person transmission rate prior to public health interventions (Table 3.2). The model was calibrated to the incident data for each scenario (Table 3.2) using the `mle2` function in R from the `bbmle` package [26,27]. The `mle2` function estimates parameters using maximum likelihood estimation, using optimization techniques based on the Nelder-Mead and Brent algorithms [27].

To determine the relative contribution of person-to-person transmission in the outbreak, the VTEC model (based on the best fit parameters) was used to calculate the proportion of model simulated cases that arose due to person-to-person transmission. Furthermore, we compared the person-to-person transmission rate pre- and post-intervention in scenario 2 to estimate how much the public health messaging intervention was projected to have reduced the person-to-person transmission rate. This was done using the pre-intervention person-to-person transmission rate, and then simulating a scenario of the outbreak where there were no public health interventions targeting person-to-person transmission. To calculate the number of cases averted by the public health intervention targeted at person-to-person transmission, we compared the outbreak size from the best-fit model scenario simulation, to the simulated scenario that assumed no additional public health intervention occurred after the closure of the point source.

Sensitivity analysis:

A Latin hypercube sensitivity analysis was performed for all model parameters. Ranges for the sensitivity analysis can be found in Table 3.1. Partial rank correlation coefficients were calculated in order to investigate how changes to the parameters influenced the model projected outbreak size.

RESULTS

Model Fit:

Table 3.3 contains the best fit parameters from the proposed VTEC model. Based on maximum likelihood estimation, scenario 2 was better able to reproduce the

observed outbreak data. Graphically, the model appeared to fit the data well both pre- and post-intervention; however, the model was not able to capture the peak incidence that occurred on day 9 and in general, appeared to fit more poorly prior to the intervention start date (Figure 3.3). Table 3.4 compares and contrasts the differences observed between the observed epidemic and the model simulations using key metrics including final outbreak size, and time to peak incidence. In scenario 1, the simulated outbreak had the same final outbreak size as the observed data (Table 3.4). However, the peak incidence occurred one day after the peak in the observed outbreak and underestimated the peak by a substantial number of cases (Table 3.4). In scenario 2, the simulated final outbreak size was overestimated, as there were 16 more cases in the model outbreak compared to the observed outbreak (Table 3.4). Similar to scenario 1, the peak incidence in scenario 2 was underestimated (with 13 fewer cases) and occurred one day later than the peak in the observed outbreak (Table 3.4). In the simulated outbreaks for both scenarios 1 and 2, there were a greater proportion of cases attributed to person-to-person transmission (8.00% in scenario 1, and 14.25% in scenario 2), compared to the observed outbreak data which estimated that 4.88% of the cases arose from person-to-person transmission (Table 3.4).

Model Intervention:

Based on the best-fit model parameters in scenario 2, the person-to-person transmission rate (β_p) post-intervention was 73.83% lower than the transmission rate prior to intervention. In the “no intervention/counterfactual” scenario, where an outbreak was simulated assuming no reduction in the person-to-person transmission rate, there were an additional 47 cases in the outbreak arising from person-to-person transmission,

with a total outbreak size of 289. Therefore, the decrease in the person-to-person transmission rate in the best-fit scenario translated to a reduction in the outbreak size by 16.47%. Additionally, in the “no intervention” scenario, the additional cases increased the proportion of cases that arose from person-to-person transmission to 28.37%.

Sensitivity analysis:

Figure 3.4 contains the results of the Latin hypercube sensitivity analysis as partial rank coefficients (PRCCs). The model was quite sensitive to changes in all model parameters (PRCC > 0.5 or < -0.5). The model was most sensitive to changes in the foodborne transmission (β_f ; PRCC = 0.98) and VTEC incubation period (δ ; PRCC = 0.83) parameters. While still highly sensitive, the model was least sensitive to changes to the duration of infection parameter (γ ; PRCC = -0.50). Increasing the values of the pre- and post- intervention person-to-person transmission rates (β_p), the foodborne transmission rate (β_f), and the incubation period parameter (δ) would translate to an increase in the VTEC outbreak size, while increasing the duration of infection parameter (γ) would lead to a decrease in the final VTEC outbreak size (Figure 3.4).

DISCUSSION

The objective of this study was to examine the potential impact of public health interventions to reduce the risk of person-to-person transmission during a VTEC outbreak. It is important to implement public health interventions that target significant routes of disease transmission; however, it is difficult to quantify the contribution of the different routes, and the subsequent impact of such public health interventions. We used a mathematical model to estimate the relative contribution of person-to-person

transmission during the course of a foodborne VTEC outbreak and then to quantify the impact the public health interventions had on the final outbreak size. In our study, we determined that person-to-person transmission may play a slightly larger role in VTEC outbreaks than previously suggested [11,18–20]. We also determined that public health interventions targeting this transmission route appeared to reduce the final outbreak size in this case study.

Despite scenario 2 having a better fit to the observed outbreak data (based on the log likelihood and visual shape of the outbreak), the model fit of scenario 1 was very similar to that of scenario 2. This suggests that differentiating between the two scenarios is challenging. While scenario 1 better estimated the overall outbreak size, the additional cases in scenario 2 arose from the increased transmission rate prior to the intervention. Given our understanding of the interventions implemented that directly targeted that transmission route, it is not realistic to assume that the person-to-person transmission rate remained constant throughout the outbreak.

Our best fit model simulation was unable to generate the observed data well prior to the intervention, where cases were underestimated. It is possible that some of the cases in the observed outbreak met the case definitions but were not actually a part of the outbreak. It is also likely that, due to our model fitting process, the pre-intervention person-to-person transmission parameter was underestimated. As previously described, this parameter value was estimated using the person-to-person transmission rate (β_p) estimated in scenario 1 (which was assumed to be the person-to-person transmission rate for the duration of the outbreak) to β_p post-intervention. Given that this constant

person-to-person transmission rate is essentially an 'average' of the pre- and post-intervention transmission rates, setting that value as the post-intervention transmission rate likely led to an underestimation of the pre-intervention transmission rate.

Furthermore, our model used a simplistic structure representing a mechanistic view of VTEC transmission during an outbreak. As persistence of VTEC in food may vary depending on factors promoting or inhibiting pathogen survival and growth, and as transmission among humans is dependent on human behaviour, it is likely that transmission of VTEC through these different routes varies through time. Therefore, the poor fit that we observed prior to intervention could indicate that the model could be improved if we were to incorporate additional variability in these parameters.

During outbreaks of enteric pathogens (including VTEC), cases commonly arise from a primary (e.g. contaminated food or water) source, followed by secondary transmission (i.e. person-to-person) that prolongs the outbreak [1,2,5–12]. The results of our study also highlight these multi-route transmission chains during outbreaks. The proportion of secondary cases estimated in our model was higher than reported in the outbreak data [23]. This could suggest that some of the cases that were transmitted through person-to-person transmission were missed or misclassified as primary cases. As there are challenges associated with determining the specific source of an infection during an outbreak, some cases reported may not have been classified as secondary infections. According to our best fit model results, approximately 14% of cases were infected via the person-to-person transmission route, which could have increased to approximately 28% had no intervention on person-to-person transmission occurred. While this upper limit is relevant to the potential proportion of cases that could have

occurred due to person-to-person transmission, it is difficult to compare this value to the literature, as VTEC outbreaks in these studies may have some level of intervention preventing secondary transmission. The proportion of secondary cases estimated by our model is qualitatively in line with current estimates on the roles of different transmission routes of VTEC and other enteric infections [11,18–20]. Several studies on the overall source attribution of VTEC infections reported that person-to-person transmission contributes approximately 10 – 13% of VTEC infections [11,18,19]. The estimates of these studies are representative of VTEC infections overall, and not specific to the proportion of cases within an outbreak. Snedeker et al. (2009) examined the rates of secondary transmissions in VTEC O157 outbreaks from parts of North America, Europe, and Japan, to characterize different transmission routes of VTEC. This study reported that approximately 20% of cases in an outbreak arose due to secondary transmission [20]. It was also found that the number of secondary cases differed significantly with the median age of cases; the younger median age group had a higher likelihood of having a high number of secondary cases [20]. It is possible that the lower estimate in our study could be due to a higher median age in the cases; however more information on the case characteristics for the described outbreak were not available. Other mathematical models also found similar proportions of person-to-person transmission during enteric outbreaks [7,9]. While Seto et al. (2007) did not directly estimate the impact of person-to-person transmission in their study, their model was only able to fit well under the assumption that person-to-person transmission contributed between 12 – 25% of the outbreak cases. The model could not be fit if the proportion of person-to-person transmission was greater than 50%, suggesting that

person-to-person transmission could not have comprised greater than 50% of the outbreak cases [7]. In the model of a *Cryptosporidium* outbreak, it was estimated that approximately 10% of the cases could have been attributed to person-to-person transmission [9]. Differences between the proportion of secondary cases in this study compared to the results in our study may be due to the different transmission dynamics of the *Cryptosporidium* parasite and VTEC bacteria, and the populations studied in the models.

Our analysis suggests that interventions that targeted person-to-person transmission reduced the size of the outbreak by approximately 16% compared to a scenario where this intervention was not used. This reduction is higher than what was reported in Seto et al. (2007), which found that interventions targeting the person-to-person transmission route could decrease the final outbreak size by 7 – 11%. Their study however examined a theoretical outbreak intervention, while we attempted to estimate the effectiveness of a real intervention [7]. Differences between our results could also be due to the structure of the compartment model. The model by Seto et al. (2007) included environment-to-person transmission of VTEC as well as waning immunity from those that recover from infection. Including an additional transmission route, and having people become susceptible to infection again could directly influence the number of subsequent infections, and thus the effectiveness of interventions.

There were multiple public health interventions used during the documented outbreak that targeted the person-to-person transmission route (including workplace exclusions, and public health messaging targeted at both the identified cases and the

susceptible population), all of which were modelled as one all-encompassing intervention. Therefore, while we cannot determine which specific intervention was most effective, we were able to determine that targeting the person-to-person transmission route during a VTEC outbreak was important for preventing secondary spread. There is evidence in the literature of other infectious diseases where interventions focused on reducing person-to-person transmission can reduce the final outbreak size [7,29–37]. Isolation or social distancing can be effective measures to prevent subsequent spread of an infection by decreasing contact between people, especially with those who come into contact with many people or people that are vulnerable to infection (e.g. children, and/or immunocompromised individuals) [29,30]. A systematic review from 2018 compared results from mathematical modelling and epidemiological studies to examine the overall effectiveness of social distancing (in workplaces) to control influenza outbreaks (which are spread primarily through person-to-person transmission) [29]. The modelling studies found that social distancing resulted in a reduction in outbreak size, as well as a delay and reduction of the peak of the outbreak [29]. Furthermore, improving hygiene practises (i.e. increased hand-washing) is a highly effective method of preventing the spread of infectious diseases including enteric infections such as VTEC [7,36,37]. Public messaging through media sources can be used as a method to relay the prevention strategies to the public. Mathematical models have shown that media campaigns can lead to a change in behaviour in the population, and lead to a reduction in the number of infected people [31–35]. While these models did not specify which disease prevention behaviours were implemented (e.g. hand-washing, isolation),

they still showed an overall change in behaviour favouring self-protection, leading to a decrease in disease transmission.

Based on the sensitivity analysis, the model was highly sensitive to changes in all of the parameters in the model. This indicates that any changes to the parameter values would lead to significant changes to the outbreak size, either increasing or decreasing the outbreak size. Therefore, all parameter values must fall within a narrow range to fit to the observed data.

Limitations:

Many assumptions and simplifications were made in order to describe the dynamics of the VTEC outbreak in this population. The model structure did not account for asymptomatic infections, due to the lack of literature on the prevalence of asymptomatic VTEC infections. We assumed that all infectious cases were symptomatic. While the model by Seto et al. (2007) differentiated between asymptomatic and symptomatic infections, the model assumed the same transmissibility for both types of cases which means that our approach is functionally similar. In our study, we assumed that the entire population of the health unit was equally at risk of infection. However, since the health unit is quite geographically large, it is likely that the true population at risk was smaller than what was considered here. There is evidence that transmission of VTEC is dependent on age, as younger children are more likely to both be infected with VTEC and transmit VTEC to others [1,2,13,20,38–43]. However, due to the lack of data on the specific age distribution of cases, the model was not age structured. Differential transmission of VTEC based on age may have led to an

improved model fit. VTEC is also commonly transmitted within-households [2,5,12,20,44]. Therefore, those individuals residing within the same household as cases are at highest risk of secondary infection. However, we did not have access to information that described the relationships between the identified cases.

Within our study, we assumed that transmission as a result of exposure to the foodborne contaminant occurred at a constant rate prior to the restaurant closure. It is likely that the risk of exposure changed over time; however, details on the exact risk of exposure were unknown. Having specific information on the transmission of VTEC through food may have improved the model fit and estimation of person-to-person transmission. This study also did not incorporate the transmission of VTEC between infected persons and food (e.g. food service workers). Incorporating this transmission route may have also improved model fit.

As previously described, all public health interventions targeting person-to-person transmission were modelled as one combined intervention. Therefore, we were not able to examine which specific intervention targeted at the person-to-person transmission route would be most effective. Furthermore, the various public health interventions were implemented at different times during the outbreak. There is also evidence that the effect of these public health interventions can vary in time, where it may take time to cause a change in behaviour, and the compliance to interventions may wane over time [29,31–34,37]. As we did not consider how the intervention changes in effect through time, the model could be missing the variability in the multiple public health interventions.

There are also limitations to our model fitting process. As previously described, the model transmission parameters in scenario 2 were fit using values estimated in scenario 1, which may have led to an underestimation of the pre-intervention person-to-person transmission rate, and an overestimation in the post-intervention transmission rate. Utilizing more sophisticated modelling approaches may have led to a better fit, in particular in the pre-intervention time. A Bayesian approach has been previously used by models to fit compartment models for diseases with multiple routes of transmission [8,9].

CONCLUSION

We found that mathematical modelling could be used to estimate the relative contribution of different transmission parameters in a disease outbreak. We created a deterministic compartment model of a VTEC outbreak in Ontario, Canada and found that targeting the person-to-person transmission route during enteric outbreaks can be an effective method of reducing the final outbreak size. Future outbreak management of VTEC and similar enteric pathogens should include strategies to prevent secondary spread through this transmission route. Prevention methods can include isolation of infected persons (e.g. social distancing) and improved hygiene techniques for those infected or at high risk for spread (e.g. in the same household as those that are infected, daycare workers). Future studies could include investigating the impact of asymptomatic infections on enteric outbreaks, estimating secondary transmission of VTEC within households during outbreaks, and examining the person-food transmission route. Our model could also be used to investigate specific interventions targeted at

person-to-person transmission, as well as investigating how improved outbreak surveillance could impact outbreak size.

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TABLES

Table 3.1: Model parameters used, with values obtained from the literature.

Symbol	Definition	Value	Range used for LHS sensitivity analysis	Source
β_f	Foodborne transmission rate	Fit to observed data	(+/- 25%)	
β_p	Person-to-person transmission rate	Fit to observed data	(+/- 50%)	
δ	Incubation period [†]	3.5 days	(2–10) days	[2,3,7,12,25,28]
γ	Duration of infection [†]	6 days	(5–10) days	[7,25,28]

[†]Inverse value is used as a rate in the model

Table 3.2: Description of two different scenarios that represent possible explanations for the observed outbreak data.

Scenario	Description of Scenario
1	<ul style="list-style-type: none"> Foodborne transmission (β_f) was present until restaurant closure (day 10) after which $\beta_f = 0$ Person-to-person transmission (β_p) was present at a constant rate over the entire duration of the outbreak
2	<ul style="list-style-type: none"> Foodborne transmission (β_f) was present until restaurant closure (day 10) after which $\beta_f = 0$ Person-to-person transmission (β_p) was present throughout the entire outbreak, but the rate was reduced after day 10 as a result of the public health interventions that were deployed.

Table 3.3: Best fit model parameters, for both model scenarios examined.

Symbol	Definition	Value
<u>Scenario 1:</u>		
β_f	Foodborne transmission rate	4.24×10^{-4}
β_p	Person-to-person transmission rate (pre and post intervention)	2.88×10^{-7}
<u>Scenario 2:</u>		
β_f	Foodborne transmission rate	4.24×10^{-4}
β_{p1}	Person-to-person transmission rate: pre intervention	1.10×10^{-6}
β_{p2}	Person-to-person transmission rate: post intervention	2.88×10^{-7}

Table 3.4: Characteristic of the observed outbreak data, compared to the two simulated model scenarios and the “no intervention” simulation.

Type of data	Final outbreak size (difference)	Peak Daily Incidence (difference)	Day of peak incidence (difference)	Number of cases infected due to person-to-person transmission (%)
Outbreak data	225	37	8	11 (4.89)
Scenario 1 - simulation	225 (0)	22 (-15)	9 (+1)	18 (8.00)
Scenario 2 - simulation	242 (+17)	24 (-13)	9 (+1)	34 (14.25)

FIGURES

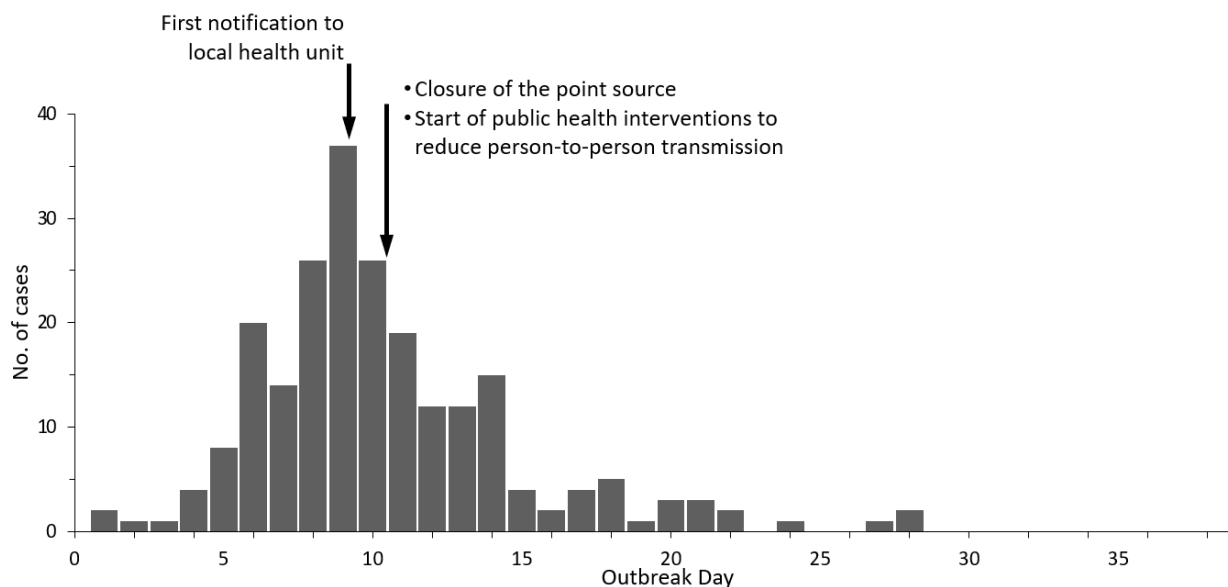


Figure 3.1: Plot of the Verocytotoxigenic *Escherichia coli* (VTEC) outbreak in Ontario, Canada. Arrows indicate relevant dates during the outbreak.

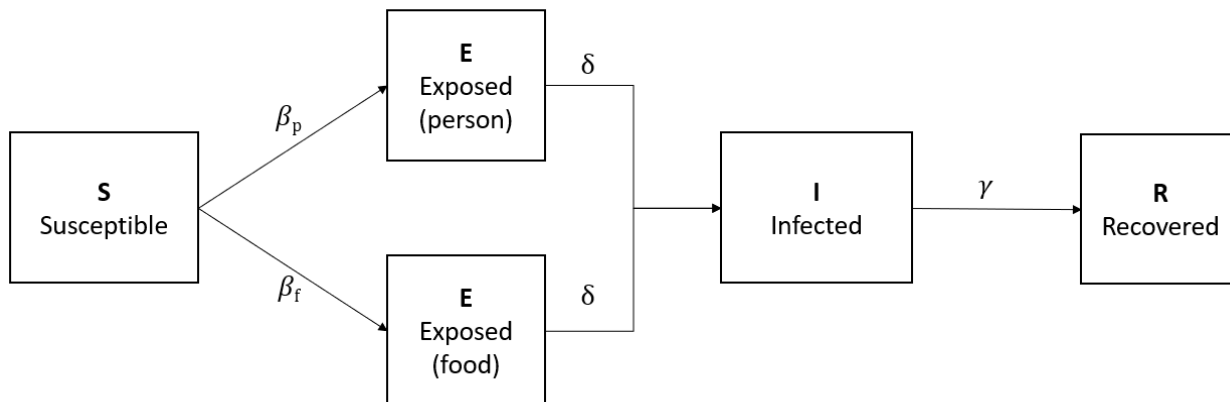


Figure 3.2: Compartment model for Verocytotoxigenic *Escherichia coli* (VTEC) transmission in humans. Susceptible people (S) are first exposed to VTEC through food (E_f) or through infected people (E_p) before becoming clinically infected (I) and infectious to others, and finally recovering (R) from infection. Greek letters above arrows indicate rates of change between the compartments.

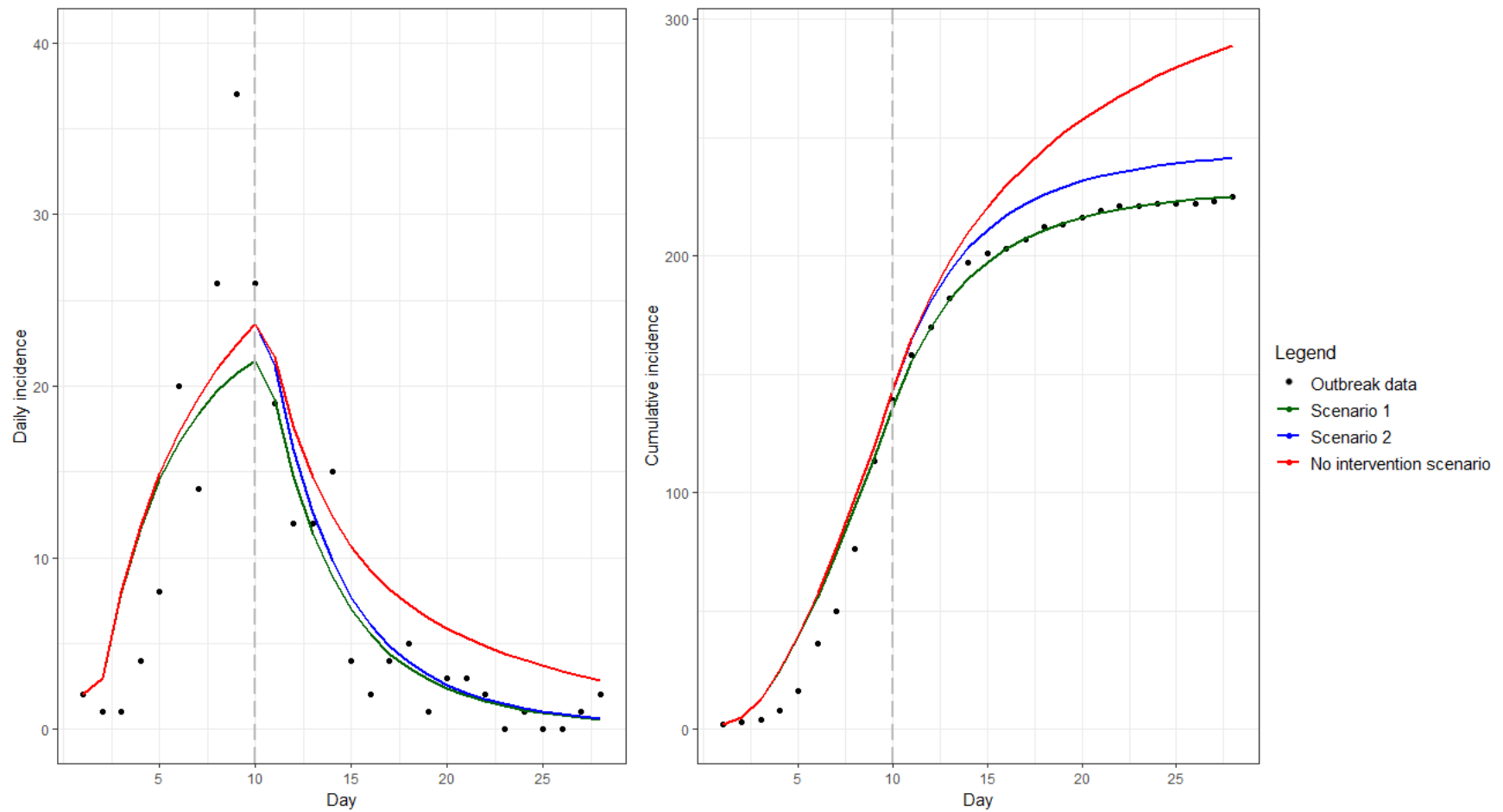


Figure 3.3: Simulation of all model scenarios compared to the observed outbreak data. Grey line indicates the start of all interventions (point source closure, and initiation of public health interventions targeted at reducing person-to-person transmission).

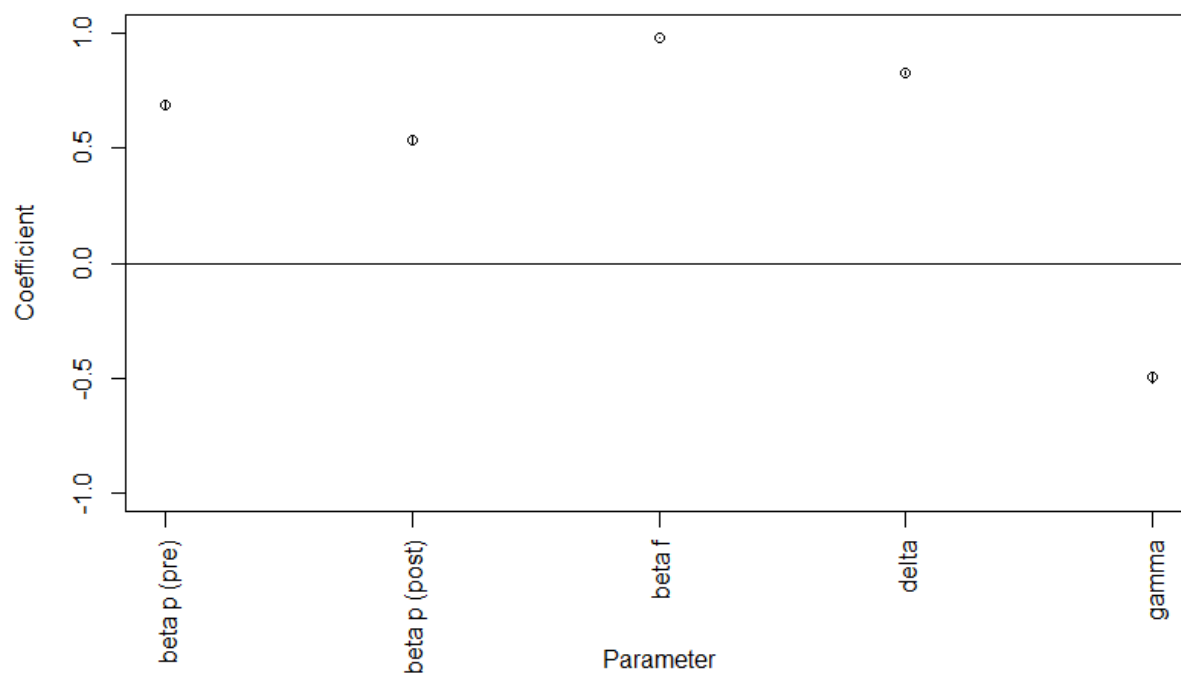


Figure 3.4: Results of the Latin hypercube sensitivity analysis for all parameters in the model, represented as partial rank correlation coefficients (PRCCs). The PRCCs represent the effect of varying each parameter on the final outbreak size.

CHAPTER 4

SUMMARY, LIMITATIONS, AND CONCLUSIONS

The objectives of this thesis were to 1) identify environmental determinants of primary human verocytotoxigenic *Escherichia coli* (VTEC) cases, 2) determine the impact of person-to-person transmission during VTEC outbreaks in Ontario, Canada, and 3) estimate the effectiveness of public health interventions to reduce person-to-person transmission during a large VTEC outbreak in Ontario. Examining how environmental factors may drive primary infections provides an understanding of the spread of VTEC between the environment and human populations. In addition to providing a different methodology to estimate the impact of multiple transmission routes in an outbreak, developing a compartmental model describing a VTEC outbreak provides an improved understanding of the role person-to-person transmission during a VTEC outbreak.

SUMMARY OF MAJOR FINDINGS

In Chapter 2, I identified hydrological factors that were associated with case occurrence of primary human VTEC infections. This included precipitation, waterflow rate, and watershed level at various time lags up to 4 weeks prior to case occurrence. However, these associations were not consistent between the five public health units studied, suggesting that either factors within the public health unit affected the association between the environmental factors and the risk of infection, or that more case information is needed to reduce the variability observed.

Chapter 3 examined the transmission dynamics of VTEC outbreaks. In this chapter, I developed a mathematical model of a VTEC outbreak in Ontario. Using the model, we estimated the proportion of cases that were attributed to person-to-person transmission in the outbreak. Furthermore, we determined that the interventions targeting person-to-person transmission appear to reduce the person-to-person transmission rate. This intervention appeared to reduce the outbreak studied by approximately 16%. These results suggest that it is important to target person-to-person transmission during outbreaks of VTEC.

LIMITATIONS

Overall, my research would have benefitted from improved surveillance case data. As enteric infections are commonly underreported, we recognize that many community infections are not captured in the surveillance data, making inference about the true disease occurrence difficult. Having these data would have provided a better representation of VTEC disease occurrence within each of the public health units.

Furthermore, this research would have benefitted from having surveillance data of VTEC from the environment, such as from water sources and agricultural farms in the surrounding areas. Having data on the levels of VTEC within nearby water reservoirs and farms would have allowed us to directly investigate if there was any association between the presence of the bacteria in water/agricultural sources and case occurrences, as well as how contamination in those locations affects the association between environmental factors and case occurrences. For the dataset examined, we were unable to reliably distinguish the cases that were attributed to foodborne sources

that may not have been driven by changes to local environmental conditions. As those cases were not removed from the study, any true association between environmental factors and case occurrences may have been lost. Having this information would have allowed for better exclusion criteria, using only cases that could have been affected by an environmental exposure.

The model that was developed in Chapter 3 did not fit the observed data particularly well in the time prior to the intervention. Model fit may have been improved by using a different model fitting process, such as a Bayesian approach. It is also likely that the lack of fit could mean that we were missing some additional components of the mechanistic biology. The specific food item responsible for this outbreak was not identified during the course of the outbreak investigation. Having had data to confirm the specific food source of the outbreak could better define the specific risk of exposure in the time period. This would allow us to more accurately model the transmission of VTEC through food. Having additional descriptive information on cases (such as the age of cases as well as which cases were family members) would allow us to explore the differential transmission of VTEC based on age and household association. If we had confirmed data on which cases were infected from food or people, we would be able to more accurately model the role of person-to-person transmission, and subsequently the effectiveness of the public health interventions. Furthermore, if we had detailed information on how the various public health interventions targeting person-to-person transmission were implemented, we would have been able to model the interventions separately. This would provide us with the ability to investigate which

specific interventions have a stronger impact on reducing person-to-person transmission during a VTEC outbreak.

FUTURE RESEARCH OPPORTUNITIES

The results of Chapter 2 can be used to inform VTEC prevention strategies. These results can help notify public health workers of times (e.g. after a heavy storm) when there is an increased risk of VTEC contamination (allowing for improved surveillance/awareness during periods of increased risk). Furthermore, this study provides an example of how case-crossover methodology can be used to examine the impact of acute environmental factors on the risk of enteric infections.

Future research should be conducted (using meta-regression methods) to examine the heterogeneity of the results found within each public health unit studied. This research would investigate if characteristics of the public health units may have impacted the variability in the direction and magnitude of the results described. Studies should also be conducted to examine interaction between the environmental effects. For instance, examining if specific precipitation patterns may impact the association between environmental conditions and VTEC occurrence. If there were also specific geographical data on the location of the human cases, as well as nearby agricultural/environment locations (e.g. farms, lakes, or rivers), geospatial analysis could be conducted to explore how proximity to these spaces might affect the association between environmental conditions and case occurrence.

The results found in Chapter 3 could be used to inform control strategies during outbreaks of VTEC or similar enteric infections. Based on the results of my study,

strategies targeting person-to-person transmission (such as improved hand-washing and isolation of infected persons) should continue to be implemented. Overall, the study is an example of how mathematical modelling can be used to investigate the relative contribution of multiple transmission routes during an outbreak. The developed model could also be used to examine other interventions during the outbreak. More research should be conducted to investigate the role of person-to-person transmission during enteric outbreaks, and how factors such as age and household clustering affect the transmission dynamics. Also, research should focus on which specific interventions and communication tools are most effective at preventing secondary spread. The model could also be used to examine how improved outbreak detection strategies (such as syndromic surveillance) can potentially further influence outbreak size.

CONCLUDING REMARKS

This research provided insight into the transmission dynamics of VTEC in Ontario, Canada. The results found in Chapter 2 can be used to further explore how environmental and hydrological factors may play a role in driving VTEC dynamics. The model we developed in Chapter 3 can be used to explore the transmission of VTEC within populations. Results from this work suggests that we do not fully understand how VTEC is transmitted at the environment-human interface, and within populations. Additionally, the results from Chapter 3 suggest that person-to-person transmission during enteric outbreaks may be an important transmission route. Therefore, that transmission route should be specifically addressed during outbreaks, regardless of outbreak source. More research needs to be conducted to understand the overall

transmission dynamics of VTEC, and to examine the how environmental/hydrological factors can impact this transmission.

APPENDICES

ADDITIONAL TABLES

Table A2.1: Univariable results from the case crossover analyses of predictors (lagged 1-4 weeks) of occurrence of verocytotoxigenic *Escherichia coli* (VTEC) infection in Toronto from January 1st, 2005 - December 31, 2013. OR: Odds ratio.

Variable		Toronto Health Region OR (95%CI) p value
Avg. minimum temperature (°C)	lagged 1 week	1.05 (0.99-1.11), p = 0.13
	lagged 2 weeks	1.04 (0.98-1.1), p = 0.23
	lagged 3 weeks	1.01 (0.95-1.07), p = 0.72
	lagged 4 weeks	0.99 (0.94-1.05), p = 0.74
Avg. maximum temperature (°C)	lagged 1 week	1.02 (0.97-1.08), p = 0.41
	lagged 2 weeks	1.02 (0.97-1.08), p = 0.46
	lagged 3 weeks	1.02 (0.97-1.08), p = 0.47
	lagged 4 weeks	1.01 (0.96-1.06), p = 0.82
Avg. weekly temperature (°C)	lagged 1 week	1.04 (0.98-1.1), p = 0.23
	lagged 2 weeks	1.03 (0.97-1.09), p = 0.33
	lagged 3 weeks	1.02 (0.96-1.08), p = 0.57
	lagged 4 weeks	1 (0.95-1.05), p = 0.94
Avg. waterflow speed in week (m ³ /s)	lagged 1 week	1.01 (0.98-1.04), p = 0.39
	lagged 2 weeks	1 (0.97-1.04), p = 0.84
	lagged 3 weeks	1.01 (0.98-1.05), p = 0.42
	lagged 4 weeks	0.97 (0.93-1.01), p = 0.09
Avg. water level in week (m)	lagged 1 week	0.93 (0.65-1.33), p = 0.7
	lagged 2 weeks	0.95 (0.7-1.28), p = 0.71
	lagged 3 weeks	0.87 (0.63-1.2), p = 0.39
	lagged 4 weeks	1.05 (0.8-1.39), p = 0.73
Avg. daily precipitation in week (mm)	lagged 1 week	0.98 (0.93-1.04), p = 0.56
	lagged 2 weeks	0.99 (0.94-1.05), p = 0.85
	lagged 3 weeks	1.01 (0.95-1.07), p = 0.84
	lagged 4 weeks	0.97 (0.91-1.03), p = 0.32
Cumulative weekly precipitation (mm)	lagged 1 week	1 (0.99-1.01), p = 0.56
	lagged 2 weeks	1 (0.99-1.01), p = 0.8
	lagged 3 weeks	1 (0.99-1.01), p = 0.89
	lagged 4 weeks	0.99 (0.98-1), p = 0.24

Table A2.1 (continued): Univariable results from the case crossover analyses of predictors (lagged 1-4 weeks) of occurrence of verocytotoxigenic *Escherichia coli* (VTEC) infection in Toronto from January 1st, 2005 - December 31, 2013. OR: Odds ratio.

Variable		Toronto Health Region <i>OR (95%CI) p value</i>
Extreme cumulative precipitation in week (mm)	<i>lagged 1 week</i>	0.95 (0.58-1.56), p = 0.85
	<i>lagged 2 weeks</i>	0.84 (0.51-1.38), p = 0.5
	<i>lagged 3 weeks</i>	0.93 (0.56-1.57), p = 0.79
	<i>lagged 4 weeks</i>	0.96 (0.57-1.64), p = 0.89
Extreme precipitation events in week (mm)	<i>lagged 1 week</i>	1.09 (0.77-1.56), p = 0.62
	<i>lagged 2 weeks</i>	0.85 (0.59-1.2), p = 0.35
	<i>lagged 3 weeks</i>	1.17 (0.82-1.66), p = 0.39
	<i>lagged 4 weeks</i>	0.77 (0.52-1.13), p = 0.18
Number of extreme precipitation days in week	<i>lagged 1 week</i>	1.05 (0.8-1.37), p = 0.75
	<i>lagged 2 weeks</i>	1 (0.76-1.31), p = 0.97
	<i>lagged 3 weeks</i>	1.05 (0.78-1.4), p = 0.77
	<i>lagged 4 weeks</i>	0.8 (0.58-1.1), p = 0.16

Table A2.2: Univariable results from the case crossover analyses of predictors (lagged 1-4 weeks) of occurrence of verocytotoxigenic *Escherichia coli* (VTEC) infection in Ottawa, Waterloo, Peel, and York from January 1st, 2005 - December 31, 2013. Bolded cells refer to statistically significant results OR: Odds ratio.

Variable		Ottawa Health Region OR (95%CI), p value	Waterloo Health Region OR (95%CI) p value	Peel Health Region OR (95%CI) p value	York Health Region OR (95%CI) p value
Avg. minimum temperature (°C)	lagged 1 week	0.94 (0.86-1.02), p = 0.13	1.06 (0.98-1.14), p = 0.17	1.06 (0.98-1.15), p = 0.16	0.98 (0.9-1.06), p = 0.59
	lagged 2 weeks	1.01 (0.92-1.11), p = 0.79	0.99 (0.92-1.07), p = 0.88	1.01 (0.93-1.1), p = 0.8	1.05 (0.97-1.14), p = 0.25
	lagged 3 weeks	1.06 (0.95-1.17), p = 0.3	1.02 (0.94-1.1), p = 0.63	0.93 (0.84-1.02), p = 0.12	0.97 (0.89-1.06), p = 0.47
	lagged 4 weeks	0.94 (0.86-1.03), p = 0.2	1.06 (0.98-1.14), p = 0.13	1.04 (0.96-1.13), p = 0.34	0.98 (0.91-1.06), p = 0.66
Avg. maximum temperature (°C)	lagged 1 week	0.98 (0.91-1.06), p = 0.61	1.06 (0.99-1.14), p = 0.08	1.07 (0.99-1.15), p = 0.1	0.98 (0.91-1.05), p = 0.55
	lagged 2 weeks	0.99 (0.92-1.07), p = 0.84	1.02 (0.95-1.09), p = 0.56	1 (0.92-1.08), p = 0.98	0.99 (0.92-1.07), p = 0.88
	lagged 3 weeks	1.02 (0.95-1.11), p = 0.56	1 (0.93-1.07), p = 0.99	0.95 (0.87-1.03), p = 0.22	1.01 (0.93-1.09), p = 0.85
	lagged 4 weeks	0.95 (0.87-1.04), p = 0.24	1.04 (0.97-1.11), p = 0.29	1.01 (0.93-1.09), p = 0.79	1.01 (0.94-1.09), p = 0.74
Avg. weekly temperature (°C)	lagged 1 week	0.96 (0.88-1.04), p = 0.3	1.07 (0.99-1.15), p = 0.09	1.07 (0.98-1.16), p = 0.12	0.98 (0.9-1.06), p = 0.55
	lagged 2 weeks	1 (0.91-1.1), p = 0.99	1.01 (0.94-1.09), p = 0.78	1.01 (0.92-1.09), p = 0.91	1.02 (0.94-1.11), p = 0.61
	lagged 3 weeks	1.04 (0.95-1.14), p = 0.41	1.01 (0.93-1.1), p = 0.82	0.93 (0.84-1.03), p = 0.15	0.99 (0.9-1.08), p = 0.79
	lagged 4 weeks	0.94 (0.86-1.03), p = 0.2	1.05 (0.98-1.14), p = 0.16	1.03 (0.94-1.12), p = 0.53	1 (0.92-1.08), p = 0.96
Avg. waterflow speed in week (m ³ /s)	lagged 1 week	1 (0.99-1.02), p = 0.69	0.99 (0.98-1), p = 0.21	1.21 (0.99-1.49), p = 0.07	0.98 (0.77-1.25), p = 0.88
	lagged 2 weeks	1.01 (0.99-1.02), p = 0.51	1 (0.99-1.01), p = 0.68	0.79 (0.57-1.1), p = 0.16	1.3 (1.06-1.61), p = 0.01
	lagged 3 weeks	0.99 (0.98-1.01), p = 0.3	1 (0.99-1.01), p = 0.85	0.89 (0.66-1.19), p = 0.43	0.9 (0.72-1.12), p = 0.34
	lagged 4 weeks	1 (0.99-1.01), p = 0.78	1 (0.99-1.01), p = 0.91	0.73 (0.49-1.08), p = 0.12	0.81 (0.64-1.02), p = 0.07

Table A2.2 (continued): Univariable results from the case crossover analyses of predictors (lagged 1-4 weeks) of occurrence of verocytotoxigenic *Escherichia coli* (VTEC) infection in Ottawa, Waterloo, Peel, and York from January 1st, 2005 - December 31, 2013. Bolded cells refer to statistically significant results OR: Odds ratio.

Variable		Ottawa Health Region OR (95%CI), p value	Waterloo Health Region OR (95%CI) p value	Peel Health Region OR (95%CI) p value	York Health Region OR (95%CI) p value
Avg. water level in week (m)	lagged 1 week	1.96 (0.28-13.9), p = 0.5	0.35 (0.08-1.51), p = 0.16	25.83 (0.68-979), p = 0.08	0.77 (0.02-25.22), p = 0.88
	lagged 2 weeks	0.95 (0.14-6.34), p = 0.96	0.88 (0.29-2.68), p = 0.83	0.02 (0-3.54), p = 0.14	80.05 (3.83-1673), p = 0.01
	lagged 3 weeks	0.25 (0.04-1.48), p = 0.13	0.98 (0.29-3.23), p = 0.97	0.14 (0-14.46), p = 0.41	0.3 (0.01-7.11), p = 0.45
	lagged 4 weeks	1.08 (0.25-4.64), p = 0.92	0.9 (0.28-2.88), p = 0.85	0 (0-1.41), p = 0.07	0.06 (0-1.55), p = 0.09
Avg. daily precipitation in week (mm)	lagged 1 week	0.93 (0.83-1.04), p = 0.22	0.96 (0.89-1.04), p = 0.33	1.01 (0.93-1.09), p = 0.89	1 (0.91-1.09), p = 0.92
	lagged 2 weeks	1.1 (1-1.2), p = 0.04	1.02 (0.95-1.09), p = 0.62	0.95 (0.87-1.04), p = 0.29	1.09 (0.99-1.19), p = 0.08
	lagged 3 weeks	0.96 (0.86-1.06), p = 0.38	0.97 (0.9-1.04), p = 0.38	1.07 (0.99-1.15), p = 0.08	1 (0.91-1.1), p = 0.96
	lagged 4 weeks	0.94 (0.85-1.03), p = 0.19	0.96 (0.89-1.04), p = 0.34	0.89 (0.8-0.99), p = 0.03	0.99 (0.91-1.08), p = 0.8
Cumulative weekly precipitation (mm)	lagged 1 week	0.99 (0.97-1.01), p = 0.19	0.99 (0.98-1.01), p = 0.28	1 (0.99-1.01), p = 0.81	1 (0.99-1.01), p = 0.92
	lagged 2 weeks	1.02 (1-1.03), p = 0.03	1 (0.99-1.01), p = 0.59	0.99 (0.98-1.01), p = 0.27	1.01 (1-1.03), p = 0.06
	lagged 3 weeks	0.99 (0.98-1.01), p = 0.39	1 (0.98-1.01), p = 0.34	1.01 (1-1.02), p = 0.07	1 (0.99-1.01), p = 0.95
	lagged 4 weeks	0.99 (0.97-1.01), p = 0.18	0.99 (0.98-1.01), p = 0.29	0.98 (0.97-1), p = 0.02	1 (0.99-1.01), p = 0.79
Extreme cumulative precipitation in week (mm)	lagged 1 week	0.54 (0.2-1.45), p = 0.22	0.41 (0.19-0.88), p = 0.02	0.74 (0.36-1.55), p = 0.43	1.24 (0.6-2.55), p = 0.57
	lagged 2 weeks	1.66 (0.76-3.63), p = 0.21	1.05 (0.56-1.99), p = 0.87	1 (0.46-2.16), p = 1	2.14 (1.03-4.47), p = 0.04
	lagged 3 weeks	0.82 (0.36-1.83), p = 0.62	0.95 (0.49-1.83), p = 0.87	2.19 (1.13-4.27), p = 0.02	1.12 (0.53-2.35), p = 0.77
	lagged 4 weeks	0.58 (0.26-1.32), p = 0.19	1.1 (0.54-2.24), p = 0.78	0.42 (0.18-0.99), p = 0.05	0.72 (0.32-1.64), p = 0.44

Table A2.2 (continued): Univariable results from the case crossover analyses of predictors (lagged 1-4 weeks) of occurrence of verocytotoxigenic *Escherichia coli* (VTEC) infection in Ottawa, Waterloo, Peel, and York from January 1st, 2005 - December 31, 2013. Bolded cells refer to statistically significant results OR: Odds ratio.

Variable		Ottawa Health Region OR (95%CI), p value	Waterloo Health Region OR (95%CI) p value	Peel Health Region OR (95%CI) p value	York Health Region OR (95%CI) p value
Extreme precipitation events in week (mm)	lagged 1 week	1.15 (0.64-2.07), p = 0.65	0.74 (0.45-1.22), p = 0.24	1.29 (0.75-2.23), p = 0.36	0.86 (0.52-1.43), p = 0.56
	lagged 2 weeks	1.02 (0.58-1.79), p = 0.94	0.77 (0.47-1.28), p = 0.31	0.8 (0.46-1.37), p = 0.41	1.36 (0.82-2.27), p = 0.23
	lagged 3 weeks	0.8 (0.45-1.41), p = 0.44	1.25 (0.79-1.98), p = 0.34	1.03 (0.62-1.71), p = 0.9	1.11 (0.69-1.81), p = 0.66
	lagged 4 weeks	0.61 (0.33-1.13), p = 0.12	0.81 (0.48-1.37), p = 0.43	0.53 (0.31-0.92), p = 0.02	0.64 (0.38-1.07), p = 0.09
No. of extreme precipitation days in week	lagged 1 week	1.11 (0.73-1.7), p = 0.62	0.8 (0.56-1.13), p = 0.2	1.21 (0.81-1.81), p = 0.35	1.03 (0.72-1.46), p = 0.89
	lagged 2 weeks	1.01 (0.67-1.53), p = 0.96	0.95 (0.69-1.32), p = 0.77	0.83 (0.55-1.25), p = 0.36	1.39 (0.97-1.99), p = 0.07
	lagged 3 weeks	0.87 (0.57-1.34), p = 0.53	1.02 (0.76-1.39), p = 0.88	1.29 (0.91-1.85), p = 0.16	1.08 (0.74-1.57), p = 0.7
	lagged 4 weeks	0.73 (0.46-1.15), p = 0.17	0.78 (0.54-1.12), p = 0.17	0.55 (0.35-0.86), p = 0.01	0.75 (0.5-1.12), p = 0.16