

**A Spatial Epidemiological Analysis of Human and Bovine Cryptosporidiosis
in Southern Ontario, from 2011 to 2014**

by

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ABSTRACT

A SPATIAL EPIDEMIOLOGICAL ANALYSIS OF HUMAN AND BOVINE CRYPTOSPORIDIOSIS IN SOUTHERN ONTARIO, from 2011 to 2014

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Cryptosporidium is a parasitic zoonotic pathogen that can result in human and animal morbidity and mortality and has been detected in Southern Ontario— an area densely populated by humans and cattle. This thesis is a study of the spatial distribution of human and bovine cryptosporidiosis across 29 Public Health Unit areas in Southern Ontario from 2011 to 2014. The overall raw farm-level prevalence of bovine cryptosporidiosis was 45% [95% CI: 42%-48%]. The raw cumulative incidence risk of human cryptosporidiosis was 6.91 [95%CI: 6.47-7.39] cases per 100,000 population. Overlapping disease clusters of both human and bovine cryptosporidiosis were identified in the Central West region of Southern Ontario. A Spatial Poisson regression showed that the incidence risk ratio of cryptosporidiosis in humans increases with increasing dairy cattle density. These findings suggest that dairy cattle play a role in the distribution of human cryptosporidiosis. Further studies on the transmission of cryptosporidiosis in Southern Ontario and the specific role of dairy cattle are warranted.

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STATEMENT OF WORK DONE

Study Design:

I was assisted by my advisory committee (Drs. Olaf Berke, David Pearl and Lise Trotz-Williams) in designing both studies.

Data Collection:

Chapter 2: The data on bovine cryptosporidiosis were abstracted from the database of the Animal Health Laboratory (AHL) at the University of Guelph and provided by Dr. Beverly McEwen.

Chapter 3: The data on human cryptosporidiosis infections in Southern Ontario were supplied by Public Health Ontario. Human population data were obtained from Statistics Canada, and cattle population data from the Ontario Ministry of Agriculture, Food and Rural Affairs.

Data Analysis:

I analyzed the data collected for each study with the assistance of Drs. Olaf Berke, David Pearl and Lise Trotz-Williams.

Results Sharing:

I prepared the manuscript for each of the research chapters and the literature review and discussion prior to receiving suggestions for edits from Drs. Olaf Berke, David Pearl and Lise Trotz-Williams. I was responsible for making the appropriate changes to each manuscript following review by the advisory committee.

I presented the research in poster format at the Canadian Association of Veterinary Epidemiology and Preventative Medicine (CAVEPM) conference in June 2017 and at the Centre for Public Health and Zoonoses (CPHAZ) One Health Symposium in May 2017.

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LIST OF ABBREVIATIONS

AHL	Animal Health Laboratory
iPHIS	Integrated Public Health Information System
OMAFRA	Ontario Ministry of Agriculture, Food and Rural Affairs
PHO	Public Health Ontario
PHU	Public Health Unit

CHAPTER ONE:

Introduction, Literature Review, Objectives, Information Sources and Methods

INTRODUCTION

Human, animal and environmental interactions are fundamental in the transmission of parasitic zoonotic diseases (Gebreyes *et al.*, 2014). In fact, as we continue to shift away from a model of emergency response towards one of prevention to combat infectious diseases, further understanding and exploration of these interactions are important for the development of effective public health measures (Heymann & Dixon, 2013). *Cryptosporidium* is a zoonotic pathogen of increasing public health concern because of its ability to cause human and animal morbidity and mortality, by contaminating our food and water. Given the ability of this parasitic agent to move between humans, animals, and the environment a One Health approach (which seeks to employ an integrated population-based understanding of health) provides an ideal framework for investigating the distribution and potential transmission of this disease (Little, 2012).

LITERATURE REVIEW

***Cryptosporidium*: A Ubiquitous Parasite**

Cryptosporidium is a protozoan parasite and the causal agent of cryptosporidiosis; a potentially lethal diarrheal disease in both humans and animals (Abeywardena *et al.*, 2015). *Cryptosporidium* was first discovered by Ernest Edward Tyzzer in 1907 when he isolated it from the gastric glands of mice (Rossel & Latif, 2013). Since then, 26 different species of *Cryptosporidium* have been identified in mammalian, amphibian, reptilian and avian hosts

(Abeywardena *et al.*, 2015). The infectious life stage of *Cryptosporidium* is a sporulated thick-walled oocyst (de Graaf *et al.*, 1999). Infections in host species occur through multiple transmission pathways (Robertson & Fayer, 2013). In general, oocysts are transmitted by the fecal-oral route either directly from host-to-host contact or indirectly through contaminated environments, such as food and water supplies (Robertson & Fayer, 2013). Hosts infected with *Cryptosporidium* shed large quantities of oocysts in their feces for extended periods of time; shedding of oocysts in infected individuals has been shown to occur up to 5 weeks after the end of diarrheal illness (Chappell *et al.*, 1999; Rose *et al.*, 1986; Rossel & Latif, 2013). These shedding patterns lead to substantial environmental contamination and the persistence of infections in both human and animal populations (Chappell *et al.*, 1999; Rose *et al.*, 1986; Madore *et al.*, 1999; Rossel & Latif, 2013).

Cryptosporidium oocysts have specific properties that contribute to the parasite's ubiquitous nature. First, the oocysts are highly persistent in the environment due to their ability to survive outside the host for long periods of time. Studies have shown that oocysts can survive in surface water for months and in soil for at least 120 days (Robertson *et al.*, 1992; Chauret *et al.*, 1995; Medema *et al.*, 1997; Fayer *et al.*, 1998; Kato *et al.*, 2004; Roberston & Gjerde, 2004). Furthermore, *Cryptosporidium parvum* oocysts have been shown to remain infectious in soil at -10°C for a period of 50 days (Kato *et al.*, 2002). Second, *Cryptosporidium* oocysts are highly resistant to common chemical disinfection methods such as chlorine, which is an important drinking water safety measure that acts as a barrier against waterborne pathogens (Korich *et al.*, 1990; Smith *et al.*, 1990; Ransome *et al.*, 1993). It is used in watershed systems and recreational pool systems. Third, *Cryptosporidium* oocysts are highly infective: ingestion of a single oocyst can result in infection (Medema, 2009). Ultimately, these properties, coupled with host shedding

patterns, allow this parasite to move efficiently through the human-animal-environmental interface.

***Cryptosporidium* in Cattle**

Although infections with *Cryptosporidium* in cattle were first reported in the early 1970's, *Cryptosporidium* did not become a significant pathogen of concern within cattle until it was reported as the sole cause of neonatal diarrhea in an experimental study conducted by Tzipori *et al.* (1983) (Pancieria *et al.*, 1971; Tzipori *et al.*, 1983). Since that time, *Cryptosporidium* has been recognized as an endemic pathogen amongst cattle populations worldwide and as a significant cause of neonatal enteritis in calves (Mosier *et al.*, 2000; Blanchard, 2012; Cho & Yoon, 2014). Globally, studies estimating the prevalence of bovine cryptosporidiosis have reported values ranging from 3.4% to 96.6% among calves; moreover, it is estimated that approximately 90% of dairy farms and 40% of beef farms are endemically infected with *Cryptosporidium* (Mann *et al.*, 1986; Olson *et al.*, 1997; Ruest *et al.*, 1998; Harp&Goff, 1998; Bendali *et al.*, 1999; Peng *et al.*, 2003; Santin *et al.*, 2004; Trotz-Williams *et al.*, 2005; Broglia *et al.*, 2008; Santin *et al.*, 2008; Brook *et al.*, 2008; Coklin *et al.*, 2009; Silverlås *et al.*, 2009; Duranti *et al.*, 2009; Imre *et al.*, 2011; Smith *et al.*, 2014; Wells *et al.*, 2015). Of the various *Cryptosporidium* species currently identified, cattle are the primary hosts of *C. andersoni*, *C. bovis*, *C. ryanae* and *C. parvum*. *Cryptosporidium parvum* is particularly unique in cattle, as it is the only species that causes clinical disease in neonatal calves.

Cryptosporidium parvum predominantly affects pre- to post-weaned dairy calves (Abeywardena *et al.*, 2015; O'Donoghue, 1995; Sivajothi, Reddy, and Rayulu, 2014). Though infections do occur in adult cattle, they are often mild or asymptomatic, making them efficient transmission vehicles (Abeywardena *et al.*, 2015; O'Donoghue, 1995). Clinical signs of

cryptosporidiosis in neonatal calves can range from acute to chronic diarrhea, and may include dehydration, inappetence, lethargy, dehydration, fever, anorexia, weight loss, depression, dullness and bloating (Thomson *et al.*, 2017). In some cases, the infection can lead to death (Abeywardena *et al.*, 2015; Thomson *et al.*, 2017). Overall, cryptosporidiosis is a source of both economic and production losses for cattle farms (Thomson *et al.*, 2017). Currently with there being no vaccine available for cryptosporidiosis and treatment being limited, prevention remains a critical intervention in the management of this calf disease especially since prevention is often more economically beneficial for cattle farms and results in better health and welfare for infected calves (Thomson *et al.*, 2017).

***Cryptosporidium* in Humans**

Human cryptosporidiosis is a widespread global disease, with human cases being reported in more than 90 countries on all continents except Antarctica (Fayer *et al.*, 1990). Globally, the prevalence of infection in humans is estimated to range from 1% to 4% in Europe and North America, and 3% to 20% in Africa, Asia, Australia, South and Central America (Medema, 2009). These estimates are likely an underestimate of the true burden of illness globally, since mild, asymptomatic and/or self-limited infections are common and often not reported. To date, human infections with *Cryptosporidium* have been associated with at least 20 of 26 known species; however, the majority of infections are caused by two species: *C. hominis* and *C. parvum* (Ryan *et al.*, 2014). Humans are the primary hosts of *C. hominis*, meaning that human infections with this specific species mostly arise through anthropogenic transmission (Ryan *et al.*, 2014). On the other hand, the host range for *C. parvum* is quite wide and human infections with this species have often been shown to arise through zoonotic transmission (Ryan *et al.*, 2014). The most common clinical manifestation of cryptosporidiosis is profuse watery

diarrhea; however, additional symptoms of stomach cramps, nausea, vomiting, low-grade fever, fatigue anorexia and weight loss have been documented (Chako *et al*, 2010). Clinical symptoms usually last 1 to 2 weeks in healthy individuals and symptom onset begins anywhere between 2 to 10 days after exposure (Chako *et al*, 2010). The clinical presentation of cryptosporidiosis is often similar in both healthy children and adults; however, children are at a higher risk for infection. In immunocompromised individuals, infections with *Cryptosporidium* result in severe chronic disease (Checkley *et al.*, 2014); often patients will exhibit chronic diarrhea, severe dehydration, weight loss and malnutrition, leading to hospitalization and death (Checkley *et al.*, 2014). Available treatment options for cryptosporidiosis are limited and often involve oral or intravenous rehydration. The United States Food and Drug Administration (FDA) has approved the use of nitazoxanide for the treatment of diarrhea caused by *Cryptosporidium* in immunocompetent patients (Rossle & Latif, 2013). Its efficacy in immunocompromised individuals has not yet been shown (Rossle & Latif, 2013). Due to the lack of available treatment for cryptosporidiosis, effective prevention and control strategies are essential for limiting the spread of this parasite.

The Role of Cattle in Zoonotic Transmission of *Cryptosporidium*

The *Cryptosporidium* species *C. parvum* is regarded as the most prominent zoonotic species of the genus because of its broad host range. Together with *C. hominis*, it accounts for 90% of all reported human cases (Xiao & Ryan, 2004). Although multiple animals have been implicated in the zoonotic transmission of cryptosporidiosis, cattle are regarded as the most important reservoir of zoonotic *Cryptosporidium* (Vermeulen *et al.*, 2017). Globally, cattle have the highest prevalence of *C. parvum* and infected calves shed a high quantity of oocysts in their feces, allowing them to contribute to both direct and indirect routes of zoonotic transmission

(Medema, 2009). Epidemiological studies conducted on small outbreaks of cryptosporidiosis in veterinary students, children and research technicians have implicated direct contact with infected calves as the likely source of exposure (Anderson *et al.*, 1982; Pohjola *et al.*, 1986; Levine *et al.*, 1988; Pritchard *et al.*, 1995; Preiser *et al.*, 2003; Smith *et al.*, 2004; Kiang *et al.*, 2006; Gait *et al.*, 2008; Gormley *et al.*, 2011; Drinkard *et al.*, 2015; Kinross *et al.*, 2015; Benschop *et al.*, 2017). For example, in a retrospective cohort study conducted by Benschop *et al.* (2017) on an outbreak of cryptosporidiosis in veterinary students, having contact with infected calves resulted in a significantly increased odds of disease; students with one week of contact had 10 times higher odds of being a case (adjusted OR = 10.61, [95% CI:1.87-108.29]) while students with two weeks of contact had 55 times higher odds of being a case (adjusted OR = 55.05, [95% CI :3.80-1931.18]) compared to students who had no contact with infected calves (Benschop *et al.*, 2017). Cattle have also been implicated in the indirect transmission of zoonotic *Cryptosporidium* through environmental contamination. Cattle manure containing *Cryptosporidium* oocysts have been noted as an important environmental source of contamination leading to foodborne and waterborne outbreaks in humans (Glaberman *et al.*, 2002; Blackburn *et al.*, 2006). Evidence from these studies have demonstrated that cattle are the main vehicle in the zoonotic transmission of this disease.

Canada and Zoonotic Cryptosporidiosis

Globally, the occurrence of zoonotic *Cryptosporidium* varies geographically. In Canada the majority of human infections of *Cryptosporidium* appear to be due to *C. hominis*; however, the presence of zoonotic infections has been observed (Ong *et al.*, 2002; Trotz-Williams *et al.*, 2006). In a study published by Murray *et al.* (2017), estimating the burden of illness of enteric pathogens associated with animal contact in Canada, it was estimated that annually, 23% of

cryptosporidiosis cases and 22% of cryptosporidiosis hospitalizations are associated with animal contact (Murray *et al.*, 2017). Given the role that animal contact can play in the burden of illness of cryptosporidiosis in Canada, understanding the potential zoonotic implications of this disease, especially in areas that have both a high density of cattle and humans, is important for prevention.

***Cryptosporidium* in Ontario**

The role of animal exposure in the occurrence of human cryptosporidiosis in Ontario has been explored in epidemiological studies to some extent (Pintar *et al.*, 2009; Ravel *et al.*, 2012; Vrbova *et al.*, 2012; Whitfield *et al.*, 2017). In a study conducted by Vrbova *et al.* (2012), 47.2% of all domestically acquired cryptosporidiosis cases in Ontario from 2007 to 2009 identified exposure to animals as the most likely source of exposure (Vrbova *et al.*, 2012). Moreover, in a study conducted by Whitfield *et al.* (2017), on-farm exposure was identified as an important transmission pathway of zoonotic cryptosporidiosis. Although these studies have indicated animal exposure as an important factor in the transmission of cryptosporidiosis in Ontario, studies on the role of cattle in the occurrence of human cryptosporidiosis in Ontario using a One Health study design are limited.

Cryptosporidium has been found to be quite prevalent amongst the Ontario beef and dairy cattle populations. In a study conducted by Dixon *et al.* (2011), on the prevalence of *Giardia* and *Cryptosporidium* on cattle farms in Waterloo, Ontario, the farm-level prevalence of *Cryptosporidium* in both beef and dairy cattle was estimated to be 63% and 64%, respectively (Dixon *et al.*, 2011). As well, the study found that in dairy cattle the zoonotic species *C. parvum* was the most frequently identified species (Dixon *et al.*, 2011). In another study conducted by Trotz-Williams *et al.* (2005), on the prevalence of *C. parvum* in dairy cattle in Southwestern

Ontario, the farm-level prevalence was estimated to be 76%. Furthermore, 41% of dairy calves were found to be shedding *C. parvum* oocysts (Trotz-Williams *et al.*, 2005). Evidence from these studies demonstrate that (1) *Cryptosporidium* is highly prevalent amongst beef and dairy cattle in the southern region of Ontario, (2) the zoonotic species *C. parvum* is common among dairy cattle—particularly dairy calves—in Southern Ontario, and (3) dairy cattle in Southern Ontario may be a significant source of zoonotic transmission of *Cryptosporidium* within the region.

In Ontario, the incidence rates of human cryptosporidiosis have been consistently higher than the national average since 2005; however, the reason(s) for these higher rates are unknown, suggesting an incomplete understanding of the pathogen's ecology, epidemiology, and transmission pathways (Public Health Ontario, 2015). Standard laboratory diagnostic procedures are not able to distinguish the different species of *Cryptosporidium* and while zoonotic infections are considered sporadic, lack of routine molecular characterization of reported human and animal cases in Ontario makes it difficult to explore the zoonotic and possible zooanthroponotic origins of human and cattle infections (Di Giovanni *et al.*, 2006). Without species level information for infections of cryptosporidiosis, alternate methods for relating human, animal and environmental interactions involved in transmission of this pathogen need to be employed in order to determine effective public health prevention methods.

Spatial epidemiological analysis provides an alternate approach for applying the One Health study design to parasitic zoonotic diseases. Spatial epidemiology is the study of the geographic patterns of the distribution of disease or the determinants of disease in specified populations or areas. By using spatial location as the link between human, animal, and environmental interactions, regional variations in disease risk due to area-level and individual-level risk factors can be explored sufficiently (Lal, 2016). Thus, focusing on Southern Ontario,

this thesis will use the three fundamental spatial methods of visualization, exploration, and modelling to investigate the geographical distribution of human and bovine cryptosporidiosis and to investigate if there is a spatial association between reported human cases, cattle cases, and the density of calves, cattle and dairy cattle.

DATA AND INFORMATION SOURCES

Laboratory data on 1,737 bovine specimens tested for *Cryptosporidium* from January 1, 2011 to December 31, 2014 were received from the Animal Health Laboratory (AHL) at the University of Guelph. The laboratory dataset included, the number of specimens tested, the number of positive *Cryptosporidium* specimens, date of specimen submission, breed of animal(s), commodity description, the city and postal code of the servicing veterinarian's clinic, and where available, the postal code of the owner of the animal(s).

Surveillance data on 1,270 confirmed human cases of cryptosporidiosis reported from January 1, 2011 to December 31, 2014 were obtained from Public Health Ontario. Cryptosporidiosis is a reportable disease in Ontario. As such, each local Public Health Unit (PHU) in Ontario is required to collect information about individuals with reportable diseases in their jurisdictions and report them to the Ministry of Health and Long-Term Care's (MOHLTC) by recording the information in the Ministry-administered integrated Public Health Information System (iPHIS) (Public Health Ontario, 2017). The surveillance dataset obtained from PHO included episode date (earliest of symptom onset date, specimen collection or report date), episode date type, age group, gender, diagnosing PHU and information on travel history for each case.

Census data on population totals for all PHU areas in Southern Ontario from 2011-2014 were retrieved from Statistics Canada (Statistics Canada, 2017). Census data on cattle population and farm area for all PHU areas in Southern Ontario from 2011-2014 were obtained from the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) (Statistics Unit, OMAFRA, & Statistics Canada, 2017).

EPIDEMIOLOGICAL AND STATISTICAL METHODS

Spatial epidemiology is an increasingly popular sub-field of epidemiology that uses spatial analytic techniques and spatial data to understand the distribution of the determinants and outcomes of disease within a specified population and area (Elliott & Wartenberg, 2004; Kirby, Delmelle, & Eberth, 2017). For the most part, spatial epidemiology research studies are descriptive and ecological by nature, focusing inherently on formulating hypotheses about the etiology of a disease within a population (Rezaeian *et al.*, 2007; Shyti, Fetahu, & Fetahu, 2015). In order to use spatial analytic techniques, spatial data—which are any type of data that have an associated spatial reference such as street address, postal code, region or country—is needed (Elliott & Wartenberg, 2004; Rezaeian *et al.*, 2007). Overall, spatial health data can be broken down into two types: point and area data. Spatial point data are data in which there is an associated precise spatial position (street address or geographical coordinates) for each item of data (Rezaeian *et al.*, 2007; Waller & Gotway, 2004a), whereas spatial area data are data aggregated by geographic area (postal code, Public Health Unit, province/territory or country) (Rezaeian *et al.*, 2007). Spatial point data can usually be aggregated into spatial area data, but spatial area data usually cannot be disaggregated into spatial point data. However, in the context of health data, often spatial area data are all that is available due to privacy concerns, which in turn is the reason that ecological study designs are more prominent in this field. For this research study, only spatial area data were available, therefore only spatial analytic techniques that are used with spatial area data will be discussed.

The spatial analytical framework can be broken-down into three fundamental analytic methods: visualization, exploration, and modelling (Pfeiffer *et al.*, 2008a). Spatial visualization is the process of displaying spatial characteristics of data using maps (Pfeiffer *et al.*, 2008a;

Pfeiffer *et al.*, 2008b; Waller & Gotway, 2004b). This analytical method is often the first step in spatial analysis as it allows for (1) the initial observation of spatial patterns or apparent patterns within a dataset and (2) the generation of hypotheses regarding these patterns that require further analytic investigation (Pfeiffer *et al.*, 2008a). Spatial exploration, the second step in spatial analysis, is the process of using statistical methods to identify and determine whether a spatial pattern actually exists (Dale & Fortin, 2014; Pfeiffer *et al.*, 2008a). This step in the analysis seeks to answer the question: if we think we see a pattern, does that pattern exist or are the data simply randomly distributed in space (Pfeiffer *et al.*, 2008a). Finally, spatial modelling is an analytical approach to explore data for cause and effect relationships in observed spatial patterns (Pfeiffer *et al.*, 2008a). In this method, spatial models or statistical models and spatial data are used to explain or predict spatial patterns (Pfeiffer *et al.*, 2008a). All three of these methods were used in this research.

Spatial Visualization: Disease Mapping

The visualization of spatial data is often communicated through maps. In the context of spatial epidemiology, disease mapping is a powerful tool that involves summarizing determinants and outcomes of disease across a geographical area (Elliott & Wartenberg, 2004; Waller & Gotway, 2004b). Though disease mapping is used for a variety of descriptive purposes, disease mapping in this research study was used to display the distribution of the disease in the population of interest, identify and highlight areas of potentially high risk and generate hypotheses as to the transmission of the disease in the population. Depending on the type of spatial data available, different mapping techniques can be used. For this research project, choropleth and isopleth maps were used to display the incidence and prevalence of

cryptosporidiosis in the cattle and human populations of Southern Ontario. Clusters of cryptosporidiosis were identified and displayed on maps as well.

Choropleth maps are a very common type of thematic map that is used to display spatial area data (Pfeiffer *et al.*, 2008b; Waller & Gotway, 2004b). For these maps, pre-defined areal units (ex: Public Health Units within a province, counties within a state or provinces/territories within a country) are assigned specific colors and/or patterns based on a data variable, allowing for similarities and differences in a particular phenomenon to be displayed (Pfeiffer *et al.*, 2008b; Waller & Gotway, 2004b). There are two main types of choropleth maps: classed and unclassed (Waller & Gotway, 2004b). Classed choropleth maps were used throughout this research. In classed choropleth maps, the full range of data values is broken down into different non-overlapping intervals based on a specific classification method (Waller & Gotway, 2004b). Pre-defined areal units are then assigned specific colors or shades of a color, based on within which interval the data value for each area falls (Waller & Gotway, 2004b). There are many different classification methods that can be used to divide data values into intervals (equal intervals, quantile, natural breaks, geometrical interval, standard deviation etc...) (Brewer & Pickle, 2002; Waller & Gotway, 2004b). The method chosen depends on the data and the specific mapping objectives. The quantile classification method was used to produce all the choropleth maps for this research (Brewer & Pickle, 2002). This divides the data value range in a manner that ideally results in an equal number of areal units falling within each class (Brewer & Pickle, 2002; Waller & Gotway, 2004). Since this method is not dependent on a specific range of data values, it is useful for comparing multiple maps built from different underlying information (Brewer & Pickle, 2002). This classification method was used in this study to allow for the visual

comparison of initial spatial patterns across years in the study period and between populations (humans and cattle).

Though choropleth maps are a popular approach used in public health, there are several issues that need to be considered when using them to display disease estimates, specifically if the goal of mapping these estimates is to gain insight into the geographical variation of disease (Waller & Gotway, 2004b). The first issue is known as small numbers problem (Berke, 2001; Cromley & McLafferty, 2012; Waller & Gotway, 2004b). When creating choropleth maps of disease estimates, there is usually variation in the denominators (ex: population size, number of farms within the area etc...) between the different areas shown on the map (Cressie, 1992). This results in some disease estimates, i.e. those with larger denominators, being more precisely estimated than others (Cromley & McLafferty, 2012; Waller & Gotway, 2004b). In this case, for disease estimates with small denominators and based on small disease counts, each case of disease will result in a huge fluctuation in the estimate resulting in artificially elevated and unreliable estimates (Berke, 2001; Cromley & McLafferty, 2012; Waller & Gotway, 2004b). This problem can obscure spatial patterns. In order to effectively compare disease rates and risks across areal units of a choropleth map, it is important to adjust for the varying denominator sizes. Empirical Bayesian smoothing is a statistical methodology that is used to counteract this imprecision and stabilize disease estimates by producing a proportion that is a weighted average of both the raw regional and global rates or risks (Cressie, 1992; Cromley & McLafferty, 2012; Marshall, 1991). The formula for the method of moments Empirical Bayes estimator is as follows (Marshall, 1991) :

$$GBS(u_i) = w(u_i)z(u_i) + [1 - w(u_i)] m^* \quad i = 1, \dots, N$$

Where:

$u_i = 1, \dots, N$ is the index and number of regions

$z(u_i)$ = the raw regional disease rate/risk

m^* = the global disease rate/risk

$w(u_i)$ = the Bayes shrinkage factor

Depending on the size of the denominator used for disease estimation, regional disease estimates based on small denominators are adjusted towards the global estimates while regional disease estimates based on large denominators remain relatively unchanged (Cromley & McLafferty, 2012). For this research study, raw disease estimates were smoothed using this method.

Though disease rates and risks may be standardized using a technique such as smoothing, caution still needs to be taken when making inferences about the risk or relative risks of disease to the population based on choropleth maps. The aggregation of health data into areal units can result in the modifiable areal unit problem (MAUP) (Waller & Gotway, 2004b). The MAUP is the second issue associated with choropleth maps and occurs when aggregating data into different areal units results in different spatial patterns depending on which areas are aggregated together. Furthermore, aggregating health data into choropleth maps can result in visual bias due to the varying shape sizes of the areal units, with larger areal units on the map tending to draw the most focus, leading to biased visual perception of the true spatial pattern.

An isopleth risk map is another type of disease map that counteracts the common pitfalls of choropleth maps by displaying data across a continuous surface, allowing for the spatial variation in the risk of disease to be easily visualized (Berke, 2005; Goovaerts, 2006). In order to produce isopleth maps from area data, spatial interpolation methods must be applied.

Interpolation is the process of using quantitative observations at known points within a region to predict quantitative values for unknown points within a region, which results in the creation of a continuous surface from a set of known values. Kriging is a geostatistical method of interpolation that factors the covariance-distance relationships of values within a region into its model for prediction of values at unknown points (Berke, 2005). The general formula for the kriging predictor is as follows:

$$\hat{Z}(s_0) = \sum_{i=1}^n \lambda_i Z(s_i)$$

Where:

$Z(s_i)$ = the sample at the i th location

λ_i = the weight for the measured value at the i th location

s_0 = the prediction location

n = the number of sampling points

For kriging, the weights λ_i depend on the distance (and eventual direction) between the locations of observations at sampling points (or region centroids) and the prediction locations, as well as the spatial dependence structure. One of the main principles that underlines kriging, is Tobler's first law of geography which states: "everything is related to everything else, but near things are more related than distant things" (Tobler, 1970). Therefore, the weights are constructed based on the covariance-distance relationship, and regional risk estimates have more influence on predictions at sites that are closer in distance than ones that are farther away (Berke, 2005).

Kriging is based on a parametric spatial model that is specified by a spatial dependence function such as the semivariogram. The semivariogram model is generally a function of three parameters: the nugget, the sill and the range, and is defined as follows (Goovaerts, 1997) :

$$\gamma(h) = \frac{1}{2} \text{Var} (Z(s_i) - Z(s_j))$$

Where:

$Z(s_i)$ = the sample at the i th location

$Z(s_j)$ = the sample at the j th location

Var = the variance

h = the distance between any two sample points

A semivariogram provides a graphical representation of the spatial autocorrelation of the observations measured at the sample locations; the graph plots the semivariance against the separation distance between sample locations (Pfeiffer *et al.*, 2008c). Semivariance reflects the degree of the relationship between points. At a separation distance of 0, the semivariance is equal to 0; however, as the separation distance between points increases, the semivariance also increases (Tobler's law)—up until a certain critical separation distance. At this critical separation distance and any distances greater than that, the average difference between points is the same. This critical separation distance is known as the range (Pfeiffer *et al.*, 2008b). The range is the value of h for which there is no longer any spatial autocorrelation between sample locations. The sill is the value of $\gamma(h)$ at the range—it is the maximum semivariance in the data (Pfeiffer *et al.*,

2008c). Though the semivariance is 0 at a separation distance of 0 (i.e. $\gamma(0) = 0$), at very small separation distances the $\gamma(h)$ will have a value that is greater than 0. This value is known as the nugget and represents measurement error and outlier variance in the data (Pfeiffer *et al.*, 2008b).

Kriging is not an automatic process, it requires the use of exploratory spatial data analysis to understand the data and produce the best fit model for the underlying spatial dependence structure. There are three main types of kriging methods used: simple, ordinary, and universal. The method employed depends on assumptions about the trend structure in the data. For the isopleth maps developed in Chapter 2 and 3, an ordinary kriging method was used since exploratory analysis of the data showed no overall trend (Cressie, 1992). The semivariogram was fitted using maximum likelihood estimation. In order to not violate the assumption of variance homogeneity, Empirical Bayes smoothed disease estimates were used instead of raw estimates. There are different semivariogram models that can be used. In these studies, a spherical model with a nugget effect was used for the semivariogram since the test used for positive diagnosis of cryptosporidiosis is not 100% specific or sensitive, meaning that the estimates were likely to be impacted by some level of measurement error.

Spatial Exploration: Cluster Detection

The investigation of disease clusters is a key method of spatial exploration. A disease cluster is here defined as an unusually high number of cases that occur close together in space. There are several statistical tests that can be used to detect a cluster—the test chosen will depend on the type of spatial data analyzed and on the assumed shape of the cluster. The flexible spatial scan statistic was used to detect disease clusters in both Chapters 2 and 3. This statistic, developed by Tango and Takahashi, is a non-parametric spatial scan statistic (Tango and Takahashi, 2005). In contrast to other spatial scan statistics, the flexible scan test does not

assume that a disease cluster is circular; instead, this test allows for the detection of irregular-shaped clusters (Tango and Takahashi, 2005). Though the flexible spatial scan statistic detects irregular shaped clusters, it also detects small circular clusters if they exist. For the flexible scan test, irregular shaped scanning windows are formed for each region by connecting it with up to $k-1$ adjacent regions (Tango and Takahashi, 2005). The scanning window increases in size from 1 to the pre-set k number of adjacent regions (Tango and Takahashi, 2005). For the scanning window that maximizes the likelihood over all scanning windows, a likelihood ratio test is employed to decide whether the number of cases in the window is in excess compared to the number of cases outside the window (Tango and Takahashi, 2005). In the studies described here, the p-value for the scan statistic was estimated using Monte Carlo simulation to find the distribution of the test statistic. The flexible spatial scan statistics was used to detect purely spatial high-risk disease clusters.

Spatial Modelling: Poisson Regression

Regression models are analytic techniques that allow for the cause and effect relationship between a dependent (outcome) variable and an independent (exposure) variable(s) to be quantified. In terms of spatial modelling, regression models are used to understand how a set of (one or more) independent variables affect the spatial distribution of a dependent variable (Pfeiffer *et al.*, 2008d). There are several different types of regression models that can be used, the method chosen depends on the nature of the data, particularly the dependent variable. A Poisson regression model is a type of regression analysis used when the dependent variable is count data (for example: the count of disease events) (Dohoo *et al.*, 2012). The general form of the model is as follows (Dohoo *et al.*, 2012):

$$E(Y) = \mu = n\lambda$$

Where:

$E(Y)$ = the expected number of cases of disease

n = the size of the population at risk

λ = the function which defines the disease incidence rate

A key assumption underlying the Poisson model is that the mean and variance are equal. If the events used in the model (counts of disease) are not independent (meaning that the occurrence of one case affects the occurrence of another case), then this assumption would be violated (Dohoo *et al.*, 2012). When the variance is greater than the mean the situation is called overdispersion (Dohoo *et al.*, 2012) and might be the result of spatially dependent data. Overdispersion in a model is a problem since it can lead to false significant results, meaning that the variance and standard error estimates from the model are not sound (Dohoo *et al.*, 2012). There are numerous causes of overdispersion, including spatial autocorrelation among observations. The amount of overdispersion in a model can be approximated by dividing the residual deviance χ^2 by the number of degrees of freedom (df) to give a dispersion parameter (Dohoo *et al.*, 2012). If the dispersion parameter is much greater than 1, this is an indication that there is overdispersion that needs to be accounted for in the model in order to allow for accurate variance estimates and p-values (Dohoo *et al.*, 2012).

Spatial correlation in observations (disease events/counts) can be accommodated in various ways. For this research study, spatial correlation was accounted for using a multilevel modelling approach—in multilevel modelling, both the fixed effects (predictor variables) and

random effects (correlation among observations) are modelled. In these studies, the spatial covariance structure for the random effects model was specified by a spherical continuous distance decay model. The spherical correlation structure forms a decay function in which observations farther away in space are less related than those that are closer in space—in agreement with Tobler’s “first law of geography” (Tobler, 1970). The semivariogram was used to determine the appropriate spatial covariance structure.

OBJECTIVES

The overall aim of this project was to investigate and identify spatial patterns and relationships of human and bovine cryptosporidiosis across the 29 Public Health Unit (PHU) areas in Southern Ontario using core spatial analytic methods. The first study, which used laboratory diagnostic data from the Animal Health Laboratory at the University of Guelph, examined the spatial distribution of bovine cryptosporidiosis in Southern Ontario from 2011 to 2014. The second study used surveillance data from Public Health Ontario, findings from the first study, and data from Ontario Ministry of Agriculture, Food and Rural Affairs to describe the spatial distribution of human cryptosporidiosis in Southern Ontario from 2011 to 2014 and investigate the relationship between the spatial distribution of human cryptosporidiosis and bovine cryptosporidiosis, cattle density, calf density and dairy cattle density.

The specific objectives of these studies were as follows:

- (1) To map the overall farm-level prevalence of bovine cryptosporidiosis in Southern Ontario from 2011 to 2014 and identify areas of high-risk for bovine cryptosporidiosis. This objective is addressed in Chapter 2.
- (2) To map and compare the geographical risk distribution of human cryptosporidiosis in Southern Ontario from 2011 to 2014 and identify areas of high risk for human cryptosporidiosis. This objective is addressed in Chapter 3.
- (3) To investigate the relationship between the spatial distribution of the incidence of human cryptosporidiosis and the farm-level prevalence of bovine cryptosporidiosis as well as the density of calves, dairy calves and cattle, using Poisson and spatial Poisson regression models. This objective is addressed in Chapter 3.

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CHAPTER TWO:

Exploring the geographical distribution of cryptosporidiosis in the cattle population of Southern Ontario from 2011-2014.

Abstract

Cryptosporidiosis is an infectious disease of relevance to the cattle industry. In Ontario, cattle farming operations densely populate the southern region of the province. Given Ontario's key role in the Canadian dairy industry and the potential impact that cryptosporidiosis can have on Ontario cattle operations, identifying areas of increased risk for bovine cryptosporidiosis is essential for initiating targeted prevention and control measures. The primary goal of this study was to use spatial analytics to explore the distribution of bovine cryptosporidiosis from 2011-2014, across the geographical areas served by the 29 Public Health Units of Southern Ontario.

Laboratory data on bovine cryptosporidiosis were collected from the Animal Health Laboratory at the University of Guelph. Using veterinary clinic location as a proxy for farm location, choropleth and isopleth maps were produced. High risk clusters of bovine cryptosporidiosis were investigated using the flexible spatial scan test. Assessment of the potential for spatial misclassification bias resulting from the use of veterinary clinic location as a proxy for farm location was conducted.

The overall raw farm-level prevalence of bovine cryptosporidiosis was 45% [95% CI: 42%-48%] (i.e 45% of 1235 farms had at least one specimen test positive for *Cryptosporidium*). A significant cluster was identified in the Central West region of Southern Ontario (relative risk 1.30 [95%CI: 1.07-1.54, p-value = 0.026]) meaning that cattle in Bruce-Grey-Owen Sound, Huron, Wellington-Dufferin Guelph, and Waterloo PHU areas were at a higher risk for infection.

Given that this area is known for their high dairy cattle density, the Central West region of Southern Ontario should be considered a target for further surveillance.

Keywords: Cryptosporidiosis, Southern Ontario, Cattle, Spatial epidemiology, Flexible scan test

INTRODUCTION

Cattle farming operations densely populate the southern region of Ontario: 97% of all Ontario dairy farms are located in Southern Ontario, a region that represents only 15% of the provincial land mass area (Bishop-Williams *et al.*, 2016; Environmental Management Branch, 2016; Statistics Canada, 2014; Statistics Canada and Canadian Dairy Commission, 2016). Geographical areas with a high density of livestock, such as Southern Ontario, are often at an increased risk for the spread of infectious diseases amongst animals (Buhnerkempe *et al.*, 2014). On a broader scale, the occurrence of zoonotic infectious diseases within these areas pose an additional risk to both human health and the environment, especially where there is also a high human population density such as that in Southern Ontario. Thus, identifying areas at risk for infectious diseases is important for targeted control and prevention of these diseases in human and animal populations.

Cryptosporidium is a ubiquitous protozoan parasite and the causal agent of cryptosporidiosis, a potentially lethal diarrheal disease that is known to affect cattle and a wide range of hosts (Abeywardena *et al.*, 2015; O'Donoghue, 1995). Of the 26 species of *Cryptosporidium* currently identified, cattle are the primary hosts of *C. andersoni*, *C. bovis*, *C. ryanae* and *C. parvum*. Epidemiologically, *C. parvum* is often regarded as the most important species because of its primary role in the occurrence of cryptosporidiosis in young calves and in zoonotic infections in humans (Fayer, *et al.*, 2008; Peter *et al.*, 2015). Infections in cattle occur through the transmission of oocysts by the fecal-oral route, either directly from host-to-host contact or indirectly through contaminated environments including food and water supplies (O'Donoghue, 1995; Peter *et al.*, 2015). *Cryptosporidium* predominantly affects pre- to post-weaned dairy calves and is a primary cause of neonatal diarrhea (Abeywardena *et al.*, 2015;

O'Donoghue, 1995; Sivajothi *et al.*, 2014). Though infections do occur in adult cattle, they are often mild or asymptomatic (Abeywardena *et al.*, 2015; O'Donoghue, 1995; Sivajothi *et al.*, 2014). Clinical signs of cryptosporidiosis in cattle can vary from acute to chronic diarrhea, dehydration, fever, anorexia, weight loss, depression, dullness, and bloating (Abeywardena *et al.*, 2015). In some cases, complications from cryptosporidiosis can result in mortality (Ouchene *et al.*, 2016). Currently, there is no effective treatment available for cryptosporidiosis and prevention remains a critical intervention in the management of this calf disease (Peter *et al.*, 2015).

Several studies on the prevalence of *Cryptosporidium* in Canadian beef and dairy cattle have been conducted in British Columbia, Manitoba, Ontario, Québec and Prince Edward Island (Coklin *et al.*, 2009; Mann *et al.*, 1986; Olson *et al.*, 1997; Ruest *et al.*, 1998; Trotz-Williams *et al.*, 2005a; Trotz-Williams *et al.*, 2007). Farm-level and within-farm (calf-level) prevalence estimates have been reported to range from 36.4% to 88.7% and 6.2% to 64%, respectively, suggesting that *Cryptosporidium*, mostly *C. parvum* (Trotz-Williams *et al.*, 2006) is quite ubiquitous in Canadian cattle herds (Coklin *et al.*, 2009; Mann *et al.*, 1986; Olson *et al.*, 1997; Ruest *et al.*, 1998; Trotz-Williams *et al.*, 2005a). In fact, in a study conducted by Trotz-Williams *et al.* (2005a), using 500 dairy calves on 51 farms in Southwestern Ontario, the farm and overall calf-level prevalence of *Cryptosporidium* were 76% and 40.6%, respectively, confirming that this parasite is quite common in the densely livestock-populated area of Ontario (Trotz-Williams *et al.*, 2005a). Given the key role that Ontario plays in the Canadian dairy industry, the potential impact that cryptosporidiosis can have on Ontario cattle operations, and the public health risk posed by zoonotic transmission of this parasite, identifying areas at increased risk for bovine cryptosporidiosis is essential.

The goal of this study was to explore the spatial distribution of bovine cryptosporidiosis from 2011 to 2014, using laboratory diagnostic records from the University of Guelph's Animal Health Laboratory (AHL), across the areas served by the 29 local public health units (PHUs) of Southern Ontario. The specific objectives were to (1) map the geographical risk distribution of bovine cryptosporidiosis, (2) to identify areas at high risk for bovine cryptosporidiosis, if any, and (3) to estimate spatial misclassification bias associated with using servicing veterinarian addresses as a proxy for farm locations.

MATERIALS AND METHODS

Study Area

Southern Ontario [Figure 2.1] is the primary and most densely populated part of Canada with respect to cattle farming, with an estimated cattle population of 1,622,949 in 2014 (Statistics Unit and Statistics Canada, 2015). Located south of Algonquin Park, and stretching approximately 600km in the north to south and east to west direction, Southern Ontario houses approximately 89% and 94% of Ontario's human and bovine populations, respectively, in only 15% of the provincial land mass area (Bishop-Williams *et al.*, 2016). The risk of bovine cryptosporidiosis over the years 2011-2014 was investigated for the 29 PHU areas of Southern Ontario.

Data Collection

Diagnostic laboratory data on 1,737 bovine specimens (from 1,235 farms in Ontario—both dairy and beef) tested for *Cryptosporidium* from January 1, 2011 to December 31, 2014,

were obtained from the Animal Health Laboratory (AHL) at the University of Guelph. The specimens were from symptomatic cattle. The laboratory dataset included variables indicating the number of samples tested per farm (range 1-5), number of positive *Cryptosporidium* specimens, date of submission, breed, commodity description, the servicing veterinarian's clinic city and postal code, and, where available, the farm owner's postal code. No street addresses were available in the dataset.

The AHL uses standard sucrose wet mount testing for ante mortem diagnosis of *Cryptosporidium*. This is a diagnostic test that identifies the presence of *Cryptosporidium* but does not provide species identification of the parasite. This diagnostic method involves concentrating a fecal sample with a Sheather's solution prior to detecting the presence of oocysts in the prepared sample by microscopy (Trotz-Williams *et al.*, 2005b). The sensitivity and specificity of this diagnostic method have been reported to be 88.6% [95% CI: 80.1%-94.4%] and 93.8% [95%CI: 86.0%-97.9%], respectively (Trotz-Williams *et al.*, 2005b).

Estimation of Prevalence

Case-level data (specimens tested) were aggregated to the farm-level using submission ID, which was unique to each farm from which specimens were submitted to the AHL. Farms tested for bovine cryptosporidiosis were assigned and aggregated to their respective PHU area based on the servicing veterinarian's clinic postal code since the postal code of farms were only available for a small proportion, 30% (n=360), of all farms tested in the study area over the study period (n=1207). The 2015 postal code conversion file (PCCF, a digital file that links all valid Canadian postal codes to Statistics Canada's 2011 standard Census geographic areas) was obtained from Statistics Canada (Statistics Canada, 2016). Using R version 3.2.3, the laboratory

dataset was joined with the PCCF in order to obtain the corresponding geographical coordinates for the centroid of the veterinarian’s postal code. Since the PCCF can contain multiple records for a single postal code, the single link indicator (SLI) was used to create a one-to-one relationship (R Core Team, 2016; RStudio Team, 2016). The single link indicator identifies the geographic area with the majority of dwellings. Using the geographical coordinates, a point map was created to determine from which PHU area each farm specimen was submitted.

For each PHU area, the true raw period farm-level prevalence of bovine cryptosporidiosis (i.e., the percentage of tested farms in each PHU area with at least one positive *Cryptosporidium* test) was determined for the four-year study period. The term “true” prevalence refers here to estimates adjusted for diagnostic sensitivity and specificity. First, the overall apparent (unadjusted) farm-level disease prevalence over the four-year study period was estimated for each area from the total number of positive farms divided by the total number of tested farms in that PHU area. Using the known sensitivity and specificity of the diagnostic test, the true farm-level prevalence of bovine cryptosporidiosis within each PHU area was then estimated. These adjusted (true) prevalence estimates were used for all subsequent analyses and are hereafter referred to as *prevalence* or *farm-level prevalence*. The general formula used was (Dohoo, Martin, and Stryhn, 2012):

$$TP = \frac{AP + Sp - 1}{Se + Sp - 1}$$

Where:

TP = the estimated true prevalence

AP = the apparent prevalence

Sp = Specificity of the diagnostic test

Se = Sensitivity of the diagnostic test

Approximate and exact confidence intervals were estimated for each prevalence estimate. For PHU areas with sample sizes ≤ 200 tested farms, exact confidence intervals (CIs) adjusted for the sensitivity and specificity were estimated using Blaker's method (Reiczigel, Foldi, and Ozsvari, 2010). For areas with sample sizes ≥ 200 , the approximate CIs adjusted for sensitivity and specificity were estimated using the normal approximation method (Rogan and Gladen, 1978).

Using R package *spdep*, the raw farm-level prevalence for each PHU area was smoothed using empirical Bayesian smoothing in order to standardize the estimates for spatial and regional variation in the sample size (Berke, 2001; Bivand *et al.*, 2013; Hampton *et al.*, 2011). The general formula is as follows (Marshall, 1991):

$$GBS(u_i) = w(u_i)z(u_i) + [1 - w(u_i)] m^* \quad i = 1, \dots, N$$

Where:

$u_i = 1, \dots, N$ is the index and number of regions

$z(u_i)$ = the raw prevalence

m^* = the global prevalence

$w(u_i)$ = the Bayes shrinkage factor

For the smoothing, it was assumed that the farm-level prevalence of bovine cryptosporidiosis followed a binomial distribution. Parallel boxplots were used to show the shrinkage effect that resulted from smoothing the raw prevalence estimates.

Mapping and Spatial Analysis

Choropleth and Isopleth Mapping

Using R package *maptools*, the raw and smoothed farm-level prevalence estimates were visualized by creating choropleth maps which were used as an initial description of the spatial variation of bovine cryptosporidiosis across Southern Ontario (Bivand and Lewin-Koh, 2013). Though choropleth maps are useful for initial visualization of the spatial variation in the risk, issues of visual bias created by the aggregation of data into administrative regions of varying sizes makes it difficult to effectively interpret the spatial pattern (Berke, 2001; Goovaerts, 2006). Therefore, isopleth risk maps for the overall study period, based on Bayesian adjusted prevalence estimates, were also produced using R package *geoR* in R v3.2.4 (Ribeiro *et al.*, 2001). The geostatistical prediction method of kriging, a method that allows for the interpolation of regional data onto a continuous surface was used to create the isopleth maps (Berke, 2005). The general formula is as follows:

$$\hat{Z}(s_0) = \sum_{i=1}^n \lambda_i Z(s_i)$$

Where:

$Z(s_i)$ = the sample value at the i th location

λ_i = the weight for the measured value at the i th location

s_0 = the prediction location

N = the number of measured values

For kriging, the weights λ_i depend on the distance (and eventual direction) between the observations at sampling points (or region centroids), and the prediction locations, as well as the spatial dependence structure. These weights are constructed so that regional risk estimates have more influence on predictions at sites that are closer in distance and any clusters of points that contain redundant information are downplayed (Berke, 2005). Kriging is based on a parametric spatial model that is specified by a spatial dependence function such as the semivariogram (Berke, 2005). In this study, the semivariogram was estimated by maximum likelihood estimation.

Cluster Detection

The flexible spatial scan statistic was used to detect high-risk clusters of bovine cryptosporidiosis for the overall study period (Tango and Takahashi, 2005). For the purposes of this study, a disease cluster is defined as an unusually high number of cases that occur close together in space. The flexible scan statistic detects purely spatial disease clusters of irregular and circular shapes, using a likelihood ratio. Irregular shaped scanning windows are formed for each region by connecting that region with up to $k-1$ adjacent regions. The scanning window increases in size from 1 to the pre-set k number of adjacent regions including the region in question. For the scanning window that maximizes the likelihood over all scanning windows, a likelihood ratio test is employed to decide whether the number of cases in the window is in excess compared to the number of cases outside the window. In this study, the null hypothesis was that the risk of bovine cryptosporidiosis within the window was the same as outside the window. Since the flexible spatial scan test was used to detect hot-spots of disease clusters, the alternative hypothesis was that the risk of bovine cryptosporidiosis was higher within the

window compared to outside the window (i.e. a one-sided test). Locations of detected clusters were indicated on a choropleth map.

R v3.2.4 package *smerc* was used to conduct the spatial scan test (French, 2015). For the scan test, a Bernoulli model was used with the raw prevalence estimates. A significance level $\alpha = 0.05$ was chosen and 999 Monte Carlo simulations was used to estimate the p-value. The pre-set maximum cluster size of $k=5$ regions was selected as an alternative to the default setting of 10; given that the number of PHUs in Southern Ontario is small ($n=29$), a k number of 10 might cover more than 50% of the total population size (i.e total number of farms). All statistical analyses were conducted in R v.3.2.4 and RStudio v1.0.44 (R Core Team, 2016; RStudio Team, 2016).

Assessment of Spatial Misclassification

An assessment of the potential for spatial misclassification resulting from the use of veterinary clinic location as a proxy for farm location was conducted. For the subset of tested farms that included both the veterinarian and farm location, farms were assigned and aggregated to their respective PHU area based on the farm's postal code. For each PHU area, the proportion of farms that were located in the same or adjacent PHU area were determined and compared, as a measure of misclassification, to the proportions assigned based on clinic location.

RESULTS

Twenty-eight of the 1235 tested farms were removed from the study because their related address was from outside the defined study area. The overall raw farm-level prevalence of bovine cryptosporidiosis in Southern Ontario was estimated to be 45% [95%CI: 42%-48%]; 541 of the 1207 farms included in the study tested positive. The raw farm-level prevalences of the 29

PHU areas ranged from 0% [95%CI: 0-8] to 62% [95%CI: 35%-87%], with the highest raw prevalence estimates being found in the areas served by Chatham-Kent and Peterborough County PHUs and the lowest in those served by Hamilton-Wentworth and Middlesex-London PHUs [Table 2.1]. No samples had been submitted for testing from farms in four PHU areas: Halton, Niagara, Windsor-Essex and the City of Toronto.

The smoothed farm-level prevalence showed less variability, ranging from 38% to 53% [Table 2.1]. The highest smoothed prevalence estimates were observed in the Ottawa-Carleton and Waterloo PHU areas, while Simcoe-Muskoka and Kingston-Frontenac-Lennox-Addington PHU areas had the lowest smoothed prevalences. The shrinkage effect on the regional prevalence estimates can be seen in the parallel boxplot shown in Figure 2.2: the extreme high and low prevalences shrink towards the distribution center [Figure 2.2].

Based on the choropleth maps for the raw and smoothed prevalences, PHU areas with higher farm-level prevalence of bovine cryptosporidiosis were predominately located in the Central West, Southeast and East regions of Southern Ontario [Figures 2.3 and 2.4]. Two areas of potential increased risk for bovine cryptosporidiosis were also visually identified in the isopleth map [Figure 2.5]. Two of the areas spanned the Central West and Southeast regions while the third potential high-risk area was located in the East region of Southern Ontario; these areas overlapped PHU areas that had higher farm-level prevalence estimates.

One statistically significant high-risk cluster of cryptosporidiosis was identified; this was in the Central West region of Southern Ontario; the relative risk of this cluster was 1.30 [95%CI: 1.07-1.54, p-value = 0.026]. The PHU areas of Bruce-Grey-Owen Sound, Wellington-Dufferin Guelph, Huron and Waterloo were within the cluster, suggesting that the cattle population in these areas were at a higher risk for infection [Figure 2.6].

Three hundred and sixty of the 1207 farms located in all of the 29 PHU areas were used for the spatial misclassification analysis. Of these 360 farms, 54% of clinic locations were within the same PHU area as the cattle owner while 27% of the clinics were within an adjacent PHU area to that of the cattle owner. The remaining 19% of clinic locations were located in PHU areas that were neither adjacent nor within the same PHU area as the cattle owner. In 5 of the 29 PHU areas (Hastings-Prince Edward, Kingston-Frontenac-Lennox-Addington, Lambton, Peterborough and Renfrew), all cattle farms were located within the same PHU area as the clinics from which sample had been submitted for testing. In 4 areas, York, Haliburton-Kawartha-Pine Ridge, Middlesex-London, and Niagara, none of their farms were located within the same PHU areas as the veterinarians that submitted the samples for testing. Overall, in 16 PHU areas, $\geq 50\%$ of the farms were located within the same PHU area as the submitting veterinarian [Table 2.2].

DISCUSSION

The primary goal of this study was to describe and visualize the distribution of bovine cryptosporidiosis at the farm-level in the areas served by the 29 PHUs of Southern Ontario using spatial epidemiology methods. Results from the analysis indicate that bovine cryptosporidiosis is not only highly prevalent in Southern Ontario cattle populations but also quite widespread. Overall, farms positive for bovine cryptosporidiosis were found in all but six PHU areas, and the raw farm prevalence for Southern Ontario was estimated to be 45%, with PHU area-specific prevalence estimates ranging from 0% to 62% of farms. Given the large number of cattle farms concentrated in Southern Ontario and how common calf diarrhea is on cattle farms, the detection of cryptosporidiosis amongst the cattle population in this area is not surprising (Trotz-Williams *et al.*, 2005a).

A high-risk cluster of bovine cryptosporidiosis (RR 1.30, p-value=0.026) was identified in the Central West region of Southern Ontario. The cluster encompassed the PHU areas of Bruce-Grey-Owen Sound, Wellington-Dufferin Guelph, Huron and Waterloo, indicating that cattle within these areas are at a higher risk for bovine cryptosporidiosis than would be expected compared to the risk outside of the cluster. The Central West region of Southern Ontario is known for having a high density of dairy cattle, which may potentially explain the higher bovine cryptosporidiosis risk seen in the cattle population (Statistics Unit, OMAFRA, & Statistics Canada, 2017). Cryptosporidiosis predominantly affects dairy calves, causing severe diarrhea that can lead to death (Abeywardena *et al.*, 2015; O'Donoghue, 1995). Given the associated production loss that can result from this disease, infection, prevention and control strategies to reduce bovine cryptosporidiosis need to be targeted to this area.

This study was subject to some limitations due to the data source used for analysis and the process for spatial aggregation. The prevalence of bovine cryptosporidiosis for Southern Ontario was determined exclusively using the laboratory data from the AHL at the University of Guelph. For this reason, there could possibly have been a bias in the estimated prevalences due to difference in veterinary testing practices. Some veterinarians may test more actively or conduct this test in-house compared to other veterinarians meaning that the resulting prevalence estimates from this source of diagnostic data may reflect veterinarian testing practices and not the actual geographical distribution of bovine cryptosporidiosis in Southern Ontario. As well, in Southern Ontario, the AHL is not the only facility capable of providing diagnostic testing for cryptosporidiosis and proximity may play a role in where veterinarians decided to submit their samples for testing. Thus, the estimated prevalences may potentially be biased towards veterinary clinics in public health unit areas that are in closer proximity to the AHL.

Furthermore, since in most instances it is highly uncommon for cattle diarrhea cases to result in diagnostic testing; submissions to the AHL for diagnostic testing usually occurs because of uncommon signs, multiple calves in a herd presenting with diarrhea, or herd outbreaks. Due to this limitation, the estimated prevalences may only reflect severe and unusual presentations of bovine cryptosporidiosis and not the prevalence of bovine cryptosporidiosis in the general cattle farm population of Southern Ontario. It is likely that these estimates underestimate the true burden of cryptosporidiosis in the cattle farm population of Southern Ontario.

Another study limitation involved the method used to aggregate specimens of bovine cryptosporidiosis into PHU areas. The postal codes of the veterinary clinics which had submitted the specimens were used, as a proxy for farm location, to geographically aggregate the data. Since postal codes can span multiple PHU areas, a single link indicator provided by Statistics Canada's PCCF was used to create a one-to-one relationship between the postal code and a PHU. The actual street addresses of the clinics were not available; therefore, it was not possible to ascertain that all the clinics (and thus the farms) were aggregated to the correct PHU areas. Based on the misclassification assessment between owner and veterinarian location, using location of veterinary clinic as a proxy for the assumed location of exposure (farm location) may have resulted in approximate 46% of the farms being misclassified. However, this misclassification is likely to be non-differential since farms were not more likely to be misclassified based on disease status. Non-differential misclassification blurs the true spatial pattern of the disease making it more difficult to identify a cluster. Therefore, since a cluster was identified in this study, it is likely that the risk in the population in the identified cluster is higher than what was estimated.

Though the study was subject to some limitations it is important to note that first, using laboratory diagnostic data, this study provided a raw estimate of the current burden of bovine cryptosporidiosis in Southern Ontario cattle farm population. Second, this is the first study to describe the spatial distribution of bovine cryptosporidiosis amongst the Southern Ontario cattle farm population. Finally, a high-risk cluster of bovine cryptosporidiosis was identified in the Central West region of Southern Ontario—an area known for having a high density of dairy cattle (Statistics Unit, OMAFRA, & Statistics Canada, 2017). Given the known association between neonatal calf diarrhea and *Cryptosporidium*, and the impact this parasite can have on Ontario dairy operations, further studies that seek to understand the transmission patterns of this disease within and between the animal and human populations of this area, and the potential epidemiological factors that contribute to these patterns, are necessary.

CONCLUSION

Cryptosporidiosis is an infectious disease of relevance to the cattle industry because it has the potential to result in economic and production loss directly through cattle mortality and indirectly through decreased milk production, increased susceptibility to other diseases, treatment cost and poor growth (Peter *et al.*, 2015; Senturk *et al.*, 2016). In addition, there is a known risk of zoonotic transmission of *Cryptosporidium* in cattle to humans, with a resulting impact on public health (Trotz-Williams *et al.*, 2006). Overall, the results from this study demonstrate that *Cryptosporidium* is quite prevalent on cattle farms across Southern Ontario. Given the relationship between high livestock density and infectious diseases in cattle, and the implications of this relationship on both human health and the environment, further targeted

surveillance studies should be conducted to understand the distribution and transmission of this disease among animals on Southern Ontario cattle farms.

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TABLES**Table 2.1-** Raw and smoothed (empirical Bayesian) true¹ farm-level prevalence estimates of bovine cryptosporidiosis for the 29 Public Health Unit areas of Southern Ontario from 2011-2014

Public Health Unit Area		Prevalence in %		Farms
Number	Name	Raw [95% CI]	Bayesian	Number tested
Mid North Region				
3557	Renfrew County and District	30 [15-51]	40	29
Southwest Region				
3531	Elgin-St Thomas	46 [14-79]	45	9
3540	Chatham-Kent	62 [18-90]	47	7
3542	Lambton County	44 [9-82]	45	7
3544	Middlesex-London	0 [0-8]	44	2
3552	Oxford County	46 [40-52]	46	228
3568	Windsor-Essex County	N/A	N/A	0
Central-West Region				
3533	Bruce-Grey-Owen Sound	39 [27-52]	42	62
3539	Huron County	57 [42-70]	50	53
3554	Perth District	48 [31-66]	46	35
3565	Waterloo Region	56 [47-64]	53	147
3566	Wellington-Dufferin-Guelph	47 [39-55]	47	164
South-Central Region				
3530	Durham Region	50 [30-74]	46	21
3536	Halton Region	N/A	N/A	0
3553	Peel Region	35 [15-43]	40	43
3560	Simcoe Muskoka District	24 [9-42]	38	27
3570	York Region	30 [18-47]	39	42
3595	City of Toronto	N/A	N/A	0
Niagara Peninsula				
3527	Brant County	27 [4-71]	43	7
3534	Haldimand-Norfolk	43 [30-59]	44	50
3537	Hamilton-Wentworth	0 [0-7]	43	3
3546	Niagara Region	N/A	N/A	0
Southeast Region				
3535	Haliburton-Kawartha-Pine Ridge	41 [5-85]	45	5
3538	Hastings and Prince Edward Counties	44 [29-62]	44	38
3541	Kingston-Frontenac-Lennox and Addington	24 [9-42]	38	27
3555	Peterborough County	62 [35-87]	48	14
East Region				
3543	Leeds-Grenville-Lanark District	44 [32-55]	44	76
3551	Ottawa Carleton	60 [44-74]	51	45
3558	Eastern Ontario	35 [24-48]	40	66

¹Prevalence estimate adjusted for sensitive and specificity of diagnostic test

Table 2.2 – Proportion of veterinary clinics located in the same or adjacent Public Health Unit area as the farm owner, Southern Ontario from 2011-2014

Owner's Public Health Unit Area		Proportion of veterinary postal codes (%)		Number of Farms
Number	Name	Within same PHU	Within an adjacent PHU	
Mid North Region				
3557	Renfrew County and District	100	0	5
Southwest Region				
3531	Elgin-St Thomas	67	0	3
3540	Chatham-Kent	N/A	N/A	N/A
3542	Lambton County	100	0	3
3544	Middlesex-London	0	21	57
3568	Windsor-Essex County	N/A	N/A	N/A
Central-West Region				
3533	Bruce-Grey-Owen Sound	80	13	15
3539	Huron County	67	11	9
3552	Oxford County	33	11	18
3554	Perth District	55	45	10
3565	Waterloo Region	60	40	15
3566	Wellington-Dufferin-Guelph	70	29	91
South-Central Region				
3530	Durham Region	67	0	6
3536	Halton Region	N/A	N/A	N/A
3553	Peel Region	63	38	8
3560	Simcoe Muskoka District	71	30	8
3570	York Region	0	0	1
3595	City of Toronto	N/A	N/A	N/A
Niagara Peninsula				
3527	Brant County	30	30	10
3534	Haldimand-Norfolk	43	43	7
3537	Hamilton-Wentworth	17	83	6
3546	Niagara Region	0	100	2
Southeast Region				
3535	Haliburton-Kawartha-Pine Ridge	0	33	3
3538	Hastings and Prince Edward Counties	100	0	1
	Kingston-Frontenac-Lennox and		0	2
3541	Addington	100		
3555	Peterborough County	100	0	1
East Region				
3543	Leeds-Grenville-Lanark District	91	9	22
3551	Ottawa Carleton	19	81	22
3558	Eastern Ontario	91	9	35

FIGURES

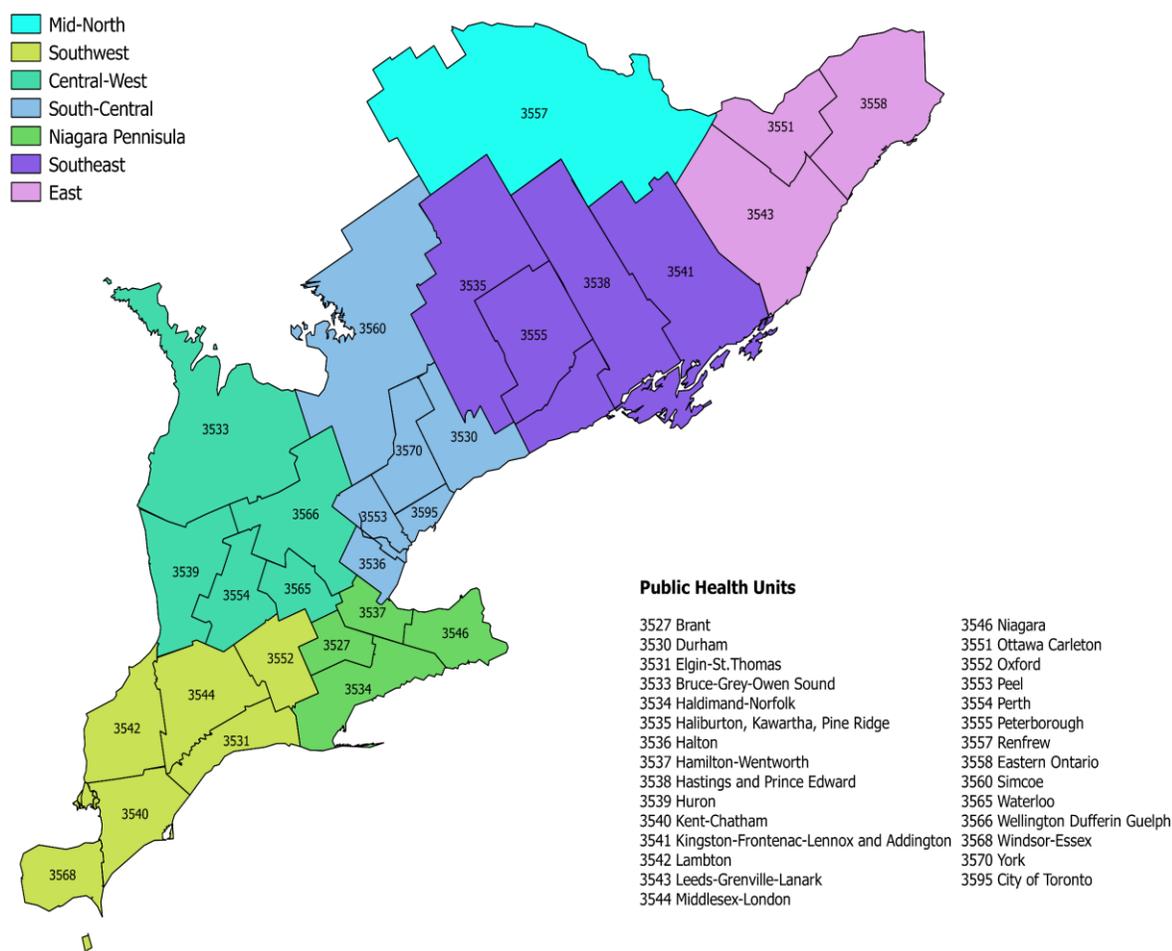


Figure 2.1- Map of the regions of Southern Ontario and areas within those regions served by individual Public Health Units in Southern Ontario (Ontario Ministry of Government and Consumer Services)

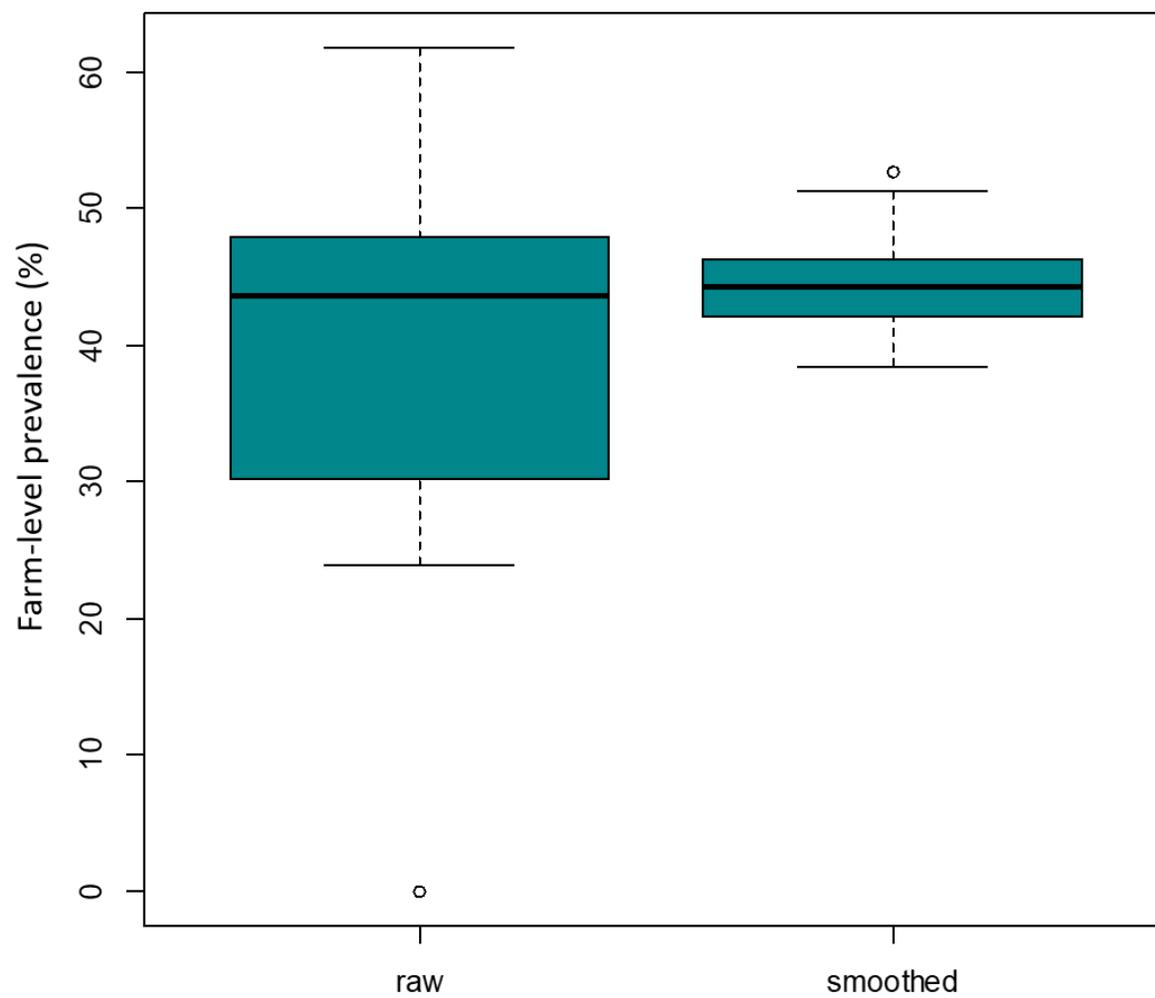


Figure 2.2- Parallel boxplot of raw and Empirical Bayesian smoothed farm-level prevalence of bovine cryptosporidiosis in Southern Ontario from 2011-2014

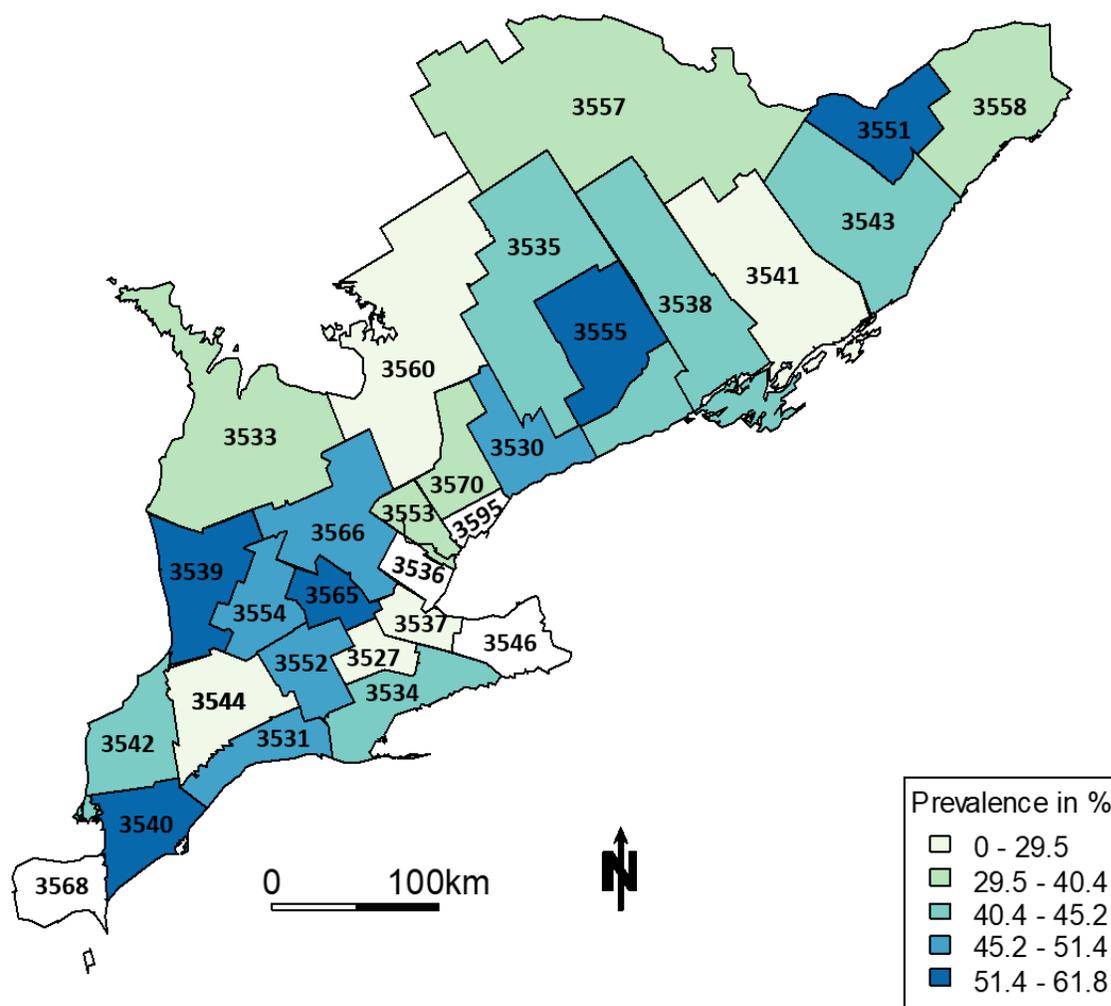


Figure 2.3- Choropleth map of the spatial distribution of raw farm-level prevalence of bovine cryptosporidiosis in 29 Public Health Unit areas of Southern Ontario from 2011-2014

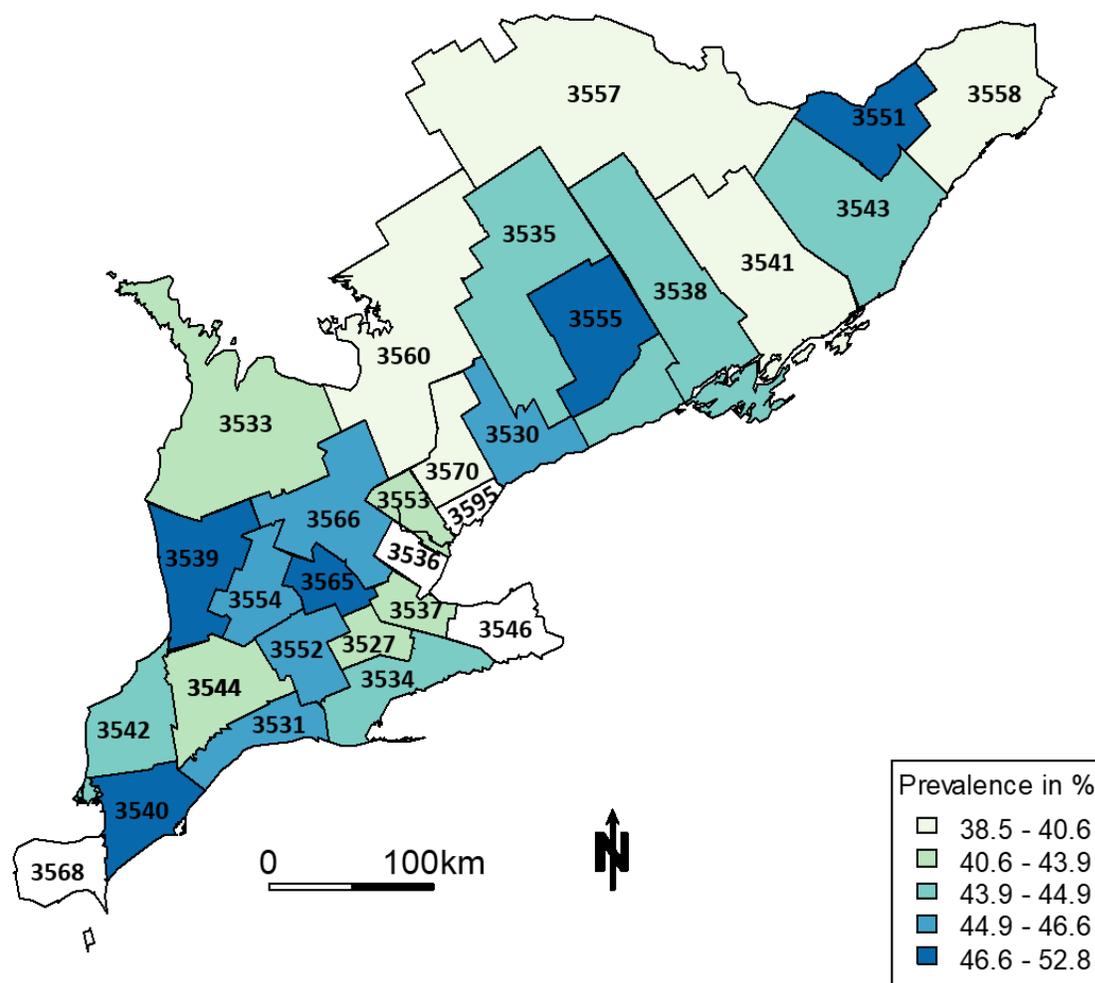


Figure 2.4- Choropleth map of the spatial distribution of smoothed farm-level prevalence of bovine cryptosporidiosis in 29 Public Health Unit areas of Southern Ontario from 2011-2014

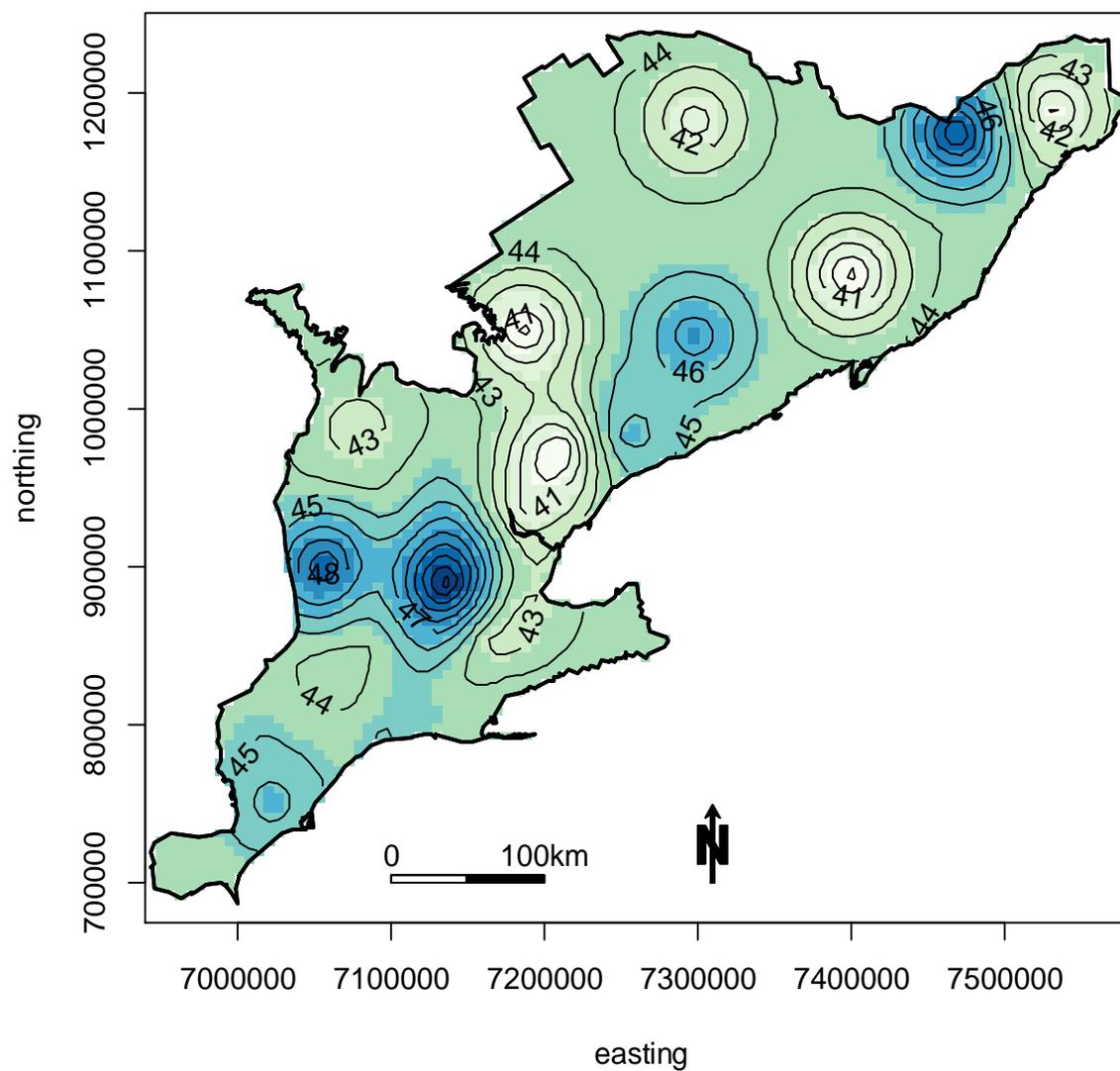


Figure 2.5 - Isopleth map of the latent risk distribution of cryptosporidiosis among cattle of Southern Ontario from 2011-2014.

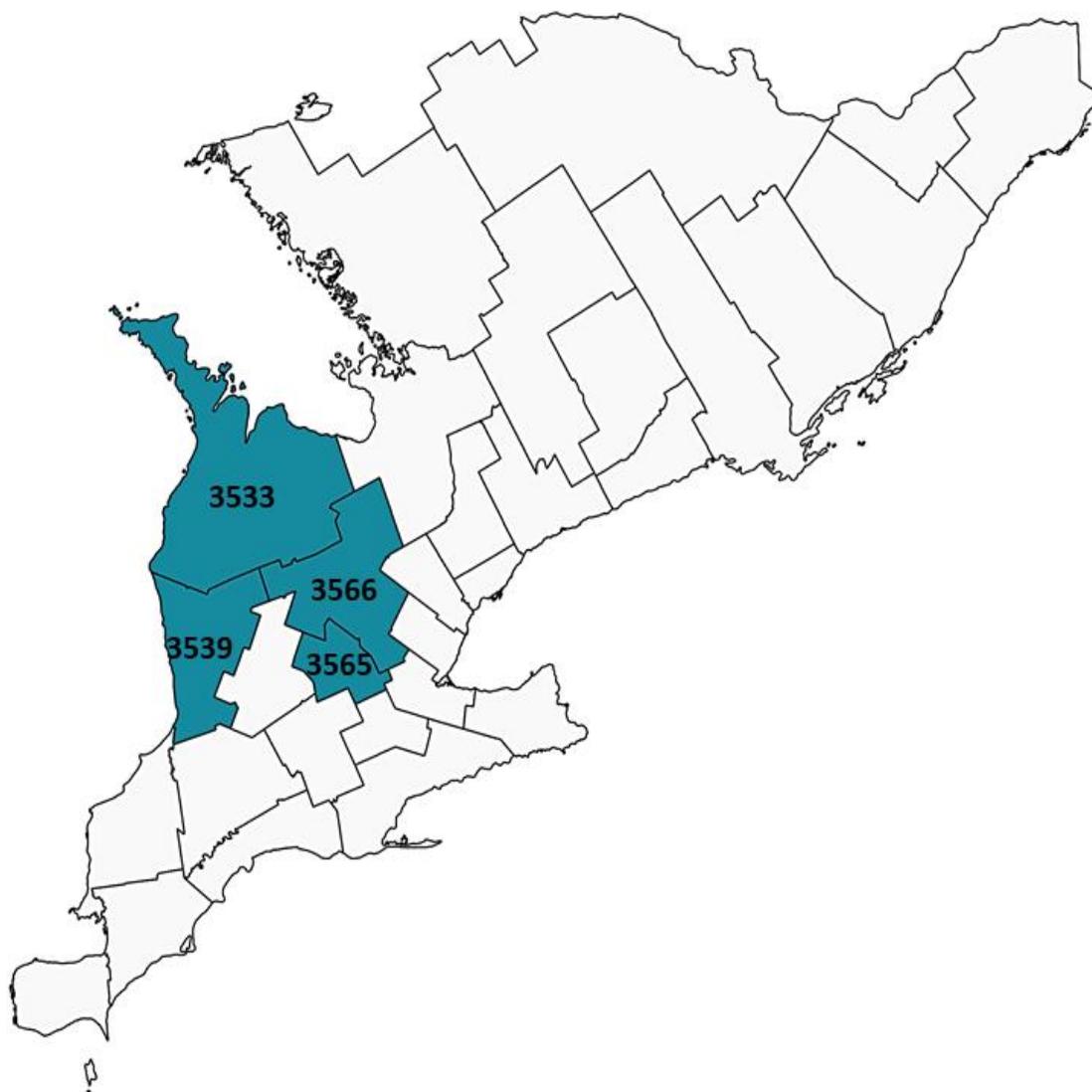


Figure 2.6- Location of high-risk cluster of bovine cryptosporidiosis in the 29 Public Health Unit areas of Southern Ontario from 2011-2014

CHAPTER THREE:

Exploring the geographical distribution of cryptosporidiosis in the human population of Southern Ontario from 2011-2014.

Abstract

Cryptosporidium is a protozoan parasite of increasing global public health concern because of its ability to cause lethal diarrheal disease in both humans and animals via contaminated food and water supplies. In Canada, the majority of human cryptosporidiosis cases are due to *C. hominis*; however, the presence of zoonotic *C. parvum* has been observed. Since 2005, the incidence of cryptosporidiosis in Ontario has been consistently higher than the national average; however, it is not understood why, suggesting an incomplete understanding of the pathogen's ecology, epidemiology, and transmission pathways. The goal of this study was to explore the spatial distribution of human cryptosporidiosis across the 29 Public Health Unit (PHU) areas of Southern Ontario from 2011-2014.

Surveillance data on human cryptosporidiosis were obtained from Public Health Ontario. Choropleth maps were used to display the distribution of both raw and smoothed incidence rates of human cryptosporidiosis. Geostatistical kriging was used to develop a risk map of human cryptosporidiosis. High rate clusters of human cryptosporidiosis were identified using the flexible spatial scan test. Poisson and spatial Poisson regression models were used to determine the relationship between the incidence of human cryptosporidiosis and cattle density, dairy cattle density, calf density and the smoothed farm-level prevalence of bovine cryptosporidiosis at the PHU level.

The annual raw incidence rate of reported human cryptosporidiosis in Southern Ontario ranged from 1.62 [95% CI: 1.41-1.86] to 1.82 [95%CI: 1.60-2.06] cases per 100,000 population

between 2011 and 2014, with an overall raw cumulative incidence rate of 6.91 [95%CI: 6.47-7.39] cases per 100,000 for the four-year study period. High-risk clusters of human cryptosporidiosis were identified in each year. The relative risk for the clusters ranged from 2.03 [95%CI: 1.63-2.55] to 6.87 [95% CI: 5.07-9.30]. A relationship was found between the incidence of cryptosporidiosis and the density of dairy cattle. Based on the findings of this study and the previous study, the Central West region would be an ideal ecological system to conduct further targeted surveillance to identify potential environmental factors that may be contributing to the higher burden of cryptosporidiosis in the human and bovine populations in that region.

Keywords: Cryptosporidiosis, Southern Ontario, Spatial epidemiology, Flexible scan test

INTRODUCTION

Cryptosporidium is a protozoan parasite of increasing global public health concern because of its ability to cause lethal diarrheal disease in both humans and animals via contaminated food and water supplies (Mosier & Oberst, 2000). In North America, *Cryptosporidium* is one of the most reported enteric protozoan pathogens and has emerged as a major cause of diarrheal outbreaks in humans (Fletcher *et al.*, 2012; Nichols, 2000). To date, more than 20 species of *Cryptosporidium* have been identified since its discovery in 1907, but only two primarily cause infections in humans: *C. hominis* and *C. parvum* (Abeywardena *et al.*, 2015). While humans are the primary host for *C. hominis*, *C. parvum* has been documented in a wide range of mammalian hosts, of which cattle are considered the primary reservoir due to the high prevalence of *C. parvum* in the cattle population (Medema *et al.*, 2009). Human infections with *Cryptosporidium* occur through the transmission of oocysts by the fecal-oral route, either directly through contact with infected individuals or animals, or indirectly through the consumption of contaminated food or water (Abeywardena *et al.*, 2015).

In Canada, where the disease in humans is reportable, the majority of human cryptosporidiosis cases are due to *C. hominis*, but infections with *C. parvum* have also been observed (Ong *et al.*, 2002; Trotz-Williams *et al.*, 2006; Xiao & Feng, 2008). Since 2005, incidence rates of human cryptosporidiosis in Ontario have been consistently higher than the national average; however, it is not understood why, suggesting an incomplete understanding of the pathogen's ecology, epidemiology, and transmission pathways (Public Health Ontario, 2015). Standard public health laboratory diagnostic procedures are not able to distinguish the different species of *Cryptosporidium*, and while zoonotic infections are considered sporadic, the lack of routine molecular characterization of reported human and animal cases in Ontario makes it

difficult to explore the direct zoonotic and possible zooanthroponotic origins of human and animal infections. In the absence of species level information for *Cryptosporidium* infections, alternate methods for exploring distribution of this disease in the population and investigating any possible causative effects of human, animal and environmental interactions involved in transmission of this parasite need to be employed. Thus, given the high density of both humans and cattle in Southern Ontario, the goal of this study was to explore the spatial distribution of human cryptosporidiosis across the 29 Public Health Unit (PHU) areas of Southern Ontario from 2011-2014, using human cryptosporidiosis surveillance data obtained from Public Health Ontario (PHO). The specific objectives of this study were to (1) map the geographical risk distribution of cryptosporidiosis in humans, (2) identify areas at high-risk for human cryptosporidiosis, if present, and (3) investigate the data, using Poisson and spatial Poisson regression analyses, for any relationships between the distribution of the incidence of human cryptosporidiosis and the smoothed farm prevalence of bovine cryptosporidiosis, as well as the density of calves, dairy cattle and cattle (beef and dairy) in the 29 PHU areas of Southern Ontario.

MATERIALS AND METHODS

Data Collection

Human cryptosporidiosis is a reportable disease in Ontario. As such, it is mandated that all laboratory-confirmed cases of cryptosporidiosis in people be reported to the PHU serving the area in which the case resides. The PHU records details of each case of any reportable disease in a database administered by the Ministry of Health and Long-Term Care (MOHLTC): the

Integrated Public Health Information System (iPHIS) (Public Health Ontario, 2017). Information on case demographics, exposures, risk factors and outcomes are reported through iPHIS.

A surveillance dataset with about 1,270 confirmed cases of cryptosporidiosis reported from January 1, 2011 to December 31, 2014 was obtained from iPHIS via PHO. The surveillance dataset included episode date (earliest of symptom onset date, specimen collection or report date), episode date type, age group, sex, diagnosing PHU area and travel history (during the incubation period) for each case. Census data on population totals for all PHU areas in Southern Ontario from 2011-2014 were obtained from Statistics Canada (Statistics Canada, 2017). Census data on cattle population (beef and dairy), calf population, dairy cattle population and farm area from 2011-2014 for all areas served by the PHUs in Southern Ontario were obtained from OMAFRA (Statistics Unit, OMAFRA, & Statistics Canada, 2017). Farm-level bovine cryptosporidiosis prevalence (i.e the number of farms with at least one bovine sample positive for cryptosporidiosis) data were obtained from the previous chapter.

Estimation of Incidence and Cattle Density

Using R version 3.2.3, the surveillance dataset was cleaned for analysis (R Core Team, 2016; RStudio Team, 2016). Any confirmed cases of cryptosporidiosis with a history of travel outside of Ontario during the disease incubation period were excluded from analysis (n=422). Cases that had been reported from PHU areas outside Southern Ontario (n=91) were also excluded. All remaining cases (n=757) were then aggregated by year and PHU area for analysis. Raw incidence rates of human cryptosporidiosis for each PHU were determined for each year of investigation and for the entire study period. The annual incidence rates were estimated as the number of human cases of cryptosporidiosis divided by the population total for each PHU area. The cumulative incidence for the entire four-year study period was estimated as the total number

of human cases of cryptosporidiosis divided by the average population over the four-year study period. Since the frequency of human cryptosporidiosis is very low, confidence intervals for incidence rates were estimated using the Wilson score interval method (Wilson, 1927). Cattle densities were calculated as the average number of cattle per PHU area from 2011 to 2014 divided by the total farm area (per 100 hectares) in each PHU area.

Mapping and Spatial Analysis

Choropleth and Isopleth Mapping

Choropleth maps of the raw incidence of human cryptosporidiosis were used to initially visualize the spatial variation in the risk of human cryptosporidiosis across Southern Ontario over the study period. The raw incidence estimates were then smoothed using empirical Bayesian smoothing and again displayed using choropleth maps (Marshall, 1991). Isopleth risk maps based on Bayesian adjusted incidence estimates were produced using the geostatistical prediction method of kriging, a method that allows for the interpolation of regional data onto a continuous surface (Berke, 2005). The mapping and smoothing techniques were conducted using Rv3.2.4 and R packages *maptools*, *spdep* and *geoR* (Bivand *et al.*, 2013; Bivand and Lewin-Koh, 2013; Ribeiro *et al.*, 2001; R Core Team, 2016; RStudio Team, 2016).

Cluster Detection

A flexible scan test based on Poisson model was used to detect high-risk disease clusters (Takahashi *et al.*, 2008). The R v3.2.4 package *smc* was used to conduct the spatial scan test using a significance level of $\alpha = 0.05$, 999 Monte Carlo simulations and a pre-set k of 5 regions as the maximum cluster size (French, 2015). The pre-set maximum cluster size of $k=5$ regions was selected as an alternative to the default setting of 10; given that the number of PHUs in

Southern Ontario is small ($n=29$), a k number of 10 might cover more than 50% of the total population size.

Modelling

Non-spatial and spatial Poisson regression models were applied in order to determine whether calf density, cattle density, dairy cattle density and spatially smoothed farm-level prevalence of bovine cryptosporidiosis were associated with the cumulative incidence of human cryptosporidiosis within PHU areas of Southern Ontario. Based on the semivariogram generated for the isopleth risk maps, a spherical correlation model was used for the spatial random effects Poisson model. The spherical correlation structure forms a decay function in which observations farther away in space are less related than those that are closer in space—in agreement with Tobler’s “first law of geography” (Tobler, 1970). A manual model-building approach using a backward elimination process was used to select the final model. The model and variables included in the model, were considered to be significant if they had a p-value of less than the significance level of $\alpha=0.05$. Confounding was assessed throughout the model-building process. If the removal of a variable resulted in a 30% or greater change in the model coefficients the variable was retained in the model regardless of its significance.

Potential collinearity between explanatory variables was evaluated using Pearson pairwise correlations between all variables. A cutoff value of $p= |0.8|$ was used to identify pairs of explanatory variables which were overly correlated. Linearity of the continuous predictor variables in the model were tested visually using a Lowess curve. Any predictor variables that did not meet the assumptions were either subjected to transformation or categorized if transformation was unsuccessful. Prior to building the model, all explanatory variables were screened using a liberal $\alpha=0.3$.

In order to assess the overall fit of the Poisson regression model, the deviance residuals were plotted against the model predicted values and visually assessed for normality using a normal Q-Q plot. Potential overdispersion in the Poisson regression model was also assessed by dividing the residual deviance χ^2 by the number of degrees of freedom (df) to give a dispersion parameter, with a dispersion parameter of >1 indicating overdispersion that needed to be accounted for in the model in order to allow for accurate estimates. All statistical analyses were conducted in R v.3.2.4 and RStudio v1.0.44 (R Core Team, 2016; RStudio Team, 2016).

RESULTS

The overall annual raw incidence of human cryptosporidiosis in Southern Ontario remained stable over the study period, ranging from 1.62 [95% CI: 1.41-1.86] to 1.82 [95%CI: 1.60-2.06] cases per 100,000 population between 2011 and 2014, with an overall raw cumulative incidence risk of 6.91 [95%CI: 6.47-7.39] cases per 100,000 for the entire study period [Table 3.1]. The raw incidence risk as well as smoothed risk of human cryptosporidiosis varied across the areas served by the 29 PHUs, with higher incidences being consistently observed in the Huron County, Perth District, Oxford County and Bruce-Grey-Owen Sound PHU areas, while lower incidences were consistently observed in the City of Hamilton and Peel Region PHU areas. The smoothed incidence estimates ranged from 0.37 to 16.29 while the crude incidence estimates ranged from 0 to 23.50 cases per 100,000 population between 2011 and 2014.

Variation in the geographical distribution of the incidence of human cryptosporidiosis can be seen in the choropleth and isopleth maps [Figure 3.1 to Figure 3.6]. It appears that, over the study period, PHU areas with higher incidences were consistently concentrated in the Central West region of Southern Ontario while PHU areas with lower incidences were consistently

concentrated in PHU areas in the South-Central region [Table 3.1 and Figure 3.5]. The isopleth risk maps displayed no evident directional trend in the human risk of cryptosporidiosis at any point of analysis of the study period.

High-risk clusters of human cryptosporidiosis were identified for all time periods [Figures 3.7 and 3.8; Table 3.2]. The clusters were consistently located in Central West, East and Southeast regions of Southern Ontario. The relative risk for the clusters ranged from 2.03 [95%CI: 1.63-2.55] to 6.87 [95%CI: 5.07-9.30] [Table 3.2].

Based on the Pearson's correlation test between each pair of explanatory variables, only calf density (beef and dairy) and cattle density (beef and dairy) exceeded the threshold value of 0.8. Therefore, cattle density was excluded from the model due to potential collinearity with calf density. The lowess curves for all variables tested (calf density, dairy cattle density, and spatially smoothed farm prevalence of bovine cryptosporidiosis) showed a non-linear relationship with the outcome (log cumulative incidence rate of human cryptosporidiosis). The spatially smoothed farm prevalence of bovine cryptosporidiosis was successfully transformed using a quadratic transformation ($p < 0.001$), while the remaining two independent variables were categorized into four groups using Jenks Natural breaks algorithm. For calf density these groups were: very low (0.0 – 4.81), low (4.82 – 10.63), medium (10.64 – 15.61) and high (15.62 – 28.0). For dairy cattle density these groups were: very low (0.0 – 3.22), low (3.23 – 7.66), medium (7.67 – 15.87) and high (15.88 – 20.36). Univariable models were run with each of the independent variables and the log cumulative incidence rate of human cryptosporidiosis. Each of the univariable models was significant at the $p = 0.3$ significance level, therefore all independent variables were included in the initial model during model-building.

The final non-spatial Poisson regression model produced by backward selection indicated a significant relationship between the cumulative incidence rate of human cryptosporidiosis and the density of dairy cattle in Southern Ontario. According to the model, the cumulative incidence risk of human cryptosporidiosis in low, medium and high dairy cattle density PHU areas were 1.92 [95% CI: 1.62-2.27], 3.04 [95%CI: 2.55-3.54] and 8.68 [95%CI: 6.07-12.06] times greater, respectively, than in very low dairy cattle density PHU areas [Table 3.3]. The dispersion parameter in the residuals was estimated to be approximately 27.7 indicating the presence of overdispersion in the model. In addition, the deviance residuals were not normally distributed, indicating that the data poorly fit the model [Figure 3.9].

The spatial Poisson regression model produced by backward selection also indicated a significant relationship between the spatial distribution of the cumulative incidence risk of human cryptosporidiosis and the spatial distribution of dairy cattle density in Southern Ontario. According to the model, the cumulative incidence risk of human cryptosporidiosis in medium and high dairy cattle density PHU areas were 2.25 [95%CI: 1.08-4.66] and 7.48 [95%CI: 2.81-19.92] times greater, respectively, than in very low dairy cattle density PHU areas [Table 3.3]. There was no significant difference between the cumulative incidence risk in low dairy cattle density PHU areas compared to very low dairy cattle density PHU areas. The Q-Q plot of the model residuals showed that the spatial Poisson regression model fit the data well [Figure 3.10].

DISCUSSION

The overall goal of this study was to use surveillance data supplied from PHO to explore the spatial distribution of human cryptosporidiosis from 2011-2014 across the areas served by the 29 PHUs of Southern Ontario. Throughout the study period, the global annual raw incidences

of human cryptosporidiosis remained relatively stable, with the incidences ranging from 1.62 [95% CI: 1.41-1.86] to 1.82 [95%CI: 1.60-2.06] cases per 100,000 population between 2011 and 2014. The choropleth maps of the raw and smoothed incidence rates demonstrated that the occurrence of human cryptosporidiosis was widespread over the study period. Overall, PHU areas with higher incidences occurred consistently in the East and Central West regions of Southern Ontario, while PHU areas with lower incidences were consistently concentrated in the Niagara Peninsula and the South-Central region of Southern Ontario (Table 3.1; Figures 3.1 – 3.4). The isopleth risk maps consistently indicated that populations in PHU areas in the Central West region might be at an increased risk for infection.

This study found evidence of human clusters of cryptosporidiosis for each year of the study period. Over the four-year study period, these clusters were also consistently located in the Central West and East regions of Southern Ontario, confirming the likelihood of an increased risk for infection amongst the population in these regions of Southern Ontario. Due to the location of high-risk disease clusters, the higher incidences of human cryptosporidiosis in those locations, and the potential zoonotic nature of *Cryptosporidium*, it was hypothesized that the incidence of human cryptosporidiosis would be predicted by either the smoothed farm prevalence of bovine cryptosporidiosis or the density of cattle, specifically dairy cattle. The (non-spatial) Poisson regression model indicated that there was indeed a significant relationship between human incidence of cryptosporidiosis and the density of dairy cattle. The spatial Poisson regression model also indicated that there was a relationship between human cryptosporidiosis and dairy cattle density. However, the quantile plot of the deviance residuals of the non-spatial Poisson regression model showed that the residuals were overdispersed, and the data were also found to fit the model poorly. Overdispersion in a model can result in too many significant

results and an underestimation of the standard error. Thus, based on the results of the spatial model, which was a better fit for the data than the non-spatial model, it can be concluded that there is a relationship between the spatial distribution of cryptosporidiosis in the human population and the density of dairy cattle in Southern Ontario.

The findings of this study, in which a relationship was found between the distribution of human cryptosporidiosis and density of dairy cattle, suggest that the transmission of cryptosporidiosis amongst the population in Southern Ontario may not be solely driven by anthropogenic transmission. Cryptosporidiosis is a disease commonly seen in cattle, particularly in pre- to post-weaned dairy calves (Sivajothi *et al.*, 2014). Previous studies estimating the prevalence of *Cryptosporidium* in Canada have demonstrated that the parasite is quite ubiquitous in Canadian dairy cattle herds (Coklin *et al.*, 2009; Olson *et al.*, 1997; Ruest *et al.*, 1998; Trotz-Williams *et al.*, 2005). In Southern Ontario specifically, the prevalence of *C. parvum* in dairy calves has been estimated at 40.6% (Trotz-Williams *et al.*, 2005). Furthermore, as reported in Chapter 2, analysis of the spatial distribution of bovine cryptosporidiosis in Southern Ontario has demonstrated that cattle cases of cryptosporidiosis are quite widespread within this geographical region. Though it has been noted that human infections of cryptosporidiosis in Canada are mostly due to the anthropogenic species *C. hominis*, sporadic human infections with zoonotic *C. parvum* have been detected in Canada and more specifically Southern Ontario (Budu-Amoako *et al.*, 2012; Trotz-Williams *et al.*, 2006; Xiao & Feng, 2008). Given that Southern Ontario is an ecological system with high densities of both humans and cattle within a relatively small geographical area, and considering the relationship found between the distribution of human cryptosporidiosis and the density of dairy cattle in this study, the likelihood that some human

infections are the results of zoonotic transmission cannot be ruled out and should be further explored and understood.

Genotyping of *Cryptosporidium* isolates from human infections, or even identification at the species level, is not routinely done by the provincial and national public health laboratories, making it difficult to understand the relative importance of anthropogenic vs. zoonotic transmission of this parasite. Based on the findings of this study and the findings reported in Chapter 2, the Central West region in Southern Ontario is an ecological environment that has higher raw estimates of human as well as bovine cryptosporidiosis in comparison to other regions in Southern Ontario. If the findings of this study are accurate, in that there is a relationship between the distribution of human cryptosporidiosis and the density of dairy cattle, the Central West region would be an ideal area in which to conduct further targeted and advanced surveillance such as routine molecular testing of positive *Cryptosporidium* isolates from human and bovine cases.

There are some limitations that need to be considered when interpreting the results of this study. First, the human incidence of cryptosporidiosis for each PHU area was estimated using reportable disease surveillance data. Since those data do not capture all cases, but only symptomatic cases that seek medical attention and are tested for *Cryptosporidium* infection, the true number of cryptosporidiosis cases in the study region and within individual PHU areas is likely underestimated (Majowicz *et al.*, 2005). Second, PHU area-specific incidences were estimated based on the number of cases reported to each PHU. Cases are assigned to, and reported to, each PHU according to the area of residence of the individual. Therefore, the PHU area to which a case is assigned may not represent the PHU area in which a case has been exposed. It follows that there is a chance that some cases were spatially misclassified in this

study with respect to area of exposure and acquisition of infection; however, this misclassification most likely to be non-differential (Majowicz et al., 2005). Finally, there were some limitations with the data used as inputs in the model. The farm prevalence estimates used in the model were taken from Chapter 2. These prevalence estimates were produced using laboratory diagnostic data and were subject to misclassification bias. This bias may have resulted in this variable not being associated with the cumulative incidence of human cryptosporidiosis.

CONCLUSION

Southern Ontario is an ecological system with a high density of both humans and cattle within a relatively small geographical area. For infectious agents like *Cryptosporidium* that are at the human, animal and environmental interface, densely populated regions such as Southern Ontario are often at an increased risk for the spread of infectious disease between animals and humans. Though the majority of the burden of illness attributable to cryptosporidiosis in humans in North America is reportedly from anthropogenic transmission, *Cryptosporidium parvum* is a zoonotic pathogen. This study described the spatial relationship between the human incidence of cryptosporidiosis and the density of dairy cattle, detected high-risk spatial clusters of disease, and identified the Central West and Eastern regions as a geographical area where the human population is at a higher risk for infection. In the previous chapter, the Central West region of Southern Ontario was also identified as a geographical area where the cattle population is at a higher risk for infection. Based on the findings of this study and of the study described in the previous chapter, the Central West region would be an ideal ecological system to conduct further targeted surveillance and One-Health epidemiological studies to identify potential environmental

factors that may be contributing to the higher burden of cryptosporidiosis in the human and bovine populations in that region.

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Table 3.1 – Raw and smoothed (adjusted) Bayesian incidence estimates of human cryptosporidiosis (per 100,000 population) in the 29 Public Health Unit areas in Southern Ontario, 2011-2014

Public Health Unit		2011		2012		2013		2014		2011-2014	
Number	Name	Raw [95%CI]	Adj.*	Raw [95%CI]	Adj.*	Raw [95%CI]	Adj.*	Raw [95%CI]	Adj.*	Raw [95%CI]	Adj.*
Mid North Region											
3557	Renfrew	0.95 [0.17-5.37]	1.16	1.89 [0.52-6.90]	1.84	2.83 [0.96-8.33]	2.57	5.65 [2.59-12.33]	4.50	11.38 [6.51-19.90]	10.99
Southwest Region											
3531	Elgin-St Thomas	2.23 [0.61-8.12]	2.02	6.66 [3.05-14.53]	5.43	4.43 [1.72-11.40]	3.73	4.43 [1.72-11.39]	3.56	17.81 [10.96-28.93]	16.70
3540	Kent-Chatham	0.94 [0.17-5.31]	1.15	1.88 [0.52-6.86]	1.83	2.83 [0.96-8.32]	2.57	1.89 [0.52-6.89]	1.87	7.50 [3.80-14.80]	7.45
3542	Lambton	2.28 [0.78-6.72]	2.11	2.29 [0.78-6.73]	2.17	4.59 [2.10-10.01]	4.03	3.07 [1.19-7.89]	2.75	12.18 [7.50-19.79]	11.80
3544	Middlesex-London	1.10 [0.47-2.59]	1.15	1.09 [0.47-2.56]	1.13	1.95 [1.03-3.71]	1.93	0.64 [0.22-1.90]	0.75	4.86 [3.21-7.36]	4.90
3552	Oxford	6.44 [3.12-13.30]	4.99	9.10 [4.95-16.76]	7.53	12.69 [7.56-21.30]	10.25	6.29 [3.05-12.99]	4.99	34.97 [25.48-47.99]	32.56
3568	Windsor-Essex	1.75 [0.85-3.62]	1.74	0.50 [0.14-1.82]	0.58	0.25 [0.04-1.41]	0.35	0.50 [0.14-1.81]	0.63	3.00 [1.72-5.25]	3.10
Central-West Region											
3533	Bruce-Grey-Owen Sound	9.23 [5.59-15.23]	7.53	9.21 [5.58-15.20]	8.06	11.02 [6.97-17.42]	9.51	10.40 [6.49-16.65]	8.53	39.99 [31.38-50.96]	38.04
3539	Huron	14.87 [7.82-28.26]	9.09	23.50 [14.00-39.44]	16.29	8.41 [3.59-19.69]	6.09	8.44 [3.61-19.76]	5.57	54.53 [38.83-76.56]	47.66
3554	Perth	12.97 [7.04-23.87]	8.68	7.73 [3.54-16.86]	6.06	15.39 [8.80-26.90]	11.45	14.06 [7.85-25.17]	9.56	50.57 [37.00-69.11]	45.47
3565	Waterloo Wellington-	3.44 [2.17-5.43]	3.29	0.94 [0.40-2.21]	0.98	1.69 [0.89-3.21]	1.69	1.12 [0.51-2.43]	1.17	7.26 [5.29-9.96]	7.25
3566	Dufferin-Guelph	5.12 [3.05-8.60]	4.61	5.79 [3.57-9.41]	5.40	3.95 [2.21-7.07]	3.72	5.68 [3.50-9.22]	5.14	20.86 [16.10-27.03]	20.36
South-Central Region											
3530	Durham	0.96 [0.44-2.09]	1.00	0.94 [0.43-2.06]	0.97	0.62 [0.24-1.60]	0.67	1.07 [0.52-2.21]	1.12	3.67 [2.45-5.51]	3.72
3536	Halton	0.58 [0.20-1.71]	0.67	0.38 [0.10-1.38]	0.45	0.56 [0.19-1.63]	0.62	1.27 [0.62-2.63]	1.31	2.90 [1.76-4.79]	2.98
3553	Peel	0.60 [0.30-1.18]	0.63	0.37 [0.16-0.86]	0.39	0.43 [0.20-0.94]	0.46	0.21 [0.07-0.62]	0.26	1.64 [1.08-2.49]	1.68
3560	Simcoe	0.77 [0.30-1.98]	0.84	0.57 [0.19-1.67]	0.63	0.93 [0.40-2.19]	0.98	2.40 [1.40-4.10]	2.35	4.81 [3.26-7.10]	4.85
3570	York	0.75 [0.38-1.48]	0.79	0.55 [0.25-1.21]	0.58	0.27 [0.09-0.80]	0.31	0.53 [0.24-1.16]	0.58	2.16 [1.44-3.24]	2.20
3595	City of Toronto	0.59 [0.36-0.96]	0.61	0.80 [0.53-1.22]	0.81	0.94 [0.64-1.37]	0.94	1.42 [1.05-1.94]	1.43	3.85 [3.17-4.66]	3.86
Niagara Peninsula											
3527	Brant Haldimand-	0.71 [0.13-4.02]	0.94	1.41 [0.39-5.13]	1.45	0.70 [0.12-3.95]	0.88	1.38 [0.38-5.05]	1.49	4.25 [1.95-9.28]	4.43
3534	Norfolk Hamilton-	2.71 [0.92-7.99]	2.39	8.15 [4.29-15.50]	6.79	3.62 [1.41-9.32]	3.20	0.00 [0.00-3.48]	0.53	14.45 [8.89-23.47]	13.81
3537	Wentworth	0.93 [0.40-2.19]	0.99	0.37 [0.10-1.35]	0.44	0.55 [0.19-1.62]	0.61	0.54 [0.19-1.60]	0.64	2.43 [1.42-4.15]	2.51
3546	Niagara	1.58 [0.77-3.26]	1.58	0.67 [0.23-1.98]	0.74	1.79 [0.91-3.54]	1.79	1.34 [0.61-2.92]	1.38	5.42 [3.64-8.07]	5.45
Southeast Region											
3535	Haliburton-Kawartha-Pine Ridge	2.27 [0.88-5.83]	2.13	1.69 [0.57-4.96]	1.68	6.73 [3.85-11.76]	5.97	6.15 [3.43-11.00]	5.27	17.01 [11.91-24.28]	16.46
3538	Hastings and Prince Edward	4.26 [2.07-8.80]	3.68	2.44 [0.95-6.28]	2.32	4.89 [2.48-9.64]	4.37	4.90 [2.48-9.66]	4.23	16.45 [11.30-23.93]	15.89
3541	Kingston-Frontenac-Lennox and Addington	2.54 [1.08-5.94]	2.36	3.52 [1.71-7.27]	3.28	1.50 [0.51-4.42]	1.53	1.99 [0.78-5.13]	1.96	9.64 [6.17-15.05]	9.50
3555	Peterborough	2.17 [0.74-6.37]	2.03	2.88 [1.12-7.41]	2.67	2.15 [0.73-6.32]	2.07	3.57 [1.53-8.36]	3.14	10.83 [6.56-17.87]	10.56
East Region											
3543	Leeds-Grenville-Lanark	2.37 [0.92-6.09]	2.20	6.50 [3.63-11.64]	5.78	5.91 [3.21-10.88]	5.25	4.73 [2.40-9.33]	4.11	19.52 [13.90-27.42]	18.81
3551	Ottawa Carleton	1.86 [1.16-2.99]	1.85	2.60 [1.75-3.86]	2.57	0.85 [0.43-1.69]	0.88	1.48 [0.88-2.48]	1.49	6.91 [5.40-8.84]	6.91
3558	Eastern Ontario	4.43 [2.33-8.41]	3.90	4.89 [2.66-9.01]	4.49	6.85 [4.08-11.49]	6.16	6.84 [4.07-11.47]	5.93	23.11 [17.38-30.73]	22.34

**Adj. denotes adjusted (smoothed) Bayesian incidence estimates

Table 3.2- Location of high-risk clusters of human cryptosporidiosis in Southern Ontario, 2011-2014

	Cluster Number	Public Health Unit areas included within identified cluster	Relative Risk [95%CI]	p-value
2011	Cluster 1	3533, 3539, 3554, 3565, 3566	3.71 [3.04-4.52]	0.001
	Cluster 2	3558, 3543, 3541, 3538	2.10 [1.46-3.03]	0.03
2012	Cluster 1	3533, 3539, 3554, 3566	5.34 [4.21-6.78]	0.001
	Cluster 2	3558, 3543, 3541, 3538, 3551	2.03 [1.63-2.55]	0.001
	Cluster 3	3552, 3531	4.82 [3.02-7.73]	0.001
2013	Cluster 1	3533, 3539, 3554	6.87 [5.07-9.30]	0.001
	Cluster 2	3535, 3538	3.79 [2.69-5.53]	0.001
	Cluster 3	3558, 3543	3.45 [2.27-5.24]	0.001
2014	Cluster 1	3533, 3539, 3554, 3566	4.63 [3.61-5.94]	0.001
	Cluster 2	3558, 3543	2.81 [2.01-3.92]	0.001
	Cluster 3	3557, 3535, 3555, 3538	3.24 [2.18-4.82]	0.001
2011-2014	Cluster 1	3533, 3539, 3554, 3566	4.90 [4.32-5.54]	0.001
	Cluster 2	3558, 3543, 3541, 3538	2.48 [2.11-2.92]	0.001

Table 3.3. Model parameters and their p-values for the Poisson and spatial Poisson regression model describing the effect of cattle density and farm prevalence of bovine cryptosporidiosis on human cases of cryptosporidiosis in Southern Ontario, 2011-2014.

Model	IRR	95%CI	<i>p</i>
Poisson Regression*:			
intercept	4.03 x 10 ⁻⁵	3.5 x 10 ⁻⁵ – 4.6 x 10 ⁻⁵	<0.001
Low dairy cattle density	1.92	1.62-2.27	<0.001
Medium dairy cattle density	3.04	2.55-3.54	<0.001
High dairy cattle density	8.68	6.07-12.06	<0.001
Spatial Poisson Regression*:			
intercept	1.58 x 10 ⁻⁵	7.4 x 10 ⁻⁶ – 3.4 x 10 ⁻⁵	<0.001
Low dairy cattle density	1.27	0.83-1.94	0.300
Medium dairy cattle density	2.25	1.08-4.66	0.044
High dairy cattle density	7.48	2.81-19.92	<0.001

*Reference category is very low dairy cattle density.

FIGURES

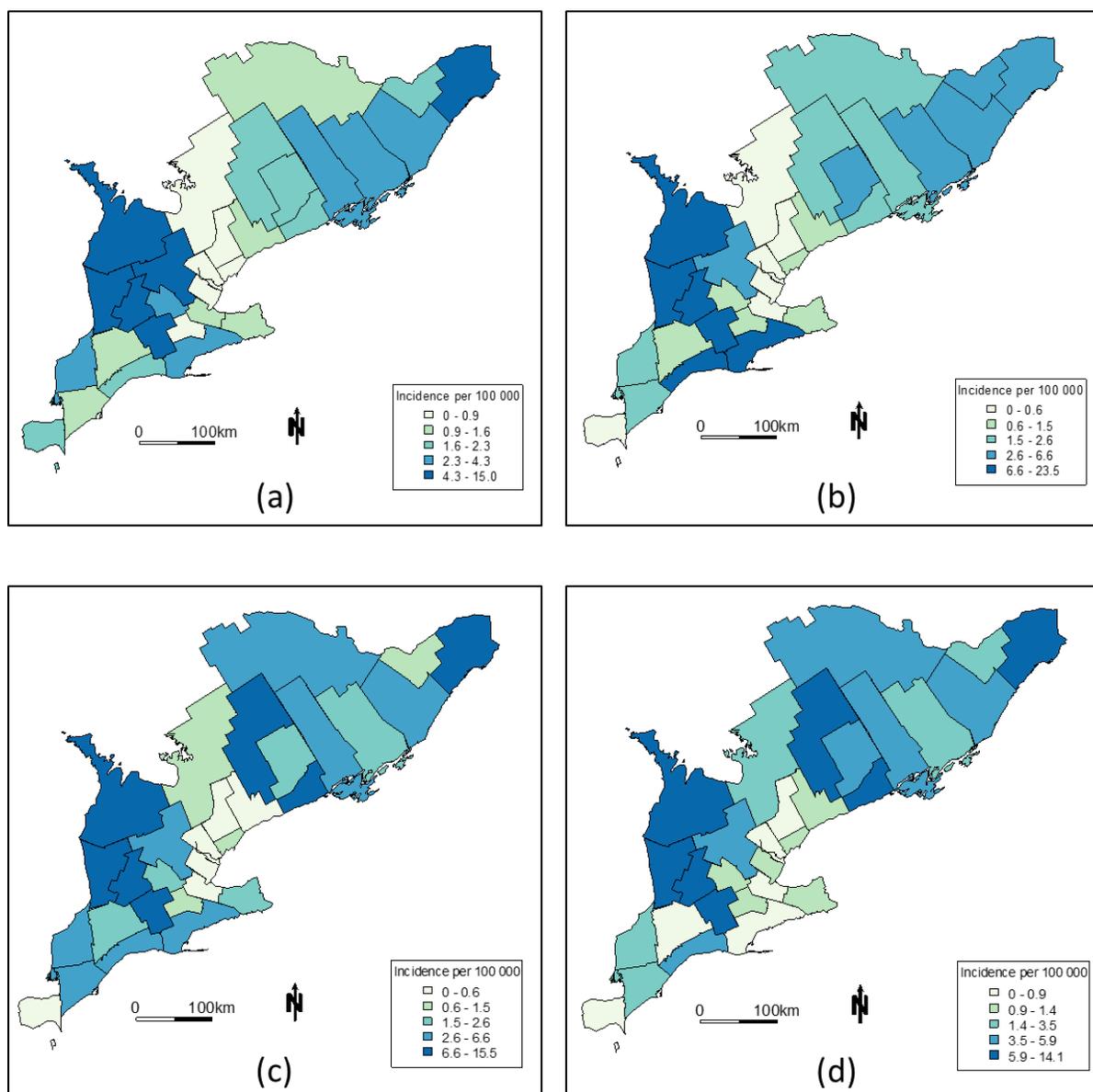


Figure 3.1 – Choropleth maps of the spatial distribution of raw annual incidence of human cryptosporidiosis in 29 Public Health Unit areas of Southern Ontario in (a) 2011, (b) 2012, (c) 2013, and (d) 2014.

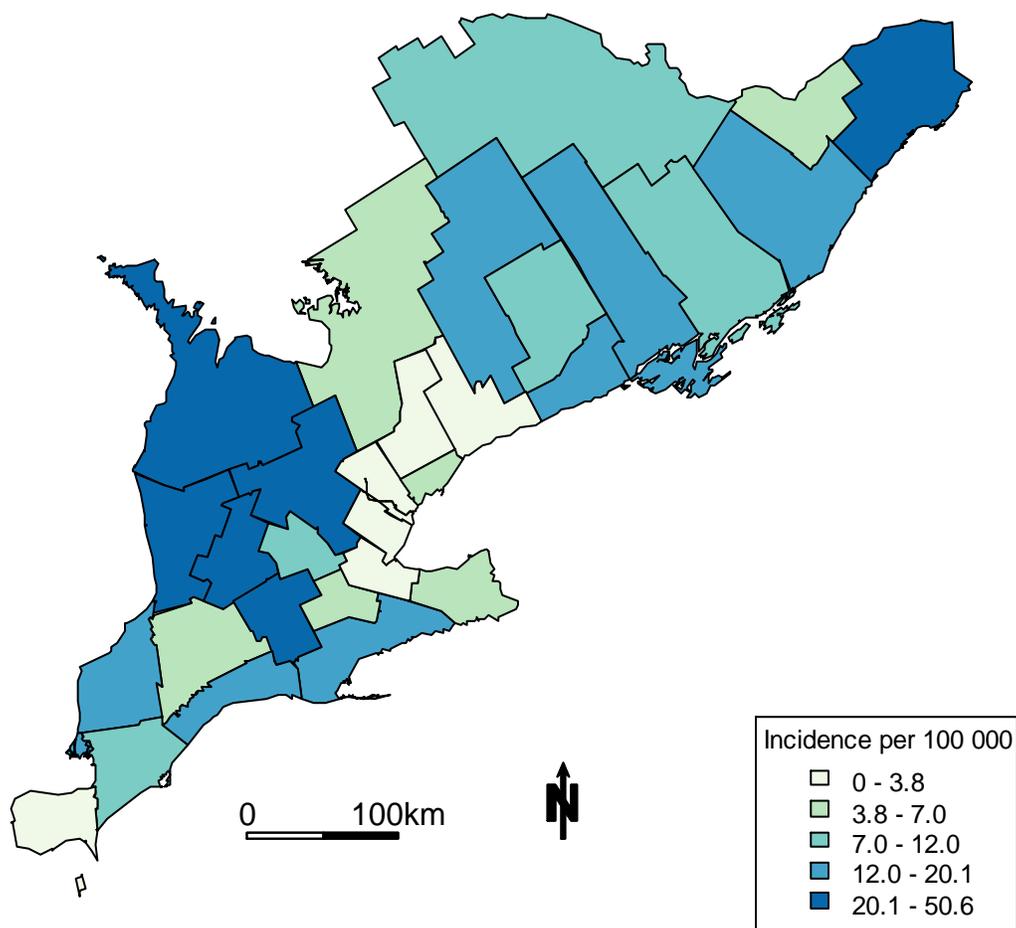


Figure 3.2 - Choropleth map of the spatial distribution of raw cumulative incidence of human cryptosporidiosis in 29 Public Health Unit areas of Southern Ontario from 2011-2014.

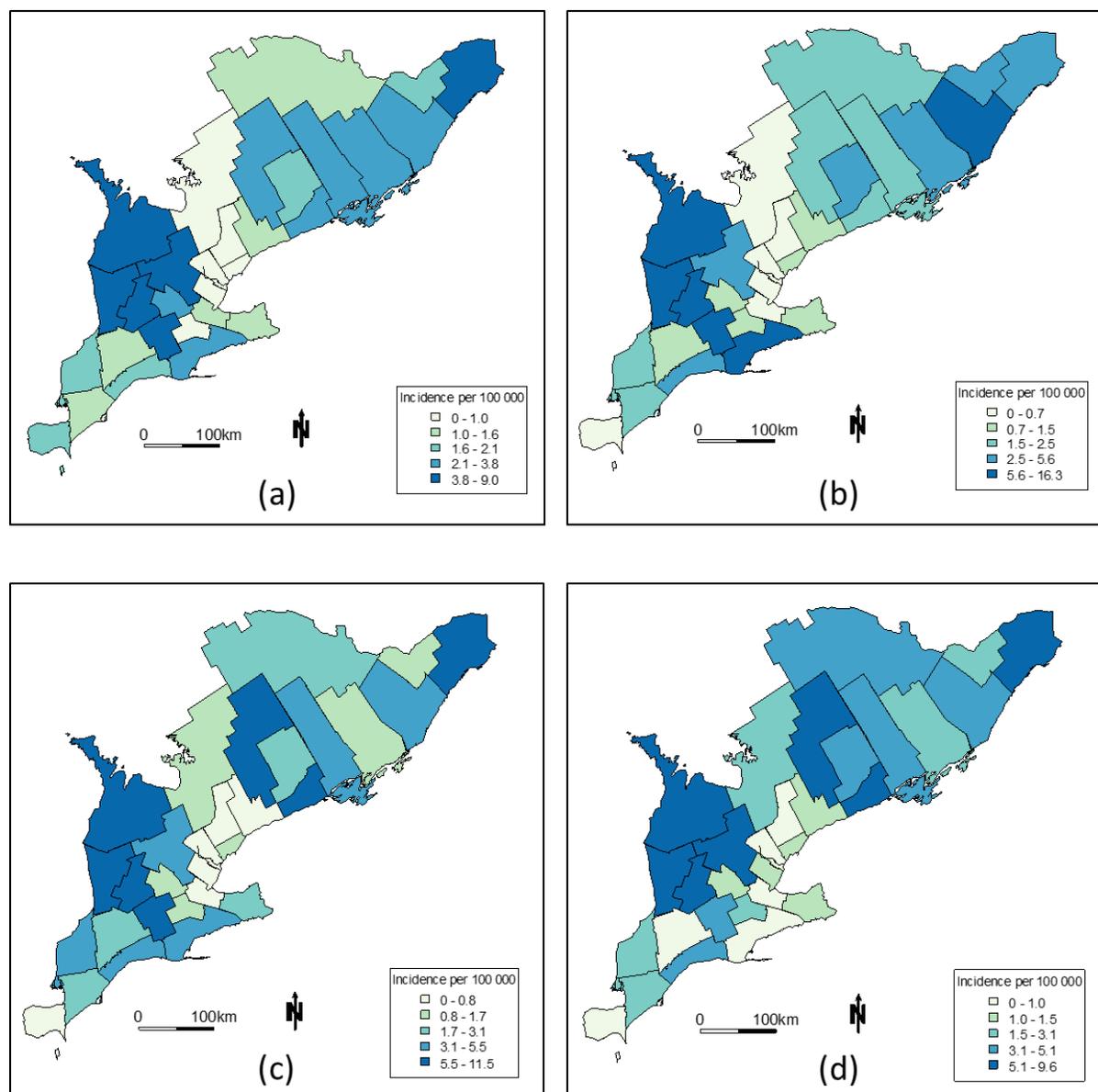


Figure 3.3 - Choropleth maps of the spatial distribution of smoothed annual incidence of human cryptosporidiosis in 29 Public Health Unit areas of Southern Ontario in (a) 2011, (b) 2012, (c) 2013, and (d) 2014.

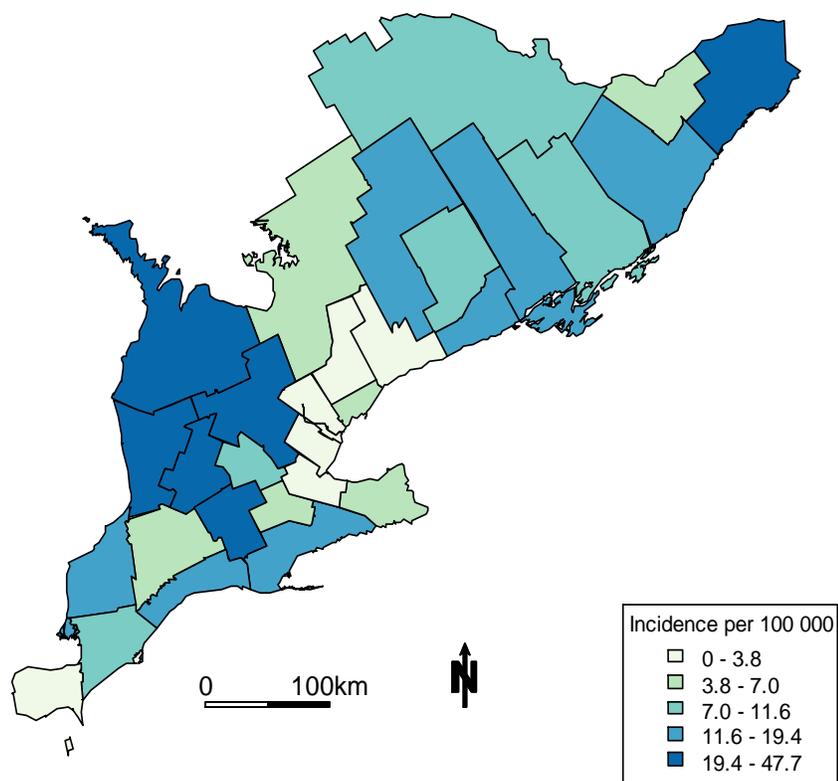


Figure 3.4 - Choropleth map of the spatial distribution of smoothed cumulative incidence of human cryptosporidiosis in 29 Public Health Unit of Southern Ontario from 2011-2014.

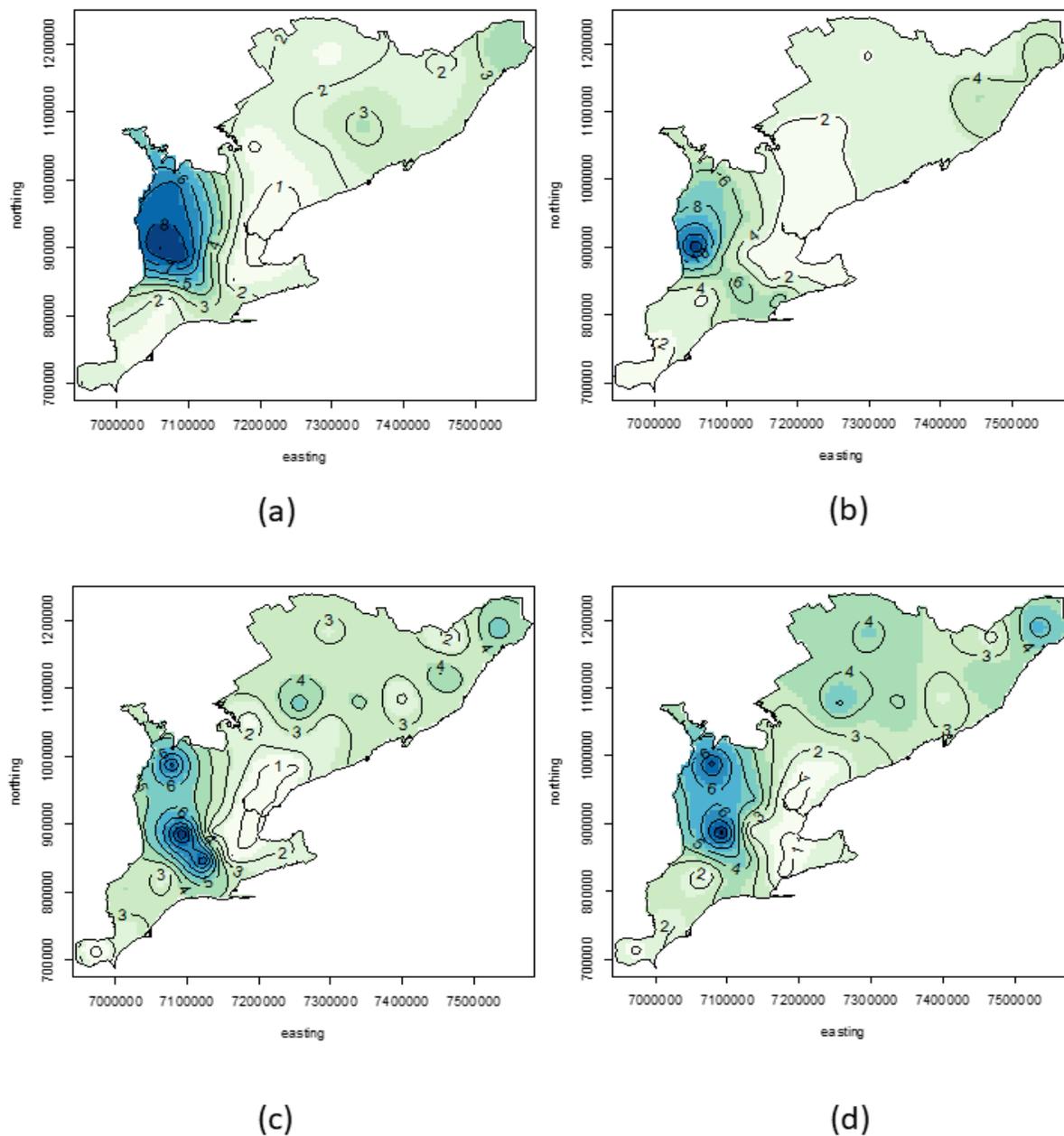


Figure 3.5. Isopleth maps of the annual latent risk distribution of human cryptosporidiosis infections in Southern Ontario in (a) 2011, (b) 2012, (c) 2013, and (d) 2014.

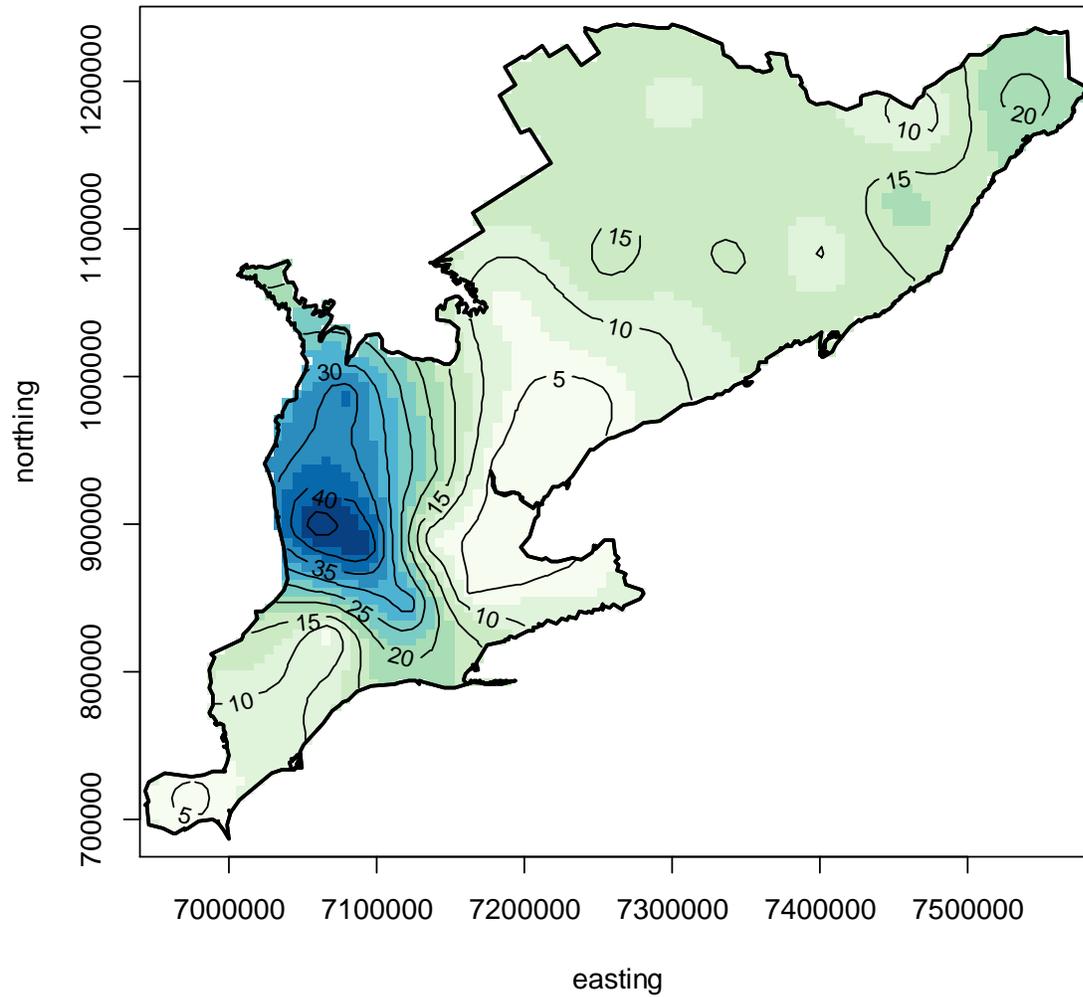


Figure 3.6. Isopleth maps of the latent cumulative risk distribution of human cryptosporidiosis infections in Southern Ontario from 2011-2014.

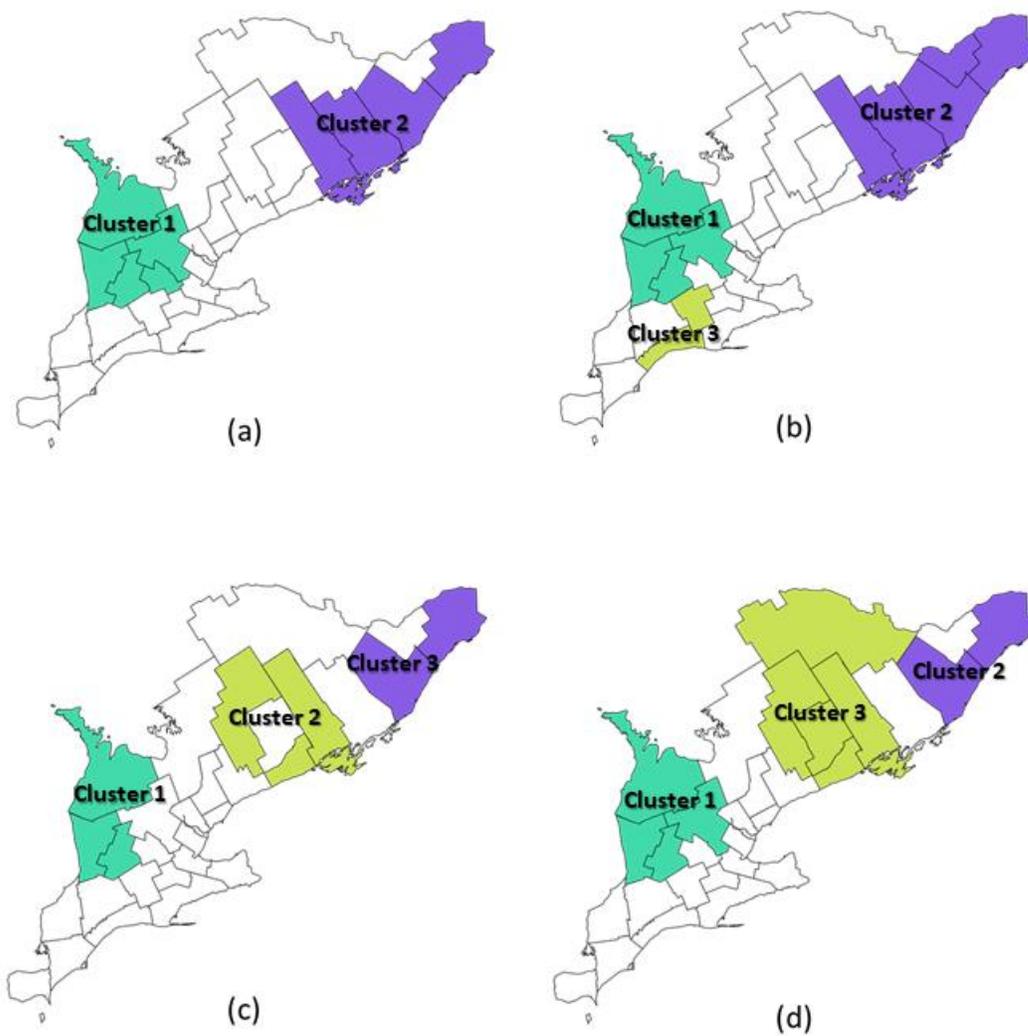


Figure 3.7- Location of high-risk clusters of human cryptosporidiosis, Southern Ontario in (a) 2011, (b) 2012, (c) 2013, and (d) 2014.

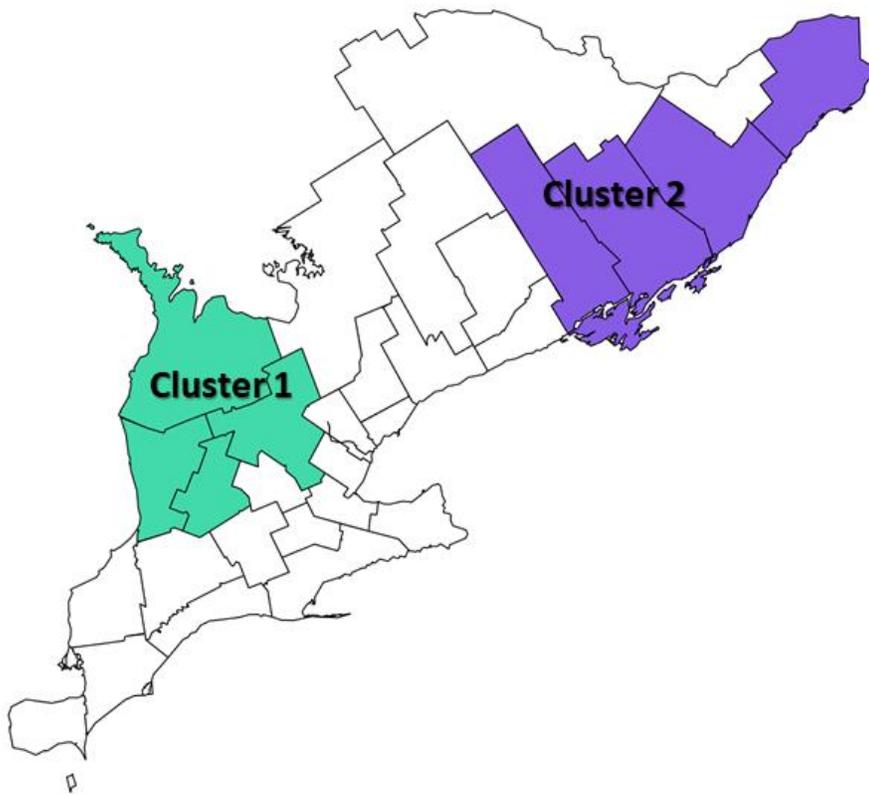


Figure 3.8 - Location of high-risk clusters of human cryptosporidiosis, Southern Ontario, over the four years from 2011-2014.

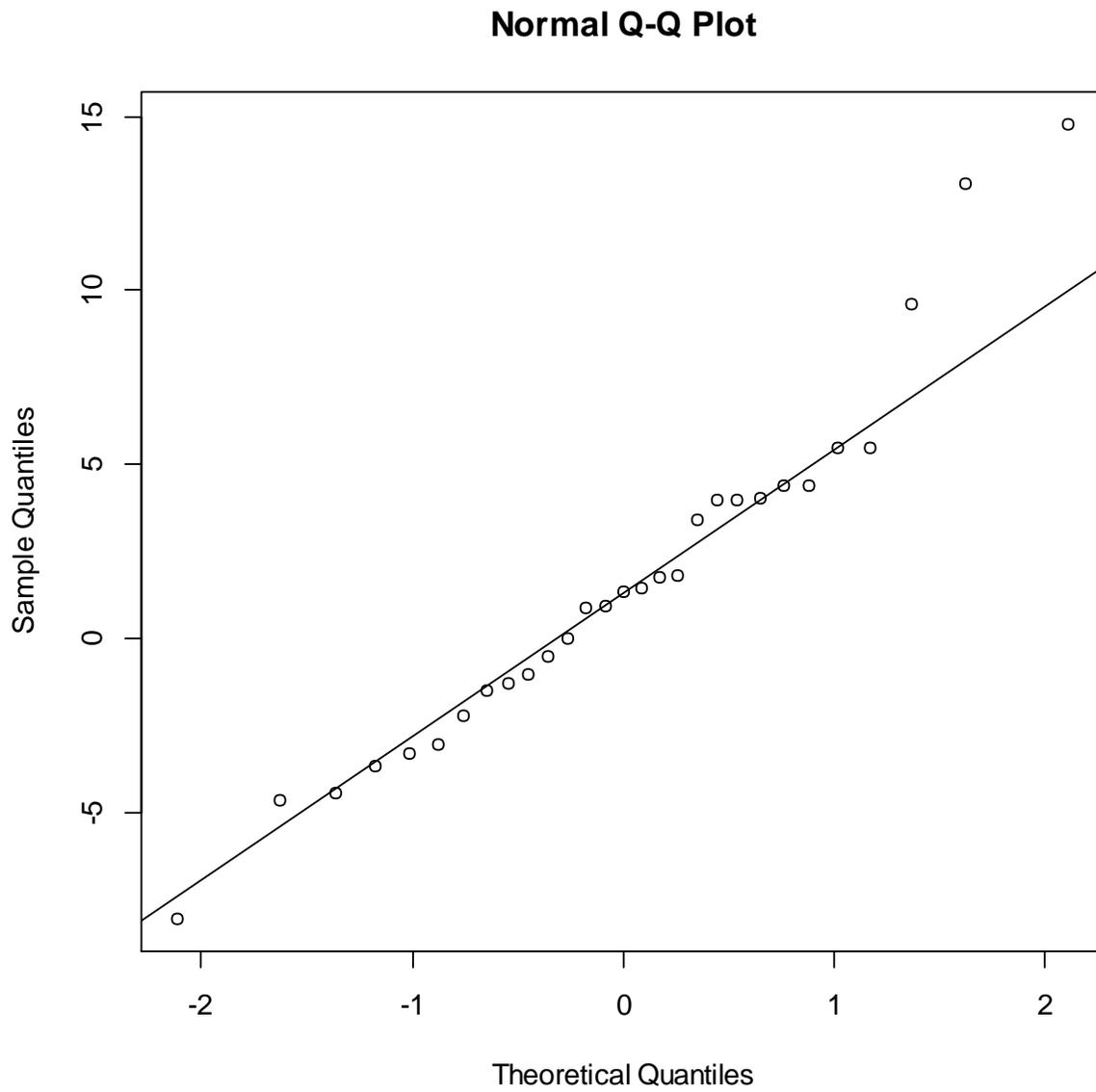


Figure 3.9 - QQ-plot of the deviance residuals from the (non-spatial) Poisson regression model.

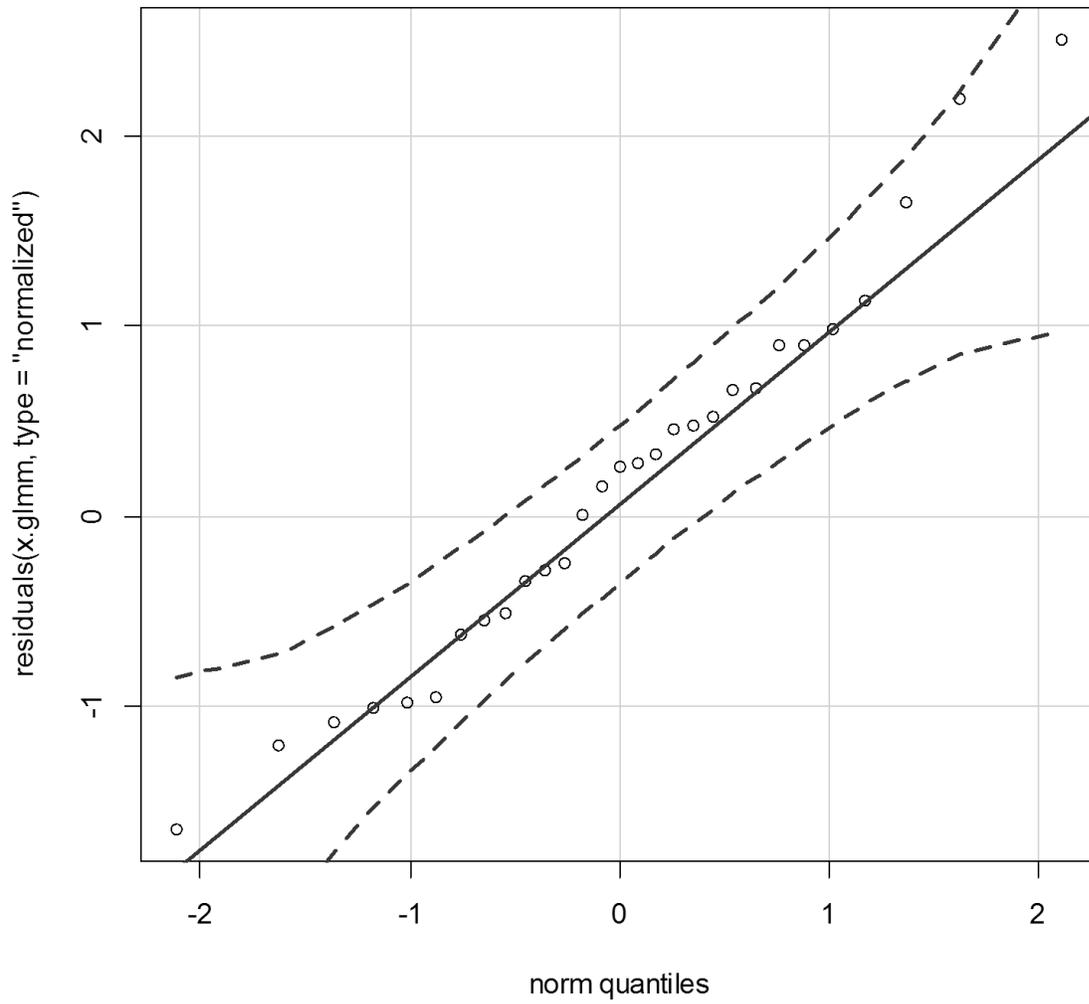


Figure 3.10 - QQ-plot of the residuals from the spatial Poisson regression model.

CHAPTER FOUR:

Discussion, Limitations, Future Directions and Conclusions

DISCUSSION

The overall goal of this thesis was to explore spatial patterns of, and the spatial relationship between, human and bovine cryptosporidiosis across the 29 Public Health Unit (PHU) areas in Southern Ontario from 2011 to 2014, using the core spatial analytic methods of visualization, exploration and modelling. In chapter 2, the spatial distribution of the farm prevalence of bovine cryptosporidiosis in Southern Ontario was described using choropleth and isopleth maps. The raw farm-level prevalence of bovine cryptosporidiosis for Southern Ontario was estimated to be 45% [95%CI: 42%-48%] and ranged from 0% [95%CI: 0%-8%] to 62% [95%CI: 35%-87%] across the 29 PHU areas of Southern Ontario. The choropleth map of the spatial distribution of the farm-level prevalence showed that in Southern Ontario, bovine cryptosporidiosis is highly prevalent and widespread [Figure 2.3]. Furthermore, a high-risk cluster of bovine cryptosporidiosis was identified in the Central West region of Southern Ontario.

In chapter 3, the spatial distribution of human cryptosporidiosis for each year of the study period and for the overall study period were described using choropleth and isopleth maps. The global raw cumulative incidence of human cryptosporidiosis was 6.91 [95%CI: 6.47-7.39] cases per 100,000 population over the four-year study period. The choropleth maps of the spatial distribution of the incidence of cryptosporidiosis demonstrated that the disease is quite widespread within the human population of Southern Ontario, with all but one PHU area reporting domestic cases for each year in the study period [Figures 3.1 – 3.4]. Higher annual

incidences of human cryptosporidiosis were consistently observed in PHU areas in the Central West region of Southern Ontario; moreover, the isopleth risk maps identified PHU areas in the Central West region as being at a higher risk of infection [Figures 3.5 – 3.6]. Using the flexible spatial scan test, high risk disease clusters were detected for each year of the study period. Over the four-year study period these clusters were consistently located in the Central West and East regions of Southern Ontario, with relative risks of disease that ranged from 2.03 [95%CI: 1.63-2.55] to 6.87 [95%CI: 5.07-9.30]. When Poisson and spatial Poisson models were used to determine if there was a relationship between the spatial distribution of human cryptosporidiosis and cattle density, calf density, dairy cattle density and farm-level prevalence of bovine cryptosporidiosis, dairy cattle density in Southern Ontario was found to be a predictive factor in the distribution of human cryptosporidiosis.

Burden of Cryptosporidiosis among Southern Ontario Cattle Farms

Previous epidemiological studies on the prevalence of *Cryptosporidium* on cattle farms in Southern Ontario have identified cryptosporidiosis as a highly prevalent and potentially endemic disease amongst the cattle farm population in several regions of Southern Ontario (Trotz-Williams *et al.*, 2005; Dixon *et al.*, 2011). The results of this study, specifically the estimated overall (global) raw farm prevalence of 45% and the widespread distribution of cryptosporidiosis in 23 of the 29 PHUs, further supports these findings. Of the approximately 3,731 dairy farms registered in Canada, one third are located in Southern Ontario (Statistics Canada, 2014; Statistics Canada and Canadian Dairy Commission, 2016). Given that *Cryptosporidium* predominantly affects pre- to post-weaned dairy calves, the widespread detection of cryptosporidiosis in Southern Ontario is not surprising (Abeywardena *et al.*, 2015; O'Donoghue, 1995).

The farm-level prevalence estimates for this study slightly differed when compared to the estimates from studies conducted by Trotz-Williams *et al.* (2005) and Dixon *et al.* (2011). These differences are likely due to study design, population and geographical area. Those studies used a sample-based study designs (active sampling) to determine farm-level prevalence and focused on only dairy or beef cattle respectively in specific regions of Southern Ontario, while this study used passive surveillance data (i.e. laboratory diagnostic data) to estimate the prevalence among cattle in general—both dairy and beef—in Southern Ontario.

This is the first study to (1) estimate the farm-level prevalence of bovine cryptosporidiosis for all Southern Ontario and (2) use passive surveillance data (i.e. laboratory diagnostic data) to estimate the farm-level prevalence of bovine cryptosporidiosis in Ontario.

Spatial Distribution of Bovine Cryptosporidiosis in Southern Ontario

Given the nature of bovine cryptosporidiosis, it was expected that the spatial distribution of the farm-level prevalence of bovine cryptosporidiosis might be similar to the distribution of cattle density—specifically dairy cattle density—in Southern Ontario. The isopleth risk map [Figure 2.5] indicated that the risk of bovine cryptosporidiosis might be slightly higher in two regions in Southern Ontario: Ottawa-Carleton area and the Central West region. These two areas are known for high dairy cattle density in comparison to other regions in Southern Ontario (Statistics Unit, OMAFRA, & Statistics Canada, 2017). A statistically significant high-risk disease cluster of bovine cryptosporidiosis was identified in the Central West region of Southern Ontario, confirming that the risk of bovine cryptosporidiosis is indeed higher, by 30%, in the cattle population in this area as compared to the overall risk in the area outside the cluster.

Spatial Patterns of Human Cryptosporidiosis in Southern Ontario: Association with Cattle

The association between cattle density and the occurrence of human cryptosporidiosis has been demonstrated in multiple epidemiological studies on outbreaks in specific populations and on source attribution studies of waterborne and foodborne outbreaks. Further, previous spatial epidemiological studies on the association of spatial patterns of cryptosporidiosis have found an association between the spatial distribution of cattle or livestock density and the distribution of human cryptosporidiosis (Jagai *et al.*, 2010; Pollock *et al.*, 2010). For example, in a study conducted by Jagai *et al.* (2010), it was found that increased spatial exposure to cattle was a significant risk factor for human infections with *Cryptosporidium* in United States (Jagai *et al.*, 2010). As well, in a spatial temporal epidemiological study on sporadic human cryptosporidiosis cases in Scotland, zoonotic *Cryptosporidium*, *C. parvum*, caused human infections predominantly in rural areas and areas with high ruminant livestock density (Pollock *et al.*, 2010). The findings from this study, specifically the association of dairy cattle density in Southern Ontario with the spatial distribution of human cryptosporidiosis, further supports the global body of evidence that cattle—specifically dairy cattle—play an important role in the transmission of this disease among humans and furthermore amongst the Southern Ontario population.

The spatial distribution of human cryptosporidiosis in Ontario has been described in surveillance reports by Public Health Ontario; however, this is the first study to conduct an in-depth spatial analysis on human cryptosporidiosis in Southern Ontario. The body of literature concerning the role of cattle in the transmission dynamics of human cryptosporidiosis in Ontario is limited (Trotz-Williams *et al.*, 2006). In fact, to the author's knowledge, only one other study has analyzed human and cattle cryptosporidiosis data in Ontario together as a means of assessing

the importance of dairy cattle in the occurrence of human cryptosporidiosis (Trotz-Williams *et al.*, 2006). Although this study cannot draw a direct causal link between the occurrence of cryptosporidiosis in cattle and in humans, it does demonstrate that there is an association between dairy cattle density and human cryptosporidiosis. Future studies should further explore this relationship in order to develop relevant public health prevention strategies.

LIMITATIONS AND GENERALIZABILITY

Interpreting the results of this thesis should include consideration of data sources and the spatial aggregation level. First, passive surveillance data, specifically laboratory diagnostic data from the AHL at the University of Guelph, were used to determine the farm prevalence of bovine cryptosporidiosis and to study the spatial variation of the farm-level prevalence in Southern Ontario. In Southern Ontario, AHL is not the only facility capable of providing diagnostic testing for cryptosporidiosis. As well, since cryptosporidiosis is a common disease amongst calves, it is often diagnosed based on common clinical signs. In fact, in most instances it is highly uncommon for cattle diarrhea cases to result in diagnostic testing; submissions to the AHL for diagnostic testing usually occurs because of uncommon signs, or two calves in a herd presenting with diarrhea or because of larger herd outbreaks. Due to the use of this data source, it is very likely that the estimates provided in this study are an underrepresentation of the true burden of bovine cryptosporidiosis in Southern Ontario and only represent the “tip of the iceberg” of this disease. As well, since the estimated prevalence may only reflect cryptosporidiosis in a specialized cattle farm population in Southern Ontario (i.e. farms with severe and unusual presentations of the disease) these results may not be generalizable to the overall cattle farm population of Southern Ontario. Furthermore, there could possible have been a bias in the estimated prevalences due to difference in veterinary testing practices. Some veterinarians may

test more actively than others meaning that the resulting prevalence estimates from this source of diagnostic data may reflect veterinarian testing practices and not the actual geographical distribution of bovine cryptosporidiosis in Southern Ontario.

Second, cattle farms were spatially aggregated into their respective PHU areas using the postal code of the veterinarian who submitted the specimen for testing since the actual address of the cattle farm wasn't available. In Canada, individual postal codes, especially in rural areas, can span multiple PHU areas. In order to aggregate cattle farms into one PHU area, a single link indicator provided by Statistics Canada's PCCF was used to create one-to-one relationships between the postal code and a PHU. Further details on this method are outlined in Chapter 2. Due to using this method to spatially aggregate the data, it is likely that some farms were assigned to the wrong PHU; however, any spatial misclassification that resulted from this is likely to have been non-differential. Non-differential misclassification blurs the true spatial pattern of the disease making it more difficult to identify a cluster. Therefore, since a cluster was identified in this study, it is likely that the risk in the population in the identified cluster is higher than what was estimated.

Third, since each owner's postal code was only available for a subset of specimens, their veterinarian's postal code was used as a proxy for farm location. Based on the distance assessment between owner location and veterinarian location, using veterinarian location as a proxy for the assumed location of exposure (farm location) may have resulted in the spatial misclassification of over 40% of the farms.

Finally, in Chapter 3, passive reportable disease surveillance data were used to determine the incidence of human cryptosporidiosis and to study the spatial variation of human cryptosporidiosis in Southern Ontario. Passive surveillance data, even for reportable diseases, is

subject to underreporting (Majowicz *et al.*, 2005), meaning that the incidence rates calculated are likely to have been underestimates. However, since the data received from PHO were likely to have been proportionally representative to the true number of cases in each underlying population, these data were nevertheless useful for understanding the spatial epidemiological patterns in Southern Ontario.

FUTURE RESEARCH

Based on the research conducted in Chapters 2 and 3, and the information provided in both the literature review and discussion chapters, the following future research should be considered:

- **Targeted Surveillance:** The findings of this study indicate that the Central West region had higher incidence and prevalence of human and bovine cryptosporidiosis, respectively. Targeted surveillance within this region may be needed in order to further understand the transmission dynamics of cryptosporidiosis in this area and to identify potential environmental factors that may also be contributing to higher rates of cryptosporidiosis in this area. This information is important for ensuring that proper public health measures can be put in place to prevent the transmission of this disease.
- **One Health Studies:** Cryptosporidiosis is a parasitic zoonotic disease. Thus, future studies trying to understand the transmission dynamics amongst a population should employ a One health study design. Further One health studies on this disease in Southern Ontario will be key in fully understanding the nature of this disease in this region.

CONCLUSION

Cryptosporidiosis is an infectious disease that requires a One Health approach to adequately describe and understand transmission dynamics within human and animal populations. This thesis used a One Health approach of linking animal and human populations to their environments to better understand spatial patterns and associations between human and bovine cryptosporidiosis in Southern Ontario. The findings from this research suggest that dairy cattle play an important role in the distribution of human cryptosporidiosis. The information contained in this thesis should encourage further One Health studies to understand the transmission of *Cryptosporidium* in Southern Ontario, and the specific role dairy cattle play in this transmission.

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