THE SPATIAL AND MOLECULAR EPIDEMIOLOGY OF LYME DISEASE IN EASTERN ONTARIO

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Table of Contents

List of Tables

Supplemental Tables

List of Figures

Supplemental Figures

Abbreviations

AP	*Anaplasma phagocytophilum*
AUC	Area under the receiver operating curve
BB	*Borrelia burgdorferi*
BM	*Borrelia miyamotoi*
BMD	*Borrelia miyamotoi* disease
BO	*Babesia odocoilei*
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CLD	Chronic Lyme disease
CLyDRN	Canadian Lyme disease Research Network
CSD	Census subdivision
DA	Dissemination area
DNA	Deoxyribonucleic acid
DTV	Deer tick virus
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
ENM	Ecological niche model
EOH	Eastern Ontario Health Unit
FSA	Forward sortation area
GIS	Geographic information system
HGA	Human granulocytic anaplasmosis
IB	Immunoblot
IgG	Immunoglobulin G
IgM	Immunoglobulin M
KFL	Kingston, Frontenac, Lennox, and Addington Health Unit
LD	Lyme disease
LGL	Leeds, Grenville, and Lanark Health Unit
LISA	Local indicators for spatial association
MLST	Multi-locus sequence typing

MTTT	Modified two-tiered testing
NML	National Microbiology Laboratory
ON-Marg	Ontario Marginalization Index
OR	Odds ratio
OspC	Outer surface protein C
OTT	City of Ottawa Health Unit
PCR	Polymerase chain reaction
PHAC	Public Health Agency of Canada
PHO	Public Health Ontario
PHU	Public health unit
POW	Powassan virus
PTLDS	Post-treatment Lyme disease syndrome
qPCR	Quantitative polymerase chain reaction
RIO	Rurality Index for Ontario
RNA	Ribonucleic acid
RPDB	Registered Persons Database
RST	Ribosomal sequence type
s.l.	Sensu lato
s.s.	Sensu stricto
SDM	Species distribution model
ST	Sequence type
STTT	Standard two-tiered testing
TBRF	Tick-borne relapsing fever

Abstract

Lyme disease is an emerging tick-borne illness in Canada, with human case numbers increasing 15- to 20-fold since Lyme disease became nationally notifiable in 2009 until the present. In Ontario, Canada's largest province by population, average Lyme disease incidence across the province is similar to that of national estimates. However, in eastern Ontario, which is near tick endemic regions in the northeastern Unites States, Lyme disease incidence is disproportionately higher compared to the rest of the province.

The objectives of this thesis are to identify environmental Lyme disease risk areas in Ontario, to explore spatiotemporal trends in Lyme disease emergence, and to identify neighbourhood-level socioecological risk factors for Lyme disease. In addition, this thesis also aims to assess the risk of other tick-borne illnesses that are transmitted by the blacklegged tick, *Ixodes scapularis*, which is also the main vector for Lyme disease in Canada.

Using maximum entropy species distribution modelling to correlate blacklegged tick occurrence data with environmental variables, predictive risk models for *I. scapularis* and the Lyme disease pathogen, *Borrelia burgdorferi*, were developed. The model prediction was used to classify low and high environment risk areas and, using a case-control epidemiological study, we assessed that residence in risk areas was a strong predictor of Lyme disease. However, this relationship was modulated by socioecological factors linked to higher overall rurality of the locality of home residence. Spatial cluster analyses further revealed that human Lyme disease cases clustered in regions with the high numbers of reported *B. burgdorferi*-infected ticks in the environment. Many individuals residing in large metropolitan regions, like the City of Ottawa, reported tick exposures

outside their public health unit of residence; however, local clusters of Lyme disease were also detected in suburban regions near conservation areas, trails, and urban woodlands. The prevalence of other tick-borne pathogens was low, although several pathogens of public health significance including *Borrelia miyamotoi* and *Anaplasma phagocytophilum* were detected at multiple sites surveyed for ticks between 2017-2021.

Overall, this thesis identify patterns in Lyme disease emergence (and potentially other tick-borne illnesses), defines environmental risk areas for Lyme disease in Ontario, and highlights important socioecological risk factors for Lyme disease in eastern Ontario.

Acknowledgements

First and foremost, I would like to thank my thesis supervisor, Dr. Manisha Kulkarni, for her contribution to this work and her overwhelming support and encouragement over the years. Your support is the reason I was able to complete this thesis, despite the many challenges that were brought on by countless administrative hurdles and a global pandemic. I will be forever grateful for your understanding, flexibility, kindness, and mentorship over the years.

At the start of this thesis, I was among the first students in Dr. Kulkarni's lab working on Lyme disease research, but as time passed, I had the privilege of working with so many talented and dedicated individuals, whom I must thank for their assistance and contribution to my thesis. I would like to thank Dr. Roman McKay for coordinating the field surveillance and helping with the many laboratory assays, Dr. Benoit Talbot for field work and always helpful feedback, and the rest of the wonderful students who have donned the white overalls in the field: Mr. James Logan, Ms. Claudia Duguay, Ms. Katarina Ost, Ms. Olivia Facchin, and many more!!

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1

1 INTRODUCTION

Would you be surprised to learn that Lyme disease (LD) is the most common vector-borne illness in Canada? From 2009–2021, public health units have reported 14,616 human cases of LD across Canada (1). In approximately a decade, Canada saw nearly a 20-fold increase in the reported numbers of LD cases, leading to the identification of LD as a priority emerging vector-bone illness by the Government of Canada (1, 2). The 'Federal Framework on Lyme Disease Act, 2017' identified key government departments and agencies, including the Public Health Agency of Canada (PHAC) and the Canadian Institutes of Health Research (CIHR), to support and fund LD research with the aim to prevent and control the spread of LD in Canada (2). Co-funded by PHAC and CIHR, the Canadian Lyme Disease Research Network (CLyDRN) was established in 2018 to continue to address critical knowledge gaps on LD emergence and to support national collaboration (3).

My research was conceptualized to meet research initiatives proposed by the 'Federal Framework on Lyme Disease Act' and the Government of Ontario's 'Provincial Framework and Action Plan concerning Emerging Vector-Borne Diseases Act, 2015' and is nested within several CIHR- and CLyDRN-funded projects led by my thesis supervisor, Dr. Manisha Kulkarni (4). The main goals of these projects were to link entomological and epidemiological LD surveillance data within a spatial analytic framework, focusing on eastern Ontario. My role was to develop environmental risk models for LD, conduct and support LD surveillance activities through field and laboratory studies, design epidemiological studies looking at determinants of LD, and analyze human and tick surveillance data for eastern Ontario.

My thesis addresses timely and important public health questions on the risk of LD and other tick-borne illnesses in Ontario, Canada, within the context of where tick vectors and their pathogens are currently emerging. The novelty of my thesis also stems from the use of multidisciplinary methods to identify LD risk in the Canadian setting from numerous viewpoints – entomological, epidemiological, and molecular. My thesis applies a One Health approach to better understand and tackle the complex challenge of tick-borne illnesses at the human-animal-environment interface, leading to more comprehensive monitoring and control of LD and other tick-borne illnesses in newly emerging areas (5).

1.1 THESIS STRUCTURE

Chapter 1: Introduction

The introductory chapter of my thesis describes my research objectives and explores the conceptual framework and entomological risk model forming the foundation of my work. It describes the epidemiological trends associated with LD emergence in Canada and, more specifically, LD emergence in Ontario where my research is situated. I also describe the current LD surveillance systems in place in Canada and briefly discuss the specific research initiatives set by the national and provincial frameworks concerning tick-borne illnesses that have inspired my work and provided funding.

Chapter 2: Background

The second chapter provides a more thorough discussion of the concepts explored throughout my thesis as well as supporting background information for readers unfamiliar with LD and tick-borne illnesses. More specifically, I discuss LD clinical manifestations, diagnosis, and treatment, blacklegged tick phenology, ecology, and the transmission of tick-borne pathogens, *Borrelia burgdorferi* biology and genetic diversity in the context of LD severity, and, finally, a brief overview of other emerging tick-borne illnesses of public health concern.

Chapter 3: Methods

The specific methods for each research objective are presented in the corresponding articles; however, this third chapter provides a more descriptive overview of the methods used throughout my thesis. First, I describe the study area and my data sources. I discuss the regression models and spatial methods that I used to explore the link between human LD incidence and tick surveillance data, the design and analysis of a matched case-control study looking at determinants of LD, and

the development of a species distribution model for blacklegged ticks using maximum entropy modelling. Lastly, I also provide a brief overview of the laboratory and molecular analyses that I developed and used to generate data for my research.

Chapters 4-7: Articles

The next four chapters contain my thesis articles. For each article, I include a short preface with the article citation, coauthor contributions, ethics approvals received, and any supplemental materials or associated appendices. In my first article, published in *PLOS One*, I develop maximum entropy species distribution models for the blacklegged tick and the LD-causing pathogen to identify predicted environmental risk areas for LD in Ontario, Canada. In my second article, published in *Vector-Borne and Zoonotic Diseases*, I design a matched case-control study to assess determinants of LD and the association between LD infection and residence in model-predicted environmental risk areas. In my third article, published in *BMC Public Health*, I explore the larger spatiotemporal trends associated with LD emergence in Ontario, Canada with specific focus on four public health units in eastern Ontario that have the highest provincial incidence of LD. In this paper, I also focus on tick exposure location rather than home residence to get a better understanding of where individuals are encountering ticks and becoming exposed to LD. My fourth article, an unpublished short report to be submitted for peer-review in the coming months, looks at the prevalence of other tick-borne pathogens and the genetic diversity of *B. burgdorferi* in eastern Ontario. This article provides an important update on other emerging tick-borne illnesses of public health significance in Ontario, Canada and the bacterial sequence types that are found throughout this region, which may play a role in LD severity and diagnosis.

Chapter 8: Discussion

In my last chapter, I provide a summary of my research findings and discuss how these fit into our current understanding of LD emergence in Canada. I also outline how my thesis fits within the context of my other published works and contributions as well as the research that has emerged from my published articles. Lastly, I present some limitations of my research objectives and propose areas that require further research.

1.2 OBJECTIVES AND CONCEPTUAL FRAMEWORK

1.2.1 Thesis Objectives

The overall goal of my thesis was to fill critical knowledge gaps on the epidemiology and process of LD emergence in eastern Ontario, with the aim to develop practical risk assessment tools (e.g., risk maps for LD and emerging tick-borne illnesses) to help improve prevention and control of LD in a Canadian setting, where LD is rapidly emerging and posing a growing risk to a larger population number.

As shown in Figure 1.1, my thesis uses a multidisciplinary approach, combining methods in ecology, molecular biology, and epidemiology, to assess human LD risk in Ontario, Canada. My thesis contributes to current research efforts directed at mitigating the risks of LD and other tick-borne illnesses by addressing the following research questions: Where in the environment are ticks present and what factors contribute to their distribution and spread? What is the prevalence of infection with tick-borne pathogens in newly endemic tick populations? Where are humans

encountering ticks and what are determinants of LD in Ontario? These research questions are critical in a setting like Ontario, Canada where LD and other tick-borne illnesses are still emerging and where diagnosis and treatment of tick-borne illnesses largely depend on an underlying knowledge of the local risk.

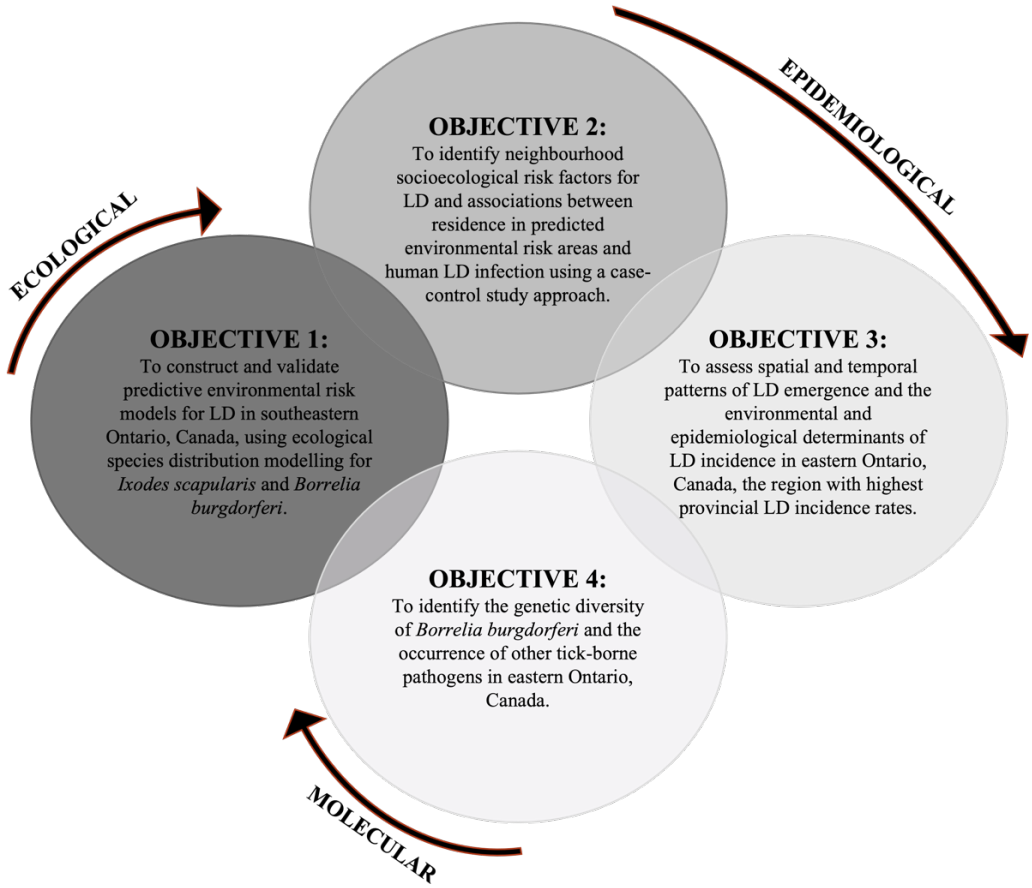

Figure 1.1 Multidisciplinary approach and specific thesis objectives.

To address these research questions, my thesis looks at four specific objectives:

1. To construct and validate predictive environmental risk models for LD in southern and eastern Ontario, Canada, using ecological species distribution modelling for *Ixodes scapularis* and *B. burgdorferi*.

2. To identify neighbourhood socioecological risk factors for LD and associations between residence in predicted environmental risk areas and human LD infection using a case-control study approach.
3. To assess spatial and temporal patterns of LD emergence and the environmental and epidemiological determinants of LD incidence in eastern Ontario, Canada.
4. To identify the genetic diversity of *B. burgdorferi* and the occurrence of other tick-borne pathogens in eastern Ontario, Canada.

1.2.2 Conceptual Framework

The scope of my thesis and the various research components and variables used for analysis were delineated from the conceptual model shown in Figure 1.2, which I adapted for LD from a risk model for malaria and water-related vector-borne diseases (6). At the core of this conceptual framework is the coupling of hazard and vulnerability to assess the risk of vector-borne diseases. This model encourages risk management from both societal and environmental perspectives and was first defined in the context of climate change by the Intergovernmental Panel on Climate Change (IPCC) and the World Health Organization (WHO) (7, 8). Hazard and vulnerability have a wide range of definitions according to the context under investigation (e.g., natural disaster risk, infectious disease risk, and vector-borne disease risk). For my thesis, I adapted definitions proposed by Kienberger and Hagenlocher (2014) and reformulated them to fit the context of LD based on the ecology and life cycle of ticks and my understanding of LD transmission and the factors that contribute to LD infection.

In the context of vector-borne diseases, Kienberger and Hagenlocher (2014) defined hazard as "the potentiality of disease occurrence in a geographic area or time frame, and is characterized by location, magnitude, frequency, and probability" (6). For LD and tick-borne illnesses, hazard can be modeled as the probability of an infective tick bite, which can in turn be approximated by the density of infected ticks (or infected nymphs) in the environment (9-11). The nymphal stage generally poses the highest risk for human LD infection due to peak nymph activity during summer months when people are more likely to participate in outdoor activities in tick habitats and the small size of nymphs, which make them less likely to be removed before transmission of pathogens. The density of infected ticks in the environment is affected by various ecological factors including tick life cycle and questing behaviour, availability and number of animal hosts, climate changes, land use, forest fragmentation, and speed of spread, as well as pathogen dynamics such as vector competence, transmission efficacy, and duration of infection in ticks.

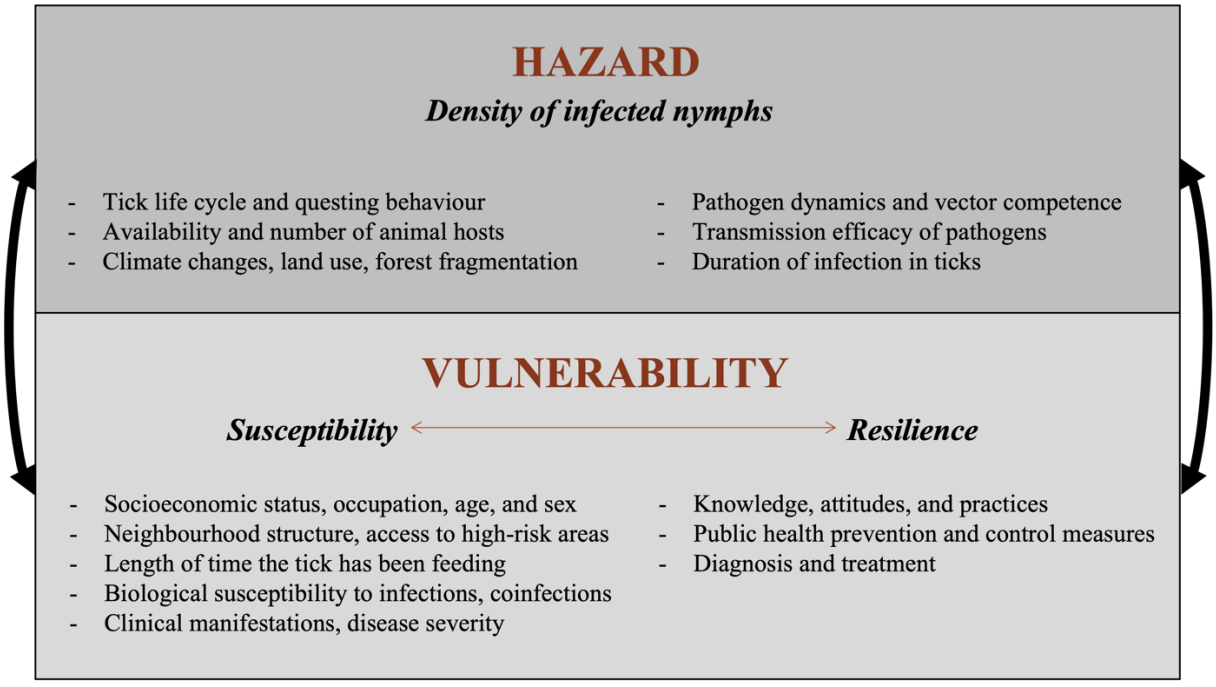

Figure 1.2 Overall conceptual model of the thesis.

Kienberger and Hagenlocher (2014) define vulnerability as “the predisposition of the society and its population to the burden of vector-borne diseases”. They further break this down into two components – differences in disease susceptibility among the population and resilience or adaptation to vector-borne diseases (6). Susceptibility to LD can be affected by risk factors such as socioeconomic status, occupation and engaging in risk behaviours, neighbourhood structure and access to high environmental risk areas, as well as biological elements such as length of time the tick has been feeding, biological susceptibility to infections, LD clinical manifestations and disease severity, and possible coinfections. Resilience to LD, on the other hand, can be thought of as individual knowledge, attitudes, and practices related to ticks and tick-borne illnesses, public health prevention and control measures, and the diagnosis and treatment of LD and other tick-borne illnesses.

1.2.3 Linking the Conceptual Model to Thesis Objectives

In my thesis, I address various aspects of LD risk management that are outlined in my conceptual framework. My first objective is centered on assessing the hazard component by identifying tick ecological habitat suitability across Ontario and the biotic and abiotic factors that contribute to this. This will help identify the distribution of ticks in Ontario and, by proxy, the predicted environmental risk for LD. While humans can become exposed to LD through adventitious ticks that are dispersed by migratory birds or other animal hosts, the risk of acquiring LD is higher in regions where ticks are well established in the environment (11). Understanding the ecological conditions that favour tick establishment is therefore a key factor for assessing environmental risk for LD and other tick-borne illnesses.

My second and third objectives focus on human susceptibility to LD by identifying risk factors that contribute to higher LD incidence in Ontario. First, using a matched case-control study approach, I aim to identify socioeconomic and ecological determinants for LD and the impact of residence in high model-predicted environmental risk areas for LD. It is often assumed that exposure to ticks occurs peri-domestically; however, evidence for this stems from studies in the USA and Europe, where tick populations are endemic in regions with a high human population density and may not be reflective of the Canadian setting. Furthermore, there are currently no other studies looking at socioeconomic determinants of LD in Canada. This objective will provide novel evidence for LD risk factors and help contribute to our understanding of LD exposure near the home and the characteristics of neighbourhood with high LD incidence.

My third objective builds off the previous two by linking human disease surveillance data and tick surveillance data to understand where humans are most likely exposed to ticks. I use spatial methods to identify clusters of human cases and infected ticks and I identify socioecological factors that contribute to higher LD incidence in four eastern Ontario health units with the highest provincial incidence of LD. This objective also focuses on more accurately assessing the location of tick exposure and the characteristics of these regions rather than those of an individual's home residence, in contrast to previous studies in Ontario.

My fourth objective looks at LD resilience by assessing the genetic diversity of *B. burgdorferi* and the distribution of sequence types in eastern Ontario. There is evidence that the severity of LD and the diagnosis and treatment of various LD stages are associated with specific *B. burgdorfe*ri

genotypes. Identifying the diversity of bacterial sequence types may help inform health care practitioners of the potential risks for different clinical manifestations and guide the development of new diagnostic testing that can identify currently circulating bacterial genotypes. Furthermore, my fourth objective also aims to identify the prevalence of other tick-borne pathogens in ticks and the rate of coinfections with *B. burgdorferi*, which can also have significant public health impacts.

The thesis objectives, data sources, outcomes of interest and the analytic approach used for each research objective is summarized in Table 1.1.

Table 1.1 Summary of data sources, outcomes of interest, and overall analytic approach for each thesis objective.

Objective	**Data Sources**	**Analytic Approach**
Objective 1	Active tick surveillance data	Maximum entropy species distribution modelling to identify predicted probability of tick occurrence and biotic and abiotic factors associated with tick habitat suitability
Objective 2	Reportable disease surveillance data and environmental model generated in objective 1	Conditional logistic regression model for matched case-control study looking at socioecological risk factors for LD and residential exposure to predicted high-risk areas
Objective 3	Reportable disease surveillance data and passive tick surveillance data	Negative binomial regression model looking at determinants of LD and cluster analyses (spatial scan statistic, local indicators of spatial autocorrelation) to assess link between environmental risk and human LD infection
Objective 4	Active tick surveillance data	Descriptive analysis of distribution and prevalence of other tick-borne pathogens and genetic diversity of *B. burgdorferi*

1.3 Lyme Disease Emergence in Canada

1.3.1 What is Lyme Disease?

LD is a vector-borne inflammatory disease, with varying clinical manifestations, that is initiated after a bite from a tick infected with the *Borrelia burgdorferi* bacterium (or other members of the *B. burgdorferi sensu lato* complex) (12, 13). The tick vectors acquire *B. burgdorferi* by feeding on wildlife reservoirs of the bacteria such as rodents and birds during their earlier developmental stages and subsequently transmit the infection to new hosts during their next blood meal (12, 14). In Canada, the vectors for LD are the eastern blacklegged tick, *Ixodes scapularis*, east of Manitoba and the western blacklegged tick, *Ixodes pacificus* west of the Rocky Mountains (15). While *I. scapularis* has been attributed to LD infections in Alberta and Saskatchewan, there are currently no endemic populations of the blacklegged tick in these regions and reported infections are likely due to adventitious ticks transported by migratory birds (16, 17). Currently, there are no reported LD cases or blacklegged tick populations in the northernmost provinces or territories (15).

Over the past decade, there has been a substantial increase in the number of locally acquired LD cases in part due to climate changes, which have increased the abundance and geographic range of blacklegged tick populations across Canada and the frequency of tick-human interactions (18). In newly emerging regions, prevention, diagnosis, and timely treatment of LD are dependent on surveillance systems and public health measures aimed at identifying environmental risk areas where tick vectors are found as well as knowledge dissemination to the public and medical community to inform about the risk of acquiring LD and other tick-borne illnesses.

1.3.2 Epidemiology of Lyme Disease

There were an estimated 2,634 cases of LD reported in Canada in 2019, which is a substantial increase from 144 cases in 2009 when LD became nationally notifiable (1). The increase in LD case numbers is consistent with the growing geographic spread of *I. scapularis* populations in Canada but may also be affected by factors that increase exposure to ticks, including forest fragmentation and the building of homes in new LD risk areas, and changes in knowledge of LD risk and prevention methods (19-22). Over 94% of LD cases reported in Canada were acquired from three provinces: Ontario, Quebec, and Nova Scotia, with the latter having the highest provincial LD incidence at 85.6 cases per 100,000 population compared to the national incidence of 7.0 cases per 100,000 population (15). However, LD incidence varies substantially at finer geographic scales, with some regional health units in these provinces reporting rates well over the national and provincial estimates (23, 24). High incidence rates of locally acquired LD correspond with regions where reproducing tick populations are found in the environment (11).

LD cases are reported year-round; however, most are reported between May and November, with symptom onset occurring predominantly during the summer months of June, July, and August (15, 23). The seasonality of LD is attributed to two main factors: the two-year life cycle of ticks in which spring and summer months correspond to peak activity of smaller nymphal ticks that are less likely to be detected and the increased probability of human-vector interaction during warmer months (15, 25). LD symptom onset occurs on average within 30 days after a tick bite, which indicates that early spring exposure, when individuals may be less aware of the LD risk, likely contributes to the higher LD incidence over the summer months (26). Earlier springs, resulting from climate changes and milder temperatures, would also be expected to advance the seasonal

activity of tick vectors in regions where blacklegged ticks are endemic or render new regions more susceptible to the introduction and establishment of ticks, thereby increasing the risk of LD exposure (27).

LD incidence has been shown to follow a bimodal pattern with two peaks of high incidence among children (aged 5-14 years) and older adults (aged 50-69 years) (15, 23, 28). The bimodal age distribution for LD incidence has also been identified in Europe and the United States and is believed to be due to age-related behaviours and frequency of exposure to ticks (29-32). More than half of reported LD cases in Canada are male, which is also attributed to behavioural and occupational differences between the sexes, with women more frequently avoiding high-risk regions or health-related risk behaviours (33, 34). In a recent national survey, Canadians reported a high level of LD awareness, but most did not report adopting protective behaviours such as conducting tick checks, wearing protective clothing, and applying insect repellent (35). Recent data from Quebec shows that Canadians are still in the first stages of adopting preventive behaviors and that rates of adoption of specific behaviors vary widely from low (e.g., 4.3% of respondents put clothes in the dryer to eliminate ticks after being outdoors) to high (e.g., 83.3% of respondents regularly complete lawn maintenance such as mowing) (36).

1.3.3 Risk Factors for Lyme Disease

There are several epidemiological risk factors that predict LD incidence; however, many are not well defined within a Canadian context where LD is still emerging. Known determinants of LD include demographic characteristics such as age and sex, which in turn are associated with an individual's knowledge, attitudes, and practices towards LD exposure risk (35). Additional risk

factors for LD include frequenting tick habitats (e.g., trails and woodlands), pet ownership, time spent outdoors, participating in activities such as gardening, and working outdoors (35, 37-41).

In Europe and the United States, there is also evidence that LD infection is associated with metrics of socioeconomic status including higher average levels of education and income, which may be linked to residence in rural or suburban locations where ticks are more abundant, cottage ownership, or outdoor activities such as camping, gardening, walking the dog, or hiking in woodland habitats (30, 42). There is considerable evidence that home/yard and neighbourhood characteristics contribute to LD risk. Neighbourhood and yard risk factors for LD include residential property near woodlands, larger property size, presence of trees and shrubs, and seeing animals in the yard (40, 43-45). Recently, Fischhoff *et al.* (2019) conducted a systematic review and meta-analysis looking at yard-level, neighbourhood-level, and broader-level risk factors for LD in the United States and found that neighbourhood risk factors where most strongly associated with LD infection (45).

This highlights the need to better understand where individuals are exposed to ticks and to characterize sources of variation in risk to apply risk-reducing interventions at multiple scales. In Canada, there is currently limited knowledge on the socioeconomic and neighbourhood risk factors for LD. Research in this area is especially important as blacklegged ticks are expanding into increasingly peri-urban and domestic areas.

1.4 Lyme Disease Emergence in Ontario

1.4.1 Blacklegged Tick Expansion

In the late 1970's, *I. scapularis* ticks were first identified in Canada on deer populations in Long Point Provincial Park, Ontario, along the northern shore of Lake Erie (46). Long Point remained the only site in Canada with stable reproducing *I. scapularis* populations until the early 1990's (47). With an increase in the mean annual degree days above 0°C, the range expansion of the blacklegged tick was documented across Ontario, with populations establishing along Lake Erie, Lake Ontario, and the St. Lawrence River, with a hot spot in eastern Ontario (48-51). A model based on 19 years of tick surveillance data predicted the northern range of *I. scapularis* to advance at 46 km/year, with variations between 35-55 km/year depending on temperature (52). Recent evidence supports the rapid range expansion of blacklegged ticks in Ontario, although the speed may not be uniform as site colonization by *I. scapularis* occurs at a heterogenous rate behind the range front (50, 51).

1.4.2 Epidemiology of Lyme Disease in Ontario

In 2019, there were 1,168 cases of LD reported in Ontario, representing 44.3% of all cases in Canada (15). The provincial incidence of LD is approximately 8 cases per 100,000 population, although more recent estimates suggest the incidence has risen above 11.2 cases per 100,000 population (53). Eastern Ontario was identified as a hot spot for LD from which the range front of the blacklegged tick is expanding northward (50). Clustering of LD cases and *B. burgdorferi*-infected blacklegged ticks were also detected in the Kingston and Gananoque regions along the St. Lawrence River (51). The eastern region of Ontario has the highest incidence rate of LD with

128.8 cases per 100,000 population in the Leeds-Grenville and Lanark Health Unit (LGL), 87.2 cases per 100,000 population in the Kingston, Frontenac, and Lennox and Addington Health Unit (KFL), 28.6 cases per 100,000 population in the Hastings and Prince Edward County Health Unit (HPE), 18.1 cases per 100,000 population in the City of Ottawa Health Unit (OTT), and 13.5 cases per 100,000 population in the Eastern Ontario Health Unit (EOH) (23).

Other tick-borne pathogens and a higher seropositivity of tick-borne anaplasmosis in humans were also detected in eastern Ontario compared to other regions of the province (54-56). While the risk of other tick-borne illnesses is currently low in Ontario, the abundance and continued increase in *I. scapularis* populations may pose a growing risk for other emerging tick-borne illnesses as well as for LD.

1.5 Lyme Disease Surveillance

1.5.1 Human Reportable Disease Surveillance

In Canada, the National Notifiable Disease Surveillance System (NNDSS) has been in place since 1924 to facilitate the control of diseases under surveillance and to identify trends in the occurrence of communicable diseases (57, 58). In 1991, the federal government, in collaboration with the provinces and territories, created standardized case definitions for all diseases under national surveillance to enhance comparability across jurisdictions, with new editions released periodically to update the list of national notifiable diseases and their definitions (59). LD became nationally notifiable in 2009 following evidence of blacklegged tick establishment in regions of Canada (59).

The NNDSS collected data on the annual number of confirmed and probable LD cases reported to provincial and territorial public health organizations by clinicians or through provincial laboratories along with basic information on patient age, sex, and episode date (60). In 2010, the PHAC initiated the Lyme Disease Enhanced Surveillance (LDES) system in collaboration with provincial public health organizations to collect more detailed data on LD cases in Canada. The LDES captures additional details on possible location of tick acquisition within or outside Canada, forward sortation area of residence (FSA: the first three digits of postal code), clinical manifestations, and methods of laboratory diagnosis, although there are variations in the data provided by provinces participating in the LDES (60). Together, these disease surveillance systems help to identify trends in LD incidence, LD risk areas where people are exposed to ticks, and the types of clinical manifestations in Canada.

1.5.2 Passive Tick Surveillance

A national passive ticks surveillance program was established in Canada in 1990 to focus on the detection of blacklegged ticks (49). Passive tick surveillance involves the voluntary submission of ticks found by the public, usually as feeding stages attached to themselves or their pets, to provincial and federal health organizations via medical and veterinary clinics or local public health authorities (49). Provincial and federal laboratories collect information on the tick species, tick life stage (instar) and engorgement level, location where the tick was found, host species on which the tick was found, date of collection, as well as the submitter's city of residence, age and sex, and travel history during the period the tick was attached (49, 61). Ticks collected through passive surveillance are also sent to the National Microbiology Laboratory (NML) for pathogen testing,

though in recent years university labs have helped with testing local specimens as the number of ticks submitted by the public has increased (49).

Passive tick surveillance provides a signal of where ticks are occurring in the environment and a measure of human exposure to ticks and tick-borne pathogens (28, 62). However, passive tick surveillance is not ideal for detecting low tick densities during the early stages of tick emergence and it lacks specificity for detecting established tick populations since adventitious ticks, which occur sporadically both spatially and temporally as they are dispersed by animal hosts, may be encountered by humans and their pets (63). Additionally, passive tick surveillance is subject to a degree of bias as it varies by public health unit, with some regions intensifying public outreach efforts to detect ticks whiles other have scaled back efforts, and its detection of tick location in the environment relies on self-reported measures by the public (61).

1.5.3 Active Tick Surveillance

Active tick surveillance is another method used to collect information on LD risk by direct sampling of the environment such as parks, trail, and wooded areas near human habitation. Active tick surveillance involves the collection of ticks by animal capture (i.e., small rodent traps) or by drag sampling, which is a standardized method to capture questing ticks by dragging a 1-meter^2 flag or flannel cloth along vegetation for a specified person-time (64-66). Active tick surveillance is considered the gold standard method for measuring environmental risk for LD because it involves multiple site visits, certainty about the tick location, and a sensitive design aimed to capture host-seeking ticks or animal hosts for ticks. This type of surveillance estimates tick densities and the prevalence of tick-borne pathogens and captures precise information on

geographic locations where ticks are found (63). However, active surveillance also has some drawbacks. Notably, it is resource intensive compared to other surveillance systems and the site selection for tick dragging or animal capture are often based on earlier detection of ticks through passive tick surveillance (63).

1.6 RESEARCH PRIORITIES IN CANADA

1.6.1 Federal Action Plan on Lyme Disease

The Government of Canada developed the Federal Framework on Lyme disease in 2017 to help guide research on LD and other emerging tick-borne illnesses in Canada (2). The Federal Framework includes three pillars with the goals to track and monitor infectious disease threats, promote healthy behaviours, enhance knowledge transfer, and promote research and innovation.

The three pillars and their goals are:

1) Surveillance: to develop a national surveillance program to collect data on LD risk and track incidence rates and the burden of LD in Canada,
2) Education and awareness: to increase national awareness about LD and enhance the prevention, identification, treatment, and management of tick-borne illnesses,
3) Guidelines and best practices: to establish guidelines focusing on prevention, diagnosis, and treatment of LD in Canada.

These research initiatives were funded by the PHAC and CIHR, with a joint call for research in 2018 that led to the establishment of the CLyDRN (3).

1.6.2 Canadian Lyme Disease Research Network

CLyDRN is a multidisciplinary, patient-centered research program launched in 2018 in response to the federal action call on LD. CLyDRN's aims are to improve the diagnosis, surveillance, prevention, and treatment of LD in Canada by bringing together patients, physicians, social scientists, veterinarians, and academic and government researchers to develop solutions and strategies for filling critical knowledge gaps on LD and by facilitating engagement across all stakeholders (3). One of CLyDRN's four pillars is focused on assessing, tracking, and predicting the changing risk of LD in Canada. Key initiatives of this pillar include the establishment of a pan-Canadian surveillance program to collect and compare data across the country and the development of epidemiological case-control studies to identify determinants of LD in Canada and establish a baseline for LD risk factors, allowing future trends to be detected and studied (55).

1.7 CHAPTER SUMMARY

The goal of this thesis is to fill critical knowledge gaps on the epidemiology of LD in Ontario, Canada using a multidisciplinary approach. It aims to help improve LD risk management by identifying environmental and societal factors that contribute to the spread and establishment of blacklegged tick populations in eastern Ontario and human exposure to infected ticks. These goals were motivated by the Canadian Action Plan on LD and partially funded by the CLyDRN and CIHR as part of the national effort to help control and prevent the spread of LD and other tick-borne illnesses in Canada.

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2

2 BACKGROUND

2.1 BLACKLEGGED TICKS

2.1.1 What are ticks?

Ticks are obligate parasites that belong to the arachnid class (*Arachnida*) and the mite superorder (*Parasitiformes*) (1). Ticks are subdivided into two families – hard ticks (*Ixodidae*) and soft ticks (*Argasidae*) that have very different morphology, lifecycles, and ecology (2). Ticks are relatively large mites ranging in size from 1-5 mm when unfed and expanding up to 20 mm when engorged (2). They feed obligately on blood of vertebrate animals including amphibians, reptiles, birds, and mammals and can transmit a multitude of pathogens such as viruses, bacteria, and protozoans (2). In humans, soft ticks are vectors for tick-borne relapsing fever (TBRF) and hard ticks transmit Lyme disease (LD), anaplasmosis, babesiosis, tickborne encephalitis, and tickborne rickettsial diseases (3-5). In North America, the western blacklegged tick, *Ixodes pacificus* (found in western

North America), and the eastern blacklegged tick, *Ixodes scapularis* (found east of the Rocky Mountains), are the main vectors for LD (6, 7).

2.1.2 Blacklegged Tick Life Cycle

Like other arthropod species, ticks have multiple life stages and morphological forms (instars) during their developmental cycle and lifespan. Blacklegged ticks have four stages (egg, larva, nymph, adult), although *I. scapularis* generally has a 2-year life cycle while *I. pacificus* has a longer 3-year life cycle (8). Blacklegged ticks are "three-host" species, meaning that they feed on a new animal host during each life stage and complete the moult process off the animal on vegetation or the forest floor (2). Blacklegged ticks only take one blood meal per stage, followed by moulting, except for adult females, which oviposit following a blood meal and then die, and adult males, which rarely feed and die after mating (2).

As shown in Figure 2.1, blacklegged ticks hatch from eggs as larvae during mid- to late July and typically cluster at the site of hatching (8, 9). The larva instar, unlike the other stages, is a hexapod, measuring between 0.5-0.6 mm in size, that generally does not pose a risk of LD infection because there is no evidence of transovarial transmission of *Borrelia burgdorferi* from an infected adult female to the eggs, although other pathogens can be passed to the eggs (2, 10). Larvae can feed during the year of hatching, or they can overwinter on vegetation and seek a vertebrate host the following year (9). This results in two peaks of larval activity during August, when newly hatched larvae are feeding, and from May to June, when overwintered larvae are feeding (11). Larvae feed for up to a week and, after engorgement, drop off the animal, often in the nest of the host, where they moult into nymphs (2). The larval moulting phase depends on temperature and the timing of

the blood meal. Larvae that fed early in the year can moult in the summer while those that fed late in the summer will overwinter and moult next year, although survival is greater if moulting occurs before they overwinter (2, 12).

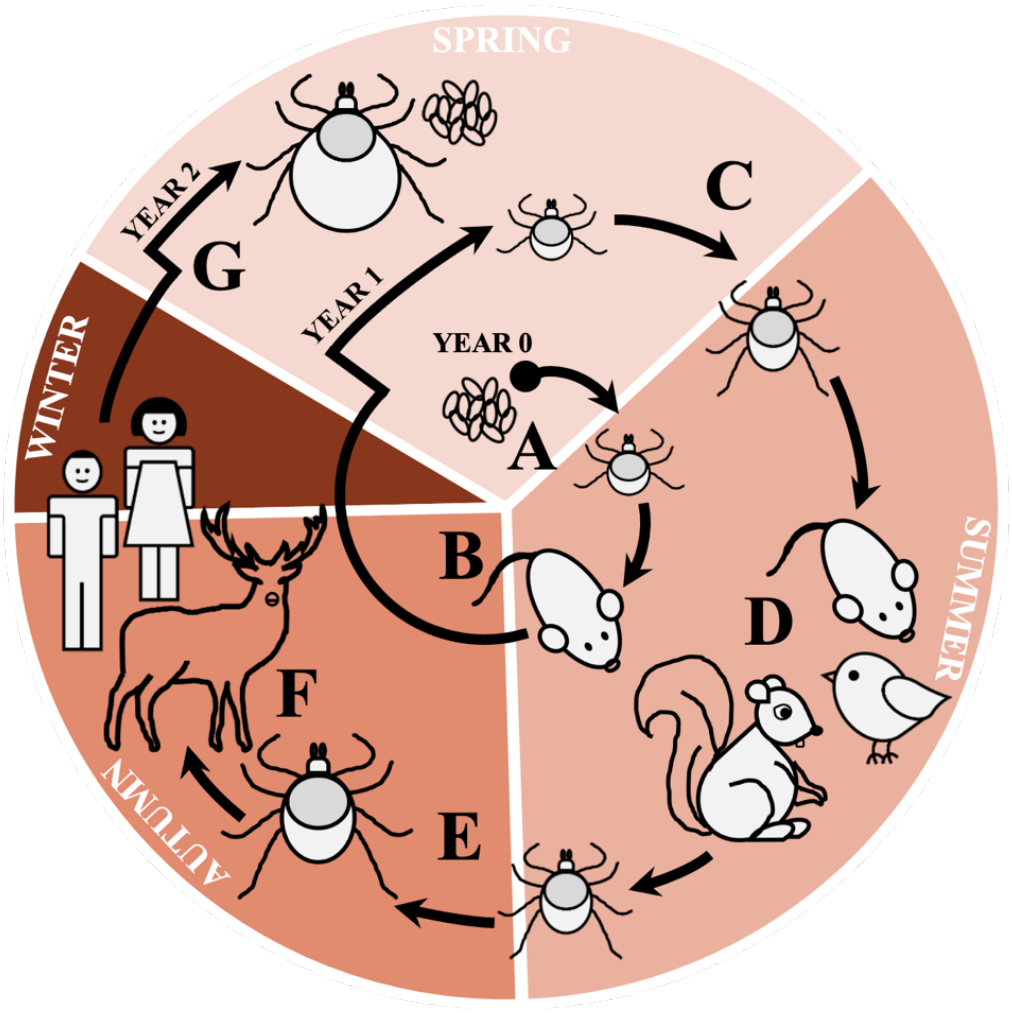

Figure 2.1 The 2-year life cycle of Ixodes scapularis. A) Eggs that were deposited by female ticks during the spring hatch into larvae, which begin to seek a host during the summer. B) Larvae that fed on a host will moult into nymphs while those that did not feed during the year of hatching will overwinter and begin host-seeking anew in the spring. C) Larvae moult into nymphs during the spring or summer, depending on when a blood meal was acquired. D) Nymphs are active during the summer and feed on a variety of vertebrate hosts, some of which are competent reservoirs for Borrelia burgdorferi and contribute to the continued cycle of bacterial transmission. E) Nymphs moult into adult ticks after acquiring a blood meal. F) Adult ticks are active during the autumn and often seek larger reproductive hosts. G) After mating and acquiring a blood meal, female ticks deposit eggs on the vegetation or forest floor before dying. This figure is adapted from the Centers of Disease Control and Prevention (CDC): https://www.cdc.gov/ticks/life_cycle_and_hosts.html

The nymph instar is a sexually immature octopod measuring approximately 1.6 mm in size (2). They are host-seeking throughout the spring and summer months, but peak nymphal activity and abundance occurs from June to July (11). Like larvae, nymphs feed for approximately a week and can moult in the year of feeding, if blood meal taken from April to July, or can overwinter and moult the next year, if blood meal taken from August to September (2, 9). Nymph survival is associated with ecological factors and climate, but it is not linked to the timing of the moult before or after overwintering and they can survive up to 16 months in the environment, longer than any other instar (2, 9, 12). Nymphs are also the most significant instar for pathogen transmission. It has been shown that *B. burgdorferi* and other pathogens are maintained through the transstadial moult (13). Therefore, if larvae acquired pathogens during their blood meal, they are transmitted to the nymphs as well (13, 14). Co-feeding between larvae and nymphs parasitizing the same host and temporal feeding of nymphs before overwintering larvae also contribute to the amplification of pathogens in the host and transmission of pathogens between instars (2).

The adult tick is also an octopod, but it is sexually mature and has two instars – males, which are 1.9-2.1 mm in size, and females, which are larger between 2.4-2.7 mm and can reach up to 10 mm when fed to repletion (2). Adult blacklegged ticks are mostly active during the autumn from September to October and as late as mid-December as well as during the spring to summer from mid-March to July (9). Females feed on a host from 6-10 days and males feed for less than 24 hours, if at all (15). Those that do not find a host during the autumn enter a quiescent state and resume host-seeking in the spring (2). Mating typically occurs before a female acquires a blood meal or on the host animal during tick feeding, after which the females drop off the host and produce one large batch of eggs numbering up to several thousand eggs between late April and

early May (2, 16). The males usually die on the host animals after mating (2). Adult ticks that have not fed and mated do not typically survive later than August (9).

2.1.3 Tick Questing Behaviour and Wildlife Hosts

Blacklegged ticks find animal hosts by questing. They crawl on vegetation (leaves, grass, brush) and then, using their extended first two legs, they grasp at a passing animal during contact (2). Ticks orient themselves to potential animal host by responding to a variety of stimuli including products of animal respiration (e.g., carbon dioxide), odours (e.g., phenols found in animal urine), changes in light and temperature, and vibrations (2, 17-19). The period of tick questing activity typically lasts from spring to fall, depending on the instar, and is affected by daylength, temperature, and relative humidity, with a warming climate predicted to extend the annual period of tick questing activity (20-22). Ticks are particularly sensitive to desiccation because they have a permeable epidermis and lose water rapidly through respiration (23). Thus, blacklegged ticks respond to microclimate conditions such as temperature and humidity to balance the risk of desiccation during questing, although it is unclear if ticks modulate questing activity during unfavourable conditions (e.g., return to moister leaf litter) or if the microclimate impacts their survival and overall density of ticks in the environment (22, 24, 25).

The height at which ticks are questing determines in part the selection of the host since ticks do not jump or leap from higher vegetation (2). Immature instars (larvae and nymphs) mostly parasitize small mammals or birds because they quest at lower heights (2). Adult ticks, on the other hand, quest higher on vegetation and prefer larger mammals that act as reproductive hosts (2, 26,

27). Despite the limited mobility of blacklegged ticks on their own, adventitious ticks can be transported by terrestrial hosts and spread great distances by migratory birds (28).

Blacklegged ticks are host-generalists and parasitize dozens of wildlife animals (26). Immature instars feed on rodents (e.g., mice, chipmunks, squirrels), birds (e.g., many species of songbirds), insectivores (e.g., lizards, shrews, opossums), lagomorphs (e.g., rabbits, hares), ungulates/cervids (e.g., deer, sheep), and other medium-sized mammals (e.g., racoons, skunks, foxes, dogs, cats) (26, 28-30). Mature instars, which are primarily seeking reproductive hosts, prefer large-sized mammals, particularly the white-tailed deer (*Odocoileus virginianus*) (2, 26, 27). All the blacklegged tick instars are capable of biting humans and pets, although humans and domestic animals represent incidental or "dead-end" hosts because they do not contribute to the enzootic or epizootic cycles of pathogen transmission (31, 32). The animal hosts on which blacklegged ticks feed also vary by geographic location. *Ixodes pacificus* ticks in western North America and *I. scapularis* ticks at lower latitudes feed largely on skinks and other lizards, while *I. scapularis* in central and eastern North America feed largely on small rodents and passerine birds (33, 34).

2.1.4 Impact of Wildlife Hosts on Pathogen Transmission

While blacklegged ticks feed on dozens of vertebrate hosts, the choice of host has important implications for amplification, dilution, and transmission of *B. burgdorferi*. Animals that are competent reservoirs become infected with *B. burgdorferi* following a tick bite and can then transmit the pathogen to naïve vectors (35). Reservoir competence is a product of infection prevalence and infectivity (36). The first reflects host susceptibility to *B. burgdorferi* and, thus, the probability that the animal will become infected (36). The latter measures the probability that

an infected animal can transmit *B. burgdorferi* to naïve vectors (36). The two measures have a strong positive correlation, meaning that animal hosts with a high *B. burgdorferi* infection prevalence are more likely to transmit the infection (36). The white-footed mouse (*Peromyscus leucopus*) is the most competent reservoir for *B. burgdorferi* and its presence in a variety of habitats contributes to the high rates of LD in central and northeastern North America (32, 37).

In the absence of the white-footed mouse, other small mammals (e.g., shrews, chipmunks) can maintain *B. burgdorferi* populations and a high disease risk (29). Non-competent reservoirs such as the white-tailed deer can also amplify LD risk by acting as reproductive hosts for mature ticks and increasing the vector population (27). In contrast, animal hosts can also have a dilution effect and reduce *B. burgdorferi* infection. Squirrels and lizards can bare a high load of parasitizing ticks, but they are not susceptible to *B. burgdorferi* infection and, as a result, have low infectivity (29, 38). Opossums and some bird species have also been shown to have a predatory effect on ticks and can efficiently remove any attached ticks before transmission of *B. burgdorferi* (39).

2.1.5 Habitat and Factors Associated with Tick Abundance

Ixodes scapularis is distributed throughout central and eastern North America, from Mexico to Canada, although tick density and *B. burgdorferi* infection rates are disproportionately higher in the Northeastern and Midwestern regions of the Unites States and central and eastern regions of Canada (40-42). *Ixodes pacificus* has a much smaller range and is only found west of the Rocky Mountains (41, 43). Blacklegged ticks inhabit a wide range of habitats from hardwood forests, mixed forests, deciduous swamps, shrublands, and woodlots (9, 11, 31, 44, 45). Their distribution across North America is discontinuous and patchy, both macro-geographically (i.e., at a large

spatial scale like states and provinces) and micro-geographically (i.e., at a small spatial scale like counties, townships, or local neighbourhoods) (46). The reason for the spatial heterogeneity in the distribution of blacklegged ticks remains unknown, although extensive ecological research has been conducted to identify the role of animal hosts and physical resources (e.g., vegetation, climate variables) that affect tick establishment (12, 27, 45, 47-49).

Investigation of biotic factors (relating to living things like plants and animals) and abiotic factors (physical elements not derived from living things like climate) affecting tick habitat suitability (Table 2.1) has been a focus of intense research over the past few decades. The steadily increasing range of *I. scapularis* across the United States and Canada suggests that much of the suitable habitat for blacklegged ticks is currently unoccupied (50-53). To date, climate remains ones of the most significant influencing factors for the expanding range front of blacklegged ticks and their establishment in the environment (20, 54, 55). Rises in minimum, maximum, and mean temperature ranges, cumulative degree days above 0°C, as well as vapour pressure and mean annual precipitation are among the climatic factors that most strongly correlate with increasing tick densities in the environment and an increase in the basic reproductive number of *I. scapularis* (20, 47, 54, 56). On a smaller scale, microclimate, such as higher average ground-level temperature and higher summer minimum and maximum daily temperatures, have also been linked to tick survival and abundance in various ecosystems (27, 49).

Blacklegged ticks inhabit a multitude of forest habitats and considerable research has also looked at the microhabitat and the factors associated with tick abundance and survival in these locations. Tick abundance had been positively associated with the presence of maple and cedar trees, which

dominate the preferred foraging forests for reproductive hosts of ticks (e.g., white-tailed deer) (48, 49, 57). Likewise, the presence of oak tree acorns, which are a food source for small rodents, has a strong association with nymphal and larval tick densities (26). The tree canopy and understory in forest habitats also play an important role in the survival of ticks by providing refuge and food for host animals, providing protection from weather extremes and microclimate, and allowing the ticks to quest on vegetation and actively find hosts (27, 47, 48, 58). Forest fragmentation, resulting in high forest edge density, is another factor associated with tick abundance because ecotone habitats (i.e., transitional area between forests and shrublands or grasslands) are very favourable for deer and small mammal communities inhabiting forest edges (e.g., mice) (59-61). However, tick abundance increases with proximity of forest patches and increasing forest cover, suggesting that ticks have greater rates of survival in regions with connectivity between fragmented forest patches (62, 63).

Table 2.1 Abiotic and biotic factors associated with increased tick habitat suitability.

ABIOTIC		**BIOTIC**	
Climate	Microclimate	Land cover	Wildlife
Degree days > 0°C	Ground-level temperature	Hardwood forest	Presence of mice
Temperature ranges	Summer temperature ranges	Understory density	Presence of deer
Annual precipitation	Soil moisture	Tree type & canopy	
Vapour pressure		Forest fragmentation	

The abundance of animal hosts in the environment is linked to tick abundance, although many uncertainties remain regarding the role these animals play in different ecosystems and in the maintenance of pathogens in enzootic and epizootic cycles (64). The white-footed mouse, the preferred host for immature blacklegged ticks, has been found to carry the largest parasitic tick load compared to other hosts, and is also the most competent reservoir for *B. burgdorferi* (35, 37).

Blacklegged tick densities and *B. burgdorferi*-infection prevalence in immature instars are strongly linked with the presence of white-footed mice in the environment (11, 37, 65). It has been hypothesized that white-footed mice thrive in habitats with lower species richness (i.e., lower overall number of species in each region), where high *B. burgdorferi* infection rates can be maintained in competent reservoirs like mice (29). In contrast, high species richness is thought to reduce pathogen transmission among wildlife due to a dilution effect caused by a higher abundance of less competent *B. burgdorferi* reservoir species (29). However, more recent studies have shown that white-footed mice are also abundant in regions with high biodiversity and that the relative abundance of white-footed mice modulates the association between species richness and infected nymphs (e.g., high biodiversity does not result in reduced nymphal infection rates if white-footed mice are also present) (27, 66, 67).

Similarly, the presence of white-tailed deer populations is strongly associated with tick abundance and the spread of tick-borne pathogens (27, 68-71). Deer/cervids are considered the most important animal host for adult blacklegged ticks, although they are not competent reservoirs for *B. burgdorferi* and largely serve as reproductive hosts (72). The relationship between deer density and tick abundance appears nonlinear as deer density above a low threshold has little impact on the abundance of ticks, and on nymphal infection rates and LD infection in humans (26, 27, 64, 73). Figure 2.2 provides a summary of the ecology of vector-host interactions, considering the biotic and abiotic factors that impact tick abundance and tick infection rates.

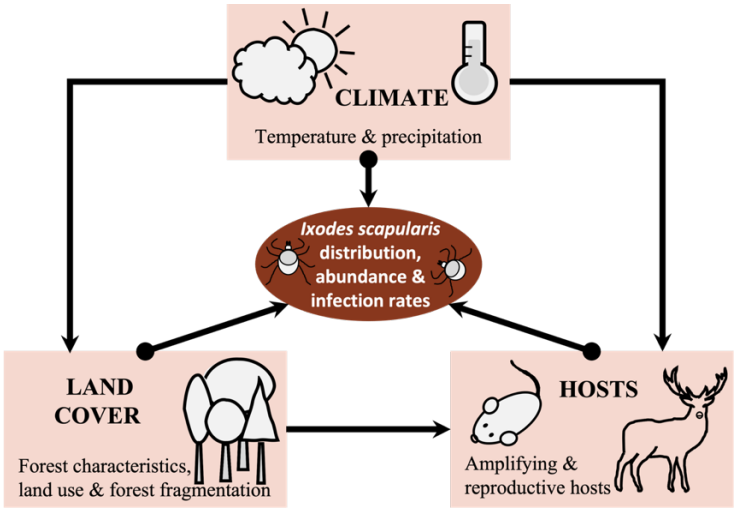

Figure 2.2 The ecology of vector-host interactions. Climate plays a central role in the distribution and abundance of Ixodes scapularis. Warming temperatures increase tick developmental rates by prolonging the duration of tick activity and moulting rates during a warmer season. Changes in climate also impact the suitability of forest habitats for ticks and the abundance of wildlife hosts. The characteristics of these forest habitats in turn impact I. scapularis abundance by providing refuge and food for wildlife hosts that ticks parasitize, proving protection for ticks from weather conditions and desiccation, and providing vegetation for ticks to quest on when they are host-seeking. Ixodes scapularis feed on many wildlife hosts including rodents, birds, lagomorphs, insectivores, mid-sized mammals, and cervids. The white-footed mouse is the preferred host for immature instars and the main reservoir for Borrelia burgdorferi. The white-tailed deer is the reproductive host for mature instars and contributes to tick abundance in the environment.

2.2 BORRELIA BURGDORFERI

Borrelia species are divided into two phyletic classes – those that cause LD and spirochetes that cause relapsing fever (32). The bacteria that cause LD belong to the *Borrelia burgdorferi sensu lato* species complex. *The B. burgdorferi s.l.* complex currently comprises 20 accepted and 3 proposed species that are transmitted between tick vectors and vertebrate hosts (74). The three species that dominate human infections are *B. burgdorferi sensu stricto* in North America and Western Europe, and *Borrelia garinii* and *Borrelia afzelii* in Eurasia (32). There is evidence that other *B. burgdorferi s.l.* species are linked to human infection, although to a lesser extent (32).

Borrelia burgdorferi s.l. spirochetes are mainly transmitted by four species of hard ticks: *I. scapularis* in eastern and central North America, *I. pacificus* in western North America, *Ixodes ricinus* in Europe, and *Ixodes persulcatus* in Asia (13). This thesis will, however, focus on *B. burgdorferi s.s.*, referred to simply as *B. burgdorferi*, as it is the causative agent of LD in Canada and the rest of North America.

2.2.1 The Epizootic Cycle

In North America, *B. burgdorferi* is maintained in an epizootic (epidemic-like) cycle, in contrast to the European enzootic (endemic-like) cycle (32, 74). The epizootic cycle consists of sharp increases in bacterial populations as tick vectors spread to new habitat, where it cycles between blacklegged tick vectors and vertebrate reservoirs including birds and rodents (32, 74). Since blacklegged tick larvae are uninfected at hatching, there is no evidence that transovarial transmission of *B. burgdorferi* occurs (32). Thus, the epizootic cycle begins when uninfected larvae acquire *B. burgdorferi* by feeding on wildlife hosts harbouring the bacteria (32, 75). After moulting to the nymphal stage, infected ticks spread the spirochetes to new reservoirs and continue the cycle of transmission (32, 75). Spirochetes are deposited into the bite wound during feeding, with longer feeding times resulting in a higher risk of pathogen transmission to the host (76, 77). The *B. burgdorferi* epizootic cycle is heavily dependent on the blacklegged tick vector and competent wildlife reservoirs for the bacteria. Non-*Ixodes* ticks are inefficient at transmitting *B. burgdorferi* spirochetes and fail to maintain the bacteria during the transstadial moult (78).

Likewise, competent wildlife reservoirs are necessary to maintain the *B. burgdorferi* transmission cycle, with the white-footed mouse consisting as the main wildlife reservoirs for *B. burgdorferi*

bacteria in North America (35, 79). Reservoirs, compared to other vertebrate hosts that ticks feed on, are animal hosts that 1) acquire *B. burgdorferi* through an infected tick bite, 2) maintain stable reproducing bacterial populations, often for the entire animal lifespan, and 3) transmit live and infectious *B. burgdorferi* bacteria via blood to ticks during feeding, enabling further spread (74). Adult blacklegged ticks do not play a significant role in the *B. burgdorferi* epizootic cycle since male ticks feed sparingly or not at all and female ticks prefer larger hosts such as white-tailed deer, which are not competent reservoirs because their immune system clears *B. burgdorferi* infections before further transmission of the pathogen to naïve hosts (73).

2.2.2 *Borrelia burgdorferi* Cellular Structure and Genome

Borrelia burgdorferi are spirochete bacteria. They are double-membrane, Gram-negative bacteria with a characteristic corkscrew or flat-wave shape, resulting from the unique helical periplasmic flagella (80, 81). The thin and long *B. burgdorferi* spirochetes move in an undulating manner, with periods of movement and pause, that are essential for transitioning between the arthropod vectors and vertebrate hosts (81). *Borrelia burgdorferi* spirochetes differ from other Gram-negative bacteria based on the structure of their outer membrane, which lacks structural components found in Gram-negative bacteria (e.g., LPS: lipopolysaccharides) and consists of a lipid bilayer made of phospholipids and glycolipids (82). In response to environmental cues, the glycolipids in the outer membrane form lipid rafts where various lipoproteins can be expressed at different stages of infection (83).

Borrelia burgdorferi has a small (~ 1.5 million bases) segmented genome comprised of one linear chromosome (910 kilobases) and 17-21 linear and circular plasmids (533-610 kilobases) (84, 85).

The chromosome contains highly conserved genes for basic bacterial function including DNA replication, transcription, translation, protein transport, motility, and chemotaxis (75, 85). The plasmids are highly variable among *B. burgdorferi* species and contain many genes with no known biological function as well as lipoproteins that play an important role in the persistence of the bacteria in ticks and hosts, and antigenic variation and immune evasion (84, 85). Differences in plasmids among *B. burgdorferi s.l.* species also contribute to the clinical variations in LD across geographic regions (82). *Borrelia burgdorferi* has a small genome compared to free-living bacteria because it does not contain genes encoding proteins involved in metabolic processes (e.g., nucleotides, amino acids, fatty acids, and enzymes) (75, 85). *Borrelia burgdorferi* spirochetes function as obligate parasites and scavenge metabolic resources from tick blood meals and the vertebrate hosts it infects (75). The *B. burgdorferi* genome also does not encode any known toxins or endotoxins, suggesting that the clinical manifestations seen in LD are a result of immune-mediated inflammation and tissue damage (75, 82).

2.2.3 The Biphasic Lifecycle

Borrelia burgdorferi has a biphasic lifecycle, where spirochetes can survive in two very different hosts – arthropod vectors and vertebrate hosts. Bacterial growth in these different environments depends on environmental factors such as temperature, pH, and nutrient availability (75, 86, 87). Notably, ticks have a higher midgut pH compared to the blood of mammals and birds and they do not regulate their body temperature (varies with ambient temperatures) (75, 86). *Borrelia burgdorferi* varies its gene expression to upregulate or downregulate protein components that enable its physiological adaptation to these different environments (Figure 2.3) (75).

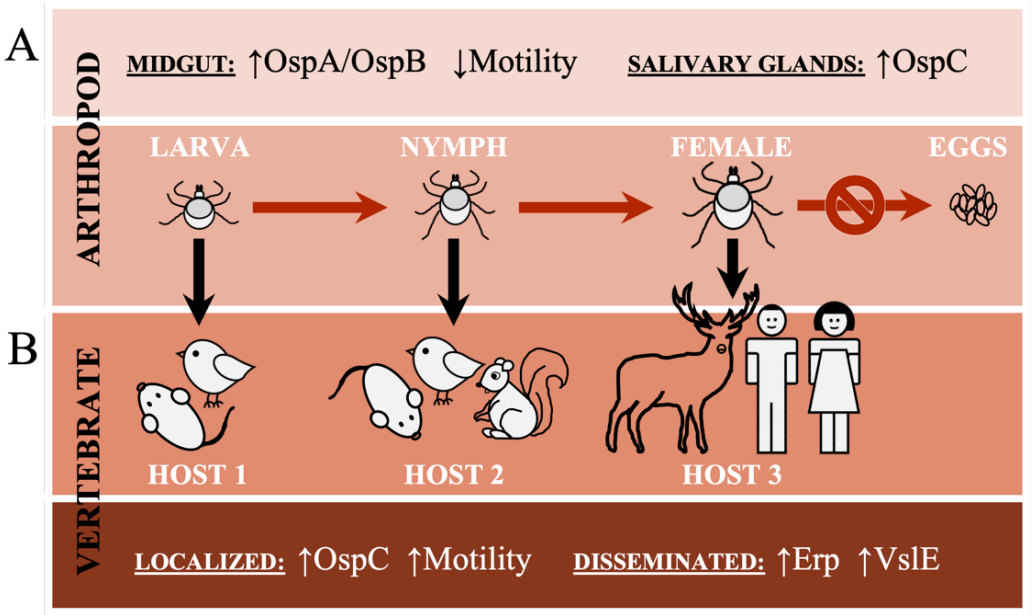

Figure 2.3 The biphasic lifecycle of Borrelia burgdorferi in the arthropod vector (A) and the vertebrate host (B). Red arrows indicate B. burgdorferi transmission across the transstadial moult and black arrows indicate B. burgdorferi transmission between vector and host. A) Borrelia burgdorferi is acquired by ticks during feeding on an infected animal host. Changes in temperature and pH, resulting from tick feeding on a blood meal, trigger differential gene expression in the bacteria that promotes survival in the tick vector. In the tick midgut, B. burgdorferi express adhesion lipoproteins to attach to the tick midgut and proliferate. The bacteria then migrate to the tick salivary glands where they express lipoproteins needed for survival in vertebrate hosts. Borrelia burgdorferi can survive in the tick midgut during the transstadial moult, after which it can be transmitted to new hosts during a subsequent blood meal. B) During their 2-year life cycle, blacklegged ticks feed on three different hosts, one at each developmental stage. Ticks attached to the host and feed to repletion for several days. At the tick bite site, B. burgdorferi bacteria avoid immune-mediated killing and proliferate abundantly before disseminating to other tissues. For hematogenous dissemination to new tissues, the bacteria express lipoproteins needed for attachment to the vascular endothelium and for evasion of the host immune system. Borrelia burgdorferi infection can be maintained for the entire lifespan of a competent animal reservoir (e.g., rodents, birds) and retransmitted by tick vectors. Non-competent animal reservoirs (e.g., deer, humans) clear the B. burgdorferi infection and do not contribute to the transmission of the bacteria to new vectors.

2.2.3.1 Infection in Arthropod Vectors

Borrelia burgdorferi respond strongly to chemotaxis stimuli in tick saliva elicited during tick feeding on a host (32, 88). The spirochetes migrate to the feeding site, enter the tick hypostome,

and can be detected in the tick midgut within 24 hours of tick attachment (32, 87, 89). *Borrelia burgdorferi* spirochetes survive in the tick midgut during the transstadial moulting period that can last up to a year in temperate zones (13). During this time, the spirochetes are alive (not dormant) but have low motility and exist in a poorly understood metabolic state due to low nutrient availability and an anoxic environment for a prolonged period in the tick midgut (32, 81, 90). The infected tick will seek another blood meal after its transstadial moult and begin the feeding cycle anew, which will trigger bacterial activity from changes in temperature and pH in the tick midgut. The nutrient-rich blood meal provides an ideal place for *B. burgdorferi* spirochetes to replicate abundantly (89). During tick feeding, *B. burgdorferi* expresses several proteins, including outer surface protein A and B (OspA and OspB), which bind to receptors in the tick midgut epithelium, as well as several lipoproteins involved in bacterial survival (e.g., resistance to oxidative stress, carbohydrate metabolism, and evasion of tick digestive and immune components) (75, 87, 91). The spirochetes have low motility in the tick midgut, and they replicate in aggregated clumps attached to midgut epithelial cells (81, 91). As tick feeding continues, *B. burgdorferi* bacteria begin to express outer surface protein C (OspC) in preparation for dissemination into a vertebrate host (32, 87). A small number of spirochetes penetrate the tick midgut epithelium into the haemocoel (primary body cavity of invertebrates containing circulatory fluid), where they subsequently enter the salivary glands (32, 91). *Borrelia burgdorferi* spirochetes are detectable in tick salivary glands after 36-48 hours of tick attachment and can be transmitted to the new host during this time (89).

2.2.3.2 Infection in Vertebrate Hosts

Borrelia burgdorferi bacteria are inoculated at the tick bite site, where its lifecycle in the vertebrate host begins. Transmission and survival of *B. burgdorferi* spirochetes in the vertebrate host depend

in part on the tick saliva, which contains anti-coagulant and immunosuppressive proteins (Salp15: 15-kDa salivary gland protein) (32, 92). Tick salivary proteins also bind to *B. burgdorferi* spirochetes through the OspC lipoprotein, allowing the bacteria to escape antibody-mediated killing and establish early infection in vertebrate hosts (32, 93, 94). After spirochetes are established in the host, OspC expression is no longer needed for persistence and its expression is downregulated (95).

In animal studies, *B. burgdorferi* is only cultured from the skin within a week of a tick bite and, when the bite area is excised within 2 days, a generalized infection is not established (96). The spirochetes appear to multiply locally for a few days before disseminating to distant tissues (32). For dissemination, *B. burgdorferi* express a set of outer surface proteins (Erp: OspEF-related proteins) that bind host proteases to digest components of the extracellular matrix between cells, allowing *B. burgdorferi* to enter the blood stream (97). Within the circulatory system, *B. burgdorferi* moves gradually along the capillary walls before escaping the vasculature (88).

At new tissue sites, *B. burgdorferi* expresses the lipoprotein VlsE (variable-major protein-like sequence expressed) that undergoes substantial antigenic variation through gene recombination (98). The variability of VlsE lipoproteins allows *B. burgdorferi* to evade antibodies generated by the host immune system and its expression is required for infection in the mammalian host (75). Despite the mechanisms employed by *B. burgdorferi* to escape detection, the host immune system still recognizes bacterial components through pattern recognition receptors expressed on tissue-resident antigen-presenting cells (32, 99). Signaling through these receptors induces the release of cytokines and chemokines that result in an inflammatory response and the accrual of more cells at

the sites of infection (82, 99). Since *B. burgdorferi* does not express any toxins or virulence factors that damage cells or tissues at sites of infection, the clinical manifestations of LD are a result of the host immune response to extracellular bacterial components (82).

2.2.4 Diversity of *Borrelia burgdorferi*

Genome analysis of *B. burgdorferi* has revealed that the organism is highly diverse (100). There are several methods of genotyping *B. burgdorferi* (101). These include classification into 1) ribosomal sequence types (RST) based on identification of the chromosomal 16S–23S rRNA intergenic spacer (IGS) region (*rrs-rrlA*), 2) IGS genotypes based on nucleotide polymorphisms, 3) *ospA* and *ospC* strain types based on analysis of sequences of the plasmid-encoded lipoproteins, and 4) sequence types (ST) based on multi-locus sequence typing (MLST) of several housekeeping genes (100, 102-105). The different genotyping methods have provided insights into various aspects of *B. burgdorferi* evolution, adaptation, and pathogenicity.

2.2.4.1 ospC Genotyping and 16S–23S (rrs-rrlA) Ribosomal Sequence Typing

The *ospC* gene, located on a circular plasmid, is the loci with the highest degree of variation. It is inherited and correlated with the *rrs-rrlA* RST through high levels of linkage disequilibrium (106, 107). RST1 corresponds uniquely to *ospC* genotypes A and B; RST2 includes *ospC* genotypes F, H, K, and N; RST3 includes the remaining *ospC* genotypes (108). The *ospC* genotypes and *rrs-rrlA* RSTs have been used as genetic markers to characterize *B. burgdorferi* isolates in epidemiological and population studies and to identify host species adaptations, likely resulting

from OspC[1]'s importance in tick to host transmission (107, 109). For example, *ospC* genotypes G, H, and A have shown a strong association with chipmunk, deer mouse, and white-footed mouse reservoirs, respectively (106). The *ospC* genotypes and the *rrs-rrlA* RSTs have also shown an association with different levels of *B. burgdorferi* pathogenicity and bacterial dissemination in the vertebrate host (109-111). Specifically, four *ospC* genotypes (K, A, B, and I) have been linked to bacterial hematogenous dissemination and greater disease severity, while RST2 and RST1strains are commonly found in synovial fluid in patients with Lyme arthritis and antibiotic refractory arthritis, respectively (108, 110, 112).

2.2.4.2 Multi-Locus Sequence Typing

More recently, MLST has been used to assess *B. burgdorferi* genotypes based on the allelic profile for eight housekeeping genes (101). The eight genes (*nifS, rplB, clpA, pyrG, clpX, pepX, recG, uvrA*) are scattered across the linear chromosome and are highly conserved (101). For each gene, single point mutations are identified by comparing the gene sequences to the existing *Borrelia* MLST database (101). A sequence type for the bacterial strain is then assigned based on the chain of eight integers that correspond to the allele number for each gene (101). While the number of single point mutations (i.e., polymorphic sites) per gene is low, the discriminatory power to identify bacterial genotypes is high when multiple gene loci are combined (100). MLST analysis is especially useful for identifying evolutionary aspects of *B. burgdorferi* and ecological processes such as dispersal and host specialization since it is based on analysis of conserved chromosomal genes that undergo slower changes. In North America, MLST analysis revealed a geographical

[1] A different nomenclature is used to distinguish between a gene encoding a protein and the name of the corresponding protein. Genes are represented by lowercase, italicized letters (e.g., *ospC*) and protein names are represented by sentence case, roman letters (e.g., OspC).

split between the Northeast and Midwest, with distinct *B. burgdorferi* STs in each region (113). The limited gene flow between the two regions suggests coincident emergence of LD originating from multiple expanding populations of the blacklegged tick (113). A similar pattern was seen in Canada, with STs in Ontario, Quebec, and Atlantic Canada emerging from populations in the Northeastern United States compared to STs in Manitoba that had similarity to those from the Midwest region (109). However, local dispersal by animals and founder effects show increasing *B. burgdorferi* ST diversity at smaller geographic scales (114). MLST analysis has also been used to delineate dispersal patterns by identifying associations between STs and forest connectivity and host specialization (106, 115).

Borrelia burgdorferi STs are also associated with pathogenicity and bacterial dissemination in vertebrate hosts (116, 117). A study of bacterial isolates from vectors and human tissues showed that *B. burgdorferi* diversity is highest in ticks and progressively decreases from skin to blood to cerebrospinal fluid (112). It is evident that only select isolates of *B. burgdorferi* are capable of hematogenous dissemination, but the exact mechanism by which this occurs are yet to be determined. Additionally, recent evidence from New York and Wisconsin has indicated that *B. burgdorferi* STs may better predict LD outcome in patients than the *ospC* and *rrs-rrlA* genes (116). However, additional studies are needed to understand the link between *B. burgdorferi* STs and pathogenicity in humans, particularly since there are an estimated 111 distinct STs in North America, a considerably larger number than that of *ospC* genotypes and RST groups (105, 114).

2.3 LYME DISEASE

LD symptoms and the frequency with which they occur can vary widely among individuals and with the bacteria causing the infection. In Europe, Lyme borreliosis is caused by *B. afzelii*, *B. garinii*, and less frequently by *B. burgdorferi s.s.*, resulting in markedly different clinical manifestations compared to North American cases where LD infection is attributed exclusively to *B. burgdorferi s.s.* and possibly the newly recognized *Borrelia mayonii* species in the Upper Midwest in the United States (118). These differences may be attributed to strain-specific ability to proliferate and disseminate throughout the body and varying inflammatory responses elicited by the bacteria and their pathogenic components (119). As is fitting to the context of this thesis, the clinical manifestations of LD and the diagnostic methods, which are discussed hereafter, will focus on infection with *B. burgdorferi s.s.* (also referred to as *B. burgdorferi*).

2.3.1 Clinical Manifestations

LD is a multisystem infection whose natural history, if left untreated, manifests in several progressive clinical stages (Figure 2.4). Stage 1 is known as early localized infection and begins shortly after infection with the spirochetal bacterium. This stage is often characterized by a skin lesion formed at the site of the tick bite and flu-like symptoms such as fatigue, myalgia, headaches, fever, and chills (82). The characteristic LD "bull's eye" rash, erythema migrans (EM), is an expanding annular erythematous skin lesion with a central clearing that expands with bacterial replication and dissemination in the dermis (120, 121). Approximately 80% of LD patients develop an EM rash during early localized infection, although it is estimated that only a third of patients develop the hallmark central clearing of the bull's eye (82, 122, 123). The remaining 18% and 2%

of LD patients develop non-specific symptoms without an EM rash and more advanced clinical manifestations characteristic of later stages, respectively (82). With or without antibiotic treatment, symptoms of early localized infection improve or resolve within weeks; however, if left untreated LD can continue to progress to a disseminated state (82).

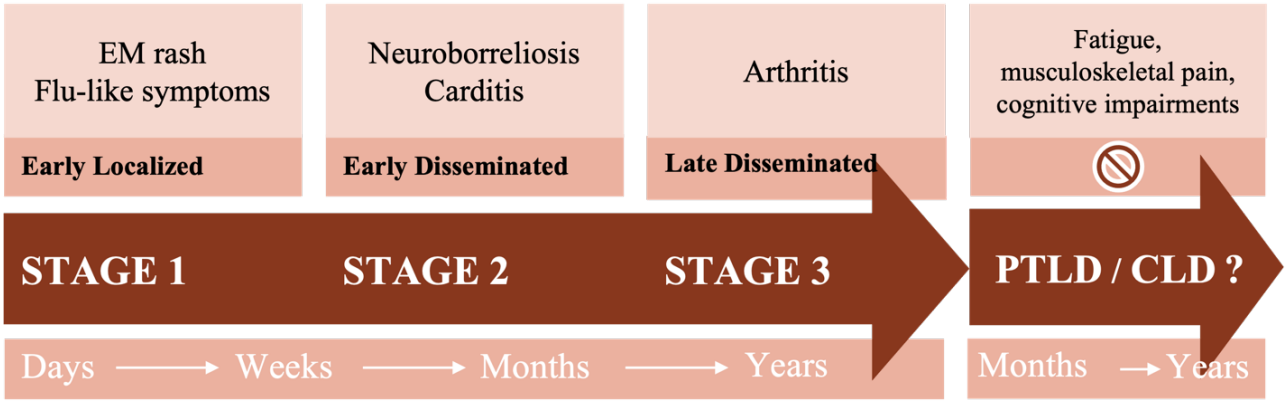

Figure 2.4 The stages of Lyme disease. Stage 1 or, early localized infection, typically occurs days to weeks after a tick bite and predominantly consists of an erythema migrans (EM) rash and flu-like symptoms. If left untreated, Stage 2 occurs weeks to months later, following hematogenous dissemination to other tissues. Lyme neuroborreliosis and carditis are rare complications of Lyme disease at this stage. Stage 3 is late disseminated infection, occurring months to years after, and predominantly consists of Lyme arthritis. Lyme disease is treated with antibiotic therapy, although a very small subset of individuals can develop post-treatment Lyme disease syndrome (PTLDS). Chronic Lyme disease (CLD) has been used to describe other non-specific symptoms resembling Lyme disease complications, but there is controversy regarding the validity of this diagnosis among physicians and scientists. This figure is adapted from Steere et al., 2016 (Steere AC, Strle F, Wormser GP, et al. Lyme borreliosis. Nat Rev Dis Primers 2016;2:16090.)

Stage 2 is early disseminated infection, which results from hematogenous dissemination of the bacteria to other parts of the skin, organs, joints, and the peripheral/central nervous system, occurring within weeks to months after a tick bite (82). At this stage, multiple EM rashes can develop throughout the body, like the secondary rash in other spirochetal infections (119). Approximately 10-15% of patients develop neurologic conditions collectively referred to as Lyme

neuroborreliosis (122). The most common clinical manifestations of neuroborreliosis are meningitis, resulting in episodic headaches and neck stiffness, neuropathies such as facial palsy, and inflammation of the spinal root nerve, resulting in motor and sensory symptoms such as numbness, tingling, and sensitivity to pain (82, 119). Less commonly, 4-8% of untreated LD patients develop Lyme carditis, although some studies suggest the prevalence maybe closer to 1% and disproportionately affects males (122, 124). Lyme carditis typically manifests as atrioventricular block and less commonly as myocarditis, resulting in tiredness, dizziness, irregular heart rate, and chest pain or difficulty breathing (82, 124).

Stage 3, or late disseminated infection, typically occurs months to years after an untreated infection. The most common clinical manifestation at this stage, found to occur in up to 60% of untreated patients, is Lyme arthritis (82, 122). On average, arthritis develops within 6 months of LD infection (range: 4 days to 2 years) and involves the localization of spirochetes in the synovial cavity, resulting in intermittent joint swelling and pain, particularly in the larger joints such as the knee (82, 119). Lyme arthritis is also associated with unexplainable clinical features such as a delayed onset and spontaneous remission and recurrence of inflammation in one or multiple joints (125). As such, Lyme arthritis is not well understood and most attempts to recover live bacterial cultures from synovial fluid have been unsuccessful, yet *B. burgdorferi* DNA can be identified by polymerase chain reaction (PCR) testing and patients respond well to antibiotic treatment (119, 125, 126). Where the spirochetal bacteria are localized in joints and how they trigger inflammation and prolonged arthritic complications remains largely unknown.

2.3.2 Complications and Controversies

2.3.2.1 Post-Treatment Lyme Disease Syndrome

Most cases of LD resolve within 2-4 weeks of antibiotic treatment; however, a small subset (~10%) of LD patients experience prolonged, persistent, and debilitating symptoms including fatigue, musculoskeletal pain, and impaired cognitive function for ≥ 6 months after antibiotic treatment (82, 122, 127). This is referred to as post-treatment Lyme disease syndrome (PTLDS) and, while risk factors for this condition are largely unknown, it is thought that patients with a disseminated infection may be more likely to experience PTLDS (82). Early diagnosis and treatment are likely to play an important role in reducing the prevalence of PTLDS. The pathogenesis of PTLDS is also not well understood, though it is hypothesized to be a result of residual damage to tissues and a central sensitization, similar to fibromyalgia, which leads to chronic pain, hypersensitivity, and fatigue (128, 129). Persistent infection with *B. burgdorferi* has not been ruled out as cause of PTLDS, but placebo-controlled randomized trials in Europe and the United States showed little clinical benefit to prolonged antibiotic treatment (130, 131).

Furthermore, studies have shown that antibiotic treatment is effective at resolving LD such that, at 12 months following treatment, debilitating symptoms are not reported at higher frequencies in LD patients compared to sex and age matched controls without a history of LD (132, 133). PTLDS is a rare complication of LD and additional research is needed to understand the pathogenesis and how to improve treatment and patient quality of life following LD infection.

2.3.2.2 Chronic Lyme Disease

In recent years, the term chronic Lyme disease (CLD) has gained popularity and is used to describe the multitude of prolonged symptoms perceived by some patients to be caused by latent LD and persistent infection with *B. burgdorferi s.l.* and sometimes even coinfection with other tick-borne pathogens (134, 135). However, unlike PTLDS, there is currently no accepted definition for CLD with objective clinical symptoms and there is substantial debate regarding the medical validity of this condition (135). CLD differs from PTLDS in one critical way: CLD refers to chronic subjective symptoms in patients who do not have clear clinical or serological confirmation of LD (119, 135). These individuals may or may not have acquired LD and are often diagnosed with LD by exclusion when no other diagnosis can be found, which is particularly significant because many rheumatologic and neurologic diseases with similar symptoms can be difficult to diagnose (135). CLD patients and advocacy groups argue that LD is severely underdiagnosed and that current guidelines for LD clinical and serological diagnosis are too restrictive (136, 137). As such, many patients with CLD, who do not have clinical findings concordant with a LD diagnosis, seek alternative testing at private laboratories that lack a standardized criteria for interpreting test results (138, 139).

Furthermore, medical professionals are generally reticent to diagnose CLD due to potential harm of long-term antibiotic use and the lack of solid evidence for persistent *B. burgdorferi* infection (135, 140, 141). While there is considerable debate regarding the validity and treatment of CLD, it is essential that patient interests are addressed without judgement and dismissal from physicians and researchers. CLD patients experience a multitude of chronic, at times debilitating, conditions

and additional efforts should be made to better understand the potential causes of this condition as well as improving LD diagnosis and treatment options.

2.3.3 Lyme Disease in Canada

There are a few recent studies that assessed clinical manifestations of LD in Canada. Gasmi et al. analyzed LD national surveillance data from 2009 to 2015 and found that a single EM rash was the most common clinical manifestation reported in 74.2% of patients followed by arthritis reported in 35.7% of patients, while the least common symptoms were multiple EM rashes (5.9%) and cardiac symptoms (3.6%) (142). Children under 15 years were most frequently diagnosed with an EM rash whereas neurologic and cardiac complications were mostly reported in adults (142). In a similar study in Ontario, Johnson et al. found that an EM rash was the most common symptom of LD reported in 69.6% of patients, of which 83.4% and 20.8% presented with a typical bull's eye rash and an atypical rash, respectively (143). The prevalence of neurological, cardiac, and arthritic symptoms was higher in the Johnson et al. study compared to the national surveillance results reported by Gasmi et al. and the frequencies observed in the United States, though this is likely due to self-reported symptoms compared to physician diagnosed conditions, which also indicates a discrepancy between patient experience and clinical diagnosis (143). In Ontario, 45.2%, 38.0%, and 16.8% of LD cases were diagnosed ≤ 30 days, 1-3 months, and > 3 months after symptom onset, respectively (143). Interestingly, early diagnosis of LD (≤ 30 days) occurred most frequently in eastern Ontario, which has the highest incidence of LD and density of ticks in the environment, whereas late diagnosis (> 3 months) occurred in regions with little to no known risk for LD (143). This shows that identification of LD risk areas and public health knowledge

translation are essential for early diagnosis, where both the public and physicians are more aware of the risk of tick-borne illnesses.

2.3.4 Lyme Disease Diagnosis

2.3.4.1 The Standard Two-Tiered Testing Protocol

In Canada and the United States, LD is diagnosed based on the guidelines established by the Centers for Disease Control and Prevention (CDC) and the Public Health Agency of Canada (PHAC) (Figure 2.5) (144, 145). A clinical diagnosis can be obtained if the patient presents with an EM rash and a history of tick exposure, either as a recent tick bite or travel/residence in an area where blacklegged ticks are endemic (144, 145). For patients without an EM rash but with a history of tick exposure and non-specific symptoms, a standard two-tiered serological testing (STTT) protocol is recommended to confirm a LD diagnosis (144, 145). The STTT algorithm consists of 1) a quantitative enzyme immunoassay (EIA) that measures total antibodies (i.e., immunoglobulin (Ig) M and G) against *B. burgdorferi* antigens, and 2) a qualitative Western immunoblot (IB) that detects presence of *B. burgdorferi* reactive IgM (only recommended for exposures ≤ 30 days) or IgG in samples that are positive or equivocal in the first step (146). When performed and interpreted in accordance with current CDC guidelines, the STTT protocol has a good sensitivity (early localized or disseminated: ~ 70%; late disseminated: 100%) and specificity (late disseminated: > 95%) (147). The detection of early localized LD is less accurate using this method and research is needed to improve diagnostic tests for early LD infection when antibody responses are not as strong.

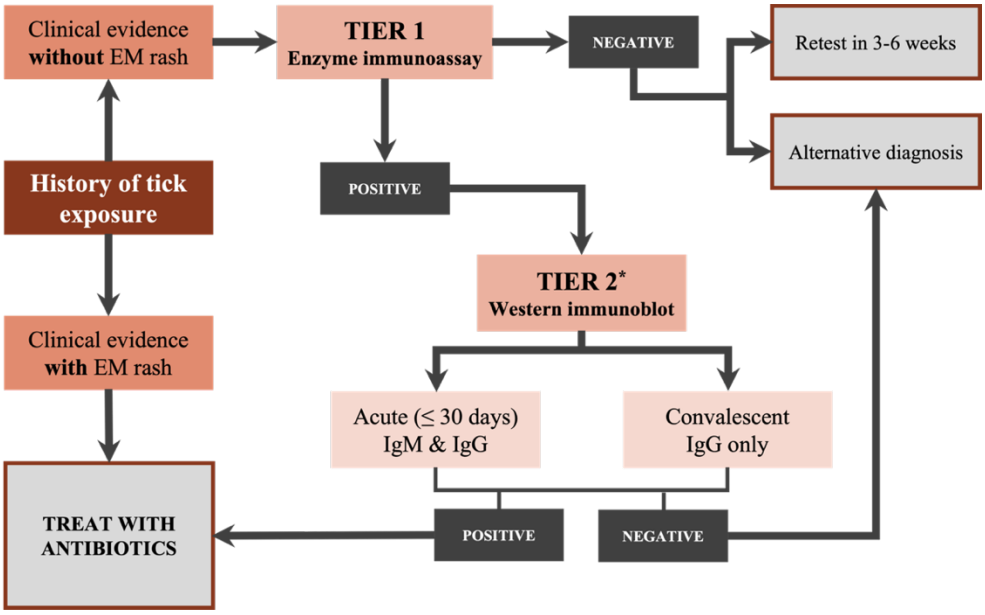

Figure 2.5 The standard two-tiered testing protocol for Lyme disease diagnosis. Lyme disease diagnosis is based on a history of tick exposure and clinical evidence of illness. Individuals with an erythema migrans (EM) rash are treated with antibiotics and do not require further serological testing. For those with clinical symptoms lacking an EM rash, serological testing is recommended via a two-tiered approach. The first-tier consists of an enzyme immunoassay that measures total antibodies against *Borrelia burgdorferi* antigen. For negative results, retesting in 3-6 weeks or an alternative diagnosis is recommended. For positive or equivocal results, a second-tier Western immunoblot is required to avoid false positives resulting from antibody cross reactivity with other bacterial, viral, or human antigen. The second-tier test administered depends on the time since infection, with IgM and IgG used for acute infections (<30 days) and IgG test used for convalescent samples (>30 days). The immunoblot is interpreted according to a standard reference proposed by the Centers for Control and Prevention (CDC) and the Public Health Agency of Canada (PHAC). Fewer than the recommended number of bands should not be interpreted as a positive test. This figure is adapted from the Public Health Agency of Canada: https://www.canada.ca/en/public-health/services/diseases/lyme-disease/health-professionals-lyme-disease.html
* The modified two-tiered testing protocol replaces the Western immunoblot with a second enzyme immunoassay.

Tier 1: Enzyme Immunoassay (EIA)

The first tier, an EIA, is used to detect total serum IgM, IgG, and IgA against *B. burgdorferi* antigens. This is a quantitative test that measures relative concentrations of serum antibodies against *B. burgdorferi* in a suspected patient compared with that in uninfected controls (although

the test result is reported as positive/negative not by the antibody titers). EIAs use a variety of *B. burgdorferi* antigens, from a single antigen to multiple antigens per assay, to capture or detect serum antibodies. The most common test, whole-cell sonicate (WCS) EIA, uses a peptide preparation consisting of multiple antigens derived from the *B. burgdorferi* bacterium (147). The WCS EIA has a high sensitivity after the acute infection (> 1-2 weeks) since it detects antibodies against multiple components of the bacterium, but lower specificity since some of the antigens can be cross-reactive with antibodies against human antigen (e.g., autoimmune diseases), viral antigen (e.g., Epstein-Barr virus), and other spirochetes (e.g., syphilis) (145, 148).

Multiple next generation EIAs have been developed using recombinant antigens and synthetic peptides derived from the *B. burgdorferi s.s.* B31 strain as well as a few other *B. burgdorferi s.l.* strains (148). These include VlsE lipoprotein, C6/IR6 peptide (invariable region 6 of VlsE), C10 peptide (conserved amino-terminal portion of outer surface protein C), and many more (148-152). These EIAs have similar or better performance than WCS EIA, with the C6 peptide EIA having the highest sensitivity (early localized LD: 65.6%, CI: 61.2% - 69.7%; late disseminated LD: 99%, CI: 95% - 100%) and less variability compared to other assays (139, 153). However, none of the EIAs demonstrated sufficient sensitivity and specificity on their own or during early acute LD to replace the STTT approach (153).

Tier 2: Western Immunoblot (IB)

The second tier, a Western immunoblot, is used to detect IgM and IgG antibodies in patients with a positive or equivocal EIA (145, 146). The IB is a qualitative test that visualizes *B. burgdorferi* reactive antibodies on a gel used to separate preselected bacterial proteins based on molecular

weight (147). Bands that appear on the gel represent binding of serum antibodies from a patient sample to specific *B. burgdorferi* proteins. The location of the bands corresponds to the protein size and the intensity of the band compared to positive and negative control samples represents the quantity of reactive antibodies (although this is not quantifiable as for the EIA) (148). In the United States and Canada, interpretation of the IB is standardized based on guidelines derived from the systematic evaluation of IBs in LD patients (148, 154, 155). A positive IgM IB requires 2 of 3 bands with molecular weight of 21/24 kDa (OspC), 39 kDa (BmpA: *Borrelia* membrane protein A), or 41 kDa (FlaB: flagellin protein B) (146, 154). A positive IgG IB requires 5 of 10 bands with molecular weight of 18 kDa (p18), 21/24 kDa (p25; OspC), 28 kDa (p28), 30 kDa (p30), 39 kDa (BmpA), 41 kDa (FlaB), 45 kDa (p45), 58 kDa (p58), 66 kDa (p66), or 93 kDa (p83/93) (146, 155). Fewer than the recommended number of bands should not be interpreted as a positive test result because, like the EIA, there is cross-reactivity of IgM and IgG antibodies with other human and bacterial antigens that may result in false positives (148). Interpretation of the IB test also depends largely on the time course of illness and stage of LD. IgM antibodies are first to appear during an infection and are generally directed against the most immunogenic antigens; therefore, the IgM IB test (with the IgG IB) is recommended for patients with signs and symptoms lasting ≤ 30 days (146). After this stage, the IgM IB has poor accuracy and a high rate of false positives (146, 148). IgG antibody responses follow IgM and are usually directed towards a larger number of bacterial antigens (147). The IgG IB is recommended for later stages of LD infection and has similar sensitivity to the EIA but a higher specificity since the exact antibody specificity against *B. burgdorferi* antigens can be visualized (148).

Multiple band combinations are considered for the IB because, in addition to cross-reactivity with non-*B. burgdorferi* proteins, antibody reactivity to specific proteins has been shown to depend on the bacterial strain (148). In the United States and Canada, commercial IB tests contain protein panels derived from a few *B. burgdorferi s.s.* variants including B31, strain 297, and isolate G39/40 (148). However, there is substantial variation in the prevalence of antibody responses against individual proteins (148). In Canada, this variation was shown to depend on year and geographic location, where different strains of *B. burgdorferi* are found, but no one protein was predictive of overall IB result (156). Individual proteins have different sensitivity and specificity characteristics and currently there is insufficient evidence to support selection of different guidelines for interpretation of the IB in different geographic regions or populations across North America (European guidelines differ from North American due to *B. burgdorferi s.l.* commercial IB tests) (156).

2.3.4.2 The Modified Two-Tiered Testing Protocol

In 2019, the modified two-tiered testing (MTTT) approach was approved by the Food and Drug Administration (FDA) and the CDC as an alternative to the STTT (157, 158). In Canada, the MTTT for LD diagnosis is not currently approved, although recent studies from Nova Scotia showed promising results and this modified testing protocol is being evaluated regionally (159, 160). The MTTT consists of a first tier WCS EIA but replaces the second tier IgM and IgG IB with a next-generation (VlsE1, C6, pC10) EIA. The benefit of the MTTT is that the time-consuming and subjectively scored IBs are replaced by another EIA, which are more easily automated, more cost-effective, and have similar or improved performance (161). In the United States, Branda et al. found that the MTTT had a higher sensitivity compared to the STTT in early

LD infection (61% versus 48%, respectively) and identical specificity to the STTT (99.5%) (151). The MTTT had superior specificity compared to a single C6 EIA (98.4%) because the cross-reactive antibodies that affect the specificity of WCS EIAs do not react with the subsequent C6 EIA (151, 162). In Canada, Davies et al. and Khan et al. showed that the MTTT algorithm was able to identify 25% and 28% more early LD cases, respectively, compared to the STTT while maintaining a comparable specificity to the STTT (159, 160). While the MTTT shows improved sensitivity compared to the STTT, early LD diagnosis remains a challenge. New MTTT protocols using chemiluminescence immunoassays (CLIA), multiplex bead assays, and improved IBs are also being evaluated compared to the currently recommended CDC testing protocols and may offer improved diagnostic capabilities (162-164).

2.3.4.3 Not Approved Testing

Microscopy and culture are often considered the gold-standard for identifying bacterial infections. *Borrelia burgdorferi s.l.* species have been successfully cultured from clinical samples including skin biopsies of EM rashes, blood, and cerebrospinal fluid (CSF) (148). However, this is not a diagnostically appropriate method because successful culture of spirochetes from patients is highly variable and depends on patient symptoms (e.g., much higher success from EM rash than CSF), it is time and labour intensive (e.g., up to 12 weeks to grow cultures), antibiotic treatment inhibits the detection of live organisms, and the sensitivity of this method is considerably lower than serology (148). Molecular detection of spirochete DNA by polymerase chain reaction (PCR) assays has similar limitations to bacterial culture since both rely on a direct measure of the pathogenic agent. PCR also has other challenges including PCR-inhibitors (e.g., other proteins/enzymes that prevent or degrade DNA) in human tissues/fluids and the selection of

specific gene targets to be amplified (also varies with *B. burgdorferi* strain) (148). PCR has higher sensitivity than culture and is often used in laboratories to identify bacterial genetic diversity and co-infections with other tick-borne pathogens (148). Although its diagnostic use is currently limited, the utility of PCR methods for LD diagnosis is growing area of research (147, 148, 153).

2.4 OTHER TICK-BORNE ILLNESSES

Ixodes scapularis ticks can transmit several tick-borne pathogens that cause diseases in humans and wildlife animals. Tick-borne pathogens of public health significance include other *Borrelia* species (e.g., *B. miyamotoi*), *Anaplasma phagocytophilum*, *Babesia* species (e.g., *B. microti*, *B. odocoilei*), Powassan virus (POW), and deer-tick virus (DTV). The prevalence of these pathogens is low but, due to the expanding range and increasing abundance of the blacklegged tick across regions of North America, other tick-borne illnesses are of growing concern.

2.4.1 Tick-Borne Relapsing Fever / *Borrelia miyamotoi* Disease

2.4.1.1 Disease Etiology

Tick-borne relapsing fever (TBRF) is caused by *Borrelia* species transmitted through the bite of soft ticks (*Argasidae*) (165). However, in the mid to late 1990s, a new *Borrelia* species, *B. miyamotoi,* was identified in hard ticks (*Ixodidae*) in Asia and Europe (166). In 2001, *B. miyamotoi* was also identified in *I. scapularis* ticks in multiple states across the northeastern United States (167). Phylogenetic analysis revealed that *B. miyamotoi* bears greater similarity to the TBRF spirochetes than to *B. burgdorferi* and is currently the only identified TBRF spirochete that infects

hard ticks (166, 168). TBRF caused by *B. miyamotoi* is commonly referred to as *B. miyamotoi* disease (BMD) to distinguish the illness from other TBRF infections (165).

2.4.1.2 Clinical Manifestations, Treatment, and Diagnosis

Clinical manifestations of BMD are like those of TBRF, and generally consist of two to three recurring episodes of high fever, with the fever lasting several days and a gap of up to two weeks between episodes (169). The febrile illness is also accompanied by fatigue, headaches, chills, myalgia, arthralgia, nausea, low blood cell count, and inflammation and liver damage (169). Unlike LD, BMD is not often associated with a rash, although several clinical case reports have identified this symptom in *B. miyamotoi* infection, including up to 18% of patients diagnosed with BMD in the United States (170, 171). In a small number of immunocompromised patients, BMD had also been linked to meningitis and other neurological complications (169, 172). BMD is effectively treated with the same course of antibiotics used for LD (165, 173).

Diagnosis of BMD is based on clinical symptoms and standard laboratory tests (e.g., peripheral blood smear, complete blood count, liver enzymes) (165). Currently, there are no approved standardized tests specifically for detection of *B. miyamotoi* infection (168). However, PCR testing and serology against *B. miyamotoi* antigen (GlpQ: glycerophosphodiester phosphodiesterase) not found in *B. burgdorferi* have been used for BMD diagnosis in Europe, the United States, and recently in Canada (174-176). Serological diagnosis remains particularly challenging due to cross-reactivity of *B. miyamotoi* antibodies with *B. burgdorferi* antigen and other relapsing fever spirochetes that have the *glpQ* gene (177-179).

2.4.1.3 Epidemiology

In the United States, *B. miyamotoi* has been detected by PCR testing (using *flaB* and *glpQ* gene targets) in *I. scapularis* ticks collected from human specimens from 19 states, with an average infection prevalence ranging from 0.5%-3.2% across the Midwest and Northeast regions (180). Unlike *B. burgdorferi*, *B. miyamotoi* is transmitted vertically from an infected adult female to the eggs, with 0.8% of *I. scapularis* larvae testing positive for *B. miyamotoi* (180). Xu et al. also found that *B. miyamotoi*-positive ticks had a high prevalence of co-infections with *B. burgdorferi*, *A. phagocytophilum*, and *B. microti* ranging from 49.3%, 8.8%, and 1.0% for dual, triple, and quadruple infections, respectively (180). The high prevalence of co-infections may be attributed to shared wildlife reservoirs such as the white-footed mouse or possibly the longer infection and transmission of *B. miyamotoi* among white-tailed deer (181, 182). Analysis of human samples revealed that seropositivity of *B. miyamotoi* ranged from 2.8% to 3.9% in New England, roughly one third the positivity rate of *B. burgdorferi*, with a small number of patients having co-infections with both pathogens (176, 183).

In Canada, the prevalence of *B. miyamotoi* is considerably lower than in the United States (184). Results from the Canadian Lyme Sentinel Network (CaLSeN), a pan-Canadian LD surveillance network launched in 2019, showed that *B. miyamotoi* prevalence in *I. scapularis* ticks was <0.1% nationally, with the provinces of Quebec and Ontario reporting the highest estimates at roughly 1.0% infection prevalence (42). A recent study looking at ticks from veterinary clinics found a somewhat higher infection prevalence of *B. miyamotoi* ranging from 1.0% (Ontario) to 4.6% (Manitoba), although data were pooled for multiple *Ixodes* tick species, some of which rarely bite humans (185). In Canada, co-infection with other tick-borne pathogens in *B. miyamotoi*-positive

samples is estimated to be < 3.0%, although *B. miyamotoi* and *B. burgdorferi* co-infection occurred more frequently than by chance (184). Similarly, in human samples, *B. miyamotoi* infection was over three times higher in patients who were seropositive versus seronegative for *B. burgdoferi* (186).

2.4.2 Human Granulocytic Anaplasmosis

2.4.2.1 Disease Etiology

Human granulocytic anaplasmosis (HGA) was identified in the mid 1990s as a tick-borne rickettsial illness in the United States (187). It was originally known as human granulocytic ehrlichiosis (HGE) before unification of some *Ehrlichia* bacterial species, which then established *A. phagocytophilum* as the causative agent of HGA (188). In North America, two genetic variants of *A. phagocytophilum* are commonly detected in *I. scapularis* ticks: the Ap-ha variant and the Ap-variant 1, with only the former being associated with HGA (187). *Anaplasma phagocytophilum* is a Gram-negative, obligate intracellular bacterium that proliferates within granulocytes (e.g., neutrophils), a subset of white blood cells (189). Within the cells, the bacteria reside in endosomes, where they prevent the fusion with lysosomes to escape granulocyte degradation and grow into a cluster called a morula (189). The bacteria induce a strong pro-inflammatory response and affect the normal function of neutrophils as microbicidal effectors and mediators of inflammation (189).

2.4.2.2 Clinical Manifestations, Treatment, and Diagnosis

Clinical features of HGA are variable; and initial manifestations are often mild and nonspecific (e.g., fever, chills, myalgia, headache) (190). Gastrointestinal symptoms such as diarrhea, nausea,

vomiting, and abdominal pain also occur in many cases with HGA (190). A rash is not typically indicative of HGA, although a recent review found that rashes occur in up to 17% of patients (190, 191). The variability of symptoms may be linked to different bacterial clades, as some *Anaplasma* species in Asia are more often associated with rashes and different clinical manifestations (190, 192). HGA is also associated with less common but severe symptoms affecting multiple organs including the heart (e.g., myocarditis), nervous system (e.g., seizures, neuropathy), kidneys (e.g., glomerulonephritis), liver, and respiratory tract (e.g., acute respiratory distress syndrome) (189, 190). Secondary opportunistic infections (e.g., *Candida*, *Aspergillus*, Herpes simplex) are also reported in HGA because of impaired immune function as well as co-infections with other tick-borne pathogens, which are reported in approximately 2.3% to 10% of HGA patients (189, 190). As many as 3% of patients with HGA develop life-threatening complications (193).

As is the case for LD and BMD, HGA is treated with doxycycline or other tetracycline antibiotics (190). Patients may require additional treatments for other complications, secondary infections, or coinfections with other tick-borne pathogens (190). There are several methods used for diagnosis of HGA. These include histology (i.e., peripheral blood smear to identify morulae in neutrophils) and immunohistology (i.e., detection of *A. phagocytophilum*-specific IgM and IgG antibodies using immunofluorescence), direct culture of *A. phagocytophilum* bacteria, and whole-blood PCR against *A. phagocytophilum* genes (Msp-2: major surface protein 2) (190, 193).

2.4.2.3 Epidemiology

Anaplasma phagocytophilum is transmitted to humans by *I. scapularis* and *I. pacificus* but is most prevalent in the Midwest region of the United States, from which HGA appears to be expanding

(4, 194, 195). *Anaplasma phagocytophilum* has also increased rapidly throughout the Northeast region. For example, in New York state, the infection prevalence in *I. scapularis* ticks increased substantially from 4.0% in 2010 to 9.2% in 2018 (196). This was mirrored by a 3.9-fold increase in HGA incidence from 2 cases per 100,000 population in 2010 to 7.6 cases per 100,000 population in 2018 (196). However, the national incidence of HGA in the United States is still low at 6.3 cases per million population, although in the Midwest and some hotspots in the Northeast the incidence of HGA is >60 cases per million population (4).

Due to the northward range expansion of *I. scapularis* in two separate clades, with one population expanding from the Midwest and another from the Northeast, *A. phagocytophilum* infection prevalence in Canada follows a similar pattern as in the United States. In Canada, *A. phagocytophilum* infection in blacklegged ticks is highest in the Prairie provinces, particularly Manitoba (5.6%) (195, 197). Recent studies have also shown an increasing prevalence of *A. phagocytophilum* in ticks in the Atlantic provinces (4.1% to 5.0%), Quebec (1.0% to 2.4%), and Ontario (1.1% to 3.5%) (42, 198). In the Thousand Islands, which share proximity to the United States, the prevalence of *A. phagocytophilum* in *I. scapularis* ticks is substantially higher and ranges between 6.0% to 9.4% (199). However, genotyping analyses revealed that these specimens and others from Ontario were predominantly part of the Ap-variant 1, which is not associated with human infection but with infection in white-tailed deer (197, 199). From 2007-2010, approximately 8.3% of infected ticks from Ontario were of the Ap-ha variant but this has increased to 19-26% from 2011-2017 (197, 200). Contrary, specimens from Manitoba and the Atlantic provinces showed a higher proportion of the Ap-ha variant (Prairies: 88-100%; Atlantic: 43-60%), which causes HGA and infection in white-footed mice (197). However, substantial knowledge

gaps remain since the Ap-ha variant is in turn composed of two distinct clades, one of which is genetically more similar to the non-disease causing Ap-variant 1 (201). Additional studies are needed to better understand the risk for human illness in view of the existing bacterial genetic diversity and the growing infection prevalence in blacklegged ticks.

2.4.3 Babesiosis

2.4.3.1 Disease Etiology

Babesiosis is an infection caused by hemoprotozoan parasites from the *Babesia* genus (202). *Babesia* parasites have a sexual stage in Ixodid ticks, where gametocytes replicate in tissues like salivary glands and produce up to 100,000 sporozoites during tick attachment to its host (202). *Babesia* parasites then have an asexual stage in erythrocytes of vertebrate animals, where infecting sporozoites, transmitted by tick vectors, replicate into trophozoites and merozoites and continue invading more red blood cells following hemolysis (202). There are over 100 species of *Babesia* identified that infect various wildlife and domestic animals including cattle (e.g., *B. bovis*, *B. divergens*), dogs and cats (e.g., *B. canis*, *B. felis*), deer (e.g., *B. odocoilei*, *B. venatorum*), rodents (e.g., *B. microti*), and currently unidentified animal reservoirs (e.g., *B. duncani*) (203, 204). However, not all *Babesia* species are zoonotic. Those that are most often associated with human babesiosis include *B. divergens*, *B. venatorum*, *B. microti*, and *B. duncani*, with the latter two contributing to infections in North America (204).

2.4.3.2 Clinical Manifestations, Treatment, and Diagnosis

Human babesiosis can be subclinical or resemble a malaria-like illness (203). Symptoms of babesiosis include a gradual onset of malaise and fatigue, intermittent high fever, chills, sweats, headaches, and myalgia (203). Less common symptoms include arthralgia, sensitivity to stimuli (e.g., touch or photophobia), neck stiffness, abdominal pain and vomiting, and difficulty breathing or coughing (203, 205). However, disease severity is considerably worse in immunocompromised individuals (203). Treatment for babesiosis resembles that for malaria and is only recommended for symptomatic infection (206). It involves a combination of antibiotic and antiparasitic drugs such as oral azithromycin with atovaquone or clindamycin plus quinine (204, 206). For patients with high parasitemia or relapsing babesiosis, three-drug artemisinin-combination therapy has also been used and, for those with severe hemolytic anemia, red blood cell exchange transfusion may be required (203, 206). Symptomatic babesiosis diagnosis is done clinically based on tick exposure history and symptoms (e.g., fever, jaundice, splenomegaly) and with laboratory testing (e.g., low red blood cell and platelet counts, elevated liver enzymes and bilirubin, hematuria) (203). The diagnosis is confirmed with blood smear microscopy, serology (i.e., immunofluorescence), or PCR testing, with the latter having the highest sensitivity (133, 203).

2.4.3.3 Epidemiology

In the United States, *B. microti* and *B. duncani* are widespread but not as prevalent as *B. burgdorferi* (207). *Babesia microti* is found in the Northeast and upper Midwest regions, where *I. scapularis* is distributed, and *B. duncani* is reported on the West coast, from California to Washington (207). In the Northeast, *B. microti* was detected in approximately 2.8% to 7.1% of ticks tested for tick-borne pathogens, which is similar to the prevalence of *A. phagocytophilum*,

whereas, in the Midwest, it was less commonly detected (0.3% to 2.9%) (207). Most human babesiosis cases are reported from New England, New Jersey, New York, Minnesota, and Wisconsin, with an estimated incidence rate of 1.0 to 14.9 cases per 100,000 population in 2019 (national incidence rate in 2019: 0.89 cases per 100,000 population) (208).

In Canada, the prevalence of *B. microti* in *I. scapularis* ticks is extremely low and most tick surveillance studies testing for zoonotic *Babesia* species are largely unable to detect this parasite or do so at extremely low frequencies (42, 199, 209, 210). A summary of *Babesia* surveillance from 2009-2013 showed that *B. microti* positivity in *I. scapularis* ticks was 0.12% and 1.6% from passive and active tick surveillance, respectively, with the majority of positive samples derived from Manitoba and a few from New Brunswick, Ontario, and Quebec (211). More recently in Ontario, *B. microti* infection prevalence in ticks submitted by the public and companion animals was 0.054% from 2011-2017 (212). Additionally, two serosurveys of Canadian blood donors were conducted in 2013 and 2018, with the former detecting no positive *B. microti* samples out of 14,000 blood donors tested and the latter detecting one positive *B. microti* sample by transcription-mediated amplification out of over 50,000 blood donor samples tested (211, 213). Overall, these data suggest *B. microti* prevalence is still currently very low in Canada, although the prevalence of this pathogen is predicted to increase with blacklegged tick abundance and favourable animal hosts (214).

Of note, *B. odocoilei* prevalence in Canada appears to be increasing and, while it is not typically associated with human infection, this parasite may have important ecological implications for wildlife (215-217). Furthermore, the association between *B. odocoilei* and human babesiosis

remains under investigation, with a recent study identifying *B. odocoilei* by PCR testing in two patients with symptoms of babesiosis (218). Other *Babesia* species may also pose a risk to humans, although it is currently difficult to determine the prevalence of these pathogens in the Canadian population since babesiosis is not currently a nationally notifiable disease and there is limited testing available for specific *Babesia* species (219).

2.4.4 Powassan Virus Disease / Powassan Encephalitis

2.4.4.1 Disease Etiology

Powassan virus disease is caused by infection with the Powassan virus, a flavivirus isolated from the brain of a patient who died of encephalitis in 1958 in Powassan, Ontario, Canada (220). Powassan virus is part of the tick-borne encephalitis virus (TBEV) complex, which comprises several related tick-borne encephalitic viruses across Asia and Europe, although Powassan is the only one found in North America (220). Unlike other tick-borne pathogens, Powassan virus is present in the tick salivary glands and is not sequestered in the tick midgut, resulting in rapid transmission of the pathogen in as little as 15 minutes of tick feeding based on animal models (221). Powassan virus is mostly transmitted by the groundhog tick, *Ixodes cookei*, although in 1997 the deer-tick virus (DTV), a subset of Powassan virus transmitted by *I. scapularis*, was identified in New England in the United States (222). The two viruses are serologically indistinguishable, with high nucleotide and amino acid similarity, and represent two prototype lineages: lineage I (Powassan virus) and lineage II (DTV) (223). For this thesis, the term Powassan virus will be used to represent both lineages, unless otherwise specified.

2.4.4.2 Clinical Manifestations, Treatment, and Diagnosis

The clinical manifestations of Powassan virus disease range from subclinical infection, symptomatic infection resembling a systemic febrile illness, and a neuroinvasive illness like that caused by other flaviviruses (e.g., West Nile virus, dengue virus, yellow fever virus, Zika virus) (168). In the United States, neuroinvasive disease, specifically encephalitis, is the most common manifestation, although this may be due to underreporting of mild or asymptomatic illness (5). Neuroinvasive illness is marked by fever, confusion, seizures, headaches, vomiting, rash, and focal neurologic deficits (224). Of those with neuroinvasive illness, Powassan encephalitis mortality ranges from 10% to 15%, and an estimated 50% of survivors are left with memory loss, weakness, and residual neurologic deficits (5, 168, 224). Currently, there is no treatment for Powassan virus disease / encephalitis and hospitalized patients only receive supportive care (168, 224). Powassan virus disease is diagnosed based on compatible clinical symptoms plus a laboratory test to distinguish the infection from other flaviviruses (224). The gold-standard laboratory diagnostic test is an enzyme-linked immunosorbent assay (ELISA) to measure IgM against Powassan virus in serum or cerebrospinal fluid (CSF) followed by a confirmatory plaque reduction neutralization test (PRNT) to measure the ability of patient serum to neutralize viral particles (224). However, these tests are mostly done at public health or research laboratories and are not point-of-care or routinely conducted on clinical specimens, as such many clinical cases of encephalitis do not have an attributable etiology (223, 224).

2.4.4.3 Epidemiology

While Powassan virus was first isolated in Canada, cases are substantially higher in the United States. From 1958 to 2000, there were 27 cases of Powassan virus disease in Canada and the United

States combined, most of which were clustered in Ontario, Quebec, New Brunswick, New York, New Jersey, and Massachusetts (223). In contrast, from 2003 to 2020, there were 165 cases of Powassan virus disease in the United States alone, which is attributed to the growing prevalence of lineage II virus in *I. scapularis* ticks, the expanding range of blacklegged ticks, and increased surveillance efforts after neuroinvasive Powassan virus become nationally notifiable in 2002 (223, 225). In the United States, Powassan virus prevalence in *I. scapularis* ticks has generally increased over the years, with an estimated 5% prevalence in parts of New England, though there is spatial and temporal variability in the positivity rates (223). In Canada, Powassan infection prevalence is lower and a recent survey of *Ixodid* ticks collected from wildlife and companion animals in Ontario found a 1.0% prevalence of Powassan virus in *I. cookei* ticks but no positive samples from *I. scapularis* ticks (226). Similarly, in the 2019 pilot year of the CaLSeN, Powassan virus lineage II (DTV) was detected in 0.6% of ticks collected through active surveillance in Nova Scotia. Overall, prevalence of Powassan virus in *Ixodid* ticks and human cases of Powassan virus disease remain low in Canada, although evidence is suggesting a geographic expansion.

2.5 CHAPTER SUMMARY

In the first part of this chapter, I described the blacklegged tick's life cycle that contributes to the seasonality of LD in temperate zones. I discussed the host-seeking behaviour of ticks and the various types of vertebrate hosts they parasitize, as well as the impact that host selection has on the amplification, dilution, and transmission of the LD-causing bacteria, *B. burgdorferi*. I also summarized the characteristics of tick habitats and the biotic and abiotic factors associated with tick densities and nymphal infection rates.

In the second part of the chapter, I discuss the epizootic cycle of *B. burgdorferi* in North America and how this relates to the blacklegged tick's life cycle. I then outline the process of bacterial infection in the arthropod vector and the vertebrate host, highlighting the key gene regulation components that contribute to this biphasic lifecycle. I end this section with an overview of the bacterial genome and the current genotyping methods and their implications for understanding host specialization, tick dispersal patterns, and pathogenicity in humans.

I then described *B. burgdorferi* infection in humans and the clinical manifestations of LD at various stages of infection as well as the complications and controversies associated with PTLD and CLD. I touch upon the recommended standard two-tiered testing method for LD diagnosis and the sensitivity and specificity of the various enzyme immunoassays and immunoblots used for serological testing. Finally, I discuss modified testing methods and other laboratory assays that have been considered previously or are in development.

In the last section of this chapter, I provided a brief overview of other tick-borne illnesses, including anaplasmosis, tickborne relapsing fever, babesiosis, and tickborne encephalitis. I discuss the bacterial, viral, and protozoan parasites that cause these illnesses, the clinical features associated with each illness, and the current prevalence of other tick-borne illnesses in North America.

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3

3 METHODS

This chapter provides a general overview of the study setting and available data sources to orient the reader ahead of the main analyses. It introduces the methods used in my thesis including maximum entropy species distribution modelling, conditional logistic regression modelling for case-control data, spatial analyses for cluster detection, and laboratory assays for identification of tick-borne pathogens and bacterial genetic diversity. The specific modelling parameters and methodological details for each thesis objective are discussed in the respective publications found in the next four chapters.

3.1 STUDY AREA

My research on Lyme disease and other tick-borne pathogens is focused on eastern Ontario, where the incidence of Lyme disease per 100,000 population is highest in the province (1). However, with collaboration from researchers at the University of Guelph and enhanced tick surveillance

over the years, I was able to expand my study areas to include the entire southern tip of the province (Chapters 4 and 5) and the National Capital Region, consisting of the cities of Ottawa, Ontario and Gatineau, Quebec (Chapter 7).

Eastern Ontario is the region between the St. Lawrence River and the Ottawa River, which is dominated by deciduous woodlands, bogs, marshes, and agricultural land. It shares water borders with Quebec to the north and the United States to the southeast. Eastern Ontario consists predominantly of rural areas, with several urban centers; the largest being the cities of Ottawa (1,017,449 population) and Kingston (132,485 population) (2, 3). Rural areas are defined by Statistics Canada as: 1) towns, villages, and populated places with less than 1,000 population, 2) rural areas of census metropolitan areas that contain estate lots, undeveloped land, agricultural land, or un-developable land, 3) agricultural land, and 4) remote and wilderness areas (4). Most rural areas in eastern Ontario are "less to least remote" on the index of remoteness, which classifies municipalities into five levels of remoteness based on geographic proximity to urban areas and the population size of the nearby urban areas (3). This creates an environment where tick populations can expand and colonize ecologically suitable regions that share connectivity and proximity to human populations.

My thesis examines tick habitat suitability in southeastern Ontario to better identify Lyme disease risk areas and ecological variables associated with *I. scapularis* presence, the patterns of Lyme disease emergence across eastern Ontario and the socioecological factors associated with Lyme disease in this region, and the current risk of other emerging tick-borne pathogens.

3.1.1 Data Sources

I used several data sources throughout this thesis that can be grouped into two broad categories: 1) surveillance data and 2) sociodemographic and ecological data.

3.1.1.1 Surveillance Data

Surveillance data on Lyme disease is collected by various provincial and national surveillance systems to monitor the distribution and abundance of blacklegged ticks in the environment and the annual number of human Lyme disease cases. These data were obtained from Public Health Ontario (PHO) and from local public health units via data sharing agreements as well as generated by the INSIGHT lab with funding from the Public Health Agency of Canada (PHAC), the Canadian Lyme disease Research Network (CLyDRN) and the Canadian Institutes of Health Research (CIHR). The three surveillance data sources that I used in my thesis are:

1) Passive tick surveillance data that contains information on *I. scapularis* ticks found by the public and submitted to local public health units and government laboratories for testing. Specifically, data was available for the location of tick acquisition based on submitter recall and/or travel history, the host on which the tick was found (i.e., human, cat, dog, other animal), the submitter's residence, age, sex, and body part where the tick was attached (if applicable), the tick species, instar, and engorgement level (an approximate indication of the length of time the tick was feeding on the host), and pathogens detected via molecular testing.
2) Active tick surveillance data that was generated by the INSIGHT lab using field sampling at sentinel locations across the study area. This data contains information on the presence, abundance, and density of *I. scapularis* ticks at specific sites surveyed (including specific

latitude and longitude coordinates collected with a handheld GPS device), the tick instar, and the pathogens detected by molecular testing at the University of Ottawa or the NML.

3) Nationally notifiable human Lyme disease case data that is reported to public health organizations by clinicians or through government laboratories reporting results of serological diagnostic testing. This data includes information on patient age, sex, episode date, serological testing results, Lyme disease status (i.e., probable or confirmed), home address or first three digits of the postal code, and possible location of tick acquisition within or outside Canada based on patient recall and travel history.

3.1.1.2 Sociodemographic and Ecological Data

Sociodemographic and ecological data were obtained from local and provincial open-source databases and were linked to surveillance data or used to calculate or derive explanatory variables. The data sources that I used in my thesis are:

1) Elevation, landcover, and land use data obtained from satellite remote sensing and classified into layers or classes (i.e., forest, marsh, bog, agricultural land, infrastructure, etc.) by the Ontario Ministry of Natural Resources and Forestry (MNRF). These data are in multilayer raster format and were available at 15- to 25-meter pixel resolution.
2) Geospatial data on national, provincial, and municipal parks and trails developed and curated by the MNRF's Land Information Ontario (LIO) program. These data contain aerial imagery and shapefiles for use in geographic information systems (GIS).
3) Canada-wide data on long-term climate averages that was generated by Natural Resources Canada. This data includes 19 bioclimatic parameters derived from temperature and precipitation records at 5 km pixel resolution and annual cumulative degree days ≥ 0°C.

4) Sociodemographic data that was obtained from the 2016 Canada Census (e.g., population density estimates, neighbourhood income quintiles, and census geographies), the Rurality Index for Ontario (e.g., measured based on access to health services), walkability and transit scores (e.g., derived from cluster analysis of connectivity, dwelling density, points of interest, and transit stops), and the Ontario Marginalization Index (e.g., four factors derived from factor analysis of 42 census indicators).

3.2 SPECIES DISTRIBUTION MODELLING

Species distribution models (SDM), also referred to as ecological niche models (ENM), are widely used in studies of ecology and conservation, and more recently for modelling diseases and disease vectors. SDMs for vector-borne diseases are of particular interest because exposure to pathogens is highly dependent on the geographic distribution and abundance of the vector (5-7). For Lyme disease, the occurrence of *I. scapularis* in the environment is the principal method used for estimating risk of human exposure to *B. burgdorferi* (8). Therefore, I aimed to develop predictive SDMs for *I. scapularis* and *B. burgdorferi* in southeastern Ontario to better understand and characterize risk areas for Lyme disease.

3.2.1 Overview of Species Distribution Models

SDMs are statistical models that use various algorithms to correlate species occurrence at specific sites with environmental or spatial characteristics of those sites (9). The statistical approaches for species distribution modelling include extensions of classical regression models, which allow for modelling complex non-linear or non-parametric geographic distributions, and decision tree

methods or complex machine learning algorithms, which can handle many climatic or environmental variables or limited *a priori* knowledge on factors contributing to a species' distribution (10, 11). The selection of the SDM approach in part depends on the type of species occurrence data that is available. This includes presence data, where the dependent variable is expressed as 1/0, coverage data, where the dependent variable is a value between 0-100%, or abundance data, where the dependent variable can be any positive number. Most SDM approaches are suitable for presence/absence and abundance data, although a drawback is that sampling factors (e.g., temperature, time of day, accessibility, etc.) can affect one's ability to detect the species so an absence does not necessarily indicate the species is not present in that location (12).

I selected maximum entropy (Maxent), a machine learning SDM approach, to model the predicted distribution of *I. scapularis* and *B. burgdorferi*-infected ticks at locations across Ontario using field surveillance data from 2014-2018. Maxent is among the most robust and frequently used SDM algorithms, which requires presence-only species data and has numerous advantages when it comes to *a priori* assumptions about the distribution of the data and efficiency with large number of parameters (11, 13).

3.2.2 Principles of Maximum Entropy

Maxent is based on the principle of maximum entropy derived from information theory[2] (14). Entropy (or Shannon entropy) is a measure of information or uncertainty (i.e., the entropy for a random variable is the amount of information needed to fully describe it, which can also be thought

[2] Information theory is the foundation of computer science and is the mathematical study of quantification, storage, and communication of information. It was pioneered by Claude Shannon's publication, *A Mathematical Theory of Communication*, in 1948.

of as the amount of uncertainty for that variable) (15, 16). Similarly, conditional entropy is defined as the entropy of one variable conditional on knowledge of another variable (i.e., the amount of uncertainty for one variable given information for another variable) (15, 16). Entropy can then be used to select probability distributions, such that the probability distribution with maximum entropy is "uniquely determined as the one which is maximally noncommittal with regard to missing information, in that it agrees with what is known, but expresses maximum uncertainty with respect to all other matters"[3] (17). In more simple terms, the distribution that best represents the state of knowledge, given certain *a priori* knowledge, is the one with maximal entropy (18). The probability with maximum entropy is calculated as (18, 19):

$$\max H(X) = -\sum_{X} p(X)\log p(X) \quad \text{subject to} \quad \sum_{X} p(X) f_i(X) = c_i \quad \text{for all constraints } f_i$$

$$\text{with solution:} \quad H(X) = \exp\left(-1 + \lambda_0 + \sum_{i} \lambda_i f_i(X)\right)$$

Where, $H(X)$ is the probability distribution with maximum entropy, $f_i(X)$ are constraints on the distribution of X, and λ_i are the values of the constraints.

3.2.3 Maxent Modelling

Maxent is a proprietary software developed by Steven J. Phillips that uses the principle of maximum entropy to identify the probability distribution of species for applications in ecology and disease modelling (14). Maxent uses species presence data and randomly selected pseudo-absences (i.e., background points) to generate the probability distribution with maximum entropy across a landscape, often conceptualized as habitat suitability or an approximation of the species' niche,

[3] The principle of maximum entropy was theorized by Thomas Jaynes in 1957 and emphasizes the link between probability theory and information theory.

constrained within a set of environmental parameters (14). The following sections describe key aspects of Maxent modelling and outputs.

3.2.3.1 Species Presence

Since the Maxent distribution can be conceptualized as an approximation of a species' ecological niche, a main assumption is that species presence points represent an unbiased sample from the species' realized niche[4] (14, 20). To ensure an unbiased representation of species presence points and pseudo-absences, I rarefied clustered presence points to avoid pseudo-replication of datapoints (as described in the Chapter 4) and I created a sampling bias file so that Maxent can select pseudo-absences from locations nearby presence points. This ensures that differences in parameters at presence and absence locations can be compared locally instead of randomly selecting a pseudo-absence that might represent suitable species habitat that is yet unoccupied due to factors such as barriers to dispersion (resulting in false negatives) (21, 22).

The bias file was created using the Gaussian kernel density sampling method, which uses a Gaussian weighing function (kernel) to estimate the probability density function based on the weighted average of neighbouring observations (21, 23). For each point, a smoothed curve (Gaussian kernel) is fitted, with the value highest at the point location and diminishing with distance within a search radius (bandwidth) (24). The values of all the kernels are then summed to create a smoothed "grid" that up-weights presence-only data points with fewer neighbours in the

[4] A realized is defined as the set of conditions used by given animal after interactions with other species (predation and especially competition) and limitations for dispersal have been considered. In contrast, a fundamental niche is defined as the entire set of conditions under which an animal can survive and reproduce itself (Soberón & Arroyo-Peña, 2017). SDM are based on locations where the species is found and, therefore, model the realized niche.

geographic landscape (24). The choice of bandwidth specifies distance decay (i.e., interactions between two locations declines as the distance between them increases), and, in ArcGIS, this search radius is calculated by default based on the spatial configuration and number of datapoints (unless it is otherwise specified) (25).

3.2.3.2 Environmental Variables

In total 41, climate, land cover / land use, and elevation variables were available or derived from satellite remoting sensing data as explained in Chapter 4. While Maxent does not have restrictions for the number of parameters that can be modelled, I elected to include only variables that contributed to the predicted distribution of *I. scapularis* or *B. burgdorferi* and that were not highly correlated or dominated by a particular feature (e.g., climate-driven only). My aim was to generate more biologically relevant models (less data mining). For each of the variables, Maxent then incorporates several "features", which are mathematical transformations of the covariates used in the model to allow complex relationship to be modeled (14). These features that can be specified include linear (a continuous variable), quadratic (square of a continuous variable), product (product of two continuous variables), threshold (piecewise constant response), binary and hinge (piecewise linear response) (14).

3.2.3.3 Model Calibration

Including large numbers of features tends to result in overfitting; therefore, I relied on previously published simulation models to select appropriate features for the *I. scapularis* and *B. burgdorferi* models based on the number of datapoints I had (26, 27). In addition, a regularization multiplier, or a penalty for overfitting, was imposed to the model. The goal is to prevent model over-

complexity and/or overfitting by controlling the chosen features used to build the model (26, 27). I selected the value of the regularization multiplier by partitioning the data into training/testing sets and evaluating the effect on the model fit (described in detail in Chapter 4).

3.2.3.4 Contributions of Variables and Model Outputs

The contribution of each predictor variable to the final model is calculated using a jackknife procedure (resampling) to assess the regularized training gain of models built using each variable individually or when each variable is omitted from the model in turn (14). Model gain is closely related to deviance for maximum likelihood estimation and indicates how closely the model is concentrated around the presence of samples (i.e., how much higher the average likelihood of the presence sample is compared to background) (28).

The Maxent output is selected by the user, with the default being the raw exponential model. The raw output, H(X), is calculated based on the solution for the distribution with maximum entropy (equation 1) and represents the exponent of the sum of a feature functions, $f_i(X)$, for a set of variables associated with a given grid cell (28). The cumulative output is based on a threshold, c, that results in a binary prediction with omission rate c% on the Maxent distribution (i.e., predicted omission) (28). The omission rate is defined as the fraction of presence localities that fall into pixels not predicted as suitable for the species and is a function of the cumulative threshold selected (28). The Maxent model can also generate a logistic-scaled output that is calculated as (28):

$$\text{logistic scaled output} = \left(\frac{\text{raw output} * \exp(\text{entropy})}{1 + \text{raw output} * \exp(\text{entropy})}\right)$$

The logistic (or cloglog) output re-scales the raw output in each grid cell to fall between 0 and 1. The output is interpreted as the probability of species occurrence or the predicted habitat suitability for the species across the landscape.

3.3 Case-Control Study Design and Analysis

3.3.1 Participant Selection

I developed a case-control study to determine whether Lyme disease is associated with residence in environmental risk areas for *I. scapularis* ticks in southeastern Ontario. Briefly, a case-control study identifies persons with a disease (Lyme disease cases) and a portion of the source population that gave rise to the cases (controls) and then retrospectively determines the exposure histories (residence in risk areas) in the two groups (29). In the analysis, the odds of Lyme disease are compared for the persons residing in high-risk areas compared to low-risk areas. The incidence rates of Lyme disease in low- versus high-risk areas cannot be estimated directly because the total population at risk of Lyme disease is unknown (29). Instead, the controls are used to approximate the exposure distribution in the source population and serve in place of the denominator in measures of disease frequency (30). The validity of case-control studies depends on the assumption that controls are representative of the source population (which also gave rise to cases), and that sampling is independent of exposure (30, 31).

For my study, I selected Lyme disease cases from a population registry (i.e., Lyme disease reportable disease surveillance) and included all probable and confirmed cases residing in the study area (southeastern Ontario) at the time of diagnosis. I chose to use population controls from the

Registered Persons Database (RPDB), which contains information on all persons registered for health insurance in Ontario, since this was the closest representation of the case source population available (i.e., Ontario residents). The controls were randomly selected from approximately 16 million records in the RPDB, although a few small exclusion criteria were applied to ensure data availability and residence in Ontario at the same times as cases (detailed in Chapter 5).

Cases and controls were matched at a 1:4 ratio based on age, sex, and index date. Matching ensures that the cases and controls do not differ on confounders such as age and gender by adjusting the sampling of the controls to have identical (for the exact matching that I used) values on these variables as each Lyme disease case (32). When matching on confounders, the adjusted odds ratio estimates are likely to be less variable (i.e., more statistically efficient) than in unmatched data, where cases and controls may be selected at a very different distribution (33, 34).

3.3.2 Matched Analysis

Case-control studies are analysed using logistic regression, but this model assumes that sampling is not stratified (except on the case or control status) (32). When cases and controls are matched on specific variables (e.g., age and sex) the observations are not independent, and they are essentially stratified by the matching variable (35). In other words, the matching process makes the controls more like the cases, which introduces a bias that needs to be controlled in the analysis (32, 36). Therefore, I used a conditional logistic regression model, which is conceptually identical to logistic regression with the exception that the estimates are conditional on the matched pairs. Conditional logistic regression models the log odds of Lyme disease as a linear function of environmental risk (*I. scapularis* habitat suitability) and other covariates (37):

$$log\left(\frac{\pi_k}{(1-\pi_k)}\right) = \alpha_k + \beta_1 x_1 + \beta_2 x_2 + \cdots \beta_i x_i$$

Where, π_k indicates the probability of Lyme disease outcome conditional on the matching sets, α_k is the contribution to the log odds of all constant terms in the *k*th matching sets (i.e., the pair effect), β_i are the regression coefficients associated with the reference group (i.e., the effect of the explanatory variables), and x_i are explanatory variables.

The regression coefficients are determined using conditional maximum likelihood estimation (38). For individual-level matching, unconditional maximum likelihood estimation should not be used because when there are many small strata for each matched pair the number of parameters (and degrees of freedom) will be very high for the number of datapoints, resulting in estimates that are invalid (32, 37). The conditional maximum likelihood uses the information contained in the matching and eliminates the nuisance stratum parameters (α_k) (37). As a result, only discordant pairs (*n*10 and *n*01 in Table 1) are considered in the estimation of the coefficients associated with predictor variables because the case-control pairs in which both the case and the control have the same exposure (*n*00 and *n*11 in Table 1) tell us nothing about the exposure-outcome association (32).

Table 3.1 Discordant and concordant matched-pairs (1:1 matching) in a hypothetical case-control study with a binary explanatory variable X (e.g., low/high environmental risk for Lyme disease).

CONTROL	**CASE**	
	X=0	X=1
X=0	*n*00	*n*10
X=1	*n*01	*n*11

The regression coefficients obtained by conditional maximum likelihood estimation are interpreted in the same way as for unmatched analysis, but the intercept is not estimable because stratum parameters are not fitted (38):

- For a continuous variable x_i, β_i represents the change in log odds of disease for a unit change of x_i when all other variables are constant.
- For a categorical variable x_i, β_i represents the log odds of disease associated with comparing levels of x_i when all other variables are constant.

3.3.3 Sample Size Calculation

The sample size for a matched case-control study was obtained using an online calculator using the formula developed by Dupont (39). In the derivation of this formula, Dupont showed that the number of cases (N) needed for a matched case-control study is a function of p_0, the probability of exposure (i.e., residence in high environmental risk areas) among controls, ϕ, the correlation coefficient for exposure between matched subject, ψ, the minimum detectable odds ratio, α, Type I error probability, and β, Type II error probability (power) (39, 40).

For my case-control study, I selected standard values of α=0.05 and β=0.90, ϕ=0.02 (suggested by Dupont when the correlation for exposure between match subjects is unknown), and conservative estimates for the minimum detectable odds ratio (ψ = 1.2) and high probability of exposure in the controls (p_0 = 0.5) to account for residence in rural and suburban areas in eastern Ontario with a high predicted probability of *I. scapularis*.

3.4 SPATIAL ANALYSES

Geographic data on blacklegged tick distribution and Lyme disease incidence tend to be spatially dependent, meaning that nearby locations have the propensity to influence each other and to have similar attributes (i.e., clustering) (41). This concept was described by the geographer, Waldo Tobler, whose First Law of Geography states, "everything is related to everything else, but near things are more related than distant things" (42). Spatial processes that give rise to clustering can be stationary or non-stationary. Stationarity is a condition describing data that has a constant mean and variance in each location (i.e., the joint probability distribution on all possible pairs of outcomes does not change with location but is dependent on the distance and direction between two locations) while non-stationarity occurs when the spatial pattern differs across the study area (43, 44). Stationary spatial processes are analysed using "global" statistics that measure spatial trends, referred to as first-order effects (43). In contrast, non-stationary spatial processes are analysed using "local" statistics that measure small-scale variation due to interactions between neighbours, referred to as second-order effects (43).

In the context of ticks and tick-borne diseases, clustering can arise from elements such as limitations for dispersal (e.g., barriers, low tick/host mobility, human accessibility) and ecological restrictions (e.g., temperature, vegetation, animal host abundance, bacterial reservoir competency). To assess clusters of *B. burgdorferi*-infected ticks and human Lyme disease cases, I used spatial methods for "local" cluster analysis because 1) the spread of ticks and tick-borne pathogens across eastern Ontario and the colonization of various woodland habitats by animal hosts is focal and varies locally (i.e., non-stationary), and 2) my objective is to identify risk areas and specific locations where clusters of *B. burgdorferi*-infected ticks and human cases are occurring (7, 45).

Through the passive tick surveillance and reportable disease surveillance systems, I had geographic data on the location of *B. burgdorferi*-infected ticks and locations where human Lyme disease cases were exposed. This data varied in accuracy from an exact location that could be converted to latitude and longitude coordinates (e.g., trail name, park, camp site, etc.) to broader or non-specific locations (e.g., neighbourhood, town, etc.). Therefore, I aggregated all locations to the dissemination area (DA), which is the finest scale at which census data is available in Canada, and I used two cluster detection methods for area-level data (i.e., DA-aggregated data) and point-level data (i.e., centroid coordinates of the DA). I used the Local Indicators of Spatial Association (LISA) statistic to detect DAs with hot spots and outliers of high Lyme disease cases and *B. burgdorferi*-infected ticks and the Kulldorff Spatial Scan statistic to detect clusters not bound by administrative boundaries.

3.4.1 Local Indicators of Spatial Association Statistic

Spatial autocorrelation is the statistical measure of the similarity of observations at spatial locations, with positive and negative values indicating that neighbouring observations are similar or dissimilar, respectively (44). The most used statistic to describe spatial autocorrelation is the Moran's *I* coefficient, which measures the similarity of values between all possible combinations of observation pairs with their degree of spatial proximity based on a spatial weights matrix (44). Moran's *I* statistic is obtained by (44):

$$I = \frac{n \sum_i^n \sum_j^n w_{ij}(y_i - \overline{y})(y_j - \overline{y})}{(\sum_i^n \sum_j^n w_{ij}) \sum_i^n (y_i - \overline{y})^2}$$

Where, n is the number of zones (i.e., DAs), $\overline{y}$ is the mean value across all locations (i.e., infected ticks or Lyme disease cases), y_i and y_j are the observed values at locations *i* and *j*, and w_{ij} is the spatial proximity matrix. Moran's *I* is a "global" test used to assess whether the data show a pattern of clustering across the entire study area, but it does not identify specific clusters (46).

The local indicators of spatial association (LISA) statistic, also referred to as the Anselin Local Moran's *I*, is a "local" test for spatial autocorrelation that decomposes the Moran's *I* statistic into contributions for each local area within the study region (47). Therefore, the sum of LISA statistics for all observations is proportional to the global Moran's *I* (44, 47). The Local Moran's *I* statistic at location *i* is given by the following equation (44):

$$I_i = z_i \sum_{j, j \neq i}^{n} w_{ij} z_j$$

Where, z_i are deviations from the mean $(y_i - \overline{y})$ and w_{ij} is the spatial weights matrix. To conceptualize the spatial relationship between zones, I used a contiguity matrix in which w_{ij} is 1 when *i* and *j* are adjacent zones (Queen contiguity: edges and corners) and w_{ij} is 0 when the zones are non-adjacent (43, 44, 48). The spatial weights were also row standardized, meaning that the weight w_{ij} was divided by the sum of the weights of all neighbouring zones (i.e., the weights sum to 1) (44, 48). Row standardization reduces bias of the imposed aggregation scheme, which can affect the number of neighbours for each zone (48).

Statistical significance of clusters is assessed through permutations, z-scores, and p-values of the observed data (48). Permutations are used to generate many random datasets, under the null hypothesis of complete spatial randomness, for which the Local Moran's *I* statistic is calculated, and an empirical distribution is generated (48, 49). The Local Moran's *I* statistic obtained from the

original data is then compared to the empirical distribution and z-scores (standard deviations) and p-values are computed to determine whether the null hypothesis is accepted (i.e., the observed spatial pattern is random) (48, 49). A high positive z-score for a zone indicates that surrounding areas have similar values (i.e., hot spots: high-high; cold spots: low-low) (48, 49). A low negative z-score indicates that the zone is a spatial outlier (i.e., high value outlier: high-low; low value outlier: low-high) (48, 49). When assessing the statistical significance of the Local Moran's *I* statistic, a false discovery rate (FDR) correction is applied to adjust for multiple inferences and Type I errors (50). The FDR correction works by estimating the number of false positives for a given confidence level from the permutation datasets, ranking the statistically significant p-values from smallest to largest, and removing the largest from the list to adjust the critical p-values and obtain a corrected 95% confidence level (48-50). The FDR correction is less conservative than traditional methods for multiple inference testing (i.e., Bonferroni correction), but these corrections also reduce the number of potential clusters detected (i.e., balance between detecting true clusters and missing potential clusters) (50). Thus, in my analyses, I elected not to report p-values associated with individual hotspots or outliers (only the overall corrected p-value (α=0.05) for the Local Moran's *I* statistic). I used the Local Moran's *I* statistic as a descriptive tool to describe the cluster types (hot spots, cold spots, outliers) alongside the Kulldoff spatial scan statistic.

3.4.2 The Kulldorff spatial scan statistic

The Kulldorff spatial scan statistic has wide application in epidemiology and is used to explore disease data for evidence of spatial patterns (43). It is often used for estimating local clustering in space and/or time, using a proprietary software called SaTScan that computes cluster sizes,

locations, cluster-specific relative risk, and statistical significance (51). The output from SaTScan can also be imported in a geographic information system (GIS) to visualize clusters on a map. I selected this method because it is established in literature for geographical surveillance of disease data, it can detect spatiotemporal clusters, and detect clusters of unknown size and location.

For each location in the input dataset (i.e., point coordinates or the administrative boundary centroid), SaTScan constructs a series of scanning windows in the geometry of circles (2-dimensional spatial data), cylinders (3-dimensional space and time data), or ellipses (irregularly shaped clusters with pre-specified angle) of varying radii that include all nearest neighbouring locations that fall within it, from zero until a pre-defined maximum of the total sample population is included (52, 53). At each location, these scanning windows of varying size are candidate clusters for which the likelihood is calculated based on the probability of observed events inside and outside the area (52). The Kulldorff spatial scan statistic then identifies the most likely cluster based on the likelihood ratio test statistic that computes the maximum likelihood value of the candidate cluster under the alternate hypothesis (i.e., rates of events are higher within the candidate cluster area than outside) to the null hypothesis (i.e., rates of events are equal within and outside the candidate cluster area) (52). The spatial scan statistic, *S*, is given by the equation (54):

$$S = \max_{Z} \left\{ \frac{L(Z)}{L_0} \right\}$$

Where, Z is the scanning window, $L(Z)$ is the maximum likelihood for Z (measures how likely the observed data are given events inside and outside the window), and L_0 is the likelihood function under the null hypothesis of spatial randomness.

Based on the type of data inputted, a different probability distribution is selected to calculate the likelihood for observed and expected (under the null hypothesis of no clustering) events inside and outside the scanning window (51, 55). For example, a Poisson-based model is used for event counts that are Poisson-distributed according to an underlying population at risk (i.e., cases of disease or incidence rates) (43, 52). Alternatively, a Bernoulli model is used for 0/1 events such as case-control data and other models are used for various types of health data (i.e., space-time permutation model for case data only, ordinal model for ordered categorical data, exponential model for survival time data, and a normal model for continuous data) (51, 55). As an example, for a Poisson model the likelihood ratio is given by (54):

$$\frac{L(Z)}{L_0} = \left\{\frac{n_Z}{\mu(Z)}\right\}^{nz} \left\{\frac{N - n_Z}{N - \mu(Z)}\right\}^{N-nz}$$

Where, N is the total number of observed cases, n_Z is the number of observed cases in scanning window Z, and $\mu(Z)$ is the number of expected cases under the null hypothesis. The number of observed and expected cases within (left part of equation) and outside (right part of equation) the scanning window can also be adjusted for population and other covariates (51).

Statistical inference is conducted by sequential Monte Carlo testing (52, 56). Briefly, hundreds of random datasets are generated using Monte Carlo sampling under the null hypothesis of no clustering. The likelihood is calculated for all candidate clusters in the same manner as for the real data. The candidate most likely clusters from the real data and the simulations are then ranked by the maximum likelihood values (56). If clusters from the real data are ranked in the top 5%, the clusters are reported as significant at a 5% level (α=0.05).

3.5 FIELD WORK AND LABORATORY ANALYSES

3.5.1 Tick Dragging

There are various methods that can be used for field collection of ticks, although their utility varies depending on the tick species targeted (i.e., one-host versus three-host ticks, species mobility and behaviour) and the goal of the surveillance efforts (i.e., presence or prevalence measures, density of nymphs or density of infected nymphs measures, phenology or human encounter measures) (57). These methods include dragging/flagging, walking survey, CO_2 traps, vacuum aspiration or leaf litter sorting, tick collection from deer, and host/small mammal trapping (57-59). The CDC-recommended methods for *I. scapularis* collection are dragging/flagging and small mammal trapping, which are suitable for identification of blacklegged tick presence, density of infected nymphs, and tick phenology (life cycle) (57).

The field surveillance data presented throughout my thesis was collected through dragging using PHO's standard operating procedures (60). Briefly, the procedure involves walking at a moderate pace while dragging a flannel cloth along the sides of trails and the vegetation to collect questing ticks. The specific protocol is described in greater detail in the following chapters. Using field dragging, I was able to generate data on the occurrence and abundance of host-seeking ticks in eastern Ontario from 2017-2021.

3.5.2 Tick Identification

Ticks collected through field dragging were brought to the INSIGHT lab at the University of Ottawa, where I collected entomological data, dissected, processed, and tested specimens. The

data collected for each specimen included species, instar, and engorgement level. This was done using microscopy and various morphological keys to identify features of each species and life stage (61-64). First, the genus is identified followed by the instar based on size and several main characteristics differentiating the genus and the female, male, nymphal, and larval stages (Figure 3.1 A-B). Each tick genus and instar have a distinct morphology; therefore, a specific morphological key is needed for each to correctly identify the species. Once an appropriate morphological key is chosen, the tick is processed through a series of questions/flowchart until the species is identified. The key characteristics of the *I. scapularis* instars are shown in Figure 3.1 C. Lastly, the engorgement level, which approximates length of time the tick has been feeding, can also be identified if applicable (i.e., for ticks submitted by the public) (Figure 3.1 D).

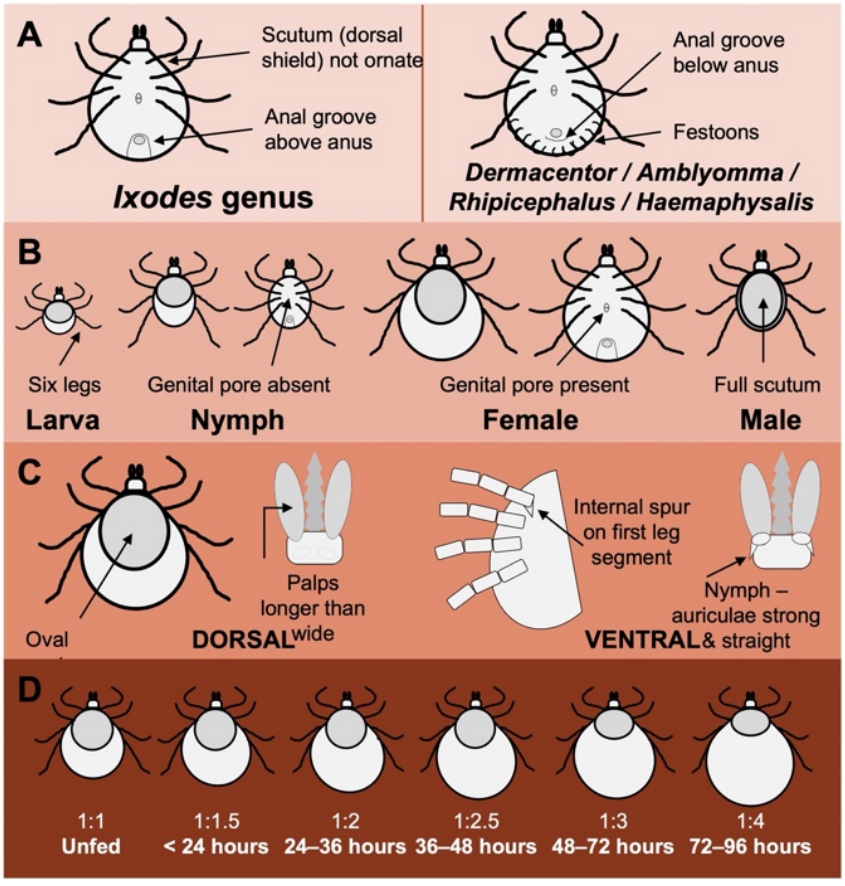

Figure 3.1 Identification of tick genus (A), instar (B), key features of Ixodes scapularis (C), and engorgement level (D).

3.5.3 Molecular Assays

Ixodes scapularis adults and nymphs were dissected and lysed to extract total genomic DNA (and RNA if testing for deer tick virus lineages). The methodology is described in detail in Chapter 7. Briefly, the process involves dissolving animal tissues and cells with lysing agents and then binding the nucleic acids to a membrane. The samples are washed free of contaminants and the DNA/RNA is eluted from the membrane using a solvent (nuclease-free water).

The DNA samples are then tested for tick-borne pathogens using quantitative polymerase chain reaction (qPCR) (or reverse transcriptase qPCR for RNA). Quantitative PCR works by using target-specific primers (i.e., short sequences of DNA that bind to the beginning and end of a gene) and a fluorescent probe for visual detection of DNA amplification during the amplification process (in real-time). The process of gene amplification occurs within a thermocycler, which is a device that heats DNA to specific temperatures for denaturation (opening the double helix), annealing (primers binding to the gene), and extension (replication of the gene). The process is repeated many times, with DNA doubling at each round. Tick samples that are positive for the pathogen will result in amplification (i.e., fluorescence intensity above background levels) and those that are negative for the pathogen will not amplify (i.e., fluorescence intensity below background levels).

For efficiency, multiple qPCR assays can be combined into one assay (i.e., duplex/triplex) and the results can be visualized using different fluorescent probes (i.e., different wavelength (colours) for each pathogen). Samples that are positive for pathogens are tested again for confirmation. Figure 3.2 shows the qPCR testing protocol for detection of tick-borne pathogens.

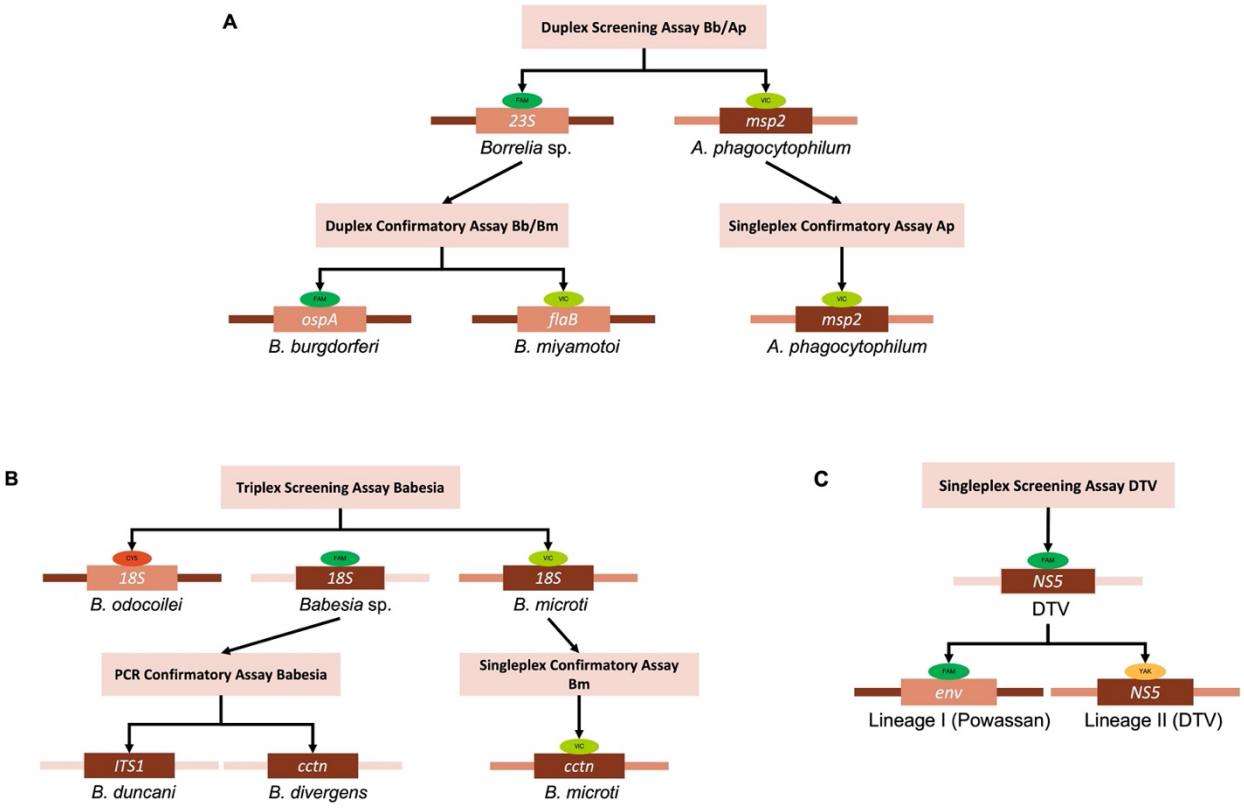

Figure 3.2 Quantitative PCR testing flowchart for Borrelia/Anaplasma (A), Babesia sp. (B), and deer tick virus lineages (C).

3.5.4 Bacterial Genetic Diversity

Nymphal ticks positive for *B. burgdorferi* were further tested for bacterial genetic diversity using multi-locus sequence typing (MLST). MLST involves the sequencing of eight bacterial housekeeping genes to determine bacterial sequence type (ST) based on the allelic combination of these genes. Each gene is first amplified using conventional PCR (as described above but without the fluorescent probe) using a nested or semi-nested approach to ensure a good quality product and verified on an agarose gel. Sequencing was then conducted by Genome Quebec using the Sanger (chain-termination) method. Briefly, the procedure involves gene amplification using conventional PCR and fluorescent chain-termination nucleotides (bases that prevent further elongation once

incorporated into the DNA copy). This creates many small DNA fragments of various size that are "aligned" using gel electrophoresis. The colour of the fluorescent marker, unique for each of the four bases, in combination with the size of the fragment results in sequencing of the gene. Sequencing is repeated in the forward and reverse directions for each gene.

The resulting forward and reverse sequences are aligned to create a consensus sequence. If there are mismatches, the sample likely contains mixed infections with multiple bacterial strains. The consensus sequence for single strains is then queried against known gene alleles using the PubMLST database that contains data on various microbial genomes including *B. burgdorferi*. Lastly, the combination of alleles for the eight genes are assigned a sequence type corresponding to the specific *B. burgdorferi* strain. The process of identifying bacterial genetic diversity and an example from the samples I tested are shown in Figure 3.3.

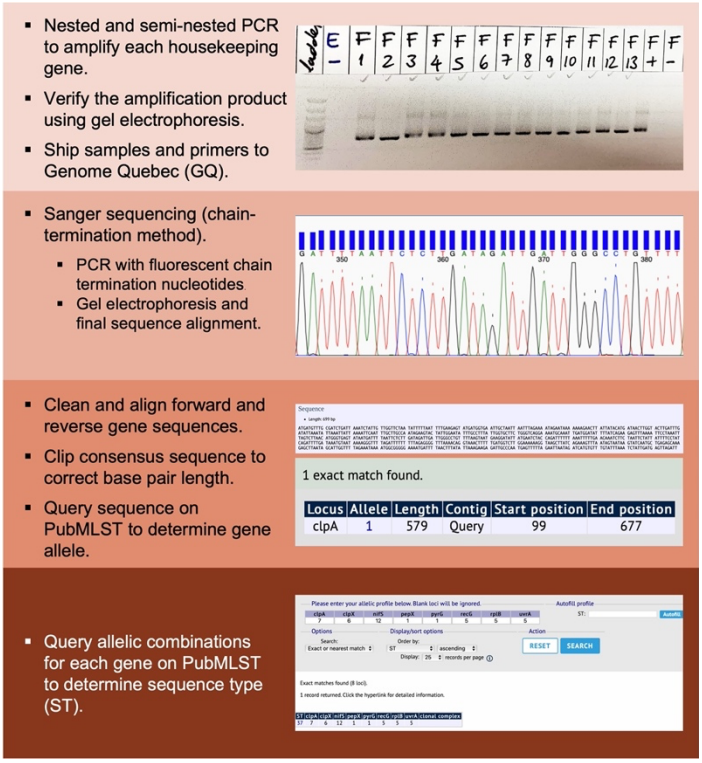

Figure 3.3 Multi-locus sequence typing (MLST) analysis of Borrelia burgdorferi genetic diversity.

3.6 CHAPTER SUMMARY

This chapter introduced the characteristics of the study setting to contextualize my thesis objectives. I also briefly described the datasets that will be used to for the specific objectives, and I introduced the main statistical, analytic, and laboratory methods that I use in the next four chapters. These include maximum entropy species distribution modelling and the considerations for model building and calibration, the design and analysis of a case-control study, and the spatial analytical methods I used for cluster detection. Finally, I also give a brief overview of the molecular assays for detection of tick-borne pathogens and the procedure for determining B. burgdorferi genetic diversity using MLST analysis.

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4

4 LYME DISEASE RISK AREAS

4.1 ARTICLE PREFACE

My first objective was to identify predicted environmental risk areas for Lyme disease in southeastern Ontario using species distribution modelling. I first conducted field and laboratory studies, with the help of colleagues at the University of Ottawa and the University of Guelph, to identify occurrence points for *Ixodes scapularis* and *Borrelia burgdorferi*. I also obtained various climate, topographical, and satellite remote sensing data to derive ecological variables used to predict species distributions. I generated two maximum entropy (Maxent) models to predict the distribution of blacklegged ticks and the Lyme disease-causing pathogen. Finally, I tested my models using two different datasets to evaluate the models' predictive accuracy.

4.1.1 Author Contributions

I conceived the research question, with guidance from my research supervisor, Dr. Manisha Kulkarni. I developed the methodology and conducted all formal analyses including data curation, modelling, and testing. I received valuable feedback on the methodology from Drs. Manisha Kulkarni, Nicholas Ogden, Claire Jardine, and Anders Knudby. Species occurrence data used for model building was collected through field sampling by the University of Ottawa team, which included Drs. Roman McKay and Benoit Talbot, James Logan, Charles Thickstun, and me, and by the University of Guelph team, which included Drs. Katie Clow and her collaborators (formally acknowledged in Dr. Clow's publication). The laboratory testing for ticks collected by the University of Ottawa was conducted in house by me and Dr. Roman McKay. All authors contributed to the editing and revision of this article.

4.1.2 Ethics Approval

Land permits were obtained from relevant authorities prior to field sampling.

4.1.3 Article Citation

Slatculescu AM, Clow KM, McKay R, et al. Species distribution models for the eastern blacklegged tick, *Ixodes scapularis*, and the Lyme disease pathogen, *Borrelia burgdorferi*, in Ontario, Canada. *PLoS One* 2020;15(9):e0238126.

4.2 ARTICLE CONTENT

4.2.1 Title

Species distribution models for the eastern blacklegged tick, *Ixodes scapularis,* and the Lyme disease pathogen, *Borrelia burgdorferi*, in Ontario, Canada.

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4.2.3 Abstract

The blacklegged tick, *Ixodes scapularis*, is established in several regions of Ontario, Canada, and continues to spread into new geographic areas across the province at a rapid rate. This poses a significant public health risk since *I. scapularis* transmits the Lyme disease-causing bacterium, *Borrelia burgdorferi*, and other pathogens of potential public health concern. The objective of this study was to develop species distribution models for *I. scapularis* and *B. burgdorferi* to predict and compare the potential distributions of the tick vector and the Lyme disease pathogen as well as the ecological factors most important for species establishment. Ticks were collected via tick dragging at 120 sites across southern, central, and eastern Ontario between 2015 and 2018 and tested for tick-borne pathogens. A maximum entropy (Maxent) approach was used to model the potential distributions of *I. scapularis* and *B. burgdorferi*. Two independent datasets derived from tick dragging at 25 new sites in 2019 and ticks submitted by the public to local health units between 2015 and 2017 were used to validate the predictive accuracy of the models.

The model for *I. scapularis* showed high suitability for blacklegged ticks in eastern Ontario and some regions along the shorelines of the Great Lakes, and moderate suitability near Algonquin Provincial Park and the Georgian Bay with good predictive accuracy (tick dragging 2019: AUC = 0.898; ticks from public: AUC = 0.727). The model for *B. burgdorferi* showed a similar predicted distribution but was more constrained to eastern Ontario, particularly between Ottawa and Kingston, and along Lake Ontario, with similarly good predictive accuracy (tick dragging 2019: AUC = 0.958; ticks from public: AUC = 0.863. The ecological variables most important for predicting the distributions of *I. scapularis* and *B. burgdorferi* included elevation, distance to

deciduous and coniferous forest, proportions of agricultural land, water, and infrastructure, mean summer/spring temperature, and cumulative annual degree days above 0℃.

Our study presents a novel application of species distribution modelling for *I. scapularis* and *B. burgdorferi* in Ontario, Canada, and provides an up-to-date projection of their potential distributions for public health knowledge users.

4.2.4 Introduction

Lyme disease is a tick-borne illness caused by the *Borrelia burgdorferi* sensu lato bacterial complex (1). In eastern North America, the bacterium is transmitted to humans through the bite of the blacklegged tick, *Ixodes scapularis* (2-4). Northward expansion of *I. scapularis* populations, at least in part attributable to climate change, is driving the emergence of Lyme disease in new regions and increasing the number of people at risk, particularly in Canada (5-7). From 2009-2017, approximately 6,000 cases of Lyme disease were reported in Canada and the national annual incidence is estimated at 2.7 cases per 100,000 population (8). However, in some locations, the incidence of Lyme disease is substantially higher, with public health units in the province of Ontario reporting estimates of 18 cases per 100,000 for the City of Ottawa (OTT), 87 cases per 100,000 for Kingston, Frontenac, Lennox and Addington (KFL), and almost 130 cases per 100,000 for Leeds-Grenville and Lanark District (LGL) in 2017 (9). In some locations in Canada, Lyme disease poses a very significant public health risk, reaching similarly high incidence (≥ 10 cases per 100,000 population per year) as observed in states in the northeastern United States (10).

The risk of contracting Lyme disease depends on several factors including human activity and behaviours as well as the density of *B. burgdorferi* infected ticks in the environment, which is determined by both the abundance of *I. scapularis* and the proportion of ticks infected with *B. burgdorferi* (11-14). The most common method used for estimating risk of exposure to *B. burgdorferi* is the identification of *I. scapularis* populations in the environment using various surveillance methods (15). In Canada, the spatial distribution of *I. scapularis* ticks has been studied via passive tick surveillance programs that rely on tick submissions from the public and healthcare providers, and by active tick surveillance that utilizes mainly drag sampling techniques to sample the environment for questing ticks (16). On a broader geographic scale, province-wide surveillance studies have shown that the distribution of *I. scapularis* populations is affected by climatic factors, which play an important role in the occurrence and abundance of arthropod vectors and in delimiting the potential range of the vector (5-7, 17, 18). At a local scale, site-level surveillance studies have shown that the distribution of *I. scapularis* is also affected by ecological factors like understory density, presence of shrubs, dominant tree type, canopy cover, proportion of forested land and forest fragmentation that are integral to the life cycle of the ticks (19-22). These factors also contribute to an adequate habitat for mammalian hosts such as white-footed mice (*Peromyscus leucopus*) and white-tailed deer (*Odocoileus virginianus*) that are integral to the developmental and reproductive cycle of the ticks, which are obligate ectoparasites (20). Ecological and climatic factors are often inter-related, and both contribute to the establishment of tick populations at different spatial scales. This has led to the use and development of complex modelling strategies to identify the geographic distribution of ticks or to make predictions about potential habitat suitability based on a wide variety of earth observation data (6, 21, 23, 24).

Species distribution models (SDM) are a variety of statistical models that relate species distribution data (e.g. occurrence or abundance at known locations) to information on the environmental or spatial characteristics of those locations (25). SDMs mainly differ in the type of species data they use, with some requiring absence/presence or abundance data (e.g. generalized linear and additive models, GLM and GAM; random forests; boosted regression trees, BRT) while others rely solely on presence-only data (e.g. maximum entropy models, Maxent; genetic algorithm for rule set production, GARP) (25). Of these, Maxent is one of the most frequently used SDMs and has shown consistently high performance compared to other models (26, 27). In this study, we used Maxent to predict the distribution of *I. scapularis* and *B. burgdorferi* in south-eastern Ontario, and to identify factors that contribute to the establishment of the pathogen, in order to more accurately estimate environmental risk for Lyme disease. Our models provide the most up-to-date environmental risk maps for Lyme disease in the province of Ontario, Canada, where the number of human Lyme disease cases is growing annually as the tick increases its range.

4.2.5 Materials and Methods

4.2.5.1 Species Occurrence Data and Study Area

Occurrence data were compiled from field collection of immature and adult *I. scapularis* ticks made by the University of Guelph and the University of Ottawa from 2015-2018 in Ontario, Canada. Both groups employed a standard field dragging protocol in which a one-meter squared white flannel cloth sheet was dragged along surface vegetation and the forest floor for three person-hours in a given site (28, 29). The drag sheets and surveyors' clothes were checked for ticks every 3 minutes (University of Guelph) or every 50 meters with step counts adjusted for each individual's walking pace (University of Ottawa) (28, 29). Latitude/longitude coordinates were recorded for

each sample location using a hand-held GPS. All selected sites were visited during the summer months (May to August) to capture the peak season for questing nymphs. In eastern Canada, the density of questing adult ticks often peaks in the spring and fall, while the density of questing nymphs peaks in the summer (5). However, various sites were revisited multiple times during the summer and fall to address other research questions. For this study, we used all sampling data available to maximize our ability to detect ticks. Locations to be sampled were selected by each University independently as described in earlier work, and included sites both known, suspected, or broadly suitable for *I. scapularis* as well as control sites, negative sites, or unsuitable *I. scapularis* sites distributed across three ecoregions (5E, 6E, 7E) of Ontario and within urban, suburban, and rural regions (24, 28-30). A total of 120 sites were sampled between 2015 and 2018 across southern, central, and eastern Ontario (Figure 4.1). *Ixodes scapularis* ticks were found at 52 locations (Figure 4.1).

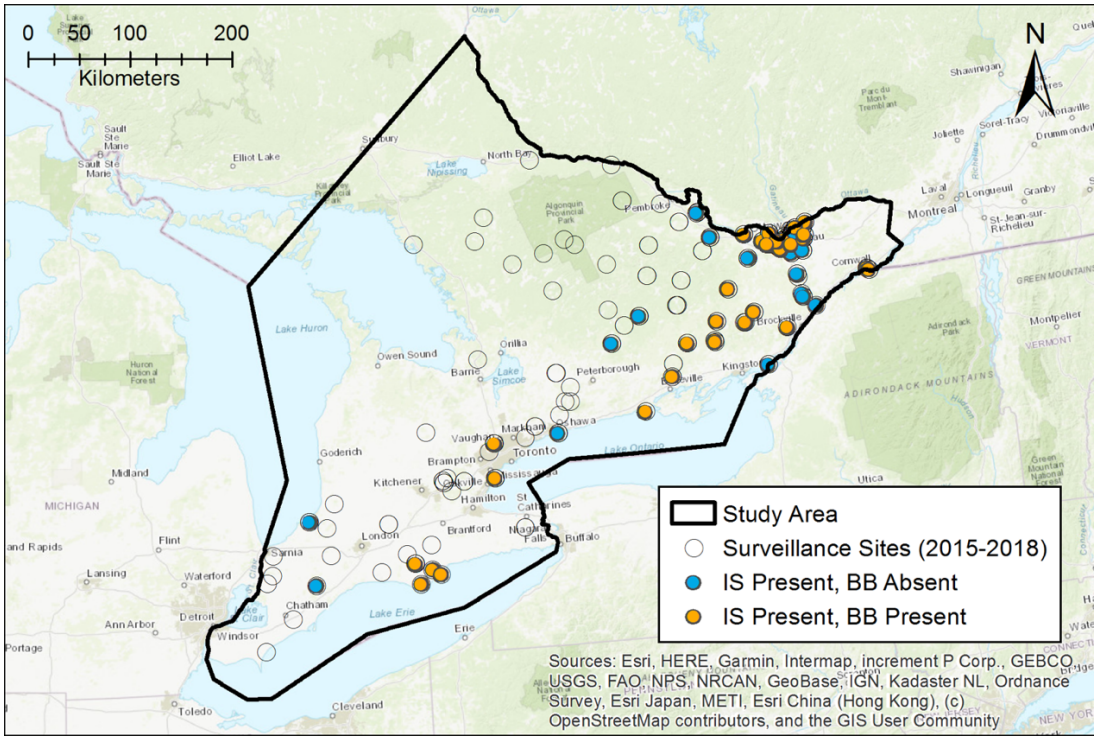

Figure 4.1 Study area and tick field sampling locations conducted between 2015-2018.Open circles show sites sampled for ticks, blue circles indicate sites where *Ixodes scapularis* (IS) ticks

were found, and orange circles show sites where *Borrelia burgdorferi* (BB) was detected through molecular testing.

All larval, nymphal, and adult ticks found in the field were collected in specimen tubes and sent to two laboratories for analysis. Ticks collected by the University of Guelph were shipped in 70% ethanol to the National Microbiology Laboratory (Public Health Agency of Canada, Winnipeg, Manitoba, Canada) for species identification. All adult and nymphal *I. scapularis* ticks were further tested for the presence of *B. burgdorferi, Borrelia miyamotoi, Anaplasma phagocytophilum,* and *Babesia microti* by real-time PCR (31, 32). Ticks collected by the University of Ottawa were identified using standard taxonomic keys and tested for the same tick-borne pathogens within the university (29, 32-36). Prior to testing, real-time PCR assays established at the University of Ottawa were validated using a panel of test samples provided by the NML to ensure comparable results between the two laboratories. Out of the 52 locations in Ontario where *I. scapularis* ticks were found, 33 sites had at least one tick positive for *B. burgdorferi* (Figure 4.1). The prevalence of the other tick-borne pathogens is low in Canada and there was insufficient occurrence data for other pathogen species to be included in this study.

4.2.5.2 Earth Observation Data and Environmental Variables

Satellite remote sensing data pertaining to climate, land cover / land use, and elevation were selected to identify areas conducive to the establishment of *I. scapularis* and *B. burgdorferi.* All grid data was projected into the NAD83 Lambert Conformal Conic projection and resampled at 100-meter resolution.

For land cover variables, we used the Southern Ontario Land Resource Information System version 3.0 (SOLRISv3.0), which is based on Landsat-7 ETM+ satellite imagery captured between 2000-2015 and classified into 30 land cover types found in Ontario at 15-meter pixel resolution. We supplemented this with the Ontario Land Cover Data Base 2000 (OLCDB2000), which is based on Landsat-7 ETM+ satellite imagery captured between 1999-2002 at 25-meter pixel resolution, for UTM zones 17 and 18. All land cover datasets were obtained from the Ministry of Natural Resources and Forestry's open data portal (https://geohub.lio.gov.on.ca). We used ArcMap 10.5.1 (ESRI, Redlands, CA) to mosaic images from SOLRISv3.0 and OLCDB2000 to create a complete grid for our study area, with precedence given to the most recent land cover data obtained from SOLRISv3.0 for any overlapping grid cells. This composite land cover raster was resampled to 100-meter resolution then reclassified into 10 land cover types that dominate southern, central, and eastern Ontario (Supplemental Table 4.1). To derive our explanatory variables, for each of these 10 land cover types we calculated 1) the proportion of each land cover type within a 1000-meter circular buffer of each grid cell, and 2) the distance of each grid cell to the land cover type, yielding a total of 20 land cover variables (Supplemental Table 4.2).

For climate variables, we used the Canada-wide long-term climate averages for 1981-2010 obtained from Natural Resources Canada (37). This dataset consists of 19 bioclimatic parameters derived from temperature and precipitation records at 5 km resolution. Additionally, we obtained grid data for annual cumulative degree days above 0℃ (DD > 0℃), calculated as the sum of all days of the year with mean surface temperatures > 0℃ as reported by climate stations within a 5 km grid. DD > 0℃ is often used as the main climatic indicator for tick establishment particularly when used in models of future climate projections (5-7).

For the elevation variable, we used the Ontario Digital Elevation Model (ODEM), which is a 3-dimensional raster dataset that captures terrain elevations for the province of Ontario at a resolution of 30 meters (available at: https://geohub.lio.gov.on.ca). In total, we derived 41 environmental variables for land cover, climate, and elevation for our *I. scapularis* and *B. burgdorferi* species distribution models; all rasters were resampled to 100-meter resolution. The complete list of the derived explanatory variables is shown in Supplemental Table 4.2.

4.2.5.3 Model Development

We used Maxent v.3.4.0 to model the potential distribution of suitable habitat for *I. scapularis* and *B. burgdorferi* in south-eastern Ontario. Maxent uses species presence data and randomly selected pseudo-absences (i.e. background points) to generate a probability distribution across a landscape, often conceptualized as habitat suitability or an approximation of the species' niche, constrained within a set of environmental parameters at presence locations (38). As such, Maxent functions under the assumption that species presence points represent an unbiased sample from the species' realized niche (38). Thus, we used species presence data derived from active field dragging because it is the most accurate method for detecting ticks in the environment through a standardized protocol. Active field dragging may capture some adventitious ticks (non-native ticks introduced most likely by migratory birds), but adventitious ticks are unlikely to play a significant role particularly if the region sampled is suspected suitable for ticks based on other surveillance reports (39, 40). Therefore, we defined presence points for *I. scapularis* as unique georeferenced locations where adult, nymph, or larvae of *I. scapularis* were found. We defined presence points

for *B. burgdorferi* as unique georeferenced locations where at least one positive *I. scapularis* specimen was found.

To further ensure our presence points represent an unbiased sample of the species' niche, we created a gridded Gaussian kernel density sampling bias file based on all 120 sites we surveyed for ticks between 2015 and 2018, following the approach described by Brown *et al.* (41). This approach was selected because it allowed Maxent to sample pseudo-absences from background with the same distribution that gave rise to presence points while accounting for higher density of sampling in specific regions. Lastly, we also explored spatial autocorrelation in the presence point data for *I. scapularis*, and for *B. burgdorferi*. We detected highly spatially clustered presence points around the city of Ottawa and in other regions of eastern Ontario with a high number of sampling locations. To avoid pseudo-replication of data points and reduce clustering, we rarefied species occurrence points by creating circular buffer zones in 1 km increments around each site and randomly selecting one location in overlapping zones (41, 42). We used nearest neighbour analysis and the z-score and associated p-value to select the buffer size that would ensure a random distribution was achieved while keeping the maximum number of data points (Supplemental Table 4.3) (41, 42).

To select ecological parameters for our Maxent models for *I. scapularis* and *B. burgdorferi*, we first evaluated the importance of the 41 variables we derived from earth observation data by grouping the variables into land cover and climate groups and running "full models" with default Maxent settings for each category. We ranked variables by their contribution to the model as measured by average loss in regularized training gain when each variable was omitted in turn and

we included explanatory variables with greater than 1% decrease in gain in our main models (43). We then used the ArcMap extension SDM Toolbox v.2.4 to derive a correlation matrix and we removed highly correlated variables (|Pearson's r| ≥ 0.7) starting with the lowest contributing variables (41, 44). For land cover, we also ensured that each variable (i.e. measured as proportion or distance) was only represented once in the final model to avoid duplicate contributions of the same variable. We analyzed land cover and climate variables separately because, while both contribute to tick establishment, climate varies much less at a fine resolution and would be underrepresented in the final model. Supplemental Table 4.4 and Supplemental Table 4.5 show the selection of the explanatory variables for the *I. scapularis* and *B. burgdorferi* models, respectively.

Using the final set of land cover and climate variables with moderate to low correlation, we selected the types of variable transformations to use in our model. In machine learning, these transformations, or functions, of the original variables (i.e. linear, quadratic, product, hinge, etc.) are called features and can be used to fit highly complex models (25). However, models with a larger number of features tend to overfit small training datasets and are more difficult to interpret (45). For models with 30-40 training points, linear-quadratic-product (LQP) features typically produce the best performance while smaller datasets perform well with linear-quadratic (LQ) features when evaluated using area under the receiver operating characteristics curve (AUC) (45). For our *I. scapularis* and *B. burgdorferi* models, we used LQP features and LQ features, respectively.

4.2.5.4 Model Calibration

To calibrate the models, we used the threshold-independent receiver operating characteristic (ROC) analysis and the threshold-dependent omission rate (46). The area under the ROC function (AUC) quantifies the probability that the model correctly ranks a random presence locality higher than a random background locality (38). Thus, AUC can be used to measure model performance (i.e. discriminatory ability) compared to random prediction. The omission rate requires a threshold to be selected in order to produce a binary prediction (i.e. suitable, not suitable) and is defined as the fraction of presence localities that fall into pixels not predicted as suitable (38). We defined the threshold as the lowest prediction value for any pixel that holds a training presence point, which indicates the least suitable environmental conditions in which a presence can be found. We compared omission rates for our testing models with the theoretical predicted omission rate of zero for this threshold (46). We used the 4-fold cross validation method described by Radosavljevic *et al.,* 2014 to partition our data and use all presence points for training and testing (46). We then generated models with different regularization values and measured AUC and omission rate to assess fit (Supplemental Figure 4.1). Regularization reduces model overfitting by ensuring empirical constraints are not fit too tightly and by removing features from the model and reducing model complexity (47). We selected the best model with a regularization value that produced the lowest omission rate (i.e. closest to the predicted value of zero) and highest AUC based on the testing data (Supplemental Figure 4.1).

4.2.5.5 Validation and Variable Contribution

To validate the predictive ability of our final models for *I. scapularis* and *B. burgdorferi*, we used two independent datasets that were generated from contemporary tick surveillance in the province.

First, we used active tick surveillance data from 2019 where 25 unique sites in southern and eastern Ontario were sampled for ticks via drag sampling over an area of 2000 m^2 per site (Kulkarni et al., unpublished data) (Figure 4.2). *Ixodes scapularis* ticks were found at 14 of these sites and *B. burgdorferi* at 8 of these sites. Second, we used contemporary passive tick surveillance data from 2015-2017 from Public Health Ontario to generate a second dataset with locations in the province where individuals have encountered ticks (Figure 4.2). We retained georeferenced records with high level of spatial certainty on location of tick acquisition (e.g. specific address, park, trail) and excluded records with imprecise locations or locations outside the study area. After rarefying data to avoid pseudo-replication of data points, we had 106 unique locations where blacklegged ticks were encountered by the public and 63 locations with *B. burgdorferi*-infected specimens across our study area. We then used AUC to assess the discriminatory ability of our models.

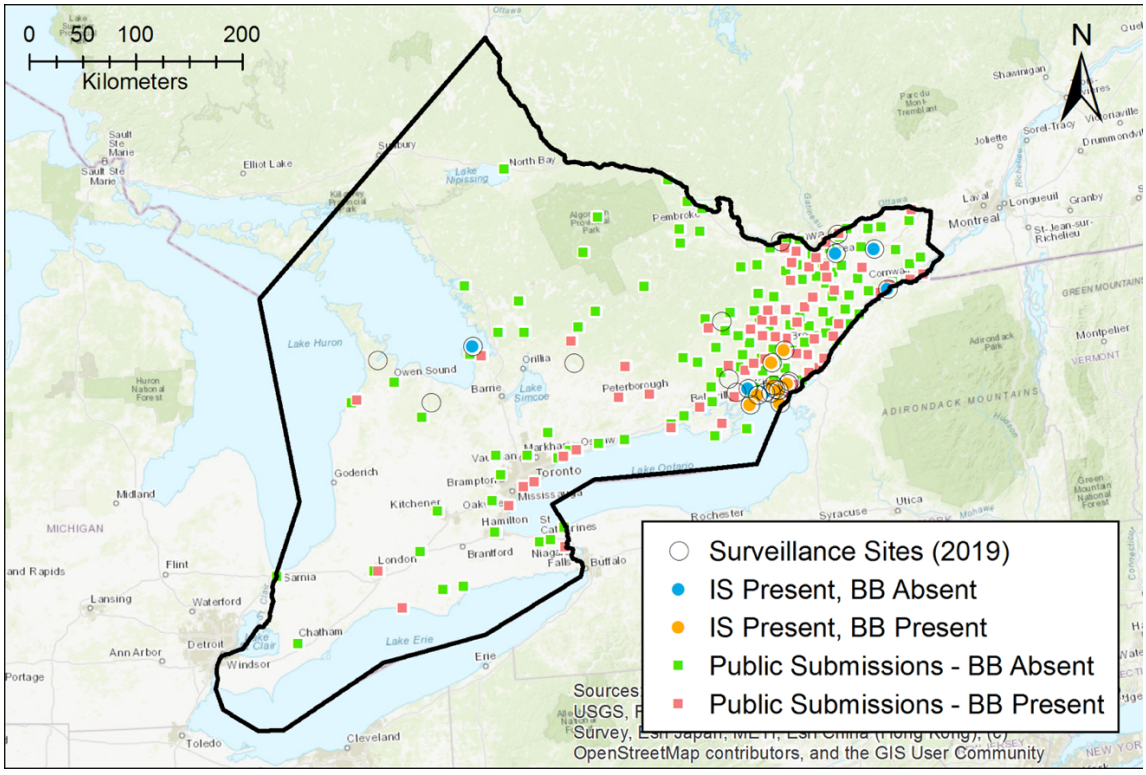

Figure 4.2 Model validation datasets: tick field sampling conducted in 2019 and publicly submitted ticks between 2015-2017. Open circles show sites sampled for ticks in 2019, blue circles indicate sites where *Ixodes scapularis* (IS) ticks were found, orange circles show sites where

Borrelia burgdorferi (BB) was detected through molecular testing, green squares show a random subset of sites where the public encountered ticks negative for *B. burgdorferi* between 2015-2017, and pink squares show a random subset of sites where the public encountered ticks positive for *B. burgdorferi* between 2015-2017.

Furthermore, we measured the variable contribution of our final models for *I. scapularis* and *B. burgdorferi* using three metrics. First, we used a jackknife procedure to assess the regularized training gain of models built using each variable individually. Second, we used a jackknife procedure to assess regularized training gain when each variable is excluded from the model in turn. Lastly, we used permutation importance obtained by randomly permuting the values of each variable on presence and background points and re-evaluating the model with these values and measuring the decrease in AUC. Independent response curves for each variable were also used to assess how each variable affects the predicted suitability of the two species.

4.2.6 Results

4.2.6.1 Ecological Niche Models for Ixodes scapularis and Borrelia burgdorferi in Ontario

Using field surveillance data, we identified 48 spatially independent locations where established populations of *I. scapularis* ticks were found; these were used as occurrence records for model development (Figure 4.1). The final niche model for *I. scapularis* included 12 environmental variables, used linear, quadratic, and product transformations of the environmental variables, imposed a regularization multiplier of 2 to avoid overfitting, and presented environmental suitability from 0 to 1 using the clog log transformation (Table 4.1, Table 4.3). The final *B. burgdorferi* model was developed using 30 spatially independent locations where ticks tested

positive for the bacterium and included 12 environmental variables, used linear and quadratic transformations of the environmental variables, and a regularization multiplier of 1.5 (Table 4.1, Table 4.4).

Table 4.1 Maxent parameters and fit metrics of the best models based on 4-fold cross validation for *Ixodes scapularis* and *Borrelia burgdorferi*.

	***Ixodes scapularis* model**	***Borrelia burgdorferi* model**
Parameter		
Presence points, *n*	48	30
Variables, *n*	12	12
Features types	linear, quadratic, product	linear, quadratic
Regularization multiplier	2	1.5
Output	clog log	clog log
4-fold cross validation		
Mean AUC	0.925	0.963
Mean omission rate	0.0415	0.10275

Our model for *I. scapularis* predicts the highest suitability for this species in eastern Ontario, particularly the regions between the cities of Kingston and Ottawa (Figure 4.3). Localized high suitability for *I. scapularis* was also predicted along the shores of Lake Ontario and to a lesser extent in areas around Lake Erie, Lake Huron, and the Georgian Bay (Figure 4.3). *Ixodes scapularis* habitat suitability was lowest in the agricultural region of southern Ontario and in the central region of Algonquin Provincial Park (Figure 4.3). Our model for *B. burgdorferi* predicted that the pathogen's range is narrower than that of the blacklegged tick and almost entirely constrained to eastern Ontario, with a few localized areas around the shores of Lake Ontario and Lake Erie (Figure 4.4).

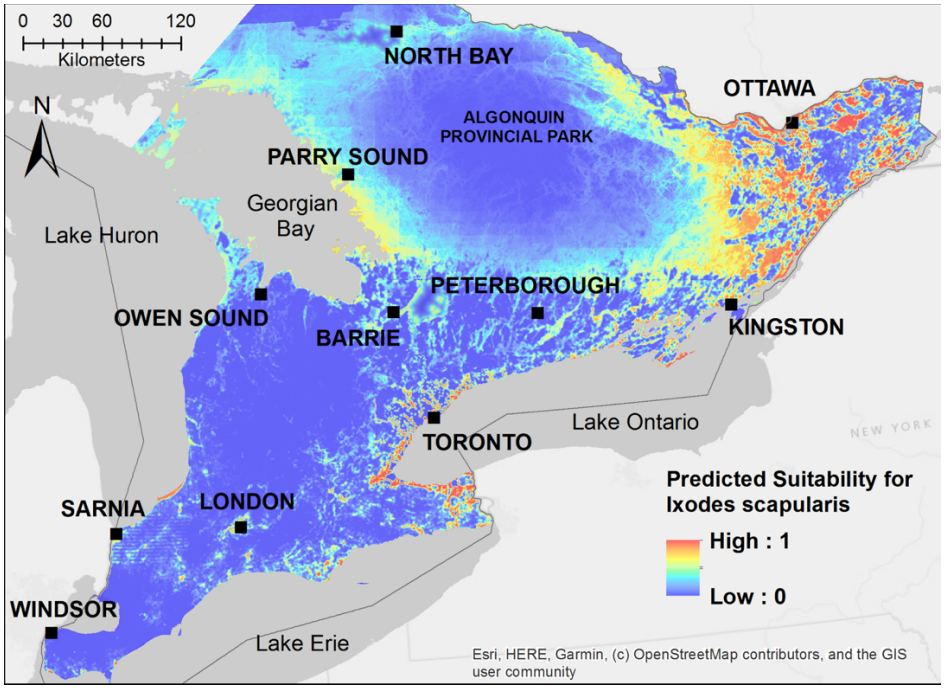

Figure 4.3 Ecological niche model for *Ixodes scapularis* derived from 48 spatially independent presence locations with established tick populations. Model output with a clog log transformation to show the predicted suitability for *I. scapularis* as a probability from 0-1.

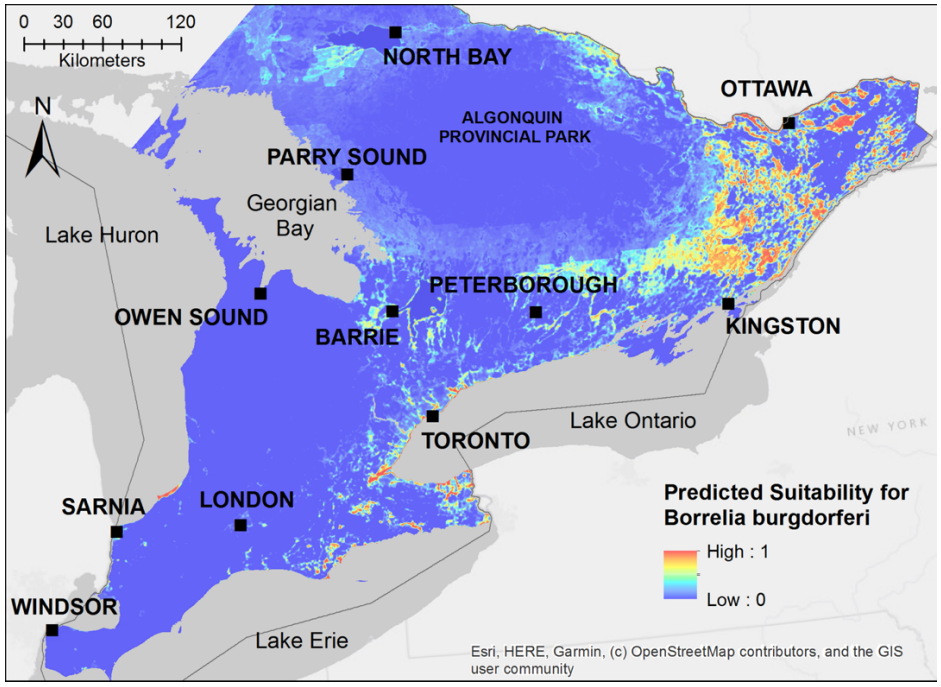

Figure 4.4 Ecological niche model for Borrelia burgdorferi derived from 30 spatially independent presence locations with established tick populations and positive specimens for the bacterium.

Model output with a clog log transformation to show the predicted suitability for *B. burgdorferi* as a probability from 0-1.

Based on model validation using occurrence points from active tick surveillance in 2019, our niche models demonstrated good discrimination of positive and background sites for both *I. scapularis* (AUC = 0.898) and *B. burgdorferi* (AUC = 0.958) (Table 4.2). Additionally, we used passive tick surveillance to generate another independent dataset consisting of ticks voluntarily submitted by the public to local health units. Based on model validation using the dataset from passive tick surveillance in 2015-2017, our niche models demonstrated good discrimination of positive and background sites for *I. scapularis* (AUC = 0.727) and *B. burgdorferi* (AUC = 0.863) (Table 4.2), although with slightly lower AUC values as would be expected given the lower precision of tick occurrence locations and possible inclusion of locations where individuals encountered adventitious ticks.

Table 4.2 Validation of *Ixodes scapularis* and *Borrelia burgdorferi* models with two independent datasets derived from active and passive surveillance activities in Ontario, Canada.

	***Ixodes scapularis* model**	***Borrelia burgdorferi* model**
Training AUC	0.950	0.981
Test AUC		
Active surveillance 2019	0.898	0.958
Passive surveillance 2010-2017	0.727	0.863

4.2.6.2 Environmental Variables Contributing to Tick Habitat Suitability and Establishment of B. burgdorferi

We used two jackknife procedures to resample our data and measure the regularized training gain when variables were omitted in turn from the models or considered in isolation in the models. For

our *I. scapularis* model, the variables whose model gain decreased the most when omitted were, in turn, elevation, proportion of agricultural land, and distance to deciduous forest (Table 4.3). Similarly, these variables also increased the model gain the most when considered in isolation, indicating they are the most informative variables in the model containing information not found in the other variables (Table 4.3). The variables with the highest permutation importance that contribute the most to model fit include distance to coniferous forest, distance to deciduous forest, elevation, and DD>0℃ (Table 4.3). Based on independent response curves, habitat suitability for blacklegged ticks increases with increasing DD>0℃ and decreases with larger distances to coniferous and deciduous forests and with higher elevation (Figure 4.5).

Table 4.3 Measures of variable contribution for covariates in the *Ixodes scapularis* model.

Variable	**Permutation importance**	**Gain without variable**	**Gain with only variable**
Distance to coniferous forest	24.7	1.1137	0.1383
Distance to deciduous forest	22.6	1.0479	0.1309
Elevation	19.1	0.9809	0.4021
DD>0°C	13.2	1.0713	0.0097
Proportion of agriculture	8.7	1.0194	0.1564
Proportion of rural or undifferentiated land	4.7	1.0578	0.0065
Precipitation of warmest quarter	2.2	1.0926	0.0154
Precipitation of wettest quarter	1.9	1.1392	0.0246
Temperature seasonality	1.4	1.1363	0.0207
Distance to water	0.8	1.1184	0.0197
Proportion of infrastructure	0.5	1.0948	0.0681
Proportion of hedge rows	0.2	1.1394	0.0176
Full model regularized training gain: 1.1494			

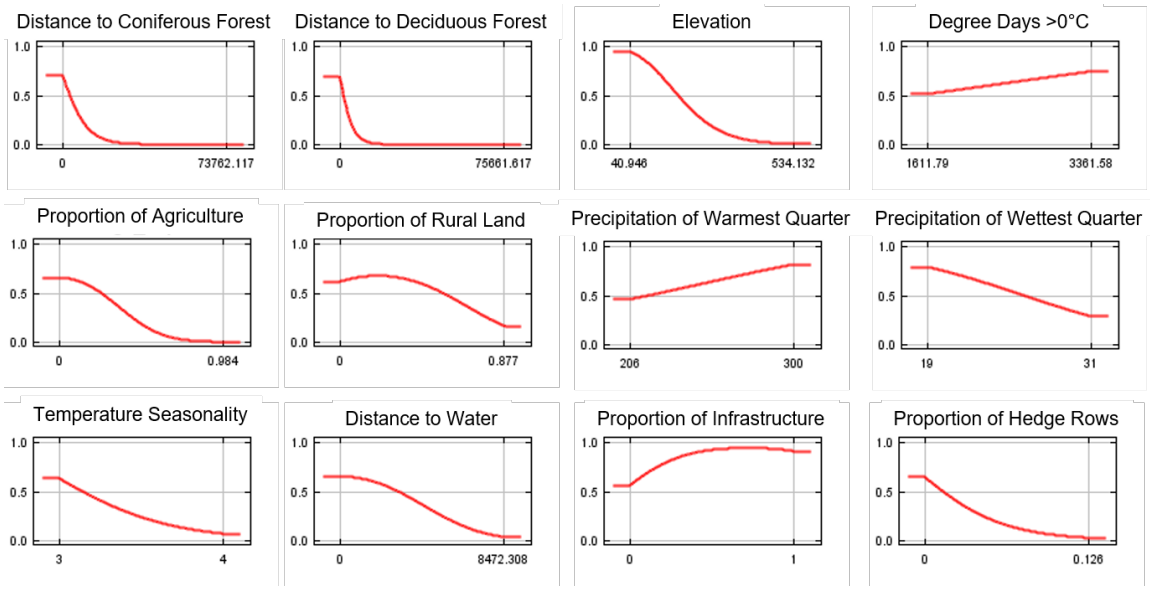

Figure 4.5 Independent response curves showing the dependence of predicted suitability for *Ixodes scapularis* on the variables modelled in turn.

Similarly, for the *B. burgdorferi* model, proportion of agricultural land, elevation, and distance to mixed treed forest were among the most informative variables (Table 4.4). However, proportion of water and proportion of infrastructure were also highly informative variables to the predicted suitability of the pathogen in Ontario (Table 4.4). Environmental suitability for this pathogen increased with higher mean spring/summer temperature and decreased with higher elevation, higher proportion of agricultural land, and larger distance to mixed treed forest based on independent variable response curves (Figure 4.6). Increasing proportions of infrastructure and surrounding area composed of open water also increased environmental suitability for *B. burgdorferi* but decreased suitability when the proportions reached a greater threshold, which likely explains why shorelines along Lake Ontario with major population centers are predicted to be highly suitable for the establishment of ticks and tick-borne pathogens.

Table 4.4 Measures of variable contribution for covariates in *Borrelia burgdorferi* model.

Variable	Permutation importance	Gain without variable	Gain with only variable
Proportion of agriculture	22.7	1.4827	0.2272
Proportion of water	18.7	1.5657	0.3319
Distance to mixed treed forest	17.4	1.6617	0.2433
Mean temperature of warmest quarter	14.5	1.6327	0.1245
Elevation	10.0	1.6289	0.4886
Precipitation of coldest quarter	6.3	1.6969	0.0917
Proportion of rural or undifferentiated land	4.9	1.6176	0.1144
Proportion of infrastructure	2.3	1.5856	0.1815
Precipitation of warmest quarter	1.7	1.6836	0.0267
Mean temperature of driest quarter	1.1	1.7207	0.0917
Proportion of coniferous forest	0.3	1.707	0.0592
Proportion of hedge rows	0.2	1.7112	0.0539
Full model regularized training gain: 1.7412			

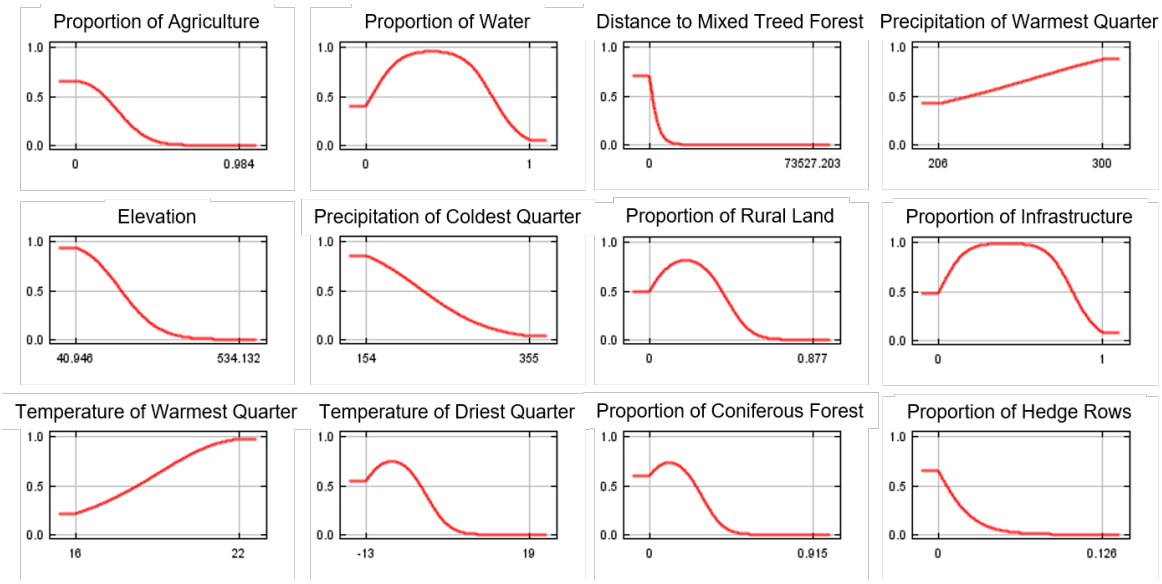

Figure 4.6 Independent response curves showing the dependence of predicted suitability for *Borrelia burgdorferi* on the variables modelled in turn.

4.2.7 Discussion

In this study, we developed environmental risk maps for Lyme disease in southern and eastern Ontario by modelling the vector's predicted habitat suitability. We also developed a second model for the Lyme disease-causing bacterium, *B. burgdorferi*, to identify the suitability of this region to sustain the transmission cycle of this pathogen. The *I. scapularis* and *B. burgdorferi* risk maps were developed using a maximum entropy modelling approach based on presence data derived from active field sampling at 120 sites across the province and environmental variables derived from high-resolution earth observation data.

Our *I. scapularis* model predicted high habitat suitability for blacklegged ticks throughout eastern Ontario and along the shorelines of Lake Ontario, where major population centers are located. This region is encompassed within the Great Lakes-St. Lawrence forest region, which is dominated by hardwood forests featuring maple, oak, birch, and pine and is home to a variety of wildlife including white-tailed deer, moose, small mammals, and migratory birds (48, 49). The predicted habitat suitability for *I. scapularis* in our model is consistent with other studies that have examined the recent distribution and expansion of *I. scapularis* in Ontario (14, 30, 50). However, our model also detected moderate habitat suitability for *I. scapularis* along the Georgian Bay and regions bordering Algonquin Provincial Park. Recent studies have shown that range expansion of *I. scapularis* populations in Ontario was limited to a northward movement of ticks in the eastern part of the province and that the odds of detecting *I. scapularis* decreased at sites located west of major endemic regions (21, 30). The limited horizontal (westward) expansion of ticks in central and eastern Ontario may be explained by the large amount of agricultural land between suitable woodland habitats as well as by a larger distance from endemic sites in the northeastern United

States, from which migratory birds are transporting ticks along the Atlantic and Mississippi flyways (21, 51). Our model complements these findings by showing the predicted distribution of *I. scapularis* in Ontario, which may extend beyond the vector's current presence, as a result of Maxent's projection into geographic areas not directly sampled for ticks (38, 52).

It is interesting that Algonquin Park and regions of northern Ontario have a lower predicted probability of blacklegged tick occurrence. This is probably a result of higher elevation, which has been shown to be a strong predictor of tick occurrence globally (21, 22, 53). However, landscape structure and connectivity as well as host abundance and diversity may also play a role in the establishment of ticks in forest habitats. It had been shown that *I. scapularis* (and its western counterpart *I. pacificus*) is predominantly found in woodlands with high canopy cover compared to open shrublands, yet on the other hand forest fragmentation contributes to higher prevalence of *B. burgdorferi* along forest edges and urban forests (22, 54, 55). It is currently unclear how landscape structure affects the colonization ability of *I. scapularis* and why regions like Algonquin Park, with very dense mixed forests, show a lower suitability for ticks. It is also possible that landscape barriers, such as a larger distance to open water and migratory bird flyways, may affect the movement of ticks to this region. Therefore, the apparent low suitability for ticks in Algonquin Park may be a result of a colonization lag that prevented us from findings ticks in this region through active surveillance. There is some evidence from our passive surveillance validation dataset that *I. scapularis* ticks have been reported in Algonquin Park by the public; however, these ticks represent a very low proportion compared to those found in eastern Ontario. It is also more difficult to interpret the accuracy of passive tick surveillance data due to possible recall bias. Overall, we believe a that Algonquin Park and the surrounding region represents a lower risk of

exposure to ticks and Lyme disease compared to areas in eastern Ontario with high tick densities and a higher prevalence of *B. burgdorferi*, though additional research is needed to explore newly emerging areas and dense forests in Ontario.

We found that distance to coniferous forest, distance to deciduous forest, elevation, and DD>0℃ were the variables that contributed most to our *I. scapularis* niche model. Our results are supported by other findings showing a positive association between tick abundance and forest/woodland habitat, warming temperatures, and lower elevations (6, 15, 21, 22, 56). In our model, total DD>0℃ was found to be an important factor for tick habitat suitability, but this variable was outweighed in importance by land cover features. In recent studies, Clow *et al.* found that the log odds of *I. scapularis* presence was correlated with increasing DD>0℃ but that ecological factors such as forest type were not significant for *I. scapularis* colonization of new sites (21, 30). A key difference is that our model used spatial measures of land cover derived from earth observation data rather than site-level descriptors; furthermore, it was calibrated on a rigorous definition of tick presence to identify environments capable of sustaining stable tick populations, where humans are most likely to encounter ticks. Cumulatively, these results indicate that climatic variables, which are more uniform over large geographic regions, are important in driving tick expansion and colonization of new areas, whereas ecological variables, which have potentially high variability at a local scale, play an important role in sustaining tick populations after initial site colonization.

Recent studies have also focused on microclimate or microhabitat to identify *I. scapularis* distributions at a local scale, and have found significant associations between nymphal and adult

tick densities with forest type, forest understory, dominant tree type, depth of litter layer, distance from trails, type of trails, and distance to roads, which support our findings for the dominance of forests in *I. scapularis* habitat suitability (15, 21, 22, 57). A local ecological niche model for *I. scapularis* in the city of Ottawa also found that distance to forests and treed land were among the strongest variables predicting the distribution of blacklegged ticks (24). Deciduous forests have been shown to be most favourable for *I. scapularis* establishment in other studies in North America, while coniferous forests were least favourable for ticks (58, 59). However, in our model distance to both types of forest were found to be important for *I. scapularis* habitat, which may reflect the importance of cedar and maple forests for tick density (22). This is also likely due to the behaviour of white-tailed deer, the main reproductive host for *I. scapularis*, which frequent forest edges that are dominated by coniferous trees such as white cedar, eastern hemlock, and white pines (20, 60).

Interestingly, in our model the predicted distribution of *B. burgdorferi* was heavily concentrated in eastern Ontario and limited to the high-probability *I. scapularis* regions. It is currently unclear if this reflects the lag-phase between tick establishment and infection with *B. burgdorferi* or whether local scale factors promote the establishment of *B. burgdorferi* in some regions of Ontario versus others. There are three hypotheses for the emergence of *B. burgdorferi*: tick first, pathogen first, or dual infection (4, 30). In Canada, studies from passive and active surveillance support the tick-first hypothesis where ticks are brought into the region by migratory birds, followed, in eastern regions, by an estimated five-year lag between tick establishment and transmission of *B. burgdorferi* (28, 51, 61). Our results support this hypothesis since the distribution of *B. burgdorferi* mirror that of *I. scapularis* but is more constrained in eastern Ontario. However, we cannot rule

out the possibility that other factors, such as abundance of white-footed mice, habitat fragmentation, or other underlying ecological and biological factors favour the establishment of *B. burgdorferi* in specific regions independently of *I. scapularis*.

Based on our results, elevation, proportion of agriculture, distance to mixed forest, proportion of water, and proportion of infrastructure are the most important and informative variables predictive of *B. burgdorferi* distribution. While there are similarities in the types of variables that contribute to both models such as distance to mixed forest, elevation, and proportion of agriculture, the distribution of *B. burgdorferi* is more dependent on the proportion of infrastructure and water than that of *I. scapularis*. In a recent study of landscape determinants for blacklegged ticks in the Ottawa-Gatineau region, Talbot *et al.,*2019 also found that distance to roads was a significant predictor of *B. burgdorferi* infection prevalence (22). The importance of infrastructure and urban development in this model may be explained by local adaptations to urbanization of the white-footed mouse, which is the main reservoir for *B. burgdorferi,* or the role of other small mammals as competent reservoirs for *B. burgdorferi* in regions of the province (62-64). Additionally, the importance of water in the *B. burgdorferi* model may represent possible habitat requirements of key reservoir species or hosts for *I. scapularis* as well as entry points of infected ticks via migratory birds (24, 51, 65).

Our *I. scapularis* and *B. burgdorferi* models showed good discrimination of positive and negative sites when validated against two independent datasets, indicating that the predicted distributions of *I. scapularis* and *B. burgdorferi* are supported by the currently available data. Our results are also consistent with human Lyme disease incidence rates in the province, with eastern Ontario

health units reporting the highest incidence rates per 100,000 population (9, 14). This further demonstrates that areas of highest environmental risk are strongly correlated with areas of highest human Lyme disease incidence, although this is not the case in all parts of the world (66). Thus, assessing environmental risk is important for informing tick and human disease surveillance, especially in areas that are predicted as suitable by the model but have limited ongoing surveillance, and to target disease prevention and control to populations living in the highest-risk regions.

Our study has several important strengths. First, we calibrated our models based on multi-year active tick surveillance data with a high degree of spatial accuracy. We modelled habitat suitability based on locations with detected tick occurrences, defined by presence of ticks in the environment and public submissions from nearby regions, to identify geographic areas where humans and animals are most likely to encounter ticks and tick-borne pathogens. Second, we used high resolution earth observation data from which we derived a large number of environmental variables on climate, elevation, and land cover that might affect the ecology of these species and we used a machine learning approach to model the predicted distribution of the species. Lastly, we rigorously validated our models with two independent datasets: active tick surveillance at new sites (i.e. not used for model calibration) and contemporary passive tick surveillance, representing sites where the public has encountered ticks.

Our study also has several limitations including the unavailability of certain variables such as forest fragmentation and other microhabitat features that are relevant on a local scale and may help explain some observed difference between the ecological requirements of *I. scapularis* versus *B.*

burgdorferi. Additionally, we were unable to account for all sampling biases that may arise from operator experience, daily conditions during dragging, and protocol variations as well as other factors that affect tick presence and/or abundance such as the distribution of tick hosts (e.g. densities of white-tailed deer and white-footed mice), barriers to host dispersal, human habitat modifications, and seasonal variations (67, 68). SDMs have also been criticized for generating more conservative estimates of a species' distribution because they are empirical models that rely on occurrence points for calibration and, therefore, cannot predict the full extent of a species' niche (23, 38, 69). Furthermore, SDMs like ours should also be interpreted with caution because they project inferences based on associations between tick occurrence and environmental variables into target geographic areas, and do not reflect tick abundance or the actual distribution of ticks (67). Our models simply predict the potential distribution of ticks and pathogens in southeastern Ontario, and thereby provide one possible measure of risk.

4.2.8 Acknowledgements

We thank the conservation authorities, naturalist clubs, and provincial parks (Ministry of Natural Resources) in Ontario for granting us site access. We also thank Drs. Robbin L. Lindsay and Antonia Dibernardo at the National Microbiology Laboratory (NML) for their contribution to the molecular analyses of tick-borne pathogens. We acknowledge Natural Resources Canada, specifically Dr. Dan McKenney, Pia Papadopol, and John Pedlar for providing climatic data. We also extend sincere appreciation to the dedicated field teams at the University of Guelph and the University of Ottawa.

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4.2.9 Author Contributions

Conceptualization: AMS, MAK.

Data curation: AMS.

Formal analysis: AMS.

Funding acquisition: MAK, CMJ, NHO.

Investigation: AMS, KMC, RM, BT, JJL, CRT.

Methodology: AMS, MAK, AJK, NHO, KMC, CMJ.

Resources: MAK.

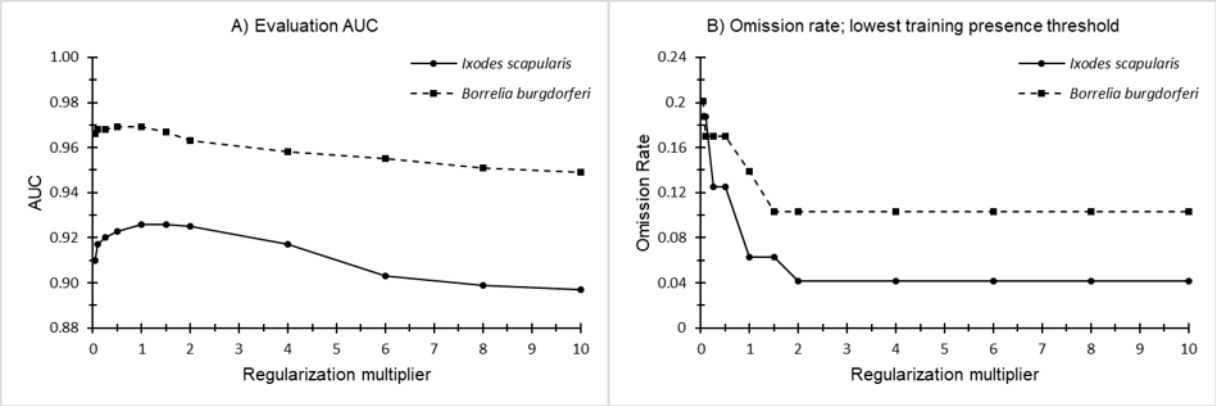
Supervision: MAK.

Visualization: AMS.

Writing - original draft: AMS.

Writing - review and editing: AMS, KMC, RM, BT, JJL, CRT, CMJ, NHO, AJK, MAK.

4.2.10 Supporting Information



Supplemental Figure 4.1 Four-fold cross validation showing the average area under the receiver operating characteristic curve (AUC) (a) and omission rate (b) for the minimum training presence threshold. The best model for *Ixodes scapularis* and *Borrelia burgdorferi* were selected to minimize omission rate and maximize AUC.

Supplemental Table 4.1 Compilation raster for land cover. Mosaic of the Southern Ontario Land Resource Information System version 3.0 (SOLRISv3.0) captured between 2000-2015 and the Provincial Land Cover Database 2000 (PLCD2000) UTM zones 17 and 18 captured between 1999-2002.

Unit Name	Source Unit Name	Source Unit Description
Water	PLC2000: *Water – deep clear*	*Water – deep clear:* Deep or clear waterbodies.
	SOLRISv3.0: *Open water*	*Open water:* Water depth > 2 meters. Lake trophic status. No macrophyte vegetation, trees or shrub cover.
Agricultural land	PLC2000: *Cropland*	*Cropland:* Areas of row crops and fallow fields.
	SOLRISv3.0: *Tilled*	*Tilled:* Agricultural fields managed as continuous annual row crops inferred from 3 observed sequential time periods over a 10-year time period. There can be as many as 2 time periods where fields are rotated with perennial crops. (e.g., hay, improved pasture).
Undifferentiated of rural land	PLC2000: *Pasture*	*Pasture:* Open grassland with sparse shrubs in rural land.
	SOLRISv3.0: *Undifferentiated*	*Undifferentiated:* Includes some agricultural features not included in tilled (i.e. orchards, vineyards, perennial crops and idle land > 10 years – out of agricultural production) as well as urban brown fields, hydro and transportation right-of- ways, upland thicket and openings within forests.
Deciduous forest	PLC2000: *Deciduous forest*	*Deciduous forest:* Largely continuous forest canopy composed primarily of deciduous species.

	SOLRISv3.0: *Deciduous forest*	*Deciduous forest:* Tree cover > 60%. Upland deciduous tree species > 75% canopy cover > 2m in height.
Coniferous forest	PLC2000: *Coniferous forest*	*Coniferous forest:* Largely continuous forest canopy composed primarily of coniferous species
	SOLRISv3.0: *Coniferous forest*	*Coniferous forest:* Tree cover > 60%. Upland coniferous tree species > 75% canopy cover > 2m in height.
Mixed forest	PLC2000: *Mixed forest*	*Mixed forest:* Largely continuous forest canopy composed of both deciduous and coniferous species. In more northerly areas, a greater component of coniferous species can be expected; in more southerly areas, a greater component of deciduous species can be expected.
	SOLRISv3.0: *Mixed forest*, *Forest*	*Mixed forest:* Tree cover > 60%. Upland coniferous tree species > 25%and deciduous tree species > 25% of canopy cover > 2m in height. *Forest:* Tree cover > 60%. Upland tree species > 75% canopy cover > 2m in height. Attribute for forest type could not be derived spectrally from Landsat automated analysis due to size of feature.
Infrastructure	PLC2000: *Settlement / Infrastructure*	*Settlement / Infrastructure:* Clearings for human settlement and economic activity; major transportation routes.
	SOLRISv3.0: *Transportation*, *Built-up area – pervious, Built-up area – impervious*	*Transportation:* Highways, roads. *Built-up area – pervious:* Urban recreation areas. (i.e., golf courses, playing fields) *Built-up area – impervious:* Residential, industrial, commercial, and civic areas.
Hedge rows	PLC2000: --	--
	SOLRISv3.0: *Hedge rows*	*Hedge rows*: Tree cover > 60%, (trees > 2m height), linear arrangement, minimum 10 meters width, maximum 30 meters width.
Marsh	PLC2000: *Intertidal marsh, Supertidal marsh, Inland marsh*	*Intertidal marsh:* Coastal marshes of the Hudson Bay-James Bay Lowlands lying between the coastal mudflats and the supertidal zone. *Supertidal marsh:* Coastal marshes of the Hudson Bay-James Bay Lowlands lying inland of both the coastal mudflats and intertidal marshes, and subject to only exceptionally high tides. *Inland marsh:* Lakeshore and inland marshes of Southern Ontario.
	SOLRISv3.0: *Marsh*	*Marsh:* Open and shrub communities. Water table seasonally or permanently at, near, or above substrate surface – tree and shrub cover <=25%. Dominated by emergent hydrophytic macrophytes.
Sparse Treed	PLC2000: *Sparse forest*	*Sparse forest:* A patchy or sparse forest canopy composed of coniferous or deciduous species or a combination of the two.
	SOLRISv3.0: *Sparse treed:*	*Sparse treed:* 60% < tree cover < 10%. Confined to the Canadian Shield in ecoregion 5E. Tree communities often situated on non-calcareous bedrock features, rapidly draining soils, or raised mineral soils.

Supplemental Table 4.2 Raster processing and derivation of land cover, elevation and climate variables.

#	Variable	Processing
	Land Cover	
1	Distance to agricultural land	Calculated distance from each grid cell to a tilled agricultural cell
2	Proportion of agricultural land	Calculated proportion of tilled agricultural cells within a 1000 m buffer
3	Distance to coniferous forest	Calculated distance from each grid cell to a coniferous forest cell
4	Proportion of coniferous forest	Calculated proportion of coniferous forest cells within a 1000 m buffer
5	Distance to deciduous forest	Calculated distance from each grid cell to a deciduous forest cell
6	Proportion of deciduous forest	Calculated proportion of deciduous forest cells within a 1000 m buffer
7	Distance to hedge rows	Calculated distance from each grid cell to a hedge row cell
8	Proportion of hedge rows	Calculated proportion of hedge row cells within a 1000 m buffer
9	Distance to infrastructure	Calculated distance from each grid cell to an infrastructure cell
10	Proportion of infrastructure	Calculated proportion of infrastructure cells within a 1000 m buffer
11	Distance to marsh	Calculated distance from each grid cell to a marsh cell
12	Proportion of marsh	Calculated proportion of marsh cells within a 1000 m buffer
13	Distance to mixed forest	Calculated distance from each grid cell to a mixed forest cell
14	Proportion of mixed forest	Calculated proportion of mixed forest cells within a 1000 m buffer
15	Distance to rural or undifferentiated land	Calculated distance from each grid cell to a rural or undifferentiated land cell
16	Proportion of rural or undifferentiated land	Calculated proportion of rural or undifferentiated cells within a 1000 m buffer
17	Distance to sparse treed	Calculated distance from each grid cell to a sparse treed cell
18	Proportion of sparse treed	Calculated proportion of sparse treed cells within a 1000 m buffer
19	Distance to water	Calculated distance from each grid cell to a water cell
20	Proportion of water	Calculated proportion of water cells within a 1000 m buffer
	Climate	
21	Bio1: Annual mean temperature	Divided values by 10 and resampled raster at 100 meters resolution

22	Bio2: Mean diurnal range	Divided values by 10 and resampled raster at 100 meters resolution
23	Bio3: Isothermality	Divided values by 10 and resampled raster at 100 meters resolution
24	Bio4: Temperature seasonality	Divided raster values by 100 and resampled raster at 100 meters resolution
25	Bio5: Maximum temperature of warmest period	Divided values by 10 and resampled raster at 100 meters resolution
26	Bio6: Minimum temperature of coldest period	Divided values by 10 and resampled raster at 100 meters resolution
27	Bio7: Temperature annual range	Divided values by 10 and resampled raster at 100 meters resolution
28	Bio8: Mean temperature of wettest quarter	Divided values by 10 and resampled raster at 100 meters resolution
29	Bio9: Mean temperature of driest quarter	Divided values by 10 and resampled raster at 100 meters resolution
30	Bio10: Mean temperature of warmest quarter	Divided values by 10 and resampled raster at 100 meters resolution
31	Bio11: Mean temperature of coldest quarter	Divided values by 10 and resampled raster at 100 meters resolution
32	Bio12: Annual precipitation	Resampled raster at 100 meters resolution
33	Bio13: Precipitation of wettest month	Resampled raster at 100 meters resolution
34	Bio14: Precipitation of driest month	Resampled raster at 100 meters resolution
35	Bio15: Precipitation seasonality	Resampled raster at 100 meters resolution
36	Bio16: Precipitation of wettest quarter	Resampled raster at 100 meters resolution
37	Bio17: Precipitation of driest quarter	Resampled raster at 100 meters resolution
38	Bio18: Precipitation of warmest quarter	Resampled raster at 100 meters resolution
39	Bio19: Precipitation of coldest quarter	Resampled raster at 100 meters resolution
40	Bio20: Degree days ≥ 0°C	Resampled raster at 100 meters resolution
	Other	
41	Elevation	Resampled raster at 100 meters resolution

Supplemental Table 4.3 Average nearest neighbour analysis to select the buffer size used to rarefy the *Ixodes scapularis* and *Borrelia burgdorferi* presence points used to develop the niche model.

***Ixodes scapularis* model**				
Buffer size	**Number of points**	**z-score**	**p-value**	**Distribution**
-	52	-3.075624	0.002101	clustered
1 km	51	-2.677671	0.007414	clustered
2 km	50	-2.357250	0.018411	clustered
3 km	48	-1.352654	0.176166	random
4 km	46	-0.895225	0.370667	random
5 km	44	-0.705652	0.480404	random
***Borrelia burgdorferi* model**				
Buffer Size	**Number of Points**	**z-score**	**p-value**	**Distribution**
-	33	-3.476831	0.000507	clustered
1 km	33	-3.476831	0.000507	clustered
2 km	32	-3.056015	0.002243	clustered
3 km	30	-1.512202	0.130482	random
4 km	29	-1.367204	0.171561	random
5 km	27	-0.976983	0.328578	random

Supplemental Table 4.4 Selection of environmental variables for the *Ixodes scapularis* model.

***Ixodes scapularis* – Land cover variable selection**					
	Gain	**% Decrease in Gain**	**Rank**	**Included in Model**	**Reason(s) Omitted**
Full Model	**1.9288**				
Infra_Prop	1.8326	4.99	1	Yes	
Rural_Prop	1.8518	3.99	2	Yes	
Elevation	1.8600	3.57	3	Yes	
Water_Dist	1.8808	2.49	4	Yes	
Decid_Dist	1.8840	2.32	5	Yes	
Conif_Dist	1.8848	2.28	6	Yes	
Infra_Dist	1.8869	2.17	-	No	Variable already in model + Strong correlation
Hedge_Prop	1.8900	2.01	7	Yes	
Agri_Prop	1.9006	1.46	8	Yes	
Water_Prop	1.9011	1.44	-	No	Variable already in model
Conif_Prop	1.9058	1.19	-	No	Variable already in model
Decid_Prop	1.9112	0.91	-	No	Limited contribution + Variable already in model
Agri_Dist	1.9178	0.57	-	No	Limited contribution + Variable already in model
Sparse_Dist	1.9185	0.53	-	No	Limited contribution
Rural_Dist	1.9186	0.53	-	No	Limited contribution + Strong correlation
Marsh_Prop	1.9191	0.50	-	No	Limited contribution + Strong correlation
Mixed_Prop	1.9211	0.40	-	No	Limited contribution
Mixed_Dist	1.9245	0.22	-	No	Limited contribution

Marsh_Dist	1.9289	-0.01	-	No	Limited contribution
Sparse_Prop	1.9289	-0.01	-	No	Limited contribution
Hedge_Dist	1.9294	-0.03	-	No	Limited contribution
***Ixodes scapularis* – Climate variable selection**					
	Gain	**% Decrease in Gain**	**Rank**	**Included in Model**	**Reason(s) Omitted**
Full Model	**0.7354**				
DD>0C	0.6829	7.14	1	Yes	
Bio11	0.7086	3.64	-	No	Strong correlation
Bio10	0.7162	2.61	-	No	Strong correlation
Bio16	0.7222	1.79	2	Yes	
Bio18	0.7236	1.60	3	Yes	
Bio04	0.7249	1.43	4	Yes	
Bio19	0.7268	1.17	-	No	Strong correlation
Bio15	0.7285	0.94	-	No	Limited contribution
Bio12	0.7307	0.64	-	No	Limited contribution + Strong correlation
Bio01	0.7308	0.63	-	No	Limited contribution + Strong correlation
Bio05	0.7325	0.39	-	No	Limited contribution
Bio13	0.7328	0.35	-	No	Limited contribution + Strong correlation
Bio03	0.7337	0.23	-	No	Limited contribution
Bio09	0.7337	0.23	-	No	Limited contribution
Bio17	0.7341	0.18	-	No	Limited contribution + Strong correlation
Bio02	0.7343	0.15	-	No	Limited contribution + Strong correlation
Bio08	0.7344	0.14	-	No	Limited contribution + Strong correlation
Bio06	0.7348	0.08	-	No	Limited contribution + Strong correlation
Bio14	0.7352	0.03	-	No	Limited contribution + Strong correlation
Bio07	0.7357	-0.04	-	No	Limited contribution + Strong correlation

Supplemental Table 4.5 Selection of environmental variables for the *Borrelia burgdorferi* model.

***Borrelia burgdorferi* – Land cover variable selection**					
	Gain	**% Decrease in Gain**	**Rank**	**Included in Model**	**Reason(s) Omitted**
Full Model	**2.4806**				
Water_Prop	2.3542	5.10	1	Yes	
Rural_Prop	2.3691	4.49	2	Yes	
Conif_Prop	2.3761	4.21	3	Yes	
Hedge_Prop	2.3896	3.67	4	Yes	
Conif_Dist	2.4139	2.69	-	No	Variable already in model
Infra_Dist	2.4203	2.43	5	Yes	

Infra_Prop	2.4224	2.35	-	No	Variable already in model
Elevation	2.4385	1.70	6	Yes	
Water_Dist	2.4450	1.44	-	No	Variable already in model
Agri_Prop	2.4509	1.20	7	Yes	
Mixed_Dist	2.4521	1.15	8	Yes	
Sparse_Prop	2.4585	0.89	-	No	Limited contribution
Marsh_Prop	2.4638	0.68	-	No	Limited contribution
Decid_Dist	2.4668	0.56	-	No	Limited contribution + Strong correlation
Agri_Dist	2.4719	0.35	-	No	Limited contribution + Variable already in model
Sparse_Dist	2.4758	0.19	-	No	Limited contribution
Mixed_Prop	2.4765	0.17	-	No	Limited contribution
Hedge_Dist	2.4783	0.09	-	No	Limited contribution + Variable already in model
Marsh_Dist	2.4788	0.07	-	No	Limited contribution
Rural_Dist	2.4808	-0.01	-	No	Limited contribution + Strong correlation
Decid_Prop	2.4812	-0.02	-	No	Limited contribution
***Borrelia burgdorferi* – Climate variable selection**					
	Gain	**% Decrease in Gain**	**Rank**	**Included in Model**	**Reason(s) Omitted**
Full Model	**0.6854**				
Bio10	0.6462	5.72	1	Yes	
Bio01	0.6605	3.63	-	No	Strong correlation
Bio19	0.6660	2.83	2	Yes	
Bio13	0.6679	2.55	-	No	Strong correlation
Bio11	0.6729	1.82	-	No	Strong correlation
Bio16	0.6735	1.74	-	No	Strong correlation
Bio09	0.6776	1.14	3	Yes	
Bio18	0.6784	1.02	4	Yes	
Bio04	0.6791	0.92	-	No	Limited contribution
Bio07	0.6797	0.83	-	No	Limited contribution
DD>0C	0.6800	0.79	-	No	Limited contribution + Strong correlation
Bio14	0.6820	0.50	-	No	Limited contribution + Strong correlation
Bio15	0.6833	0.31	-	No	Limited contribution
Bio05	0.6841	0.19	-	No	Limited contribution + Strong correlation
Bio03	0.6848	0.09	-	No	Limited contribution
Bio08	0.6851	0.04	-	No	Limited contribution + Strong correlation
Bio12	0.6852	0.03	-	No	Limited contribution + Strong correlation
Bio17	0.6852	0.03	-	No	Limited contribution + Strong correlation
Bio06	0.6853	0.01	-	No	Limited contribution + Strong correlation
Bio02	0.6854	0.00	-	No	Limited contribution

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5

5 SOCIOECOLOGICAL RISK FACTORS OF LYME DISEASE

5.1 ARTICLE PREFACE

In this next chapter, I designed a case-control study to assess the relationship between residence in model predicted environmental risk areas and Lyme disease. I used the predicted distribution of *I. scapularis* from the previous chapter, which I classified as high/low based on model thresholds, to define the Lyme disease risk areas. Reported Lyme disease cases in Ontario, which were previously linked with health administrative data at ICES, were used as the cases and matched by age and sex to population controls from the Registered Person Database. A conditional logistic regression model was used to analyze the association between residence in Lyme disease risk areas and Lyme disease, while controlling for socioecological risk factors.

5.1.1 Author Contributions

I conceptualized the study with my thesis supervisor, Dr. Manisha Kulkarni. Lyme disease cases linked to health administrative data were extracted from a previous project conducted by Dr. Beate Sander at ICES. I developed the dataset creation plan, which included defining covariates, the matching of controls to cases, and all other study design aspects. Due to data security and privacy, an ICES analyst, Michael Pugliese, conducted the matching and data linkages using the raw case and control data. I conducted the formal statistical analyses, interpretation, and manuscript writing, with methodological help and advice from Drs. Beate Sander, Kate Zinszer, Mark Nelder, Curtis Russell, and Manisha Kulkarni. All authors contributed to the editing and review of this article.

5.1.2 Ethics Approval

The use of the data in this project is authorized under section 45 of Ontario's Personal Health Information Protection Act (PHIPA) and does not require review by a Research Ethics Board.

5.1.3 Article Citation

Slatculescu AM, Pugliese M, Sander B, et al. Rurality, Socioeconomic Status, and Residence in Environmental Risk Areas Associated with Increased Lyme Disease Incidence in Ontario, Canada: A Case-Control Study. *Vector Borne Zoonotic Dis* 2022;22(12):572-81.

5.1.4 Associated Appendix

Appendix 9.1 includes the conditional logistic regression univariable analyses.

5.2 ARTICLE CONTENT

5.2.1 Title

Rurality, socioeconomic status, and residence in environmental risk areas associated with increased Lyme disease incidence in Ontario, Canada: A case-control study.

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5.2.3 Abstract

Lyme disease (LD) is the most common tick-borne illness in North America. LD is acquired through exposure to the tick vector, *Ixodes scapularis*, known as the blacklegged tick. In Canada, LD is rapidly emerging, with the establishment of *I. scapularis* in many newly endemic regions posing a growing risk to local communities. In the Canadian context, many environmental and socioeconomic risk factors for human LD infection are yet to be ascertained and the degree of risk associated with residential and community exposure to ticks is not well known. We conducted a matched case-control study in southeastern Ontario, using LD patient data from provincial laboratory databases and uninfected population controls from 2014-2018. We aimed to identify area-level risk factors for LD and associations with residence in environmental risk areas, defined as areas with high model-predicted probability of *I. scapularis* occurrence, using the neighbourhood dissemination area as the unit of analysis. Using multivariable conditional logistic regression analysis, we identified that patients with LD had higher odds (odds ratio, OR; 95% confidence interval, CI) of living in neighbourhoods with high probability of tick occurrence in the environment (OR=2.2; 95% CI:2.0, 2.5), low walkability (OR=1.6; 95% CI:1.2, 2.1), low material deprivation (OR=1.4; 95% CI:1.2, 1.7) and low ethnic concentration (OR=8.1; 95% CI:6.7, 9.9). We also found that the odds of LD infection for individuals residing in environmental risk areas was highest for those living in public health units with <250,000 population (OR=3.0; 95% CI:2.4, 3.9) compared to those living in public health units with >1,000,000 population (OR=1.5; 95% CI:1.1, 2.1). This study shows that odds of human LD infection in Ontario, Canada is higher in less urbanized areas with higher socioeconomic status and indicates that exposure to ticks around the home residence or neighbourhood is linked to increased odds of LD.

5.2.4 Introduction

Lyme disease (LD) is the most common vector-borne illness in North America (1, 2). In central and eastern North America, LD is transmitted to humans by the tick vector, *Ixodes scapularis*, also known as the blacklegged tick, which has continued to spread in recent decades due to a warming climate and favourable environmental changes that promote the expansion of tick habitat (3, 4). *Ixodes scapularis* ticks acquire the causative agent of LD, *Borrelia burgdorferi* sensu stricto, during successful feeding by larvae and nymphs on small- and medium-sized mammals or birds (5). Blacklegged ticks are mostly found in woodland habitats where the density of animal hosts is highest and where the canopy, vegetation, and leaf litter provide protection from desiccation in summer and freezing in winter (6-8). Growing changes in land use and forest fragmentation have increased the intersection between ticks and high-human population densities (9). Fragmented forest edges are also associated with higher populations of white-tailed deer (*Odocoileus virginianus*), which are reproductive hosts for adult *I. scapularis*; as well as reduced species diversity that is believed to favour the abundance of habitat-generalists like the white-footed mouse (*Peromyscus leucopus*), considered the main reservoir of *B. burgdorferi* (10, 11).

In the United States, human exposure to infected *I. scapularis* ticks mainly occurs in peridomestic environments (12-15). Survey studies conducted in the New England region have reported high percentages of domestically-acquired ticks, with 69% of participants (N=70) in Westchester County, NY, and 74% of individuals (N=4717) across Connecticut self-reporting backyard tick exposure (16, 17). Peridomestic tick exposure has been linked to various yard and neighbourhood risk factors such as lawn maintenance, fencing around the yard, type of shrubbery and trees in the yard, and presence of deer or other animals on the property as well as behavioural risk factors such

as time spent in the yard, pet ownership, and use of protective clothing or acaricides outdoors (12, 18-22). Despite evidence for peridomestic tick exposure, a recent meta-analysis looking at spatial risk factors for tick-borne diseases in 14 American states and in Canada found that the odds of acquiring tick-borne diseases was highest for neighbourhood-scale risk factors (OR=4.1; neighbourhood defined as outside the yard but within 500m of the property boundary) compared to yard-scale risk factors (OR=2.6) (23). There remains considerable variation in risk at various spatial scales and additional studies are needed to assess yard and neighbourhood characteristics that contribute to the transmission of tick-borne diseases.

In Canada, populations of *I. scapularis* were first identified in Long Point, Ontario in the early 1970s, a decade before the blacklegged tick was identified as the vector for *B. burgdorferi* transmission (24, 25). LD has been a reportable disease in Ontario since 1988 and later became nationally notifiable in 2009 (2, 26). Since 2009, the numbers of reported cases have increased more than tenfold in the last decade as the distribution of *I. scapularis* continued to spread northward (2, 27-29). It is estimated that from 2012-2022, the range expansion of *I. scapularis* is expected to increase at approximately 46 km/year, with many communities becoming newly emerging risk areas (4, 30-32). Currently, it is unclear what neighbourhood risk factors contribute to the growing incidence of LD in Canada and whether peridomestic exposure to ticks occurs to the same extent as it does in neighbouring states in the United States. Therefore, we designed a matched case-control study in Ontario, Canada to 1) identify area-level neighbourhood and socioecological risk factors for LD in a Canadian setting, and 2) determine if LD incidence is associated with residence in high environmental risk areas.

5.2.5 Methods

5.2.5.1 Study Site

The study was conducted in the southeastern region of Ontario, Canada (Figure 5.1). The area includes three ecoregions (Lake Erie–Lake Ontario ecoregion 7E, Lake Simcoe–Rideau ecoregion 6E, and Georgian Bay ecoregion 5E), defined by the Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry (NDMNRF) on the basis of bedrock, climate, physiography and vegetation, as well as several large census metropolitan areas including Toronto and the Greater Toronto Area (5,928,040 population) and Ottawa (934,243 population) (33, 34).

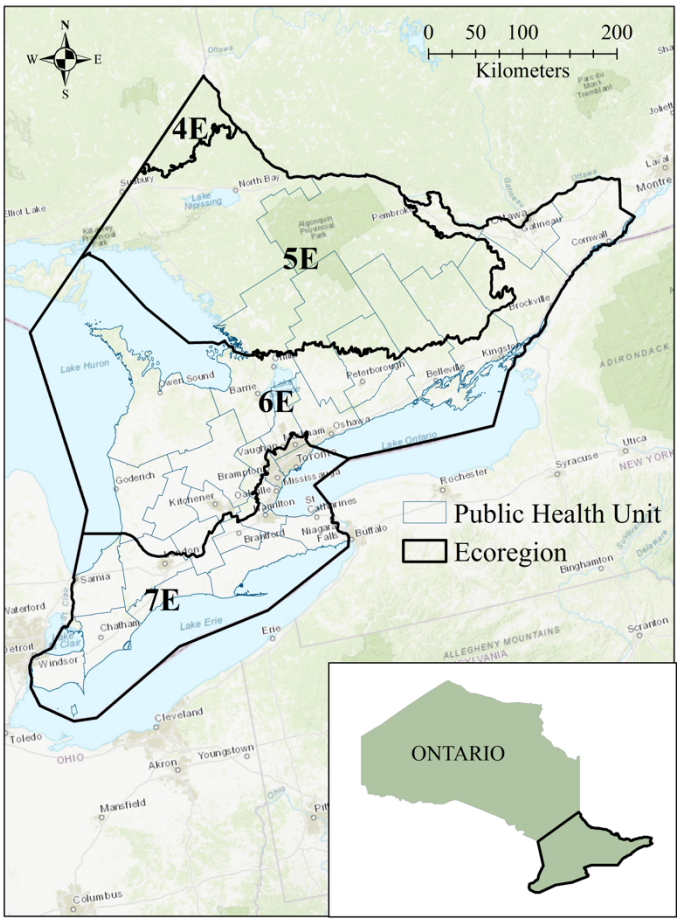

Figure 5.1 Study area map showing public health units and ecoregions in southern and eastern Ontario.

5.2.5.2 Study Population

All LD cases from 2014-2018 that resided in Ontario at the time of LD diagnosis, hereafter referred to as index date, were extracted from data held at ICES, which was previously linked to health administrative data routinely collected from Ontario's publicly funded single-payer healthcare system and used to assess healthcare costs of LD in Ontario (35). ICES is an independent, non-profit research institute whose legal status under Ontario's health information privacy law allows it to collect and analyze health care and demographic data, without consent, for health system evaluation and improvement. In this study, we included both confirmed and probable LD cases, based on the national case definition (36). Confirmed LD is defined as 1) clinical evidence of illness with laboratory confirmed *B. burgdorferi* infection via isolation of bacterium or DNA from a clinical specimen, or 2) clinical evidence of illness with two-tiered serological testing confirmation and a history of residence or travel to a known LD risk area. Probable LD is defined as 1) clinical evidence of illness with two-tiered serological testing confirmation but without a history of residence or travel to a known LD risk area, or 2) physician-diagnosed erythema migrans without laboratory evidence but a history of residence or travel to a known LD risk area. A description of clinical evidence included in the case definition, including objective signs and symptoms that may be used in a clinical diagnosis of Lyme disease, is provided in the 2016 case definition report (36). Confirmed and probable LD cases who did not reside in the study area or who had invalid health or postal code data at the index date were excluded from the study (N=85, 3.1%).

Controls, representative of the general population, were selected from the Registered Persons Database (RPDB), available through ICES data holdings. The RPDB contains demographic information on persons registered under the Ontario Health Insurance Plan (OHIP) who are eligible

for publicly funded healthcare (~16 million records), which constitutes nearly the entire population of the province due to the universal publicly funded health care system in Ontario. People appearing in the RPDB were assigned a pseudo-index date based on the distribution of index dates for LD cases. Individuals who appeared in the LD provincial reportable disease dataset from 2014-2018, who were deceased before the pseudo-index date, who had missing age, sex, date of birth, or a home postal code, who were aged over 105 years, and who were not eligible for OHIP or residing in the study area at the pseudo-index date were excluded from the RPDB source population from which uninfected controls were selected (N=2173837, 13.7%).

LD cases and controls were hard matched on age, sex and index date/pseudo-index date using a Greedy algorithm (linear matching algorithm) that generates one-to-many matched pairs sampled without replacement. These datasets were linked using unique encoded identifiers and analyzed at ICES. LD risk is seasonal and age- and sex-related, with most cases occurring in children 5-14 years, older adults 60-74 years, males, and during the spring-to-fall peaks of tick activity (2, 29). Likewise, age and sex are associated with various degrees of knowledge, risk perception, and preventive behaviours that affect exposure to ticks (37, 38). Matching balances baseline characteristics between cases and controls by selecting controls with similar distributions for age, sex, and date of residence in Ontario. This helps increase statistical efficiency by reducing the variation in the calculated odds ratio estimates that would arise had controls been randomly selected at a very different distribution for these factors (39).

5.2.5.3 Risk Factor Assessment

Exposure to environmental risk areas

A previously constructed species distribution model for *I. scapularis*, which predicts the probability of tick occurrence across a continuous landscape from 0-1 based on regional climate and land cover features, was used as a measure of environmental risk for LD in southeastern Ontario (8). This model was constructed using tick surveillance data from 2015-2018 and reflects the contemporaneous environmental risk for the study population. The model output at 100m resolution was used to calculate an average probability of *I. scapularis* occurrence per dissemination area (DA) using zonal statistics in ArcGIS v.10.5.1 (ESRI, Redlands, CA, USA). DAs are defined as small, relatively stable geographic units, composed of one or more adjacent dissemination blocks with an average population of 400 to 700 persons, bounded by features such as roads, railways, and water sources (40). We used the DA-level to approximate neighbourhood-level characteristics for subsequent analyses. The average probability of *I. scapularis* occurrence per DA was used to categorize the DA-level environmental risk for LD as low or high using the model prevalence as a threshold. The model prevalence is defined as the average probability of tick occurrence over all the background pixels (i.e., a model prevalence of 0.5 implies that the species is present in half of all the possible locations); this threshold was selected as it is one of the more suitable approaches for transforming the results of species distribution models from probabilities to categorical measures (41). The DA-level low/high environmental risk for LD was then assigned to each LD case and control based on the individual's home address using DA as the matching variable. The home address was also linked to the nearest public health unit (PHU), a larger geographic unit governed by a health agency that provides local public health services. 29 PHUs were located within our study area; however, to avoid small cell sizes, we created three PHU categories based on population size (Group 1: >1,000,000 population, Group 2: 250,000–999,999 population, and Group 3: <250,000 population) (Supplemental File 5.1).

Environmental and socioeconomic risk factors

Land cover data from the Southern Ontario Land Resource Information System version 3.0 (SOLRISv3.0) and geospatial data on provincial/municipal regulated parks and trails in Ontario, available from NDMNRF's open data portal (https://geohub.lio.gov.on.ca), were used to calculate DA-level proportion of wooded land and parks and total trail length in meters using ArcGIS v.10.5.1. Walkability and transit scores were obtained from the Canadian Active Living Environments (Can-ALE) database (https://nancyrossresearchgroup.ca/research/can-ale/) to measure DA structure and characteristics such as intersections, foot paths, points of interest (e.g., businesses, schools, shops), dwelling density, and transit stops (42). DA-level socioeconomic variables of interest included the 2008 Rurality Index for Ontario (RIO), neighbourhood income quintiles, and population density, obtained from the 2016 Canada Census (Statistics Canada) as well as the 2016 Ontario Marginalization Index (ON-Marg), available from Public Health Ontario (PHO) (34, 43). The ON-Marg was derived from a series of iterative factor analyses of 42 census-based indicators, yielding four factors (i.e., residential instability, material deprivation, dependency, and ethnic concentration) with 18 remaining indicators that represent multiple dimensions of socioeconomic status in Ontario (43). Variable definitions are listed in Table 5.1.

Table 5.1 Definition of DA-level risk factor variables.

Variable	***Type***	***Description***	***Ref***
Environmental risk for LD	Categories	Mean predicted probability of occurrence of *I. scapularis* in the environment across all pixels. Categorized as low/high risk areas for LD using the model prevalence (mean probability of tick occurrence across all background pixels) as a threshold.	(8)
Residence PHU	Categories	Nearest PHU to the home address, classified into three categories based on population size (Group 1: >	–

		1,000,000 population, Group 2: 250,000 – 999,999 population, and Group 3: < 250,000 population)	
Treed land	Percentage	Sum of pixels covered by mixed-treed land (i.e., deciduous, coniferous, mixed forest, and shrubs) divided by total number of pixels.	–
Parks	Percentage	Sum of pixels covered by regulated provincial and municipal parks divided by total number of pixels.	–
Trail length	Sum	Sum of all trail line lengths in meters.	–
Walkability score	Score 1-5	Measure of k-medians cluster of connectivity (3-way intersections and foot paths), density (dwelling density), and destinations (points of interest such as businesses, schools, shops, parks, and hospitals).	(42)
Transit score	Score 1-5	Similar measure as walkability score with the inclusion of transit stops.	(42)
Rurality index (RIO)	Score 1-100	Measure of rurality based on healthcare access. The indicators include population density, travel time to basic and advanced referral centres.	(44)
Population density	Average	2016 Canada Census population density (people/km^2).	(34)
Neighbourhood income	Quintiles	Community-specific income quintiles were derived by ranking DAs according to average household income, from lowest to highest, in each CMA, CA, or provincial residual area not in any CMA or CA, and then dividing into approximate fifths (i.e., 20% of DAs in each quintile). Average household income was adjusted for household size and sourced from a combination of self-reported census and tax file data.	(45, 46)
Residential instability	Quintiles	Measure of concentrations of people who experience high rates of family or housing instability. The indicators included are proportion of the population living alone, proportion of the population who are not youth (age 5-15), average number of persons per dwelling, proportion of dwellings that are apartment buildings, proportion of the population who are single/divorced/widowed, proportion of dwellings that are not owned, proportion of the population who moved during the past 5 years.	(43)
Material deprivation	Quintiles	Measure is closely connected to poverty, and it refers to inability for individuals and communities to access and attain basic material needs. The indicators included are proportion of the population aged 20+ without a high-school diploma, proportion of families who are lone parent families, proportion of total income from government transfer payments for population aged 15+, proportion of the population aged 15+ who are unemployed, proportion of the population considered	(43)

		low-income, proportion of households in dwellings in need of major repair.	
Dependency	Quintiles	Measure of concentrations of people who do not have income from employment. Indicators included are proportion of the population who are aged 65 and older, dependency ratio (total population 0-14 and 65+ / total population 15-64), proportion of the population not participating in labour force (aged 15+).	(43)
Ethnic concentration	Quintiles	Measure refers to high concentrations of people who are recent immigrants and/or people belonging to a 'visible minority' group (defined by Statistics Canada as "persons, other than Indigenous peoples, who are non-Caucasian in race or non-white in colour"). Indicators included are proportion of the population who are recent immigrants (arrived in the past 5 years), proportion of the population who self-identify as a visible minority.	(43)

LD: Lyme disease; PHU: Public Health Unit; DA: Dissemination Area; CMA: Census Metropolitan Area; CA: Census Agglomeration.

5.2.5.4 Statistical Analysis

Sample size calculation

The number of LD cases and controls needed for the study were calculated using Sampsize (© 2003 Philippe Glaziou, Free Software Foundation, Boston, MA, USA). 1585 LD cases and 6340 controls were needed to detect a modest difference in disease odds ratio (OR) of 1.2, assuming 50% of controls reside within a high LD environmental risk area, a correlation coefficient of ϕ=0.2 between case and control tick exposure status, and Type I and Type II error probabilities of α=0.05 and β=0.90, respectively (47). A 1:4 case to controls matching ratio was used due to the availability of controls in the RPBD and to increase power for subgroup analyses (48).

Multivariable regression analysis

Multivariable conditional logistic regression analysis was performed to calculate the OR and 95% confidence intervals (CI) for the association between human LD infection and residence in high

environmental risk areas, while adjusting for environmental and socioeconomic risk factors. A full model was generated with variables defined in Table 1; those with significant effect based on the 95% CI were retained. Non-significant variables were added back to the model one at a time to test for confounding using the percent change in estimate method. If regression coefficients changed by more than 10% the variable was kept in the model. The remaining model was tested for multicollinearity using the Spearman rank correlation coefficient to assess correlation between ordinal variables and using variance inflation factor (VIF) and collinearity diagnostics to measure overall correlation when all variables are together. Highly correlated variables (R_s>0.6, VIF>5 and proportion of variance >0.5) were removed to generate the most parsimonious model (49). Homogeneity of effect was assessed through stratified analyses and model fit was assessed by visual inspection of residuals and deviance analysis. Analyses were conducted in SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC, USA).

Sensitivity Analysis

The selection of a threshold for transforming model predictions from probabilities to presence/absence affects the classification of an individual's residence in terms of environmental risk for LD. We conducted a sensitivity analysis to test the robustness of the selected threshold on our overall results. There is currently no standard approach for selecting a threshold to categorize the output of a species distribution model, although several studies have used simulation data to test the validity of various approaches (41, 50). We selected maximization of sensitivity plus specificity as a secondary threshold because yielded good results using simulation data (41).

5.2.6 Results

5.2.6.1 Study Subjects

From 2014 through 2018, 2630 incident LD cases (confirmed: N=2284, 86.8%; probable: N=346, 13.2%) were identified in southern and eastern Ontario. Mean age of LD cases was 46.9±21.4 years, 1485 (56.5%) were males, 245 (9.3%) resided rurally (RIO>40), and 1400 (53.2%) resided in PHUs with less than 250,000 population (Table 5.2). LD cases increased annually from 224 (8.5%) in 2014 to 629 (23.9%) in 2018, with a peak of 985 (37.5%) in 2017 (Table 5.2). All cases were matched to controls (N=10,520) at a 1:4 ratio by exact age, sex, and index date. Controls had the same distribution as cases for the matched variables, but fewer resided rurally (N=486, 4.7%) and in PHUs with less than 250,000 population (N=1805, 17.2%) (Table 2).

Table 5.2 Descriptive statistics of laboratory-confirmed and probable LD cases and matched controls in southeastern Ontario, Canada (2014-2018).

	Cases ***N (%)***	***Controls*** ***N (%)***
Study Subjects	2630	10520
Confirmed LD	2284 (86.8)	
Probable LD	346 (13.2)	
Age at index date, years		
Mean ±SD	46.9±21.4	46.9±21.4
<5	69 (2.6)	276 (2.6)
5–14	256 (9.7)	1024 (9.7)
15–24	167 (6.4)	668 (6.4)
25–34	228 (8.7)	912 (8.7)
35–44	283 (10.8)	1132 (10.8)
45–54	457 (17.4)	1828 (17.4)
55–64	598 (22.7)	2392 (22.7)
65–74	419 (15.9)	1676 (15.9)
≥75	153 (5.8)	612 (5.8)
Sex		
Female	1145 (43.5)	4580 (43.5)
Male	1485 (56.5)	5940 (56.5)
Rural residence		
Yes	245 (9.3)	486 (4.7)
No	2340 (89.0)	9931 (95.3)
Missing	45 (1.7)	–
PHU of residence, population		
>1,000,000	323 (12.3)	4246 (40.4)

250,000–999,999	907 (34.5)	4469 (42.4)
<250,000	1400 (53.2)	1805 (17.2)
Index year		
2014	224 (8.5)	896 (8.5)
2015	419 (15.9)	1676 (15.9)
2016	373 (14.2)	1492 (14.2)
2017	985 (37.5)	3940 (37.5)
2018	629 (23.9)	2516 (23.9)

LD: Lyme disease; PHU: Public Health Unit.

5.2.6.2 Area-Level Risk Factors for Lyme Disease

Using the model prevalence threshold to identify low/high environmental risk areas for LD, 56.6% (N=7438) of the DAs within the study area were classified as high-risk regions (Figure 5.2). Multivariable analysis of matched case-control pairs indicated that LD patients were more likely to reside within neighbourhood with high environmental risk for LD (OR=2.2; 95% CI:2.0, 2.5), lower walkability score (OR=1.6; 95% CI:1.2, 2.1), lower material deprivation (OR=1.4; 95% CI:1.2, 1.7), and lower ethnic concentration (OR=8.1; 95% CI:6.7, 9.9) (Table 5.3). However, the association between residence in high environmental risk areas and LD incidence was influenced by the larger geographic region; thus, we stratified analyses by PHU groups with similar population sizes. The odds of acquiring LD were highest for individuals residing in neighbourhoods with higher average environmental risk that were situated within PHUs with <250,000 population (OR=3.0; 95% CI:2.4, 3.9) compared to those living in PHUs with 250,000–999,999 population (OR=2.0; 95% CI:1.7, 2.5) and PHUS with >1,000,000 population (OR=1.5; 95% CI:1.1, 2.2) (Table 5.4).

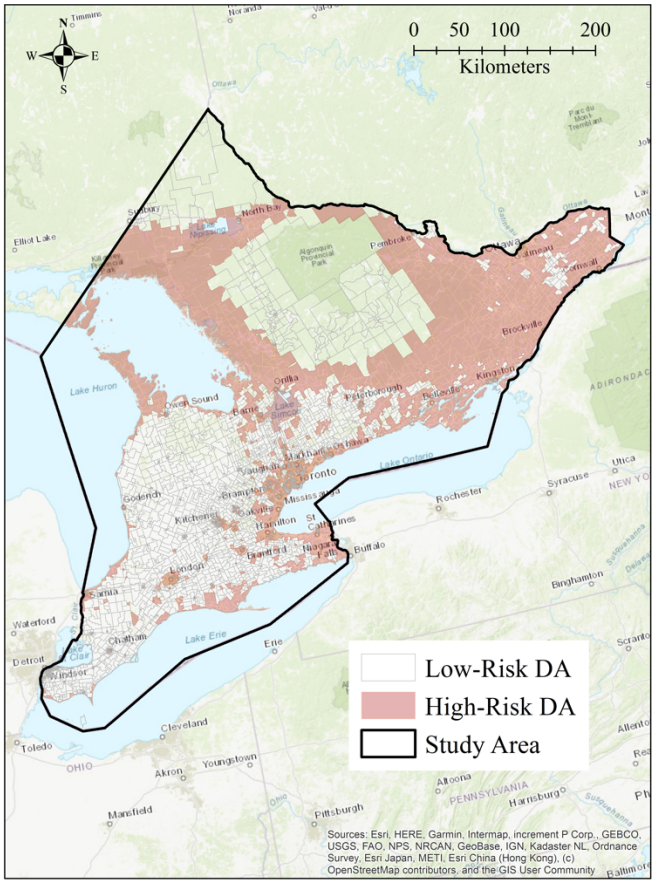

Figure 5.2 Dissemination areas (DA) with low/high environmental risk for Lyme disease in southeastern Ontario using the model prevalence threshold to categorize risk.

Table 5.3 Multivariable conditional logistic regression analysis of area-level neighbourhood and socioeconomic risk factors for LD in southeastern Ontario, Canada (2014-2018).

Variable	***Matched***		
	OR	*95% CI Lower*	*95% CI Upper*
Environmental risk for LD Low	reference	—	—
Environmental risk for LD High	2.248	2.029	2.491
Material deprivation quintile 1 (low)	1.447	1.218	1.718
Material deprivation quintile 2	1.476	1.243	1.752
Material deprivation quintile 3	1.097	0.917	1.312
Material deprivation quintile 4	1.171	0.974	1.408
Material deprivation quintile 5 (high)	reference	—	—
Ethnic concentration quintile 1 (low)	8.138	6.666	9.934
Ethnic concentration quintile 2	5.644	4.648	6.854

Ethnic concentration quintile 3	3.937	3.243	4.781
Ethnic concentration quintile 4	2.999	2.468	3.643
Ethnic concentration quintile 5 (high)	reference	—	—
Neighbourhood walk score 1 (low)	1.587	1.211	2.081
Neighbourhood walk score 2	0.802	0.612	1.049
Neighbourhood walk score 3	0.937	0.713	1.231
Neighbourhood walk score 4	0.982	0.171	1.345
Neighbourhood walk score 5 (high)	reference	—	—

LD: Lyme disease; PHU: Public Health Unit.

Table 5.4 Stratified analysis of associations between LD incidence and average neighbourhood LD environmental risk in southeastern Ontario, Canada (2014-2018).

Group (PHU Population)	***Matched***					
	Crude			*Adjusted*		
	OR	*95% CI (Lower)*	*95% CI (Upper)*	*OR_a*	*95% CI (Lower)*	*95% CI (Upper)*
>1,000,000	1.320	0.975	1.787	1.509	1.054	2.159
250,000–999,999	1.998	1.645	2.428	2.043	1.664	2.509
<250,000	3.259	2.594	4.094	3.045	2.381	3.896

LD: Lyme disease; PHU: Public Health Unit; OR: Odds Ratio; CI: Confidence Interval.
[a] Adjusted for neighbourhood walkability, material deprivation, and ethnic concentration.

5.2.6.3 Effect of Different Classification Methods for Lyme Disease Environmental Risk

The sensitivity analysis showed that using the maximization of sensitivity plus specificity threshold for classifying low/high environmental risk areas for LD resulted in more conservative estimates of LD risk. Using this approach, 38.1% (N=5012) of DAs in the study area were classified as high-risk neighbourhoods for LD. However, the OR for the association between human LD infection and residence in neighbourhoods with high environmental risk for LD, when adjusting for socioecological risk factors, was nearly identical (OR=2.1; 95% CI:1.9, 2.3). A

similar trend was also observed for stratified analyses based on PHU of residence, although the magnitude of the association was slightly attenuated (Supplemental File 5.2).

5.2.7 Discussion

Our study identified positive associations between the odds of LD and lower neighbourhood walkability scores, which are strong indicators of a lower degree of urbanization due to fewer 3-way intersections and footpaths, dwelling density, and points of interest (i.e., businesses, shops, schools). Using k-means cluster analysis, Herrmann et al. found that DAs in rural regions and outermost city regions clustered in group 1 (lowest walkability score), DAs in residential and suburban neighbourhoods were found in groups 2-4, and DAs in central business districts of major cities clustered in group 5 (highest walkability score) (42). The association between LD and lower neighbourhood walkability suggests a greater risk in peri-urban regions, suburban neighbourhoods, and rural communities, where more favourable environments exist for blacklegged ticks. In the city of Ottawa, Ontario, local surveillance at sites across the city found a higher density of *I. scapularis* and prevalence of *B. burgdorferi* in recreational trails and conservation areas/forests located in predominantly suburban and rural regions (51). The sites that had high tick densities were also associated with a larger proportion of forests as well as specific forest composition and structure (e.g., dominant tree types) (7, 52).

The inverse relationship between urbanization and LD was also observed more directly using population density (LD odds lower in PHUs with increasing population density), although LD risk does exist within an urban setting to a lesser extent. While the complete effect of urbanization on tick-borne illnesses is still unclear, recent studies in the USA and Europe found tick species and

tick-borne pathogens in urban parks, where populations are believed to be maintained by small rodents and birds (53, 54). In this study, we also found a significant positive association between LD and DA-level proportion of provincial and municipal regulated parks (univariable analysis not shown), although it should be noted the magnitude of this effect was small, likely due to the geographic scale at which the data were analyzed (i.e., many DA boundaries did not contain parks). Nonetheless, these results suggest that pockets of high-risk of tick encounters may exist in various neighbourhood structures, highlighting the importance of local-scale assessments of environmental risk to inform risk mitigation strategies. For example, landscape management options, such as ecotonal woodchip barriers along recreational trails, may constitute important interventions that can reduce LD risk in communities (55).

We found that the odds of LD were also associated with two components of the ON-Marg: lower material deprivation and ethnic concentration. While direct associations between individual components of the ON-Marg and LD incidence are difficult to interpret, these results are supported by similar findings in Ontario, where LD incidence was shown to be highest in communities with increased dependency, a measure of the non-working population including older adults 65+ year and young children under 15 years, which are the highest risk demographic groups for LD (56). Similar associations between LD and socioeconomic status were observed in the USA and Europe (57, 58). This may be in part linked to the higher socioeconomic status of suburban neighbourhoods surrounding major cities, where homes and properties sizes are larger and more green spaces are available for recreational use (59). Land “parcelization” (i.e., the division of large land blocks under single ownership into small blocks under multiple owners) is also positively correlated with favourable deer habitat and higher risk of tick-borne diseases as rural to

exurban/suburban development continues to increase (60-62). These may explain the paradoxical association between rurality and socioeconomic status.

Similar to studies in the USA, where LD incidence is higher than in Canada and ticks are largely endemic across the Midwest and Northeastern regions, we found an association between residence in communities with high tick occurrence and odds of contracting LD (13, 19, 22, 23). These results support the theory that LD environmental risk around the home and surrounding neighbourhood contributes significantly to human LD infection, presumably through peridomestic or recreational exposure to infected ticks. However, this association was weaker for individuals living in urban regions as they may be more likely to travel outside of their neighbourhood for recreational activities or to visit rural cottages in tick endemic areas. This was observed by Slatculescu et al. in eastern Ontario, where 65.2% of Ottawa residents reported LD exposure locations outside of the health unit of residence, compared to 13.9%, 4.0%, and 1.9% for residents of the three largely rural neighbouring health units (56). Similarly, Bron et al. found significant differences in risk behaviours related to yard activities and attitudes towards tick protective behaviours between the US Midwest and Northeast, two regions with very different levels of urbanization and socioeconomic status (63). It is likely that such differences also exist in regions of Ontario with varying levels of urbanization and socioeconomic status; however, these subjects warrant further investigation.

Our study has several limitations. The LD database from which we extracted LD cases did not contain tick exposure information or variables pertaining to location of tick acquisition; thus, we were unable to eliminate travel-related exposures (exposures outside Canada were not included in

the LD database at ICES). We acknowledge that at the DA-level this is a significant limitation since we cannot account for non-neighbourhood exposures, although we included subgroup analyses at the PHU-group level where local travel is less likely to affect results. Second, the human disease surveillance system allows for updates to previous data, which may differ from earlier reports. Thus, the number of LD cases at the time of data extraction may not reflect all cases diagnosed in the province; however, we do not expect this to alter the study findings. It is also possible that some LD cases in Ontario may not be captured by the provincial reportable disease surveillance system; however, recent evidence showed that under-reporting of LD in Canada is estimated to be low (64). Lastly, we cannot draw conclusions about individual-level behaviours and household features that may be linked to tick exposures around the home and neighbourhood as this study focused solely on area-level risk factors for LD. Our study also has notable strengths, as it identified novel socioecological risk factors for LD and affirms that exposure to ticks in the neighbourhood and area around the home residence is a significant predictor of LD infection in the Canadian population. Our findings provide an important overview of the risk associated with LD exposure in the community for Ontario residents, which, together with local-scale research using active tick sampling in the peridomestic environment, can help demonstrate and identify regions where Canadians are exposed to ticks and have a higher risk for LD infection. Lyme disease risk prevention is mainly conducted at a local scale, where decision making takes place and where urban planning, landscape management, and other tick control strategies are employed. Identifying community, neighbourhood, and socioeconomic risk factors for LD, such as those identified by this study, is essential for devising appropriate risk prevention strategies and increasing public health initiatives.

In Ontario, Canada, risk of human LD infection is higher in less urbanized areas with higher socioeconomic indices. Additionally, this study found that living in regions with higher occurrence of *I. scapularis* is linked to increased odds of LD; while this association was strongest in more rural communities, the association was also present in urban municipalities and cities. This may warrant the implementation of multiple public health messaging and risk mitigation strategies across different settings, to reduce the risk of residential/neighbourhood tick exposure while also focusing efforts on tick-endemic recreational spaces frequented by urban residents.

5.2.7.1 Acknowledgments

We thank Drs. Meltem Tuna and Deshayne Fell at ICES uOttawa at the Ottawa Hospital Research Institute (OHRI) for their continued administrative and technical assistance with this project. We also thank Dr. Nicholas Ogden at the Public Health Agency of Canada (PHAC) and Drs. Marc Desjardins and William Cameron at the University of Ottawa for providing guidance with the study research questions and methodology.

5.2.7.2 Author Contribution Statement

AMS and MAK conceived the study; AMS curated the data, conducted the formal analyses, and wrote the main manuscript; MP curated the data and created the dataset at ICES; AMS, BS, KZ, MPN, CR and MAK developed the methodology and contributed to the interpretation of study results; MAK and BS provided supervision and funding. All authors have read, revised, and approved the manuscript.

5.2.7.3 Author Disclosure Statement

No competing financial interests exist.

5.2.7.4 Funding

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5.2.7.5 Availability of Data and Materials

The dataset from this study is held securely in coded form at ICES. While legal data sharing agreements between ICES and data providers (e.g., healthcare organizations and government) prohibit ICES from making the dataset publicly available, access may be granted to those who meet pre-specified criteria for confidential access, available at www.ices.on.ca/DAS (email: das@ices.on.ca). The full dataset creation plan and underlying analytic code are available from the authors upon request, understanding that the computer programs may rely upon coding templates

or macros that are unique to ICES and are therefore either inaccessible or may require modification.

5.2.7.6 Ethics Approval and Consent to Participate

The use of the data in this project is authorized under section 45 of Ontario's Personal Health Information Protection Act (PHIPA) and does not require review by a Research Ethics Board.

5.2.8 Supplemental Information

Supplemental Table 5.1 Grouping of PHUs with LD cases and control subjects for stratified analysis of LD risk factors and residential exposure to ticks.

	Public Health Unit	**Population**	**Density/km^2**
Group 1 >1,000,000 population	City of Toronto	2,731,571	4334.4
	Peel Regional	1,387,744	1108.1
	York Regional	1,109,909	629.9
Group 2 250,000–999,999 population	City of Hamilton	536,917	480.6
	City of Ottawa	934,243	334.8
	Durham Regional	645,862	255.9
	Halton Regional	548,430	568.9
	Middlesex-London	455,526	137.3
	Niagara Regional	447,888	241.5
	Simcoe Muskoka District	540,249	61.4
	Waterloo	535,154	390.9
	Wellington-Dufferin-Guelph	284,461	68.6
	Windsor-Essex County	398,953	215.5
Group 3 <250,000 population	Brant County	134,943	119.5
	Chatham-Kent	102,042	41.3
	Elgin-St. Thomas	88,978	47.3
	Grey Bruce	161,977	18.8
	Haldimand-Norfolk	109,652	38.4
	Haliburton, Kawartha, Pine Ridge District	179,083	19.8
	Hastings and Prince Edward Counties	161,180	22.5
	Huron County	59,297	17.4

	Kingston, Frontenac, Lennox and Addington	193,363	29.2
	Lambton	126,638	42.2
	Leeds, Grenville and Lanark District	169,244	26.4
	Oxford County	110,862	54.4
	Perth District	76,796	34.6
	Peterborough County-City	138,236	35.9
	Renfrew County and District	103,593	6.9
	The Eastern Ontario	202,762	38.2

PHU: Public Health Unit; LD: Lyme disease.

Supplemental Table 5.2 Multivariable conditional logistic regression analysis of associations between LD incidence and average neighbourhood LD environmental risk, when using the maximization of sensitivity plus specificity threshold for classifying LD risk areas.

Group (PHU Population)	***Matched***					
	Crude			*Adjusted*		
	OR	*95% CI (Lower)*	*95% CI (Upper)*	*OR_a*	*95% CI (Lower)*	*95% CI (Upper)*
>1,000,000	1.395	1.011	1.924	1.543	1.044	2.281
250,000–999,999	2.042	1.702	2.449	2.014	1.665	2.436
<250,000	2.593	2.105	3.195	2.364	1.894	2.951

LD: Lyme disease; OR: Odds Ratio; CI: Confidence Interval.

[a] Adjusted for neighbourhood walkability, material deprivation, and ethnic concentration.

5.2.9 Chapter References

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6

6 LYME DISEASE EMERGENCE

6.1 ARTICLE PREFACE

In this chapter I aim to identify where Ontarians are exposed to ticks. I use passive tick surveillance data and human reportable disease surveillance data to analyze the spatial and temporal trends in Lyme disease emergence in eastern Ontario using spatial cluster detection methods. I also use a negative binomial regression model to identify socioecological risk factors associated with Lyme disease incidence in eastern Ontario.

This analysis is also a continuation of the previous chapter where I analysed the relationship between Lyme disease and residence in environmental risk areas. However, in this chapter I geocoded the tick exposure location, which was reported by the tick submitter or Lyme disease patient, to better understand where people are exposed to ticks, whether that is near the home or elsewhere in Ontario.

6.1.1 Author Contributions

I conceived the study design and methodology, with guidance from my thesis supervisor, Dr. Manisha Kulkarni. My thesis advisory committee members, Drs. Nicholas Ogden, Beate Sander, Marc Desjardins, and William Cameron, provided methodological guidance and advice on the interpretation of results. I conducted most of the extensive geocoding and data cleaning, with help from Ms. Claudia Duguay, as well as the formal analyses, interpretation, and manuscript preparation. All authors contributed to the editing and revision of the manuscript.

6.1.2 Ethics Approval

Ethics approval for this study was obtained from the Ottawa Public Health Research Ethics Board (no. 226-16) and the University of Ottawa Science and Health Sciences Research Ethics Board (H06-16-22). All methods were carried out in accordance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans – TCPS 2 (2018). Informed consent was not required for secondary use of data in accordance with TCPS 2 Article 5.5A. Access to Integrated Public Health Information System data was granted through data-sharing agreements with participating public health units. All data were aggregated to preserve anonymity.

6.1.3 Article Citation

Slatculescu AM, Duguay C, Ogden NH, et al. Spatiotemporal trends and socioecological factors associated with Lyme disease in eastern Ontario, Canada from 2010–2017. *BMC Public Health* 2022;22(1).

6.1.4 Associated Appendix

Appendix 9.2 includes the negative binomial regression univariable analyses.

6.2 ARTICLE CONTENT

6.2.1 Title

Spatiotemporal trends and socioecological factors associated with Lyme disease in eastern Ontario, Canada from 2010-2017.

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6.2.3 Abstract

Currently, there is limited knowledge about socioeconomic, neighbourhood, and local ecological factors that contribute to the growing Lyme disease incidence in the province of Ontario, Canada. In this study, we sought to identify these factors that play an important role at the local scale, where people are encountering ticks in their communities. We used reported human Lyme disease case data and tick surveillance data submitted by the public from 2010-2017 to analyze trends in tick exposure, spatiotemporal clusters of infection using the spatial scan statistic and Local Moran's I statistic, and socioecological risk factors for Lyme disease using a multivariable negative binomial regression model. Data were analyzed at the smallest geographic unit, consisting of 400-700 individuals, for which census data are disseminated in Canada. We found significant heterogeneity in tick exposure patterns based on location of residence, with 65.2% of Lyme disease patients from the city of Ottawa reporting tick exposures outside their health unit of residence, compared to 86.1% - 98.1% of patients from other, largely rural, health units, reporting peri-domestic exposures. We detected eight spatiotemporal clusters of human Lyme disease incidence in eastern Ontario, overlapping with three clusters of *Borrelia burgdorferi*-infected ticks. When adjusting for population counts, Lyme disease case counts increased with larger numbers of *Borrelia burgdorferi*-infected ticks submitted by the public, higher proportion of treed landcover, lower neighbourhood walkability due to fewer intersections, dwellings, and points of interest, as well as with regions of higher residential instability and lower ethnic concentration (Relative Risk [RR] = 1.25, 1.02, 0.67-0.04, 1.34, and 0.57, respectively, $p<.0001$). Our study shows that there are regional differences in tick exposure patterns in eastern Ontario and that multiple socioecological factors contribute to Lyme disease risk in this region.

6.2.4 Background

Lyme disease is a tick-borne illness caused, in northeastern and midwestern North America, by infection with the bacterium *Borrelia burgdorferi* sensu stricto, which is transmitted through the bite of the blacklegged tick, *Ixodes scapularis* (1, 2). Lyme disease is the most prevalent vector-borne illness in North America and, with the northward spread of ticks from endemic regions in the United States and southern Canada, its incidence has also increased substantially in central and eastern Canada (3-8). The continued range expansion of *I. scapularis* is in part attributed to a warming climate increasing environmental suitability for tick establishment, permitting *B. burgdorferi* transmission cycles to establish between wildlife hosts and reproducing tick populations (9-14). Ecologically, *I. scapularis* colonization depends on a complex set of factors including the presence of deciduous forests, shrubs, and forest understory, increased ground temperature and degree-days above 0°C, and a higher proportion of forest fragmentation that favours the abundance of animal hosts such as white-tailed deer and white-footed mice (15-19).

There is evidence that human infection with Lyme disease in regions where blacklegged ticks are established is correlated with the density of host-seeking *I. scapularis* and the prevalence of *B. burgdorferi* infection in ticks, although there is heterogeneity at different spatial scales (20-23). Recent studies by Ripoche et al. (2018) in Quebec and Gasmi et al. (2019) in Ontario and Manitoba showed that the cumulative number of locally acquired *I. scapularis* ticks submitted by the general public to local health units was a strong indicator of municipalities at risk of Lyme disease (24, 25). However, the extent to which ecological risk factors influence the risk of contracting Lyme disease also depends on factors that increase human exposure and accessibility to risk areas, such as location of residence, type of occupation, where people undertake leisure activities, and overall

knowledge, attitudes, and practices relating to tick exposures and preventive measures (26-28). Recently, Bouchard et al. (2018) developed an integrated social-behavioural and ecological risk map to characterize multiple components of Lyme disease risk in the Montérégie region of southern Quebec, and while individual social-behavioural variables were not significantly associated with Lyme disease cases, they found that social-behavioural variables had a markedly different distribution compared to ecological variables, indicating that various factors may have different impacts on human Lyme disease (29).

In Ontario, most studies have focused on the distribution of the blacklegged tick vector as a measure of environmental risk for Lyme disease and on ecological factors that contribute to tick establishment and northward spread (9, 10, 17, 30). However, little is known about how neighbourhood structure and other socioecological factors affect human exposure to these environmental risk areas. Tick exposure location for Lyme disease patients is also often approximated to broad geographic regions or assumed to be near the home residence, further highlighting the need for fine-scale studies and more accurate analysis of tick exposure (7, 24, 25, 31). In this study, we use human Lyme disease case data and passive tick surveillance data, consisting of ticks submitted by the public to local public health units (PHUs), to analyze tick exposure patterns for Lyme disease patients and tick submitters as well as spatiotemporal trends in Lyme disease incidence and socioecological risk factors at the smallest geographic unit for which census data are available in Canada, the dissemination area (DA). DAs are defined as small, relatively stable geographic units with a population of 400 to 700 persons bounded by features such as roads, railways, and water sources (32).

6.2.5 Methods

6.2.5.1 Study area

Our study encompassed four PHUs in eastern Ontario, Canada: Eastern Ontario Health Unit (EOH), City of Ottawa Health Unit (OTT), Leeds, Grenville, and Lanark Health Unit (LGL), and Kingston, Frontenac, Lennox and Addington Health Unit (KFL) (Figure 6.1). The region is largely rural with several population centres including Ottawa (population 934,243), Kingston (population 123,798), and Cornwall (population 46,589) (33).

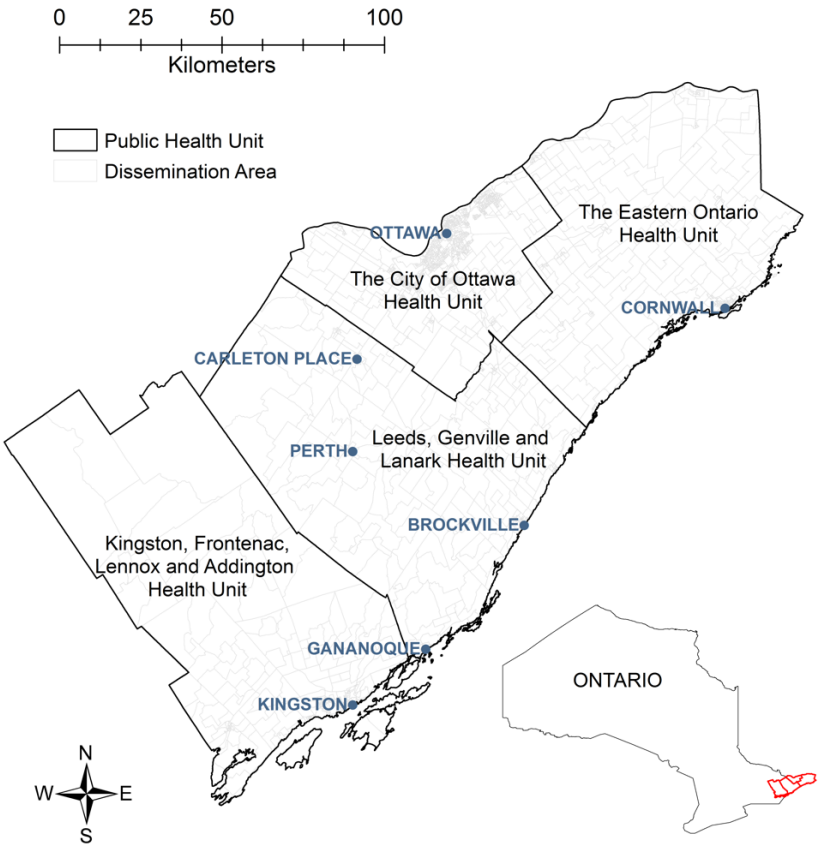

Figure 6.1 Map showing the study area and inset showing the location of the four health units in the province of Ontario, Canada.

6.2.5.2 Surveillance data

We obtained reported human Lyme disease case data (2010-2017) from the Integrated Public Health Information System (iPHIS) database, which contains patient information and laboratory test results for reportable diseases in Ontario. We defined cases as patients with confirmed or probable Lyme disease according to the national case definition for Lyme disease (see Additional file 6.1) (34). We also obtained passive tick surveillance data (2010-2017) from Public Health Ontario (PHO), which receives and identifies ticks from PHUs and healthcare providers and collates these data with real-time polymerase chain reaction (PCR) test results from the National Microbiology Laboratory. We retained all information available on patient and tick submitter home residence and self-reported travel history or location of tick exposure. For tick submissions, we retained *I. scapularis* specimens along with tick life stage and PCR test results for *B. burgdorferi*.

6.2.5.3 Exposure analysis

We geocoded the home location and the most probable tick exposure location using a Google Application Programming Interface in R v.3.5.1 (35). We defined the most probable tick exposure location as 1) the only exposure provided, or 2) the finest-scale location if multiple fields were provided and the locations were reasonably close (e.g., trail versus neighbourhood). If multiple locations with no geographic overlap were reported, we removed the case or tick from analysis. We then linked home and exposure locations with the DA geographic boundaries obtained from the 2016 Canada Census (Statistics Canada) using ArcGIS v.10.5.1 (ESRI, Redlands, CA, USA). For those with a broader exposure location that could not be easily geocoded (e.g., city/suburb level), we assigned the exposure to the nearest census division and used a weighted approach to

subsequently link each data point to a DA. The weight was derived from a maximum entropy species distribution model for *I. scapularis* developed by Slatculescu et al. (2020) and represents the average predicted habitat suitability for *I. scapularis* per DA; thus, allowing the exposure to be assigned to a biologically plausible location within the region indicated by the individual (10). For patients that provided additional notes, we created a separate variable classifying the type of exposure (e.g., occupational, recreational, cottage, and home/yard). We used frequency tables to identify characteristics of tick submitters and Lyme disease patients, tick exposure locations compared to home residence, and types of exposures. We used the Chi-Square test (or the Fisher's exact test for cells with fewer than 5 observations) and the two-sided t test ($\alpha = 0.05$) to determine if there was a relationship between categorical variables or to compare the means of two independent groups, respectively. Statistical analyses were conducted using SAS v.9.4 (SAS Institute, Cary, NC, USA).

6.2.5.4 Cluster analysis

We used SaTScan™ software (v 9.4.1 Kulldorff and Information Management Services, Inc.) to detect spatiotemporal clusters of human Lyme disease cases and infected ticks in eastern Ontario (36). This method uses a cylindrical scanning window across all spatial and temporal locations to identify regions where the observed numbers of cases or ticks exceed the expected numbers in a comparison region under the null hypothesis of spatial randomness (37). To detect clusters of human Lyme disease cases, we used a Poisson-based probability model, with patient and census population counts per DA. To detect clusters of infected ticks, we used a Bernoulli-based probability model, with the outcome being ticks testing positive and negative for *B. burgdorferi*. Since we only had DA-level exposure for most individuals, we analyzed clusters at the DA by

assigning cases and ticks the centroid coordinates for the DA in which they were exposed. Therefore, cluster size is based on the number of DAs with higher observed counts than expected counts (i.e., a cluster radius of 0 km indicates high counts in a single DA). We did not permit spatial overlap between clusters, we used a one-year minimum temporal window, and 5% or 30% maximum spatial cluster sizes for cases and ticks, respectively, were set a priori to account for the low infection rates (38). The most likely clusters were detected using a likelihood ratio test and p-values were calculated based on maximum likelihood rank using 999 Monte Carlo replications ($p < .05$ was considered significant).

We used ArcGIS v10.5.1 to generate Anselin's Local Moran's I statistic to better characterize clusters based on spatial autocorrelation among neighbouring DAs, under the null hypothesis that there is no association between values of human Lyme disease or infected ticks in nearby DAs (39). Statistical significance was determined using Z-scores ($\alpha = 0.05$). A positive value for I indicates that a DA has neighbouring DAs with similarly high or low infection rates; hence, the DA is part of a cluster. A negative value for I indicates that a DA has neighbouring DAs with dissimilar infection rates; hence the DA is an outlier. The cluster/outlier types were categorized as statistically significant cluster of high infection (high-high), cluster of low infection (low-low), outlier of high infection surrounded by primarily low infection (high-low), and outlier of low infection surround by primarily high infection (low-high). Since we are interested in detecting regions with high Lyme disease incidence and tick infection rates, we only mapped high-high clusters and high-low outliers.

6.2.5.5 Risk factor analysis

We constructed a multivariable regression model to assess the relationship between the outcome variable of DA-level number of human Lyme disease cases and multiple socioecological variables, defined in Additional file 6.1. All variables were obtained or calculated at the DA level. Lyme disease case counts and publicly submitted ticks from 2010-2017 were aggregated per DA. Ecological variables of interest included: numbers of publicly submitted *I. scapularis* ticks and nymphs, total positive *B. burgdorferi* ticks, proportion of mixed treed land and infrastructure derived from the Southern Ontario Land Resource Information System (SOLRISv3.0) from the Ministry of Natural Resources and Forestry's open data portal (https://geohub.lio.gov.on.ca), and neighbourhood walkability score obtained from the Canadian Active Living Environments (Can-ALE) database from McGill University (https://nancyrossresearchgroup.ca/research/can-ale/). Socioeconomic variables of interest included median income, commute to work duration, language knowledge, and population density obtained from the 2016 Canada Census (Statistics Canada), as well as the 2016 Ontario Marginalization Index (ON-Marg) available from PHO. The ON-Marg was derived from a series of iterative factor analyses of 42 census-based indicators, yielding four factors with 18 remaining indicators that represent multiple dimensions of socioeconomic status in Ontario (40). These factors are 1) Residential instability: a measure of high rates of family and housing instability (indicators are proportion of population who is living alone, who is not youth age 5-15, who is single/widowed/divorced and who moved during the last 5 years, and dwellings that are not owned or that are apartments), 2) Material deprivation: a measure of inability to access and attain basic material needs (indicators are proportion of population who does not have a high school diploma, who are unemployed, who are considered low income, who are single parent families, who receive government transfer payments, and whose housing is in need of major

repair), 3) Dependency: a measure of people who do not have income from employment (indicators are proportion of population who is 65 years or older, who are not participating in the labour force, and a high dependency ratio of youth (0-14 years) and elderly (65+ years) per working population 15-64 years), and 4) Ethnic concentration: a measure of recent immigrants and visible minorities (indicators are proportion of population who arrived in Canada in the last 5 years and persons, other than Indigenous peoples, who are non-Caucasian in race or non-white in colour) (40). The ON-Marg is available at a fine spatial scale (DA-level) and was selected to analyze multiple aspects of the broader socioeconomic risk factors for LD.

We used an extended Poisson regression with a negative binomial response for the outcome to account for overdispersion of Lyme disease cases per DA. Model fit was assessed by visual inspection of residuals and the deviance value. Each predictor variable was analyzed individually and only those significantly ($p < .05$) associated with Lyme disease counts were considered in the multivariable model. We also tested for correlation among potential predictors and retained those with low to moderate correlation ($|\text{Pearson's } r| \leq 0.6$). We further included the natural log of human population size as a predictor since we expect number of tick submissions and Lyme disease cases to increase with population. We selected the final model based on the Akaike Information Criterion (AIC) when compared to smaller nested models.

6.2.5.6 Sensitivity analysis

We conducted a sensitivity analysis to verify the validity of our weighted approach for tick exposure. We repeated all analyses with a smaller subset of patient (n=589) and tick (n=1,684) data that had geographically precise exposure locations and compared this with our global analyses

that also included weighted exposures by DA-habitat suitability for *I. scapularis* for broader exposure locations. Results from the sensitivity analysis are presented in the supplemental materials.

6.2.6 Results

6.2.6.1 Trends in Human Lyme Disease Cases and Tick Exposures in Eastern Ontario

There were 1,224 Lyme disease cases and 6,706 *I. scapularis* ticks acquired in Ontario between 2010-2017 among residents of our study area. We found that most tick exposures occurred within the PHU of residence, with OTT Lyme disease patients being the notable exception (Table 6.1). In OTT, 65.2% (n=215) of Lyme disease patients and 26.8% (n=404) of tick submitters reported tick exposures outside their health unit of residence compared to 13.9% (n=10), 1.9% (n=8), and 4.0% (n=16) of Lyme disease patients and 11.1% (n=71), 8.1% (n=226), and 19.2% (n=337) of tick submitters for EOH, LGL, and KFL, respectively (Table 1). Despite the greater number of outside-PHU tick exposure for OTT Lyme disease patients, within-PHU tick exposures also increased annually, indicating a growing risk at home and via travel-related exposures (Figure 6.2).

Table 6.1 Lyme disease cases and public tick submissions with exposure within and outside the public health unit of residence.

	Exposure within PHU	**Exposure outside PHU**	**P value**
Lyme disease cases, n (%)			
EOH	62 (86.1)	10 (13.9)	<.0001
OTT	115 (34.8)	215 (65.2)	<.0001
LGL	416 (98.1)	8 (1.9)	<.0001
KFL	382 (96.0)	16 (4.0)	<.0001
Tick submissions, n (%)			

EOH	567 (88.9)	71 (11.1)	<.0001
OTT	1105 (73.2)	404 (26.8)	<.0001
LGL	2580 (91.9)	226 (8.1)	<.0001
KFL	1416 (80.8)	337 (19.2)	<.0001

PHU: public health unit, EOH: Eastern Ontario Health Unit; OTT: City of Ottawa Health Unit; LGL: Leeds, Grenville, and Lanark Health Unit; KFL: Kingston, Frontenac, Lennox and Addington Health Unit.

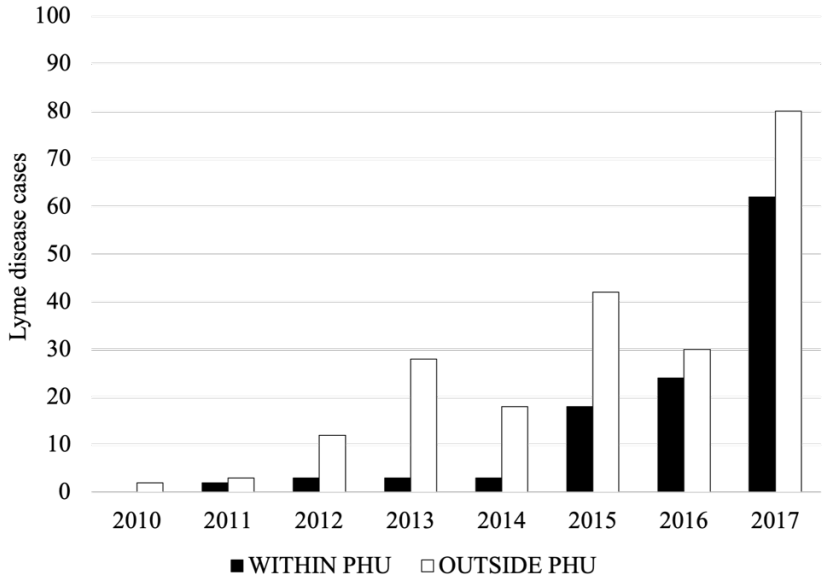

Figure 6.2 Annual Lyme disease cases and tick submissions with exposure within and outside the City of Ottawa Health Unit (OTT) from 2010-2017.

For patients who provided extra exposure notes, home/yard was most reported by patients from EOH (n=36) and LGL (n=186), whereas OTT patients reported a split between cottage exposure (n=81) and home/yard (n=70) as well as recreational activities (n=25) and occupational/school exposures (n=21). The cottage exposures indicated by OTT patients were mostly attributed to locations in LGL (n=69), KFL (n=23), and other PHUs outside our study area (n=6). For KFL patients, this type of exposure information was largely unavailable because most cases were

attributed to unspecified local tick exposure due to the high endemicity of *I. scapularis* in this region.

6.2.6.2 Spatiotemporal Clusters of Human Lyme Disease Cases and Infected Ticks in Eastern Ontario

We detected eight statistically significant spatiotemporal clusters of human Lyme disease cases (Figure 6.3). The three largest clusters were located across LGL (Cluster 1: radius 36.7 km, years 2014-2017, relative risk [RR] = 19.09, p<.0001; Cluster 2: 34.4 km, 2017, RR = 15.02, p<.0001; Cluster 4: 24.4 km, 2015-2017, RR = 5.13, p<.0001), two smaller clusters located in KFL (Cluster 3: 2.6 km, 2017, RR = 38.57, p<.0001; Cluster 5: 0.4 km, 2017, RR = 112.97, p<.0001) and OTT (Cluster 6: 0 km, 2016-2017, RR = 82.85, p<.0001; Cluster 7: 5.8 km, 2015-2017, RR = 15.02, p<.0001) and one cluster in EOH (Cluster 8: 7.9 km, 2012-2015, RR = 6.44, p=.0002). The Local Moran's I statistic detected statistically significant high-high clustering in LGL and KFL, indicating that neighbouring DAs have similarly high infection rates and Lyme disease risk is more geographically dispersed in these regions. In contrast, high-low clustering was detected in OTT, which suggests outlier DAs with high Lyme disease rates and more localized risk in the western region of Ottawa.

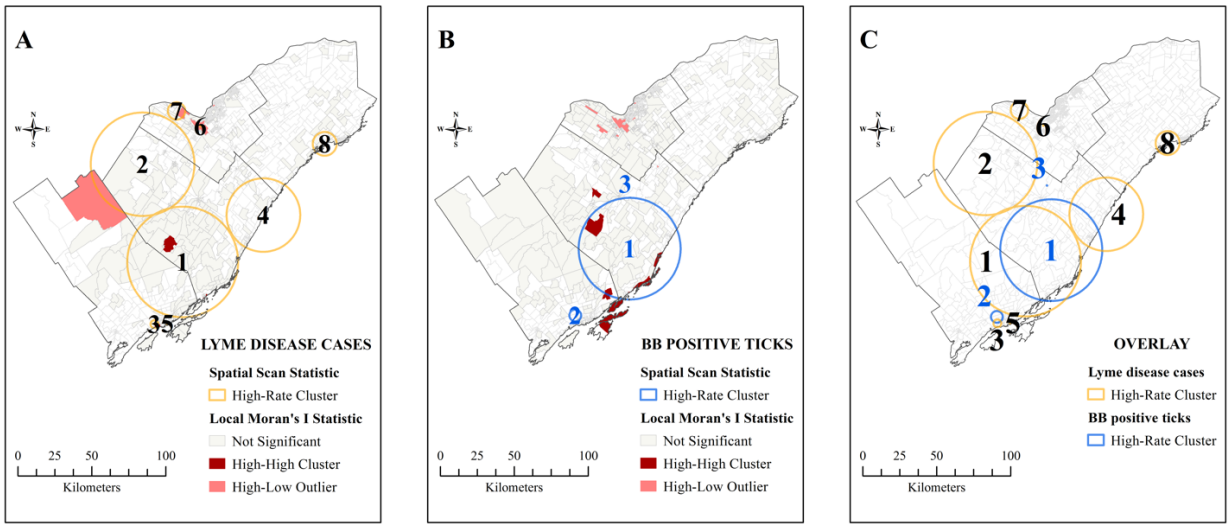

Figure 6.3 Spatiotemporal clusters of Lyme disease (A) and *Borrelia burgdorferi* infected ticks (B) in eastern Ontario, Canada, from 2020-2017. The overlay of the two shows geographical overlap between clusters (C).

We also detected three statistically significant clusters of *B. burgdorferi*-infected ticks (Figure 6.3). Two clusters were in LGL (Cluster 1: 33.7 km, 2012-2015, RR = 1.45, p<.0001; Cluster 3: 0 km, 2013-2016, RR = 3.60, p=.0490) and one in KFL (Cluster 2: 3.9 km, 2012-2013, RR = 2.25, p=.0022). The Local Moran's I statistic detected a similar pattern as for human Lyme disease cases, with statistically significant high-high clustering in LGL and KFL and high-low outliers in OTT. Clusters of *B. burgdorferi*-infected ticks overlapped geographically with clusters of human Lyme disease cases in these regions, though most clusters of *B. burgdorferi*-infected ticks preceded those of human Lyme disease cases by 3-4 years.

6.2.6.3 Socioecological Risk Factors for Human Lyme Disease

Lyme disease case counts per DA increased by approximately 25% for every unit increase in the number of *B. burgdorferi*-positive ticks submitted through passive tick surveillance (RR=1.25,

95% CI= 1.19,1.31, p<.0001). Lyme disease counts also rose significantly with increased proportion of mixed treed land cover (RR=1.02; 95% CI=1.01,1.02; p=.0001) and with various neighbourhood characteristics associated with increased rurality (residential instability: RR=1.36, 95% CI=1.19,1.56, p<.0001; ethnic concentration: RR=0.57, 95% CI=0.45,0.73, p<.0001). In contrast, Lyme disease cases counts decreased with higher neighbourhood walkability score, which is associated with increased urbanization (Table 6.2).

Table 6.2 Negative binomial regression model showing socioecological factors associated with Lyme disease case counts in eastern Ontario, Canada from 2010-2017.

Parameter	**RR**	**95% CI**		**P value**
Intercept	0.0232	0.0049	0.1090	<.0001
B. burgdorferi-positive ticks	1.2464	1.1860	1.3098	<.0001
Walk score 1	1.0000	-	-	-
Walk score 2	0.6653	0.5068	0.8733	0.0033
Walk score 3	0.2106	0.1360	0.3261	<.0001
Walk score 4	0.0619	0.0177	0.2168	<.0001
Walk score 5	0.0478	0.0058	0.3949	0.0048
Proportion treed	1.0161	1.0079	1.0244	0.0001
Residential instability	1.3588	1.1853	1.5578	<.0001
Ethnic concentration	0.5744	0.4517	0.7304	<.0001
Log population	1.5851	1.2599	1.9941	<.0001

RR: relative risk, CI: confidence intervals.

6.2.6.4 Sensitivity Analysis

For Lyme disease patients and tick submitters, particularly those from tick-endemic regions like KFL, home residence and year were strongly correlated with availability of exposure data, in part due to the lack of exposure-related data collected through disease and tick surveillance (see Additional file 6.2) (7, 31). However, weighted exposures for Lyme disease cases and ticks showed a similar geographic pattern at the DA level compared to unweighted exposures despite the

differences seen in the sources of the data (data not shown). We detected fewer clusters of Lyme disease and *B. burgdorferi*-infected ticks using the smaller dataset, although the larger patterns were consistent with our main analyses (see Additional file 6.3). We also found similar associations between Lyme disease case counts and socioecological predictors when weighted exposures were removed from analysis (see Additional file 6.4).

6.2.7 Discussion

In this study, we found heterogeneity in tick exposure patterns by public health unit of residence. Lyme disease patients and residents of the three largely rural public health units, EOH, LGL, and KFL, reported tick exposure locations near the home, consistent with comparable studies in the United States that found high levels of peri-domestic tick exposures (41-43). In contrast, individuals from the major population centre of Ottawa reported many tick exposures outside their health unit of residence as well as increasing annual exposures within the city, highlighting the heterogeneity in risk at finer spatial scales. A recent study by Tulloch et al. (2019) in the United Kingdom showed that Lyme disease incidence was associated with higher socioeconomic status (based on patients' residence postcode) and with residence in more rural regions, suggesting that socioecological factors and neighbourhood characteristics may contribute to the marked difference between OTT and other PHUs (44).

We detected several spatiotemporal clusters of Lyme disease incidence and *B. burgdorferi*-infected ticks in LGL and KFL, with high spatial autocorrelation among DA infection rates. This pattern signals the presence of foci of tick establishment and subsequent expansion from these regions (45, 46). We also detected a few smaller spatiotemporal clusters in OTT that, in contrast,

showed low spatial autocorrelation among DA infection rates, indicating this region represents a newer area of tick establishment with more localized risk in outlier regions (14). Our results differ somewhat from a recent study by Kulkarni et al. (2019) that found spatiotemporal clusters of Lyme disease and *B. burgdorferi*-infected ticks in KFL but not elsewhere in eastern Ontario (31). These differences are likely due to several factors including the spatial scale at which clusters were investigated (i.e., smaller DA, ~20,000 total number in Ontario, versus the larger forward sortation area of the postal code, with ~500 total number in Ontario), the length of study period (i.e., we include an additional year with a large increase in number of cases), and exposure characteristics (i.e., we include travel-related exposures in nearby health units and used tick exposure location instead of home location).

Lyme disease in eastern Ontario increased with higher proportion of treed land, greater numbers of *B. burgdorferi*-positive ticks submitted by the public, and lower neighbourhood urbanity indicated by less favourable walking environments, lower ethnic concentration, and higher residential instability. These results are consistent with our knowledge regarding ecological risk for Lyme disease and provide evidence that tick densities at the community-level and local neighbourhood structure and characteristics are important determinants of Lyme disease risk (30, 31, 43). The associations found in this study between human Lyme disease and area-level measures of marginalization provide the first evidence of socioeconomic risk factors for Lyme disease in the Canadian context. Factors of the ON-Marg such as ethnic concentration, a measure of recent immigrants and visible minorities (defined by Statistics Canada as persons, other than Indigenous people, who are non-Caucasian in race or non-White in colour), and residential instability, a measure of home security, home ownership and occupancy, represent multidimensional metrics of

socioeconomic status, making it difficult to draw conclusions about the contribution of individual variables on Lyme disease risk (47). In the United States, Springer and Johnson (2018) found evidence for comparable associations between Lyme disease and a relatively higher proportion of White population, higher average levels of education, lower poverty and crime rates, and increased vacant housing, of which 30% were seasonal or rental properties (48). Together with our finding that most residents from the largely urban city of Ottawa were exposed to ticks outside of their health unit of residence, often citing cottage exposures, it is possible that the association with residential instability may be in part attributed to the higher rates of non-resident home ownership in recreational destinations such as Muskoka Lakes and Rideau Lakes (49). However, further research would help to ascertain this mechanism of association and identify specific characteristics of neighbourhood structure that are driving the association between residential instability and ethnic concentration and Lyme disease risk in Ontario.

Our study has several limitations, the most significant being the sparsity of data pertaining to tick exposure locations collected via notifiable disease surveillance and passive tick surveillance data. We attempted to accurately identify tick exposure location at a fine geographic scale; however, to maximize data use for our analyses we used a weighted approach to assign most probable tick exposure locations when detailed data were unavailable. Our sensitivity analysis showed similar trends and results using a smaller fraction of data with detailed exposure, but we acknowledge that certain results such as our spatiotemporal cluster analyses may be influenced by the quality of data. Furthermore, due to regional differences in tick surveillance (i.e., cessation of passive tick surveillance submission from the public in endemic regions) we did not have complete data for publicly submitted ticks from LGL and KFL health units for later years of our study to explore

additional temporal trends. Lastly, we used the four dimensions of the ON-Marg, derived from factor analyses of many census-based indicators, to assess the link more broadly between Lyme disease and socioeconomic risk factors. Additional research is warranted to better understand the nature of these relationships and potential public health implications. This study also focused on area-level socioecological risk factors and does not capture individual-level factors pertaining to home-ownership rates, household occupancy and structure, or potential factors such as under-reporting of Lyme disease by immigrants and minorities stemming from barriers accessing health care services or barriers to social integration and engaging in activities that increase exposure to Lyme disease (i.e., camping, outdoor sports and social interaction, cottages, etc.) that may also contribute to the association between Lyme disease risk and ethnic concentration or residential instability found in this study (50, 51). On the other hand, our study has notable strengths as it represents the first analysis in Ontario, Canada looking at socioeconomic predictors of Lyme disease, which may help identify communities at higher risk of Lyme disease and to inform future studies looking at mechanisms of association and individual-level risk factors. Furthermore, we identified tick exposure location at the finest geographic unit possible and as a result we were able to detect heterogeneity in tick exposure patterns that would be overlooked at a larger spatial scale.

Our findings indicate that human Lyme disease risk is in part predicted by the spread of *I. scapularis* and *B. burgdorferi* and by various ecological and socioeconomic factors that affect access to environmental risk areas, with substantial heterogeneity in tick exposure patterns between rural and urban regions. This shows the importance of devising appropriate risk mitigation strategies and tailoring public health messaging to target different regions based on regional risk. Furthermore, it highlights the importance of ongoing Lyme disease surveillance methods and fine-

scale studies to further identify neighbourhood-level patterns and determinants of Lyme disease and other tick-borne pathogens.

6.2.7.1 Declaration

Ethical Approval and Consent to Participate

Ethics approval for this study was obtained from the Ottawa Public Health Research Ethics Board (no. 226-16) and the University of Ottawa Science and Health Sciences Research Ethics Board (H06-16-22). All methods were carried out in accordance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans – TCPS 2 (2018). Informed consent was not required for secondary use of data in accordance with TCPS 2 Article 5.5A. Access to Integrated Public Health Information System data was granted through data-sharing agreements with participating public health units. All data were aggregated to preserve anonymity.

Consent for Publication

Not applicable. This article does not contain information from any individual person that would require consent.

Conflict of Interest

The authors declare that they have no competing interests.

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Author Contribution

AMS and MAK conceived the study; AMS curated the data, conducted the formal analyses, and wrote the main manuscript; CD curated the data and geocoded exposure locations; AMS, NHO, BS, MD, DWC, and MAK developed the methodology and contributed to the interpretation of study results; MAK provided supervision and funding. All authors have read, revised, and approved the manuscript.

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Availability of Data and Materials

Tick surveillance data that support the findings of this study are available from Public Health Ontario (661 University Ave., Toronto, Ontario, M5G 1M1), but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. These data are however available from the authors upon reasonable request and with permission of Public Health Ontario. Human Lyme disease case data are available from the City of Ottawa Public Health Unit (100 Constellation Dr., Nepean, Ontario, K2G 6J8), the Eastern

Ontario Public Health Unit (872 rue Principale St., Casselman, Ontario, K0A 1M0), the Leeds, Grenville, and Lanark District Public Health Unit (458 Laurier Blvd., Brockville, Ontario, K6V 7A3), and the Kingston, Frontenac, and Lennox and Addington Public Health Unit (221 Portsmouth Ave., Kingston, Ontario, K7M 1V5), but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. These data are however available from the authors upon reasonable request and with permission of each public health unit.

6.2.8 Supplemental Information

Supplemental Table 6.1 Definition of the outcome and predictor variables evaluated in the negative-binomial regression model looking at associations between Lyme disease (LD) prevalence and socioecological risk factors in eastern Ontario.

Variable	Description
Outcome	
Total LD case counts	Number of confirmed and probable LD cases reported per dissemination area. Confirmed LD is defined as: 1) Clinical evidence of illness with laboratory confirmed *B. burgdorferi* infection via isolation of bacterium or DNA from a clinical specimen 2) Clinical evidence of illness with two-tiered serological testing confirmation and history of residence/travel to a known LD risk area Probable LD is defined as: 1) Clinical evidence of illness with two-tiered serologic testing confirmation but without history of residence/travel to a known LD risk area 2) Physician-diagnosed erythema migrans without laboratory evidence but history of residence/travel to a known LD risk area.
Predictors	
Total ticks	Number of publicly submitted *I. scapularis* adults, nymphs, and larva per dissemination area.
Total nymphs	Number of publicly submitted *I. scapularis* nymphs per dissemination area.
Total *B. burgdorferi*	Number of publicly submitted *I. scap*ularis adults, nymphs, and larva that tested positive for *B. burgdorferi* per dissemination area.
Treed land	Proportion of mixed treed land cover (i.e., deciduous, coniferous, mixed forest, shrubs) per dissemination area.

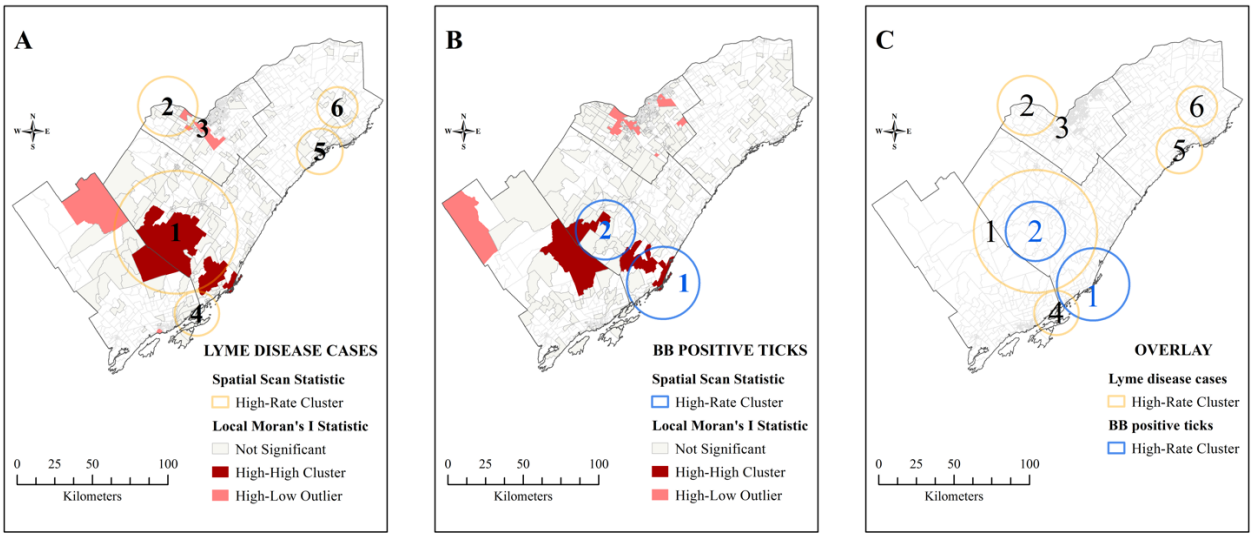
Infrastructure	Proportion of infrastructure, roads, and built-up land cover per dissemination area.
Walkability score	Score (1-5) is a measure of k-medians cluster of connectivity (3-way intersections), density (dwelling density), and destinations (points of interest) per dissemination area.
Income median	Median income range per dissemination area.
Commute to work	Categorized measure of duration of commute to work (i.e., less than 15min, 15-29min, 30-44min, 45-59min, over 60min) per dissemination area.
Language knowledge	Categorized measure of language knowledge (ie., English only, French only, both English and French, neither English nor French) per dissemination area.
Population density	Average population density per dissemination area.
Residential instability	Measure refers to area-level concentrations of people who experience high rates of family or housing instability. The indicators included are: proportion of the population living alone, proportion of the population who are not youth (age 5-15), average number of persons per dwelling, proportion of dwellings that are apartment buildings, proportion of the population who are single/divorced/widowed, proportion of dwellings that are not owned, proportion of the population who moved during the past 5 years.
Material deprivation	Measure is closely connected to poverty, and it refers to inability for individuals and communities to access and attain basic material needs. The indicators included are proportion of the population aged 20+ without a high-school diploma, proportion of families who are lone parent families, proportion of total income from government transfer payments for population aged 15+, proportion of the population aged 15+ who are unemployed, proportion of the population considered low-income, proportion of households living in dwellings that are in need of major repair.
Dependency	Measure refers to area-level concentrations of people who do not have income from employment. Indicators included are proportion of the population who are aged 65 and older, dependency ratio (total population 0-14 and 65+ / total population 15-64), proportion of the population not participating in labour force (aged 15+).
Ethnic concentration	Measure refers to high area-level concentrations of people who are recent immigrants and/or people belonging to a 'visible minority' group (defined by Statistics Canada as "persons, other than indigenous peoples, who are non-Caucasian in race or non-white in colour", consisting mainly of the following groups: South Asian, Chinese, Black, Filipino, Arab, Latin American, Southeast Asian, West Asian, Korean and Japanese). Indicators included are proportion of the population who are recent immigrants (arrived in the past 5 years), proportion of the population who self-identify as a visible minority.

Supplemental Table 6.2 Characteristics of Lyme disease cases and tick submitters with weighted versus unweighted tick exposure location.

	LYME DISEASE CASES			TICK SUBMISSIONS		
	Unweighted N=592	Weighted N=608	P value	Unweighted N=1,684	Weighted N=4,835	P value*
Age, y ± SD	48.2 ± 21.0	47.7 ± 21.3	0.6884	-	-	-
Sex						
F	241	253	.5893	-	-	-
M	348	355	.7918	-	-	-
Unknown	3	0	-	-	-	-
Residence						
EOH	70	1	<.0001	188	439	<.0001
OTT	242	67	<.0001	585	890	<.0001
LGL	235	188	.0223	478	2235	<.0001
KFL	45	352	<.0001	433	1271	<.0001
Year						
2010	13	2	.0045	10	4	.1088
2011	20	16	.5050	235	1008	<.0001
2012	43	11	<.0001	324	1065	<.0001
2013	74	22	<.0001	437	1306	<.0001
2014	61	40	.0367	159	590	<.0001
2015	91	113	.1235	51	123	<.0001
2016	69	93	.0593	174	246	.0004
2017	221	311	<.0001	294	493	<.0001

SD: Standard deviation; M: Males; F: Females; EOH: Eastern Ontario Health Unit; OTT: City of Ottawa Health Unit; LGL: Leeds, Grenville, and Lanark Health Unit; KFL: Kingston, Frontenac, Lennox and Addington Health Unit.

* Chi-Square test, Fisher's exact test, and two-sided t test as appropriate.



Supplemental Figure 6.1 Sensitivity analysis of spatiotemporal clusters of Lyme disease (A) and *Borrelia burgdorferi* infected ticks (B) based on unweighted tick exposure location. The overlay of the two shows geographical overlap between clusters (C).

Supplemental Table 6.3 Negative binomial regression model showing socioecological factors associated with Lyme disease case counts in eastern Ontario, Canada from 2010-2017 based on sensitivity analysis dataset with unweighted tick exposures.

Parameter	RR	95% CI		P value
Intercept	0.0514	0.0051	0.5163	0.0117
B. burgdorferi-positive ticks	1.1647	1.1082	1.2240	<.0001
Walk score 1	1.0000	-	-	-
Walk score 2	0.4467	0.2861	0.6975	0.0004
Walk score 3	0.5384	0.2908	0.9967	0.0488
Walk score 4	0.2942	0.0578	1.4986	0.1408
Walk score 5	0.2684	0.0244	2.9467	0.2820
Proportion treed	1.0220	1.0130	1.0311	0.0001
Residential instability	1.4384	1.1743	1.7620	0.0004
Ethnic concentration	0.5688	0.4022	0.8044	0.0014
Log population	1.4148	1.0078	1.9862	0.0450

RR: relative risk, CI: confidence intervals.

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7

7 TICK-BORNE PATHOGENS

7.1 ARTICLE PREFACE

My final thesis objective was to identify what other tick-borne pathogens are detected in blacklegged ticks across eastern Ontario. I used quantitative PCR assays to detect the infection prevalence of tick-borne pathogens of public health or veterinary importance including *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Babesia microti*, *Babesia odocoilei*, and deer tick-virus. I used descriptive statistics, owing to the low sample size which inhibited my ability to explore more complex relationships at present, and geographic information system (GIS) tools to show the prevalence of these pathogens across eastern Ontario and to summarize tick surveillance activities in this area from 2017-2021.

Furthermore, in this chapter, I explore the genetic diversity of *Borrelia burgdorferi* in eastern Ontario using gene sequencing methods to assign bacterial genotypes (multi-locus sequence typing analysis). This analysis helps contribute to our understanding of the emergence of Lyme disease

in eastern Ontario and to identify potential implications of bacterial genetic diversity on human Lyme disease outcomes.

7.1.1 Author Contributions

This study was conceived by me and my thesis supervisor, Dr. Manisha Kulkarni, with important input from members of my thesis advisory committee including Drs. Nicholas Ogden, Marc Desjardins, William Cameron, and Beate Sander. Tick specimen collection occurred over several years and was led by Drs. Roman McKay and Benoit Talbot, James Logan, Charles Thickstun, Claudia Duguay, Olivia Facchin, and me. Laboratory testing was primarily conducted by me, with assistance from Dr. Roman McKay and Ms. Olivia Facchin. This manuscript is still in production and full author list and contributions are yet to be assigned.

7.1.2 Ethics Approval

Land permits were obtained from local authorities for all sites visited between 2017-2021.

7.1.3 Article Citation

Slatculescu AM, McKay R, Talbot B, et al. The increasing infection prevalence of *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, and *Babesia odocoilei* in *Ixodes scapularis* in Ontario, Canada: Surveillance results from 2017-2021. [In production] 2023.

7.2 ARTICLE CONTENT

7.2.1 Title

The increasing infection prevalence of *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, and *Babesia odocoilei* in *Ixodes scapularis* in Ontario, Canada: Surveillance results from 2017-2021.

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7.2.3 Abstract

Background: The blacklegged tick, *Ixodes scapularis*, has become endemic in many regions of Ontario, Canada. Situated between the St. Lawrence River and the Ottawa River, the region of eastern Ontario has the highest number of blacklegged tick submissions from the public and human Lyme disease cases in the province. This study provides an epidemiological update on the infection

prevalence of tick-borne pathogens in *I. scapularis* ticks and the genetic diversity of *Borrelia burgdorferi*, the causative agent of Lyme disease, in eastern Ontario.

Methods: We conducted a multiyear surveillance study of 66 sites across eastern Ontario and the National Capital Region from 2017-2021. Over 1,500 *I. scapularis* ticks were tested for tick-borne pathogens including *B. burgdorferi*, *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Babesia microti*, *Babesia odocoilei*, and deer tick virus (DTV). We also used multi-locus sequence typing (MLST) to sequence *B. burgdorferi* housekeeping genes and identify bacterial genotypes.

Results: *Borrelia burgdorferi* was detected at 24 of 66 (36.4%) of sites sampled for ticks, of which 87.5% had an infection prevalence of 20% or higher. *Borrelia burgdorferi* infection rates per person-hour of sampling were similar in the Ottawa and Kingston regions and were associated with total site-level *I. scapularis* density (p=0.0186). *Borrelia burgdorferi* genotypes in eastern Ontario were diverse but consistent with lineages found across the Northeastern United States. We did not detect any positive specimens for *B. microti* or DTV in any years. The regional mean infection prevalence of *B. miyamotoi, A. phagocytophilum,* and *B. odocoilei* over the sampling years ranged from 0-0.7%, 1.4-4.7%, and 3.6-10.1%, respectively.

Conclusion: In eastern Ontario, *B. burgdorferi* infection prevalence in ticks increased from previous reported years. The prevalence of other tick-borne pathogens remains low, although there is a need for ongoing surveillance as substantial variation at the site-level was detected.

7.2.4 Introduction

The blacklegged tick (also known as the deer tick or *Ixodes scapularis*) is the vector of *Borrelia burgdorferi* (*sensu stricto*) (causative agent of Lyme disease) in central and eastern North America. The blacklegged tick's range has expanded northward from the Northeast and Upper Midwest over the last decade, with tick populations now endemic in many parts of central and eastern Canada (1-4). In Canada, *Borrelia burgdorferi* invasion has followed the range expansion of the blacklegged tick, with a 1- to 5-year lag between detection of the vector and pathogen via surveillance systems (5, 6). Increasing *B. burgdorferi* infection prevalence in ticks correlates with the growing incidence of human Lyme disease in Canada, which has increased more than 20-fold from 144 cases in 2009 to 3,147 cases in 2021 (6-8).

There is evidence from the United States that *B. burgdorferi* genetic diversity is linked to Lyme disease severity in humans due to differences in hematogenous dissemination by certain bacterial genotypes compared to others (9). To our knowledge, there are currently no Canadian counterpart studies assessing *B. burgdorferi* genetic diversity in clinical isolates from patients. Genetic analysis of *B. burgdorferi* housekeeping genes (multi-locus sequence typing: MLST) has revealed that *B. burgdorferi* populations in Canada largely resemble those from the Northeastern United States (10). However, there is also evidence of founder events that have skewed the genetic diversity of emerging *B. burgdorferi* populations in Canada and associations between host species (e.g., deer mouse, white-footed mouse, chipmunk) and bacterial genotypes that can result in greater persistence and transmission of specific genotypes in different habitats (10, 11). Additional studies are needed to assess the evolution of *B. burgdorferi* genotypes in Canada and associations between these genotypes and pathogenicity in humans as well as implications for Lyme disease diagnosis.

Blacklegged ticks are also vectors for other tick-borne bacterial, parasitic, and viral pathogens of veterinary and public health importance including *Borrelia miyamotoi* (*B. miyamotoi* disease: BMD), *Anaplasma phagocytophilum* (human granulocytic anaplasmosis: HGA), *Babesia microti* (human babesiosis), *Babesia odocoilei* (cervid babesiosis) and deer tick virus (DTV; DTV encephalitis) (12-16). These diseases are largely associated with febrile illness and non-specific flu-like symptoms, although severe outcomes can manifest if left untreated (17, 18). Detection of tick-borne illnesses is important as, in most cases, full recovery can be achieved with pathogen identification and early treatment or supportive care (e.g., antibiotics or antiparasitic drugs, managing clinical symptoms) (17, 18). Tick-borne pathogens not typically associated with human infection such as *B. odocoilei* also have important ecological implications for wildlife (16, 19). Additionally, *B. odocoilei* has not been closely examined as a source of human illness and it is currently unclear whether multiple *Babesia* spp. are linked with human babesiosis in Canada (20). The annual incidence of tick-borne illnesses remains low in Canada and, with the exception of Lyme disease, they are not currently nationally notifiable (21).

This study reports multi-year surveillance results for tick-borne pathogens in Ontario, Canada. Blacklegged tick abundance and the incidence of human Lyme disease are highest in the eastern region of the province (22, 23); therefore, we tested over 1,500 *I. scapularis* ticks for tick-borne pathogens collected in the Ottawa, Kingston, and surrounding regions from 2017-2021. The aims of this study are to 1) identify the overall and site-specific prevalence of tick-borne pathogens and the temporal changes occurring over 5 years, 2) identify the proportion of co-infections with multiple tick-borne pathogens in blacklegged ticks, and 3) identify *B. burgdorferi* genetic diversity in tick endemic regions of eastern Ontario through MLST analysis.

7.2.5 Methods

7.2.5.1 Study Area

Eastern Ontario comprises the geographic region between the Ottawa River and the St. Lawrence River, sharing water borders with Quebec to the north and New York state to the southeast. The region is largely marked by deciduous woodlands, bogs, marshes, and agricultural land. There are several major metropolitan areas located in eastern Ontario including Ottawa (1,135,014 population) and Kingston (172,546 population) (24). In this study, we also include Gatineau, Quebec (353,293 population) as part of the larger National Capital Region due to its close proximity to the City of Ottawa and the daily population flow between the two regions (24).

From 2017-2021, we surveyed 66 sites across eastern Ontario and the neighbouring region of Quebec (Ottawa: 34 sites; Gatineau: 13 sites; Kingston: 16 sites; other areas: 3 sites) to assess the occurrence and density of *I. scapularis* and rates of infection with tick-borne pathogens (Figure 7.1). Sites were pooled from multiple research projects and included a variety of municipal parks, provincial parks, forests, conservation areas, and recreational trails that were publicly accessible (25-29). Land access permits were obtained from the National Capital Commission, the City of Ottawa, the City of Kingston, and provincial park authorities.

7.2.5.2 Field Data

All sites were visited by teams of 2-3 trained personnel during the spring and summer months (May to June) for a combined minimum of 1.5 person-hours for small sites (e.g., municipal parks, trails) and 3.0 person-hours for large sites (e.g., provincial parks, conservation areas, trails). To detect multiple blacklegged tick instars, certain sites were visited a second time during the fall (September to October) and two sites were visited weekly for 15-minute intervals from June to October (full site descriptions available in Table S7.1). We were unable to visit all locations multiple times per season or in every year due to logistical constraints and unforeseen events (e.g., flooding, COVID-19 restrictions). Ticks were collected using the drag sampling method described by Public Health Ontario (30). Briefly, a 1-meter square white flannel cloth was dragged across the forest floor and surrounding vegetation and checked for ticks every 50 meters. If ticks were found, they were collected in plastic test tubes and marked with the geographic coordinates recorded using a handheld global positioning system (GPS) device (Garmin eTrex 20x). Collected ticks were maintained alive and transported on ice to the University of Ottawa laboratory for specimen identification and testing.

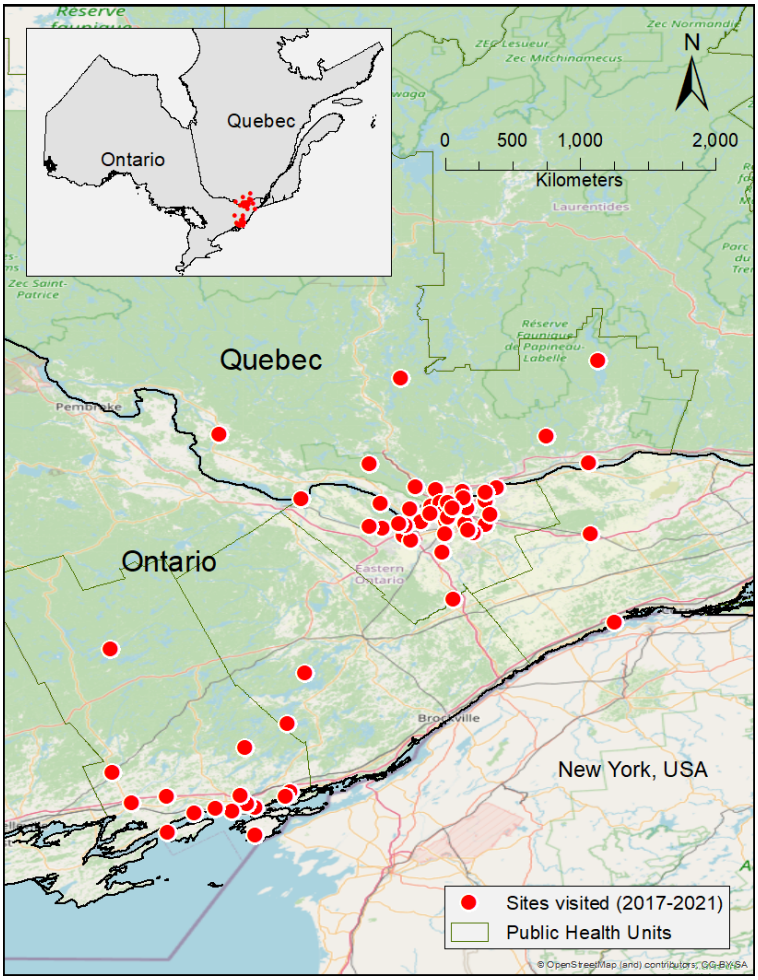

Figure 7.1 Sites visited from 2017-2021 in Ottawa-Gatineau, Kingston, and eastern Ontario

7.2.5.3 Molecular Testing

Nucleic acid extraction

Tick species and instar were identified by microscopy using taxonomic keys (31, 32). *Ixodes scapularis* nymphs and adults were transferred to individual 1.5 mL Eppendorf tubes and dissected using a scalpel (female ticks were cut in half, with one half used for testing and the other stored at -20°C). Scalpel blades were sterilized between specimens by soaking in accelerated hydrogen peroxide 0.5% (PREempt RTU) followed by 70% ethanol. Nucleic acid isolation and purification was performed using the QIAamp DNA Mini Kit (QIAGEN Inc., Mississauga, Ontario, Canada)

for samples collected from 2017-2018 and using the RNeasy Mini Kit (QIAGEN Inc.) for samples collected from 2019-2021. Briefly, dissected ticks were incubated in guanidine-isothiocyanate lysis buffer and processed as per the manufacturer's instructions, transferred to spin columns and bound to the silica-gel membrane using ethanol, washed 2-3 times to remove impurities, and eluted in nuclease-free water or buffer. During isolation of RNA, the DNase treatment was omitted, allowing residual amounts of DNA to be used for later testing. All DNA and RNA extracts were stored at -80°C.

Detection of tick-borne pathogens

Gene amplification and detection of *B. burgdorferi*, *B. miyamotoi*, *A. phagocytophilum*, *B. microti*, *B. odocoilei*, and DTV lineages I and II was conducted using quantitative polymerase chain reaction (qPCR) and reverse-transcriptase qPCR (RT-qPCR) as previously published, with a few modifications (33-35). From 2017-2021, all nymphal and adult tick samples were individually tested for three pathogens: *B. burgdorferi, B. miyamotoi* and *A. phagocytophilum*. Briefly, a duplex screening qPCR assay targeting 23S ribosomal RNA (rRNA) and major surface protein 2 (*msp2*) was used to detect *Borrelia* spp. and *A. phagocytophilum*, respectively (33). For positive specimens, a second qPCR assay was used to confirm presence of the pathogen using the following gene targets: outer surface protein A (*ospA*) for *B. burgdorferi*, glycerophosphodiester phosphodiesterase (*glpQ*) for *B. miyamotoi*, and *msp2* for *A. phagocytophilum*. For increased time efficiency, the confirmatory assays for *Borrelia* spp. were replaced in 2019 with a single duplex assay targeting *ospA* for *B. burgdorferi* and flagellin protein B (*flaB*) for *B. miyamotoi* (35). Starting in 2018, we gradually broadened our testing of tick-borne pathogens by first including detection of *B. microti* using the chaperonin-containing t-complex polypeptide 1 (CCTη) qPCR

assay developed by Nakajima et al. (36). For samples collected from 2019-2021, we also added a *Babesia* spp. triplex screening qPCR assay targeting the 18S rRNA genes of *Babesia* spp. (developed in-house by the National Microbiology Laboratory) and an RT-qPCR assay for detection of DTV lineages I and II. All positive samples were confirmed by a secondary qPCR assay. We used the SsoAdvanced Universal Probes Supermix (Bio-Rad (Canada) Laboratories Inc., Mississauga, Ontario, Canada) and the TaqMan™ Fast Virus 1-Step Master Mix (ThermoFisher Scientific, Nepean, Ontario, Canada) for all qPCR and RT-qPCR assays, respectively. Amplification was carried out using the CFX96 Real-Time PCR Detection System (Bio-Rad Inc.). After amplification and real-time data acquisition, analysis was performed using the CFX Maestro software (Bio-Rad, Hercules, California, USA).

Multi-locus sequence typing

We genotyped all nymphal ticks positive for *B. burgdorferi* using the MLST scheme (37). Only nymphal ticks were genotyped because, compared to adult instars, they are less likely to be infected with multiple bacterial strains, having only fed on a single host during the larval stage. MLST analysis was carried out according to the protocol published by Wang et al. (38). Briefly, we amplified fragments of eight chromosomal housekeeping genes (*clpA*, *clpX*, *nifS*, *pepX*, *pyrG*, *recG*, *rplB*, and *uvrA*) using nested and semi-nested PCR. All PCR assays were performed on ice using the KAPA HiFi PCR Kit (Roche Diagnostics, Laval, Quebec, Canada) and a touchdown approach to limit non-specific amplification and account for the varying melting temperatures of the primers (except for *recG*, which was tested separately with a stable annealing temperature). Amplification was carried out with the C1000 Touch Thermal Cycler (Bio-Rad Inc.) and PCR products were visualized by 1% agarose gel electrophoresis using SYBR safe (Invitrogen,

Waltham, Massachusetts, USA) on a Mini-Sub Cell GT Electrophoresis Cell (Bio-Rad Inc.). Samples were sequenced in the forward and reverse direction at the CES Génome Québec (Centre d'expertise et de services Génome Québec, Montréal, Canada).

Sequences were analyzed in BioEdit® v.7.2 (https://bioedit.software.informer.com). Briefly, forward and reverse sequences were visually inspected for quality and mixed sequences (two base peaks in one position), aligned to create a consensus sequence, and trimmed to the correct fragment size. If a mixed strain infection was detected in any locus, the entire sample was omitted from further analysis. The resulting sequences were assigned an allele number using the MLST database (http://www.pubmlst.org). The allele combination of all eight housekeeping genes for each sample was then assigned to a sequence type (ST) number in the MLST database.

7.2.5.4 Descriptive Analyses

Ixodes scapularis density and infection rates were calculated by calendar year, combining data from all field collections (spring and fall). Total *I. scapularis* density per site was calculated as the sum of all instars collected divided by the total person-hours of sampling. Nymphal density was calculated separately, as nymphs pose the highest risk of transmission of tick-borne pathogens to humans due to their small size and peak activity during the spring/summer months. Infection prevalence and infection rates were calculated as the number of ticks positive for *B. burgdorferi*, *B. miyamotoi*, *A. phagocytophilum*, *B. microti*, *B. odocoilei*, and DTV divided by the total number of *I. scapularis* tested (excluding larvae) or the total number of person-hours of sampling, respectively. Data curation was conducted in SAS v. 9.4 (SAS Institute, Cary, North Carolina, USA). We used a mixed effects model with infection prevalence as the outcome and tick density

as the predictor, while accounting for site-level repeated measures over four years, to identify associations between tick-borne pathogens and *I. scapularis* density. Patterns in *I. scapularis* density and tick-borne pathogen infection rates were mapped using ArcGIS v.10.7.1 (ESRI, Redlands, California, USA). All map figures were created using publicly available spatial data for administrative boundaries available from Public Health Ontario (https://geohub.lio.gov.on.ca), open data portals for the City of Kingston (https://opendatakingston.cityofkingston.ca/) and the City of Ottawa (https://maps.ottawa.ca/geoottawa/), and ESRI basemaps available via ArcGIS.

7.2.6 Results

7.2.6.1 Ixodes scapularis Density and Borrelia burgdorferi Infection Prevalence

Between 2017-2021, we collected 1,652 *I. scapularis* ticks over a combined 523 person-hours of sampling (2017: n=294, person-hours=131.5; 2018: n=479, person-hours=174.5; 2018: n=517, person-hours=118.9; 2021: n=362, person-hours=98.4). Mean (SD: standard deviation) *I. scapularis* density per sampling time was 2.8 ticks/person-hours (SD = 5.1) for Ottawa sites, 0.2 ticks/person-hours (SD = 0.4) for Gatineau sites, 3.5 ticks/person-hours (SD = 4.6) for Kingston sites, and 11.3 ticks/person-hours (SD = 14.2) for other sites in eastern Ontario. *Ixodes scapularis* density varied widely by site, with the highest mean density of 19.9 ticks/person-hours of sampling observed at Murphy's Point Provincial Park, located in eastern Ontario between Ottawa and Kingston. Site-specific surveillance results from 2017-2021 are shown in Table S7.2.

We tested nymphal and adult instars for various tick-borne pathogens (*B. burgdorferi*: n=1,543; *B. miyamotoi*: n=1,543; *A. phagocytophilum*: n=1,543; *B. microti*: n=1,248; *B. odocoilei*: n=846; DTV: n=846). *Borrelia burgdorferi* was detected in ticks from 24 of 66 sites (36.4%) visited from

2017-2021 (Ottawa: n=15, 44%; Gatineau: n=0, 0%; Kingston: n=8, 50%; Other: n=1, 33%). *Borrelia burgdorferi*-infection rates in ticks were 1.5 (SD=2.5) infected ticks/person-hours for Ottawa sites, 1.7 (SD=2.2) infected ticks/person-hours for Kingston sites, 3.1 (SD=4.5) infected ticks/person-hours for other sites in eastern Ontario, and no positive specimens detected in Gatineau (Figure 7.2). *Borrelia burgdorferi* infection prevalence varied by site and year of sampling but was generally high, with 21 of 24 sites having an infection prevalence over 20% (Table S7.2). Mean *B. burgdorferi* infection prevalence across all surveyed sites and years was 32.2% (n=497). Site-level *B. burgdorferi* infection prevalence was associated with *I. scapularis* density per person-hours of sampling (p=0.0186).

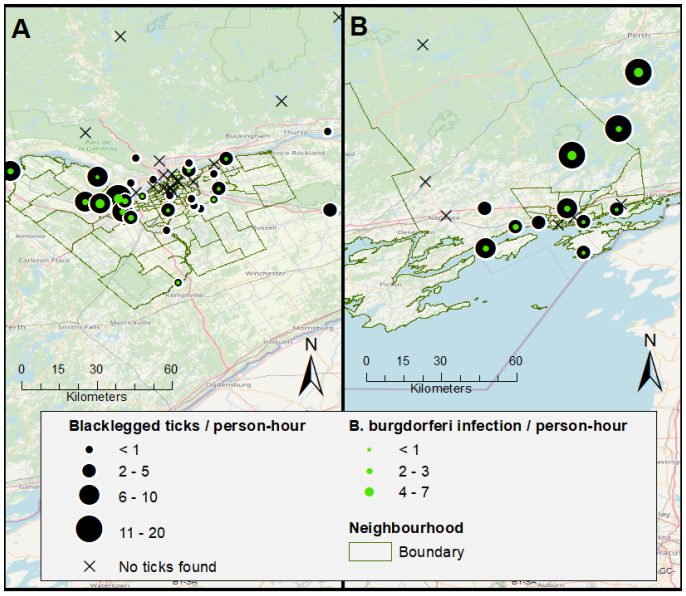

Figure 7.2 Average *Ixodes scapularis* tick density per person-hour of tick dragging and *Borrelia burgdorferi* infection rate in ticks per person-hour of tick dragging from 2017-2021 in Ottawa-Gatineau (A) and the Kingston region (B).

7.2.6.2 Borrelia burgdorferi Genetic Diversity

Overall, 69 *B. burgdorferi*-positive nymphs were analyzed by MLST (of which 24 were successfully sequenced). Of these, 7 nymphs (29.2%) had mixed *B. burgdorferi* infection and 17 (70.8%) nymphs were assigned a specific sequence type (ST) using the *Borrelia* MLST database. *Borrelia burgdorferi* genetic diversity varied substantially across the study area, with 11 unique STs (contained within 8 clonal complexes) detected for 17 nymphs assigned a ST (Table 7.1). The STs identified in our study are consistent with those occurring in the Northeastern United States and regions of Ontario. We did not detect any new STs that are not currently registered in the *Borrelia* MLST database.

Table 7.1 Sequence types (ST) and allelic profile of *Borrelia burgdorferi* in nymphs collected by active surveillance in Ontario, Canada from 2017-2021. Clonal complex (CC) was extracted from Mechai et al. (2016) (11).

Sample ID	Region	CC	ST	Allele Number							
				clpA	***clpX***	***nifS***	***pepX***	***pyrG***	***recG***	***rplB***	***uvrA***
07.03.148	Ottawa	CC3	1	1	1	1	1	1	1	1	1
13.02.367.3	Other	CC3	1	1	1	1	1	1	1	1	1
13.04.152.1	Other	CC3	3	4	1	1	1	1	6	1	7
N4.I3.05.357	Ottawa	CC3	3	4	1	1	1	1	6	1	7
N4.I3.06.213.1	Ottawa	CC3	3	4	1	1	1	1	6	1	7
N2.I2.11.211	Ottawa	CC3	4	8	1	1	1	4	6	1	7
07.W1.06.1267.1	Ottawa	CC12	12	3	3	2	4	3	4	4	4
07.06.1228.4	Ottawa	CC16	16	2	2	1	2	2	2	2	2
N2.W1.11.231.7	Ottawa	CC19	19	4	4	3	3	3	3	3	3
P4.W2.09.W.1	Ottawa	CC19	19	4	4	3	3	3	3	3	3
13.04.152.4	Other	CC34	34	8	1	1	7	1	6	1	10
N2.W3.11.244	Ottawa	CC34	14	9	1	1	7	1	6	1	10
13.02.368.2	Other	CC36	9	10	5	4	6	1	6	1	6
10.05.648.2	Ottawa	CC36	36	10	5	4	6	1	15	1	6
N1.W2.05.627	Ottawa	CC36	36	10	5	4	6	1	15	1	6
13.03.225	Other	CC37	37	7	6	12	1	1	5	5	5
KG11.05.99.1	Kingston	CC226	226	8	1	15	94	2	20	1	7

7.2.6.3 Infection and Co-Infection Prevalence with Other Tick-Borne Pathogens

Borrelia miyamotoi, *A. phagocytophilum*, and *B. odocoilei* were detected at 3 of 66 sites (4.5%), 9 of 66 sites (13.6%), and 17 of 54 sites (31.5%), respectively (Figure 7.3). *Babesia microti* and DTV were not detected in *I. scapularis* ticks collected between 2017-2021 in any region across eastern Ontario or Quebec.

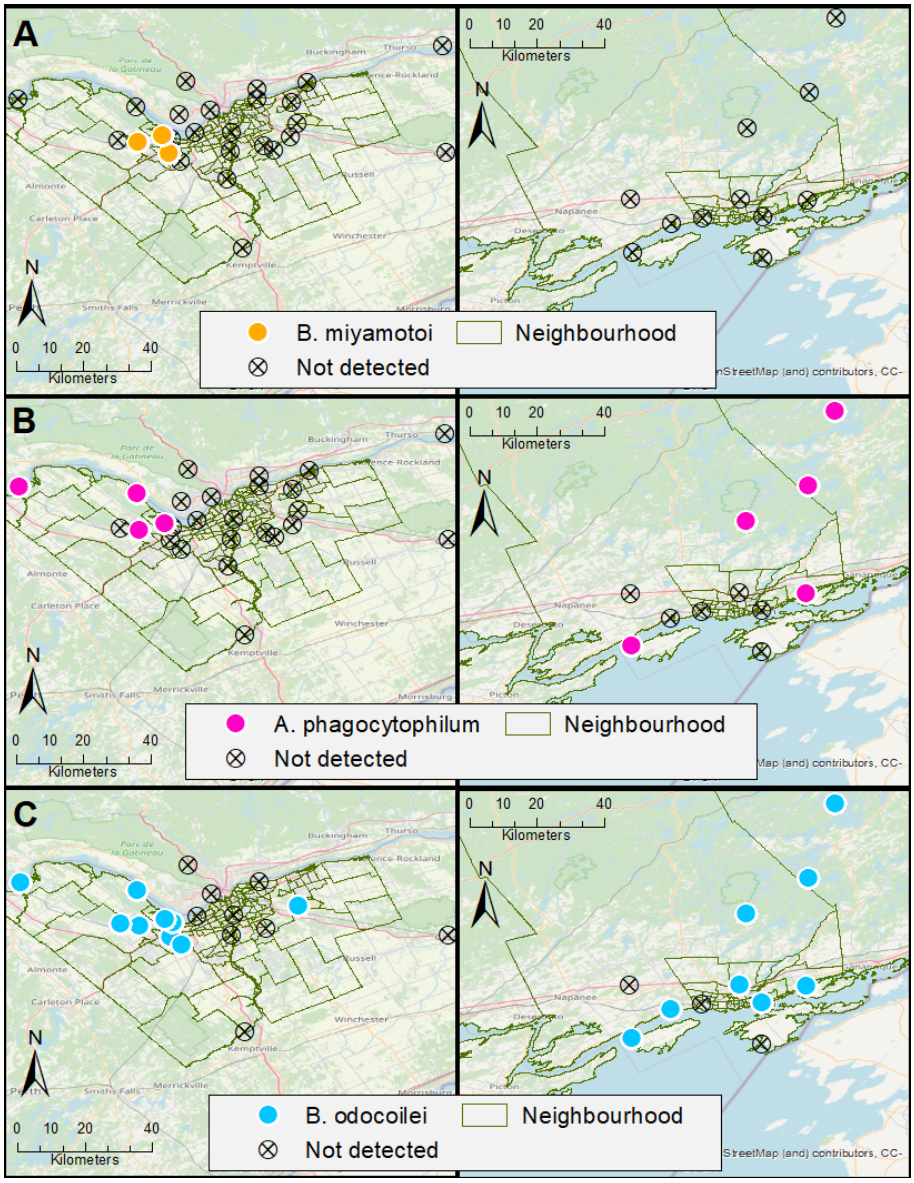

Figure 7.3 Sampled sites in Ottawa-Gatineau (left panels) and Kingston (right panels) where *Borrelia miyamotoi* (A), *Anaplasma phagocytophilum* (B), and *Babesia odocoilei* (C) were detected in blacklegged ticks between 2017-2021.

Borrelia miyamotoi was detected in each year of sampling but only in Ottawa and the mean infection prevalence in ticks over sampling years was very low (mean=0.7%, SD=0.3%; annual range: 0.4-1.1%; site range: 0-2.8%) (Table 7.2, S7.2). *Anaplasma phagocytophilum* was detected throughout eastern Ontario and mean infection prevalence in ticks from 2017-2021 was similar in Ottawa (mean: 1.4%, SD=1.9%; annual range: 0-4.3%; site range: 0-8.7%) and Kingston (mean=1.6%, SD=0.9%; annual range: 1.0-2.2%; site range: 0-3.6%) but roughly 3 times higher at Murphy's Point Provincial Park (mean=4.7%, SD=6.7%; annual range: 0-9.4%; site range: 0-10.2%) (Table 7.2, S7.2). *Anaplasma phagocytophilum* infection prevalence was also associated with *I. scapularis* density ($p<0.001$). *Babesia odocoilei* was present across eastern Ontario, with the highest mean infection prevalence observed in Ottawa (mean=10.1%, SD-2.3%; annual range: 8.5-11.7%; site range: 0-33.3%) compared to Kingston (mean=5.4%, SD=6.2%; annual range: 1.0-9.7%; site range: 0-20%) and Murphy's Point Provincial Park (3.6%) (Table 2, S2).

Table 7.2 Prevalence of infection and coinfection in ticks (only *Ixodes scapularis* nymphs and adults were tested for tick-borne pathogens) with *Borrelia burgdorferi* (Bb), *Borrelia miyamotoi* (Bm), *Anaplasma phagocytophilum* (Ap), *Babesia odocoilei* (Bo) from 2017-2021 by region.

Year	Ticks, n	Infection prevalence, n (%)				Co-infection prevalence, n (%)				
		Bb	Bm	Ap	Bo	Bb-Bm	Bb-Ap	Bb-Bo	Ap-Bo	Bb-Ap-Bo
Ottawa, ON										
2017	218	65 (29.8)	1 (0.5)	2 (0.9)	-	0	0	-	-	-
2018	413	154 (37.3)	3 (0.7)	0	-	3 (0.7)	0	-	-	-
2019	247	80 (32.4)	1 (0.4)	1 (0.4)	21 (8.5)	0	0	9 (3.3)	1 (0.4)	0
2021	94	28 (29.8)	1 (1.1)	4 (4.3)	11 (11.7)	0	2 (2.2)	0	0	0
Kingston, ON										
2019	98	30 (30.6)	0	1 (1.0)	1 (1.0)	0	0	1 (1.0)	0	0
2021	267	79 (29.6)	0	6 (2.2)	26 (9.7)	0	1 (0.4)	0	0	1 (0.4)
Gatineau, QC										
2018	11	0	0	0	0	0	0	-	-	-
2019	2	0	0	0	0	0	0	0	0	0
2021	0	-	-	-	-	-	-	-	-	-
Other (eastern ON)										
2017	55	23 (41.8)	0	0	-	0	0	-	-	-
2019	138	38 (27.5)	0	13 (9.4)	5 (3.6)	0	5 (3.6)	2 (1.4)	0	1 (0.7)

Co-infection with two or more tick-borne pathogens was detected in 26 (1.7%) *I. scapularis* ticks across all study areas and years. Co-infection between *B. burgdorferi* and *B. odocoilei* was the most common, accounting for 12 of 26 (46.2%) ticks with mixed pathogens (Ottawa: n=9, annual range: 0-3.3%; Kingston: n=1, annual range: 0-1.0%; eastern ON: n=2, 1.4%) (Table 7.2). Co-infection with *B. burgdorferi* and *A. phagocytophilum* (Ottawa: n=2, annual range: 0-2.2%; Kingston: n=1, annual range: 0-0.4%; eastern ON: n=5, annual range 0-3.6%) and with *B. burgdorferi* and *B. miyamotoi* (Ottawa: n=3, annual range: 0-0.7%) were observed in 8 of 26 (30.8%) and 3 of 26 (11.5%) ticks with mixed pathogens, respectively (Table 7.2). We also detected 1 tick (3.8%) with *A. phagocytophilum* and *B. odocoilei* co-infection and 2 ticks (7.7%) with a triple co-infection between *B. burgdorferi*, *A. phagocytophilum*, and *B. odocoilei* (Table 7.2).

7.2.7 Discussion

In this study, we present multi-year tick surveillance and tick-borne pathogen testing results from across eastern Ontario and the National Capital Region. We identified *I. scapularis* tick densities and the infection prevalence of *B. burgdorferi*, *B. miyamotoi*, *A. phagocytophilum*, *B. microti*, *B. odocoilei*, and DTV in ticks collected by drag sampling from 2017-2021 at 66 sites in Ottawa, Gatineau, Kingston, and other regions in eastern Ontario. We also explored the genetic diversity of *B. burgdorferi* in infected nymphs using MLST analysis. To our knowledge, this is the largest multi-year assessment of tick-borne pathogens in host-seeking ticks in eastern Ontario and provides an important baseline for future surveillance studies. Additionally, we identified the geographic distribution of tick-borne pathogens at a finer scale, which provides a valuable risk

estimate for tick-borne illnesses and serves as a complement to human and animal surveillance studies reporting the prevalence of tick-borne pathogens.

Ixodes scapularis density per person-hours of tick dragging varied widely by site but was highest in the Kingston-Frontenac region followed by the Ottawa and Gatineau regions. Other locations sampled in eastern Ontario included two sites in the Eastern Ontario Public Health Unit, which had relatively lower tick densities, and one site in the Leeds, Grenville, and Lanark Public Health Unit, which was a significant outlier (i.e., Murphy's Point Provincial Park) and had the largest tick density of any sites sampled. Sites with high *I. scapularis* densities were classified as forests, conservation areas, provincial parks, and recreation trails mostly located in suburban and rural areas previously predicted to have high habitat suitability for ticks (27, 29, 39). Sites with low to moderate *I. scapularis* densities included municipal parks and recreational trails in regions with lower forest density and higher proportion of agricultural land use, where ticks and their animal or reproductive hosts are less abundant (27, 39, 40).

Borrelia burgdorferi-infection prevalence in ticks was correlated with *I. scapularis* tick densities; however, there were variations among the different sites. Lower infection rates in sites with high tick density are likely due to an estimated 5-year lag time between *I. scapularis* and *B. burgdorferi* establishment as well as possible annual changes in animal host abundance and reservoirs for *B. burgdorferi* (5, 41). On the other hand, high infection prevalence found in low tick density sites may reflect a small sample size of ticks in that location and factors related to field sampling such as month of tick dragging, weather conditions affecting questing ticks, and site accessibility (e.g., flooding, inability to visit each site in all study years). Previous studies looking at *B. burgdorferi*

in ticks submitted by the public to local public health units showed that infection prevalence tended to be higher in the Kingston region, where clusters of *B. burgdorferi*-infected ticks and human Lyme disease cases were detected, with a northeastern spread of 54 km from 2010-2016 (6, 42). In this study, we found that mean *B. burgdorferi*-infection rates per person-hour of sampling were similar in the Kingston (1.7 *B. burgdorferi*-infected ticks/person-hour) and Ottawa (1.5 *B. burgdorferi*-infected ticks/person-hour) regions, which may indicate stabilization and establishment of *B. burgdorferi* from 2019-2021 in more northern regions.

Using MLST analysis, we also found that *B. burgdorferi* genotypes in eastern Ontario were highly diverse, which is consistent with earlier studies that have analyzed *B. burgdorferi* genotypes in Canada using MLST (10). Most STs found in our study belong to four of the largest clonal complexes (CC34, CC3, CC36, CC12) in North America (clonal complexes include highly related STs), which also supports the finding of high bacterial diversity in eastern Ontario (11). All *B. burgdorferi* STs found in our study were previously detected throughout the Northeastern United States and no new lineages were identified in ticks collected between 2019-2021 (43). Similarly, *B. burgdorferi* STs found in ticks collected in the Thousand Islands region of eastern Ontario from 2006-2010 were found to be direct lineages from the Northeastern United States compared to the novel STs detected in southern Ontario and Manitoba, where founder effects arising from refugial populations may have occurred (43). However, our sample size was small, and we cannot fully capture the evolution of *B. burgdorferi* bacterial strains in eastern Ontario.

A study by Hanincova et al. (2013) found that *B. burgdorferi* STs cultured from patient samples in the Northeast and Midwest United States were associated with different pathogenic properties

in humans (9). The STs identified in our study were predominantly those that Hanincova et al. linked to disseminated infection (e.g., ST3, ST9, ST16, ST36) or both disseminated and localized infection (e.g., ST1, ST4, ST12, ST14, ST19, ST34), with only two STs in our study linked to localized infection (e.g., ST37, ST226). While this is an interesting finding, we cannot speculate that there is a greater risk for disseminated Lyme disease in eastern Ontario without clear clinical evidence of these associations. Furthermore, Johnson et al. (2018) found that a larger proportion of individuals with Lyme disease were diagnosed with early localized disease in eastern Ontario, where there is a higher degree of knowledge regarding Lyme disease risk (44). Together, these observations suggest that identification of environmental Lyme disease risk areas is essential for increasing awareness and early diagnosis and treatment of Lyme disease.

In this study, we also assessed the prevalence of other tick-borne pathogens in eastern Ontario and the National Capital Region. We did not detect any positive specimens for *B. microti* or DTV and a very low infection prevalence of *B. miyamotoi* in ticks. These findings are in line with previous tick surveillance efforts, seroprevalence studies conducted in blood donors, and wildlife studies, which together indicate there is currently a low risk of human infection with *B. mircoti*, *B. miyamotoi*, and DTV (28, 33, 45-49). The infection prevalence of *A. phagocytophilum* in ticks was higher than all other tick-borne pathogens associated with human illness (second to *B. burgdorferi*), and two sites (#9, #13) had an *A. phagocytophilum*-infection prevalence near 10%, which is higher than previously reported estimates in Ontario (50, 51). Like *B. burgdorferi*, *A. phagocytophilum* was associated with site-level *I. scapularis* density and co-infection with *B. burgdorferi* and *B. odocoilei* (both double and triple co-infections among these pathogens were detected). The three pathogens likely infect common small mammals and deer that blacklegged

ticks parasitize, resulting in similar patterns of emergence (51, 52). Lastly, we detected a moderate to high *B. odocoilei*-infection prevalence in host-seeking ticks. *Babesia odocoilei* causes cervid babesiosis and may have implications for local wildlife in eastern Ontario (16, 19, 53). It is currently not associated with human infection; however, a recent study detected *B. odocoilei* by PCR in two patients with symptoms of babesiosis (20). Further investigation on the potential of *B. odocoilei* to cause human babesiosis is needed and as is continued surveillance of this tick-borne pathogens.

This study has several limitations. First, there were differences in our sampling at the various sites throughout the study period. We were unable to visit all locations every year because of logistical constrains and, due to the total number of sites, the month of sampling varied and may have affected the total number of collected tick specimens. We also pooled data from multiple projects to increase sample size, which we accounted for using sampling person-time, but this may contribute to annual differences in the site-specific analyses. While the field dragging logistics are not likely to have a major impact on the overall study conclusions, we cannot dismiss the effects of a small tick sample size for some sites, which can skew the infection prevalence of tick-borne pathogens. Second, due to the development of new laboratory assays and the expansion of our testing protocols over several years, we did not test for all tick-borne pathogens during all study years. This limited our ability to detect temporal trends in the prevalence of tick-borne pathogens. Furthermore, our MLST analysis was also limited by a small sample size and difficulties successfully amplifying the eight gene loci for all *B. burgdorferi*-positive nymphal specimens, which is not uncommon when sequencing potentially low concentrations of bacterial DNA derived from ticks or animal issues (e.g., non-cultured *B. burgdorferi* samples) (11, 38). Lastly, and most

importantly, our results provide a surveillance report, and we cannot infer the implication of *B. burgdorferi* genotypes on Lyme disease outcomes or the direct population risk for other tick-borne infections without human surveillance data or supporting clinical evidence.

Nevertheless, our study provides an important epidemiological update on the prevalence of tick-borne pathogens in host-seeking ticks in eastern Ontario, where tick populations are becoming endemic. Our results have shown an increase in the prevalence of *B. burgdorferi* and *A. phagocytophilum* in more locations throughout eastern Ontario as well as moderate prevalence of *B. odocoilei*, which is not commonly reported on due to unknown implications for human health. However, the overall prevalence of other tick-borne pathogens (except *B. burgdorferi*) remains low in eastern Ontario.

7.2.8 Supplemental Information

Supplemental Table 7.1 Description of sites visited from 2017-2021.

Site ID	Site Name	No. Years Visited	Person-Hours	Site Type
Ottawa, ON				
1	Britannia Conservation Area	4	18.4	Conservation area / forest
2	Rideau River Provincial Park	2	9.9	Provincial park
3	Rideau River Eastern Pathway	3	14.0	Recreational trail
4	Berryl Gaffney/Maple Hill Park	3	14.6	Municipal park
5	Dominion Arboretum & Fletcher Wildlife Garden	3	14.7	Municipal park
6	Heritage Park	3	15.7	Municipal park
7	Greenbelt Pathway West	4	26.0	Recreational trail
8	Pine Grove/Conroy Pit	2	14.3	Municipal park
9	South March Highlands Conservation Forest	4	21.0	Conservation area / forest
10	Morris Island Conservation Area	2	11.5	Conservation area / forest
11	Stoney Swamp	3	22.2	Conservation area / forest

12	Petrie Island	2	4.6	Conservation area / forest
15	Meadowbrook Park	3	9.3	Municipal park
16	Prescott & Russel Recreational Trail	4	17.3	Recreational trail
17	Pinhey's Point Park	3	14.7	Conservation area / forest
18	Brown's Inlet Park	3	9.8	Municipal park
19	Fairmont Park	3	10.9	Municipal park
20	Carling Campus North Access	3	11.5	Recreational trail
21	Shirley's Bay	4	16.6	Recreational trail
22	Beacon Hill	4	12.6	Recreational trail
23	Hog's Back Park	3	9.0	Municipal park
24	Carp Hill	3	10.5	Conservation area / forest
25	Findlay Creek/Greely	3	10.0	Municipal park
26	Rockcliffe Park	2	6.5	Municipal park
27	Mer Bleue Bog	3	11.0	Municipal park
28	Brewer Park	2	3.5	Municipal park
29	Strathcona Park	2	3.5	Municipal park
30	Black Rapids Creek	3	10.0	Recreational trail
31	Ottawa River Pathway/Remic Rapids	2	3.5	Recreational trail
32	Orleans Trail along Ottawa River	2	4.0	Recreational trail
34	Bank Street Golf Course	1	2.6	Recreational trail
Kingston, ON				
KG1	Kashwakamak Lake	2	4.0	Conservation area / forest
KG2	Channelview Park	2	6.0	Municipal park
KG3	Little Cataraqui Creek	2	4.0	Conservation area / forest
KG4	Lake Opinicon (QUBS)	2	4.6	Conservation area / forest
KG5	Perth Road	2	4.5	Recreational trail
KG6	Roblin	2	5.4	Conservation area / forest
KG7	Napanee	2	4.5	Recreational trail
KG8	Parrot's Bay	2	4.0	Conservation area / forest
KG9	Amherst Island	2	6.0	Farm land / forest
KG10	Wolfe Island	2	4.5	Recreational trail
KG11	Arrowhead Beach Park	2	6.0	Recreational trail
KG12	Belle Island (Cataraqui Park)	2	5.5	Conservation area / forest
KG13	Grass Creek Park	2	4.0	Recreational trail
KG14	Marshlands Conservation Area	2	3.5	Conservation area / forest
KG15	ON Route Napanee	2	5.4	Forest
KG16	Lemoine Park	1	3.0	Conservation area / forest
Gatineau, QC				
G1	Parc du lac Beauchamp	1	4.0	Recreational trail
G2	Parc National de Plaisance	2	6.0	Recreational trail
G3	Forêt Boucher	1	6.0	Conservation area / forest
G4	Parc de la Gatineau - Chelsea	3	7.0	Recreational trail

G5	Parc de la Gatineau – Lac Philippe	1	3.0	Recreational trail
G6	Centre touristique du Lac Simon (SEPAQ)	1	3.0	Recreational trail
G7	Cycloparc PPJ	1	3.0	Recreational trail
G8	Réserve écologique de la Forêt la Blanche	1	3.0	Conservation area / forest
G9	Mont Ste-Marie	1	3.0	Recreational trail
G10	Guillot Park	3	6.7	Recreational trail
G11	St-Dominique Park	3	7.6	Recreational trail
G12	Parc du marais de Touraine	1	2.6	Recreational trail
G13	Desjardins Park	1	1.6	Recreational trail
Other sites				
13	Murphy's Point Provincial Park	2	11.7	Provincial park
AMS1	Casselman	1	2.0	Recreational trail
AMS3	Upper Canada Migratory Bird Sanctuary	1	2.0	Conservation area / forest

Supplemental Table 7.2 Active surveillance of *Ixodes scapularis* ticks in Ottawa-Gatineau, Kingston, and other regions of eastern Ontario from 2017-2021.

Site ID	Year	Person-hours	Ixodes scapularis abundance (n)				Ixodes scapularis density per person-hours (n)		Infection prevalence (%)					
			Larvae	Nymph	Adults	Total	Nymph	Total	Bb	Bm	Ap	Bmi	Bo	POW
Ottawa, ON														
1	2017	8.4	0	0	15	15	0	1.8	13.3	0	0	-	-	-
	2018	6.0	0	0	1	1	0	0.2	0	0	0	0	-	-
	2019	2.0	0	0	1	1	0	0.5	0	0	0	0	0	0
	2021	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
2	2017	6.9	0	1	0	1	0.1	0.1	0	0	0	-	-	-
	2019	3.0	0	5	0	5	1.7	1.7	40.0	0	0	0	0	0
3	2017	6.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2018	6.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
4	2017	6.6	0	0	1	1	0	0.2	0	0	0	-	-	-
	2018	6.0	0	0	1	1	0	0.2	0	0	0	0	-	-
	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
5	2017	6.7	0	0	0	0	0	0	n/a	n/a	n/a	-	-	-

	2018	6.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
6	2017	7.7	0	0	1	1	0	0.1	0	0	0	0	0	0
	2018	6.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	-	-
	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
7	2017	8.0	1	6	10	17	0.8	2.1	43.8	0	0	-	-	-
	2018	12.0	51	30	81	162	2.5	13.5	34.2	1.8	0	0	-	-
	2019	3.0	0	12	14	26	4.0	8.7	11.5	0	0	0	11.5	0
	2021	3.0	0	25	14	39	4.2	6.5	15.4	2.6	0	0	10.3	0
8	2017	8.3	3	0	0	3	0	0.4	n/a	n/a	n/a	n/a	n/a	n/a
	2018	6.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
9	2017	7.2	0	2	23	25	0.3	3.5	32.0	0	0	-	-	-
	2018	6.0	0	0	38	38	0	6.3	34.2	2.6	0	0	-	-
	2019	3.0	0	8	40	48	2.7	16.0	45.8	0	0	0	4.2	0
	2021	4.8	0	18	28	46	3.0	7.7	43.5	0	8.7	0	15.2	0
10	2017	8.5	0	3	26	29	0.4	3.4	34.5	0	3.4	-	-	-
	2019	3.0	9	34	6	49	11.3	16.3	12.5	0	0	0	10.0	0
11	2017	13.2	4	11	11	26	0.8	2.0	31.8	0	0	-	-	-
	2018	6.0	1	2	16	19	0.3	3.2	22.2	0	0	0	-	-
	2019	3.0	0	0	17	17	0	5.7	58.8	0	0	0	11.8	0
12	2017	2.6	0	0	12	12	0	4.7	8.3	0	0	-	-	-
	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
15	2017	3.8	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2018	3.5	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
16	2017	6.3	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2018	6.0	0	0	8	8	0	1.3	12.5	0	0	0	-	-

	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2021	3.0	1	0	0	1	0	0.3	n/a	n/a	n/a	n/a	n/a	n/a
17	2017	5.7	11	0	8	19	0	3.4	50.0	0	0	-	-	-
	2018	6.0	0	0	19	19	0	3.2	36.8	0	0	0	-	-
	2019	3.0	18	21	1	40	7.0	13.3	9.1	0	4.5	0	4.5	0
18	2017	4.3	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2018	3.5	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
19	2017	4.4	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2018	4.5	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
20	2017	4.0	0	0	6	6	0	1.5	50.0	0	0	-	-	-
	2018	6.0	0	0	1	1	0	0.2	0	0	0	0	-	-
	2019	1.5	0	0	14	14	0	9.3	71.4	0	0	0	7.1	0
21	2017	4.0	0	0	46	46	0	11.5	43.5	2.2	2.2	-	-	-
	2018	5.0	3	7	141	151	1.4	30.2	45.3	0	0	0	-	-
	2019	3.0	0	2	34	36	0.7	12.0	38.9	2.8	0	0	13.9	0
	2021	4.6	0	1	4	5	0.2	0.8	20.0	0	0	0	0	0
22	2017	1.2	0	0	15	15	0	12.9	13.3	0	0	-	-	-
	2018	6.0	0	0	4	4	0	0.7	0	0	0	0	-	-
	2019	2.0	0	1	1	2	0.5	1.0	0	0	0	0	0	0
	2021	3.4	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
23	2017	1.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2018	6.0	0	0	2	2	0	0.3	0	0	0	0	-	-
	2019	2.0	0	0	1	1	0	0.5	0	0	0	0	0	0
24	2017	1.5	0	0	18	18	0	12.0	5.6	0	0	-	-	-
	2018	6.0	0	7	32	39	1.2	6.5	38.5	0	0	0	-	-

	2019	3.0	0	10	17	27	3.3	9.0	40.7	0	0	0	7.4	0
25	2017	2.0	0	0	3	3	0	1.5	0	0	0	-	-	-
	2018	6.0	0	0	1	1	0	0.2	0	0	0	0	-	-
	2019	2.0	0	0	1	1	0	0.5	0	0	0	0	0	0
26	2018	4.5	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
27	2018	6.0	0	4	10	14	0.7	2.3	21.4	0	0	0	-	-
	2019	2.0	0	0	3	3	0	1.5	0	0	0	0	33.3	0
	2021	3.0	0	1	2	3	0.2	0.5	33.3	0	0	0	0	0
28	2018	1.5	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
29	2018	1.5	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
30	2018	3.0	0	0	8	8	0	2.7	62.5	0	0	0	-	-
	2019	3.0	0	0	5	5	0	1.7	20.0	0	0	0	0	0
	2021	4.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
31	2018	1.5	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
32	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2021	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
34	2021	2.6	0	1	0	1	0.4	0.4	0	0	0	0	0	0
Kingston, ON														
KG1	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2021	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
KG2	2019	3.0	0	1	0	1	0.3	0.3	100	0	0	0	0	0
	2021	3.0	0	21	14	35	3.5	5.8	20.0	0	2.9	0	8.6	0
KG3	2019	2.0	0	13	3	16	6.5	8.0	43.8	0	0	0	0	0
	2021	2.0	0	10	10	20	5.0	10.0	25.0	0	0	0	10.0	0

KG4	2019	2.0	0	23	5	28	11.5	14.0	21.4	0	3.6	0	0	0
	2021	2.6	0	26	17	43	4.3	7.2	7.0	0	2.3	0	9.3	0
KG5	2019	2.5	0	10	0	10	4.0	4.0	40.0	0	0	0	0	0
	2021	2.0	0	1	68	69	0.3	17.3	55.1	0	2.9	0	8.7	0
KG6	2019	3.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2021	2.4	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
KG7	2019	2.5	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2021	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
KG8	2019	2.0	2	6	2	10	3.0	5.0	25.0	0	0	0	12.5	0
	2021	2.0	0	8	0	8	4.0	4.0	37.5	0	0	0	0	0
KG9	2019	3.0	0	20	0	20	6.7	6.7	30.0	0	0	0	0	0
	2021	3.0	0	47	15	62	7.8	10.3	30.6	0	3.2	0	11.3	0
KG10	2019	3.0	0	4	0	4	1.3	1.3	75.0	0	0	0	0	0
	2021	1.5	0	4	0	4	2.7	2.7	0	0	0	0	0	0
KG11	2019	2.0	0	4	1	5	2.0	2.5	20.0	0	0	0	0	0
	2021	4.0	0	18	2	20	4.5	5.0	20.0	0	0	0	20.0	0
KG12	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2021	3.5	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
KG13	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2021	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
KG14	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2021	1.5	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
KG15	2019	2.4	0	1	0	1	0.4	0.4	0	0	0	0	0	0
	2021	3.0	0	6	0	6	2.0	2.0	0	0	0	0	0	0
KG16	2019	3.0	1	5	0	6	1.7	2.0	0	0	0	0	0	0
Gatineau, QC														
G1	2018	4.0	0	2	2	4	0.5	1.0	0	0	0	0	-	-
G2	2018	4.0	0	1	4	5	0.3	1.3	0	0	0	0	-	-

	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
G3	2018	6.0	0	0	1	1	0	0.2	0	0	0	0	-	-
G4	2018	3.0	0	0	1	1	0	0.3	0	0	0	0	-	-
	2019	2.0	0	0	1	1	0	0.5	0	0	0	0	0	0
	2021	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
G5	2018	3.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
G6	2018	3.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
G7	2018	3.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
G8	2018	3.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
G9	2018	3.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
G10	2018	3.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2019	2.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2021	1.7	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
G11	2018	3.0	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
	2019	2.0	0	1	0	1	0.5	0.5	0	0	0	0	0	0
	2021	2.6	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
G12	2021	2.6	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
G13	2021	1.6	0	0	0	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
Other sites														
13	2017	7.7	2	35	20	57	4.5	7.4	41.8	0	0	-	-	-
	2019	4.0	2	104	23	129	26.0	32.3	29.9	0	10.2	0	3.9	0
AMS1	2019	2.0	0	2	1	3	1.0	1.5	0	0	0	0	0	0
AMS3	2019	2.0	0	7	1	8	3.5	4.0	0	0	0	0	0	0

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8

8 DISCUSSION

The main goal of my thesis was to assess the risk of Lyme disease and other tick-borne pathogens in Ontario using a multidisciplinary approach. To do so, I adapted a risk model for vector-borne diseases, in which risk is defined based on two main components: hazard and vulnerability (1). Conceptually, hazard encompasses "the potentiality of disease occurrence in a geographic area or time frame, and is characterized by location, magnitude, frequency, and probability", which I estimated based on the distributions of *I. scapularis* ticks and *B. burgdorferi*-infected ticks (1). Vulnerability is "the predisposition of the society and its population to the burden of vector-borne diseases", which I assessed by identifying socioecological risk factors for Lyme disease and the prevalence of specific *B. burgdorferi* bacterial types and other tick-borne pathogens (1).

In this thesis, I developed two species distribution models for *I. scapularis* and *B. burgdorferi* to predict their potential distributions across Ontario based on climatic parameters and land cover features. I then classified model predicted environmental risk as low/high for persons living in southeastern Ontario and measured the relationship between residence in predicted high-risk areas

and the odds of Lyme disease. I also analyzed provincial surveillance data to identify locations where Ontarians are exposed to ticks, irrespective of whether individuals reside in those areas, to better understand the environmental and socioeconomic risk factors associated with Lyme disease incidence and the spatiotemporal trends in Lyme disease emergence. Finally, I assessed the prevalence of other tick-borne pathogens transmitted by *I. scapularis* ticks and the genetic diversity of *B. burgdorferi,* which may be correlated with different clinical Lyme disease outcomes.

In this final chapter, I will review the main results and contextualize these findings in view of what is currently known as well as highlight the contributions of my work on Lyme disease research in Canada. Finally, I will discuss some general limitations and provide recommendations to address other remaining knowledge gaps.

8.1 Environmental Risk Areas for Lyme Disease

In Chapter 4, I developed predictive species distribution models for *I. scapularis* and *B. burgdorferi* using Maxent modelling. Geographical areas of high predicted suitability for ticks and the Lyme disease-causing pathogen were focused mainly on the region of eastern Ontario and along the shorelines of Lake Ontario and Lake Erie. The Maxent model for blacklegged ticks predicted some moderate suitability for ticks around Algonquin Provincial Park and towards the western areas bordering Lake Huron and Georgian Bay. On the other hand, the Maxent model for *B. burgdorferi* was predominantly restricted to the eastern part of the province. These findings reflect the introduction of *I. scapularis* (and *B. burgdorferi*) in Canada from neighbouring US

states with established tick populations (2, 3). In the United States, *I. scapularis* tick populations are endemic in most parts of the northeastern coast (4, 5).

A recent reanalysis of species distribution models for *I. scapularis* in the United States suggested that the "rimming" distribution of the blacklegged tick along the Atlantic coastal edge may be due to model specifications rather than a reflection of the species distribution (4, 6). The argument made by Peterson and Raghavan was that the use of validation thresholds that utilize sensitivity (i.e., omission error, or presence locations that fall within non-suitable regions) and specificity (i.e., commission error, or absence locations that fall within suitable regions) for species distribution models places undue weight on absence points (6). This is particularly relevant since absences are either pseudo-absences (background) or locations where the species was not detected at a particular time/place (not necessarily a true absence). Peterson and Raghavan suggested that under omission-based thresholds the predicted distribution of *I. scapularis* appears simpler and more filled-in (6).

My models also predicted a "rimming" distribution for *I. scapularis* and *B. burgdorferi*, although this was not likely due to the criticism suggested by Peterson and Raghavan since I used the lowest omission rate threshold to ensure the models captured even the least suitable regions were ticks or pathogens could be detected. It is possible that the fuller distribution for blacklegged ticks in the United States using similar omission thresholds may be due to differences in the endemicity of ticks in the two regions. Many locations in Ontario represent newly emerging regions where ticks have not fully colonized the available habitat. A study by Talbot et al. looking at genetic microsatellite markers to determine the connectivity and differentiation of blacklegged ticks in

Ontario and Quebec found substantial genetic clustering, indicating patchy genetic differentiation and no clear spatial pattern in the spread of ticks (7). Talbot et al. propose that the gene flow patterns of sampled blacklegged ticks in Ontario were correlated with the movement of mammalian hosts and long distance transport by migratory birds (7). Thus, the "rimming" pattern predicted by my Maxent models likely reflects the clustered and patchy distribution of ticks at the leading edge of their distribution in Canada. Similarly, a multiyear field study by Clow et al. found that *I. scapularis* colonization, or the "filling-in" of woodland habitat, in Ontario is occurring heterogeneously and does not consistently align with the range front expansion (8). The invasion of ticks was found to occur at approximately at 46 km/year, but this was only observed in eastern Ontario (8). It is likely that the large proportion of agricultural land and dense forest in in central and western regions may also provide a barrier to the horizontal distribution of ticks in Ontario.

The study by Clow et al. could not establish a clear temporal relationship between invasion of *I. scapularis* and *B. burgdorferi* due to low sample size, although their results were consistent with a tick-first hypothesis because sites where *I. scapularis* was recently emerging were still negative for *B. burgdorferi (8)*. This was further supported by evidence that, in eastern Ontario, the first human case of Lyme disease was reported on average 2.2 years after the first tick and 1.1 years after the first *B. burgdorferi*-positive tick, with an approximate 1-year lag between each phase (9). Thus, the smaller predicted distribution for *B. burgdorferi* in my analysis is consistent with earlier reports, in which *I. scapularis* invasion and colonization precedes pathogen detection.

Peterson and Raghavan also showed that the occurrence data used for species distribution modelling had an important effect on the predicted *I. scapularis* distribution, with established tick

occurrence data resulting in a narrower "rimming" distribution along the Atlantic states compared to established and reported occurrence data resulting in a wider filled-in tick distribution (6). For my models, I used established tick occurrence data based on sampling at specific sites to ensure the accuracy of the tick presence locations used for model building. It is possible that this modelling choice may have contributed to a narrower predicted distribution for *I. scapularis* and *B. burgdorferi* in southeastern Ontario. However, my models showed good discrimination ability for *I. scapularis* and *B. burgdorferi* presence when tested against tick data submitted by the public, although the AUC value was lower than for test data derived from field sampling of ticks. Passive tick surveillance data, though subject to recall bias and lower accuracy for tick location, has also been used for local species distribution modelling in Ottawa with good discrimination of positive and negative sites (10). It is possible that a combination approach, using established and reported tick occurrence data, may improve future modelling approaches.

Maxent species distribution modelling uses presence locations, sampled from the species' realized niche, to predict the species' potential niche across geographic space (11-13). While there exists a risk of contracting Lyme disease outside of predicted suitable locations for *I. scapularis*, the entomological risk is significantly higher in regions with high tick density (14-16). A recent study by Burrows et al. evaluated the ability of my *I. scapularis* Maxent model to predict the density of blacklegged ticks and, therefore, by proxy its value for measuring human Lyme disease risk (17). Burrows et al. found that the number of ticks per meter square in locations predicted to be suitable by my model was over 30 times higher than in regions predicted to be non-suitable (17). A moderate correlation between tick density and predicted habitat suitability was detected, although the correlation between density and suitability was strongest in more well-established tick regions

like the Kingston area (17). At the leading edge of the blacklegged tick's distribution in the Ottawa area, several sites were detected having high suitability/low density (17). Based on an ecological classification tool proposed by Clow et al., these sites were characterized as emerging; thus, indicating that the Maxent model classified these locations as suitable and that tick abundance in these locations may increase in coming years (17-19).

The Maxent predicted species distribution models for *I. scapularis* and *B. burgdorferi* are consistent with the body of evidence on the expansion and colonization of blacklegged ticks in Ontario. Studies by Burrows et al. and Kulkarni et al. further corroborate the utility of these entomological risk maps for assessing human Lyme disease risk.

8.2 SOCIOECOLOGICAL RISK FACTORS

An objective of my thesis was to evaluate the vulnerability (a measure of susceptibility to disease and population resilience) of Ontarians for acquiring Lyme disease by determining socioecological risk factors. In Chapters 5 and 6, I used two complementary methods to evaluate area-level risk factors associated with human Lyme disease near the home and by exposure to tick habitats, respectively. This is an important distinction because many Canadian studies reporting on human Lyme disease and passive tick surveillance either assume a broad geographic location for tick exposure (e.g., at the census subdivision or public health unit), which does not accurately reflect the local areas where people are encountering ticks, or a residential exposure to ticks (e.g., using forward sortation area of the postal code or home address) (9, 20-22). In the northeastern United States, where blacklegged ticks are largely endemic, there is evidence for peri-domestic exposure

to ticks (23-25). However, in Canada this may not be the case because ticks are still emerging in many regions. For example, in Ontario, there is substantial heterogeneity in the distribution of ticks and human Lyme disease cases, with some health units reporting above national estimates for human Lyme disease incidence or high counts of tick submissions from the public, while other heath units report little to no cases or ticks in the environment (20, 26).

My thesis provides insight into the tick exposure patterns seen in Ontario. In Chapter 5, I show that individuals who reside in high predicted environmental risk areas (based on the Maxent predicted distribution of *I. scapularis*) have 2.2 times higher odds of acquiring Lyme disease compared to individuals who reside in low predicted environmental risk areas. This increases to 3 times higher odds of Lyme disease if the individual resides in rural communities (public health units with overall population <250,000 people). Likewise, in Chapter 6, I show that the number of reported Lyme disease cases increases with abundance of ticks found in the environment and with rurality (e.g., low neighbourhood walk scores, residential instability). I also found that most individuals residing in rural regions reported tick exposures near the home whereas residents from urban regions reported a large percentage of tick exposures in the neighbouring rural health units. The link between Lyme disease and rurality has also been documented in the United States, Europe, and globally, suggesting that residence or travel to largely rural areas is a significant ecological risk factor for Lyme disease (27-29). Eastern Ontario is a largely rural region but not very remote from population centers, which may explain why this region has the highest provincial incidence of Lyme disease due to its high habitat suitability for ticks and closeness to metropolitan areas for travel, work, and other recreational activities (30).

My results, however, also suggest there is a growing risk for Lyme disease within more urban and suburban environments. Both *I. scapularis* and *B. burgdorferi* species distribution models predicted pockets of high environmental risk for Lyme disease within suburban areas of metropolitan regions. Lyme disease was also associated with residence in urban regions (e.g., public health units with >1,000,000 population), although the odds of infection were lower compared to more rural residence. Furthermore, the number of locally acquired infections in metropolitan regions like Ottawa increased annually. In my analyses of ecological risk factors for Lyme disease, proportion of treed land, number of parks, and moderate neighbourhood walkability score, which is a measure of connectivity (three-way intersections), density (dwellings), and destinations (points of interest), were found to be associated with Lyme disease in Ontario. It is possible that Lyme disease risk in suburban locations may be due to increasing development and forest fragmentation, maintenance of tick populations by small mammals and birds in local parks/trails, or larger lot sizes and outdoor recreational spaces (31-33). A recent study in Europe found that urban and peri-urban woodlands had a high density of nymphs, with a *Borrelia* infection prevalence around 10%, which suggests that these regions might pose an even higher risk for Lyme Borreliosis due to high human traffic and probability of tick encounters (33).

In Chapter 5, I further identified associations between Lyme disease and measures of higher socioeconomic status based on dimensions of the Ontario marginalization index, specifically the lowest quintiles of material deprivation, which is a measure of poverty and access to basic needs. High socioeconomic status is likely linked to larger properties sizes and access to parks and trails in suburban or peri-urban regions where individuals can become exposed to ticks, as discussed previously (28, 34). In my analyses, Lyme disease was also associated with lower ethnic

concentration, which is a measure of recent immigrants and visible minorities, although the mechanism behind this association requires additional investigation and may be related to rurality, access to care, risk/protective behaviours, knowledge of risk, or underlying socioeconomic factors. Interestingly, in the United States, Moon et al. found that disseminated Lyme disease was more strongly linked with lower socioeconomic status due to reduced access to care (35). Therefore, it is difficult to draw individual-level conclusions about Lyme disease risk from area-level associations. However, my thesis provides the first evidence of a link between Lyme disease and socioeconomic status in Canada as well as other population and neighbourhood characteristics.

8.3 Growing Risk for Lyme Disease in Ontario

Provincial surveillance data from 2010-2017 indicated that human Lyme disease incidence in Ontario has increased annually, with two public health units in eastern Ontario (LGL: Leeds, Grenville, and Lanark; KFL: Kingston, Frontenac, Lennox and Addington) having the highest cumulative incidence per 100,000 population (9, 20). The risk for Lyme disease in these regions has been documented over multiple years and has resulted in cessation of passive tick surveillance efforts (i.e., acceptance of public tick submissions) in 2014 because of evidence that *I. scapularis* populations were endemic in these regions (36). In Chapter 6, I showed that spatiotemporal clusters of *B. burgdorferi*-infected ticks and human Lyme disease cases were localized primarily in LGL and KFL, which is expected given the results from previous surveillance studies. However, I also detected more recent clusters of Lyme disease cases and *B. burgdorferi*-positive ticks in the western part of Ottawa, which interestingly were found to be spatial outliers as they consisted of dissemination areas (DA) with high numbers of reported cases and tick submissions surrounded

by other DAs of low infection. These regions represent locations along the Greenbelt zone, with several suburban and urban woodlands like conservation areas, forests, and local trails.

Results from our multi-year surveillance across eastern Ontario, presented in Chapter 7, indicated that, from 2017-2021, there was a narrowing of the gap between the density of *I. scapularis* ticks collected and the percentage of ticks testing positive for *B. burgdorferi* among surveillance sites in Ottawa and Kingston. In a recent analysis, Burrows et al. used a field indicator tool to determine the risk for Lyme disease at various sites in Ottawa (18). Most surveillance sites were classified as "high-stable", indicating the presence of reproducing tick populations in successive sampling cohorts, while other were classified as "emerging" (i.e., reproducing tick populations may be developing) or "low-stable" (i.e., *I. scapularis* present although immature instars indicative of reproducing population not detected) (18). Cumulatively, these results indicate that the risk for Lyme disease is present and growing in several regions in Ottawa, particularly at sites with stable tick populations and *B. burgdorferi* infection.

The *B. burgdorferi* genotypes detected in Ottawa and Kingston were very diverse but represented sequence types that were previously detected in the northeastern United States. For the analysis of bacterial genotypes, I had a small sample size; therefore, it is not possible to fully determine if founder effects have resulted in novel genotypes in eastern Ontario since the introduction of ticks in this region from neighbouring US states. However, the detection of similar *B. burgdorferi* genotypes in the northeastern United States and eastern Ontario is supported by the *I. scapularis* genetic analysis conducted by Talbot et al. which showed a patchy distribution of ticks resulting from the movement of animal hosts (7). *Borrelia burgdorferi* is likely introduced in Canada via

similar animal movements and infected ticks. The similarity in bacterial genotypes also raises potential questions regarding Lyme disease severity in Ontario since the sequence types found in the northeastern United States have been linked to disseminated stages of disease (37). However, the clinical link between *B. burgdorferi* genotypes and disease severity has yet to be assessed in a Canadian setting.

8.4 Emergence of Other Tick-Borne Pathogens

In Chapter 7, I presented results for the multi-year tick surveillance projects conducted at sentinel sites across eastern Ontario from 2017-2021. Molecular testing for tick-borne pathogens revealed no positive samples for *Babesia microti*, the causative agent of human babesiosis. *Babesia microti* has previously been detected in ticks from humans and companion animals in Ontario by Nelder et al.; however, at a very low prevalence and mostly in southern regions of Ontario (38). Similarly, in our surveillance sites, I did not detect positive specimens for deer tick virus lineage I or lineage II, which is consistent with the very low prevalence of this virus in ticks reported by previous studies (39, 40).

Tick-borne pathogens transmitted by *I. scapularis* ticks that we detected in eastern Ontario included *Borrelia miyamotoi*, and *Anaplasma phagocytophilum*, and the wildlife pathogen *Babesia odocoilei*. Of these, *B. miyamotoi* infection in ticks was found to be very low (annual prevalence ≤1%) and was only detected in the west end of Ottawa. It is possible that greater sampling efforts in Ottawa, including more sites within a small geographic radius and multiples years of surveillance, may have allowed us to detect the presence of this rare pathogen. The infection

prevalence of *B. miyamotoi* in Ontario has not increased substantially from earlier reports (38, 40-42). *Anaplasma phagocytophilum* was detected at moderate levels across eastern Ontario, although site-specific infection prevalence varied widely. *Anaplasma phagocytophilum,* the causative agent of human granulocytic anaplasmosis, has been detected in ticks across Ontario and generally follows a similar distribution as *B. burgdorferi*, with a higher prevalence in eastern Ontario (43). Serological testing has also indicated low *A. phagocytophilum* infection levels in humans (43).

The current risk for infection with other tick-borne pathogens is relatively low. However, there is still a level of risk in any region where *I. scapularis* is present. The increase in prevalence of *A. phagocytophilum* suggests this may be one of the more concerning pathogens, although it is possible that the apparent increase in tick infection prevalence may be a result of increased testing. Overall, my thesis provides a baseline for the prevalence of other tick-borne pathogens in eastern Ontario at a fine geographic scale, where individuals are most likely to encounter ticks.

8.5 Limitations and Research Recommendations

8.5.1 Application of Species Distribution Models for Risk Mapping

Species distribution models are widely used in ecology but have become increasingly popular for disease modelling. In the context of ticks and tick-borne pathogens, a quick literature search will yield numerous studies on the distribution of ticks and the effects of climate projections on the predicted species distribution. Maxent is a popular choice for species distribution modelling though it is subject to several limitations, the most important of which is the bias that may result

from use of presence-only data because occurrence points may have inherent biases in geographic space due to non-uniform sampling (44). These biases will persist in the modeling and evaluation phase, which also used presence data or splits the original data into training and testing sets (44). Furthermore, the use of presence-only data may generate more conservative distributions for the species because it is based on the species' realized niche, which may be subject to unmeasured factors such as barrier to dispersion, availability of animal hosts, etc. (45).

Given these limitations and the complexity of Maxent (many users often rely on default settings), an area of future investigation should focus on the comparison of various species distribution models for predicting the distribution of *I. scapularis* ticks. While some studies have used simulation data to compare modelling algorithms, the application of multiple methods to real data would help provide greater confidence in the predicted distributions of ticks and tick-borne pathogens as conclusions would not be based solely on one type of model (44). This is especially important since the model specifications have considerable impact on the resulting predicted distribution (6).

The variables selected for Maxent modelling also have significant effect on the predicted distribution due to the scale at which variables influence the species. For example, climate data is best for modelling large-scale distributions because climate does not vary locally, topographical data is suitable for large to small scale predictions, and satellite remote sensing data on land cover, land use, and vegetation index is best for small scale predictions (12). Ticks have limited mobility (though they can be transported large distances by birds and large mammals), and the colonization of suitable habitat occurs at a small geographic scale. However, limited data on micro-habitat

conditions and biotic factors (i.e., animal hosts) are available for wide use in species distribution modelling. Therefore, Maxent models should be used to make more general predictions about a species' potential distribution, while additional studies at the local level are needed to better understand the process of tick colonization.

8.5.2 Analysis of Risk Factors for Lyme Disease

Key research questions that I addressed in my thesis was to determine where Ontarian are exposed to ticks and what were some associated risk factors. I found elevated risk associated with home residence in predicted environmental risk areas and clustering of Lyme disease cases in various rural and urban locations in eastern Ontario. However, additional research is needed to further explore these associations by looking more directly at peri-domestic exposure to tick, for example the risk of encountering ticks in the backyard compared in other parts of the home neighbourhood. In my Chapter 5 and 6 analyses, I also found a strong link between Lyme disease and measures of rurality, which was not unexpected given the suitability of these environments for ticks, but to a lesser extent there was also a link between Lyme disease and urban/ suburban environments. This is an important finding that warrants additional research to understand the neighbourhood structure and features where ticks can be found. Urban development may also have an impact on *I. scapularis* occurrence and the overall risk for Lyme disease. I touch briefly on these concepts using area-level indicators for walkability, which is a multidimensional measure of various aspect of urban environments such as density of dwellings, connectivity by three-way intersections, and points of interest. Additional studies could explore these elements at a smaller neighbourhood scale.

My thesis also looked at associations between Lyme disease and socioeconomic risk factors. This has not previously been studied in Ontario (or other regions in Canada) and my thesis is among the first works that show evidence for this association. The mechanism of this association is unclear, as there is contradictory evidence on this topic. A hypothesis may be that higher socioeconomic status is related to larger property size in more newly developed regions (suburbs or peri-urban locations) where ticks are found. However, on the other hand severity of Lyme disease might vary with socioeconomic status based on factors such as access to care and delayed diagnosis. Furthermore, the link between ethnic concentration, a measure of recent immigrants and visible minorities, might also have an impact on Lyme disease exposure and diagnosis. While my thesis provides evidence for these links with Lyme disease, it is difficult to draw conclusions based on area-level associations. Additional studies are needed to further explore this at the individual level and to provide more insight into the possible mechanisms behind these associations.

8.5.3 Detection of Bacterial Genotypes and Tick-Borne Pathogens

In my analysis of *B. burgdorferi* genetic diversity and the molecular detection of other tick-borne pathogens, I encountered substantial limitations from low sample size. The low sample size was a result of limited number of specimens collected at various sites and in different years, low infection prevalence of pathogens, possible low yield of bacterial DNA from nymphs, and other processes that can increase DNA degradation (e.g., long term storage of collected specimens, freeze-thaw cycles during other testing, performance of PCR assays, etc.). While many of these limitations could not be completely avoided (unfortunate drawback of laboratory work), the results that I presented in my thesis provide very valuable information for public health risk assessment in the absence of human disease surveillance data. Lyme disease is the only nationally notifiable tick-

borne illness in Canada (other tick-borne illnesses are recently reportable in select provinces); therefore, assessing the wider human health risk associated with other tick-borne illnesses is not possible without clinical data. To fill critical knowledge gaps, additional disease surveillance systems and clinical studies are needed to determine the incidence of other tick-borne illnesses in Canada. Similarly, clinical evidence is essential to assess the impact of *B. burgdorferi* genotypes on Lyme disease severity and clinical manifestations within the Canadian context.

8.6 IMPLICATIONS FOR PUBLIC HEALTH

The work presented in this thesis fills critical knowledge gaps on the epidemiology of Lyme disease and other emerging tick-borne illnesses in Ontario and provides practical tools for public health knowledge users. First, the species distribution models for *I. scapularis* and *B. burgdorferi,* which I developed in this thesis, are the first 100-meter high-resolution predictive environmental risk models for LD in Ontario. They are a valuable tool for public health because the models show a heterogeneous risk for LD across the province, which can help inform the public about the likelihood of encountering ticks in different regions and to help tailor public health messaging in the highest risk-areas. The case-control study that I conducted further indicates that environmental risk models can be used to predict human LD outcomes and that residence in such risk areas is associated with an increase in LD.

From an ecological and research standpoint, these species distribution models, which were developed using satellite remote sensing data on land cover and regional climate averages, can be generalized to other areas across Canada where similar environmental parameters are available

and to help understand the influence of abiotic and biotic factors driving the spread of *I. scapularis* and *B. burgdorferi* in new habitats. Importantly, my models have been used to identify new tick surveillance sites in regions predicted to be moderately to highly suitable for blacklegged ticks and to identify local sites where the density of ticks is expected to increase (e.g., suitable tick habitat in the process of colonization).

In this thesis, I also provide the first evidence for socioecological risk factors for LD in a Canadian context. While many individuals are increasingly aware of the risk of encountering ticks in forest habitats and during outdoor activities such as camping or hiking, fewer may be aware of the risk within their communities. The identification of socioeconomic risk factors for LD such as higher indices of wealth and lower urbanity may be linked to increased LD risk, presumably due to larger property sizes in suburban and rural regions, new home development and land parcellation, and larger proportion of parks, trails, and green spaces within the community. Increased public health messaging regarding the risk of locally acquired LD can be enhanced through community outreach, particularly in suburban regions of larger metropolitan centres such as Ottawa where clusters of human LD cases have been identified. Additionally, landscape management options can be targeted to such high-risk communities to mitigate the risk of tick exposures. More research is needed to fully understand the association between socioeconomic factors and LD; however, these results show the importance of LD risk management from socioeconomic, behavioural, and environmental perspectives.

Lastly, in this thesis, I provide a multi-year assessment of the prevalence of other tick-borne pathogens that are transmitted by the blacklegged tick. With the rapid spread of *I. scapularis* across

Ontario and other regions in Canada, there is a growing public health concern linked to the emergence of other tick-borne illnesses. The pathogen surveillance results presented in Chapter 7 have been shared with local public health units, PHO, and PHAC to monitor the emergence of tick-borne pathogens, which has increased over the years. This work, in conjunction with other veterinary and passive tick surveillance efforts across the province, has had a notable impact on public health with the recent announcement on July 1st, 2023, by the Government of Ontario to include Babesiosis, Anaplasmosis, and Powassan Virus as reportable diseases of public health significance in Ontario (46). The pathogen surveillance results also serve as a valuable baseline for tracking the growing prevalence of other tick-borne pathogens spread by *I. scapularis* across Ontario.

8.7 CONCLUSIONS

My thesis used a multidisciplinary approach to assess the risk for Lyme disease in eastern Ontario. I used ecological methods to develop species distribution models for *I. scapularis* and *B. burgdorferi* to provide updated environmental risk maps for Lyme disease. The utility of these maps for predicting tick abundance and, by proxy, human risk for Lyme disease has been assessed by other studies. I also employed the use of these maps in epidemiological studies to determine if residence in high-risk areas increases the odds of Lyme disease as well as to assign tick exposure locations to the most biologically probable location based on habitat suitability for ticks. The species distribution models that I generated in my thesis provide important information on the locations where individuals are most likely to encounter ticks, which is especially essential in regions that have limited surveillance in place or are not considered current risk areas. The maps

can also be used to target disease prevention and control strategies for individuals living in the highest-risk regions.

My thesis also confirms that individuals are exposed to ticks near the home in areas where tick populations are still emerging. The preliminary evidence of peri-domestic tick exposure raises interesting questions on Lyme disease risk, particularly in urban and suburban environments, that warrant additional research. My spatiotemporal cluster analysis further indicates that the range front of *I. scapularis* tick populations is expanding into new regions in a relatively short time frame, with more urban locations in the Ottawa region becoming suitable for tick colonization. These regions pose a significant public health risk as stable levels of *B. burgdorferi* infection in ticks has been detected over multiple years as well as an increase in the prevalence of multiple tick-borne pathogens that cause human disease or animal disease including *B. burgdorferi*, *B. miyamotoi*, *A. phagocytophilum*, and *B. odocoilei*.

Finally, in my thesis I also provide evidence for socioeconomic determinants of Lyme disease. The link between Lyme disease and socioeconomic risk factors is understudied in Canada but is important for identifying populations that are at higher risk of contracting Lyme disease or other tick-borne illnesses. My analyses provide evidence for an area-level association, which should be confirmed by additional studies, although the findings indicate that risk for Lyme disease is not uniform across eastern Ontario.

Overall, this thesis highlights the importance of devising appropriate risk mitigation strategies for different populations and tailoring public health messaging for the specific regional risk. My thesis

also shows the importance of ongoing Lyme disease surveillance, particularly for emerging tick-borne illnesses where human disease data is limited, and the necessity of fine-scale studies to further identify neighbourhood-level patterns and socioecological determinants of Lyme disease.

8.8 CHAPTER REFERENCES

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9

9 APPENDICES

9.1 CHAPTER 5 APPENDIX

In Chapter 5, I present the multivariable analysis of area-level neighbourhood and socioeconomic risk factors for LD. In Appendix 9.1, I also include the univariable analyses for all variables considered in the model building stage.

Appendix 9.1 Univariable conditional logistic regression analyses of area-level neighbourhood and socio-economic risk factors for Lyme disease in southeastern Ontario, Canada (2014-2018).

VARIABLE	MATCHED			
	OR	**95% CI lower**	**95% CI upper**	**P-value**
NEIGHBOURHOOD				
Environmental risk for LD Low	reference	—	—	<.0001
Environmental risk for LD High	2.400	2.183	2.639	
Neighbourhood walk score 1 (low)	4.126	3.229	5.271	<.0001
Neighbourhood walk score 2	1.349	1.050	1.732	
Neighbourhood walk score 3	1.044	0.810	1.347	
Neighbourhood walk score 4	0.939	0.694	1.250	
Neighbourhood walk score 5 (high)	reference	—	—	

Neighbourhood transit score 1 (low)	2.905	2.196	3.845	<.0001
Neighbourhood transit score 2	1.212	0.920	1.598	
Neighbourhood transit score 3	0.982	0.739	1.304	
Neighbourhood transit score 4	1.076	0.780	1.483	
Neighbourhood transit score 5 (high)	reference	—	—	
Proportion of provincial/municipal parks	1.034	1.031	1.036	<.0001
Trail length	1.020	1.010	1.027	<.0001
Rurality index ≤ 39 (urban)	reference	—	—	<.0001
Rurality index > 40 (rural)	2.167	1.843	2.548	
Residence PHU >1,000,000 population	reference	—	—	<.0001
Residence PHU 250,000 – 999,999 population	2.691	2.350	3.082	
Residence PHU <250,000 population	10.376	9.037	11.914	
Population density	0.999	0.989	0.999	<.0001
SOCIOECONOMIC				
Neighbourhood income quintile 1 (low)	reference	—	—	<.0001
Neighbourhood income quintile 2	1.290	1.103	1.509	
Neighbourhood income quintile 3	1.820	1.569	2.112	
Neighbourhood income quintile 4	1.694	1.457	1.969	
Neighbourhood income quintile 5 (high)	2.092	1.811	2.418	
Residential instability quintile 1 (low)	reference	—	—	<.0001
Residential instability quintile 2	1.765	1.544	2.018	
Residential instability quintile 3	1.884	1.647	2.155	
Residential instability quintile 4	1.212	1.048	1.402	
Residential instability quintile 5 (high)	0.845	0.728	0.979	
Material deprivation quintile 1 (low)	2.303	1.974	2.687	<.0001
Material deprivation quintile 2	2.360	2.019	2.758	
Material deprivation quintile 3	1.849	1.572	2.176	
Material deprivation quintile 4	1.611	1.360	1.907	
Material deprivation quintile 5 (high)	reference	—	—	
Dependency quintile 1 (low)	reference	—	—	<.0001
Dependency quintile 2	1.439	1.241	1.668	
Dependency quintile 3	1.880	1.624	2.176	
Dependency quintile 4	2.303	1.911	2.541	
Dependency quintile 5 (high)	3.202	2.787	3.679	
Ethnic concentration quintile 1 (low)	12.776	10.686	15.276	<.0001
Ethnic concentration quintile 2	7.190	5.994	8.623	
Ethnic concentration quintile 3	4.462	3.705	5.374	
Ethnic concentration quintile 4	3.199	2.647	3.866	
Ethnic concentration quintile 5 (high)	reference	—	—	

9.2 CHAPTER 6 APPENDIX

In Chapter 6, I present the multivariable negative binomial regression analysis of area-level neighbourhood and socioeconomic risk factors for LD. In Appendix 9.2, I also include the univariable analyses for all variables considered in the model building stage.

Appendix 9.2 Univariable negative binomial regression analyses of socioecological factors associated with Lyme disease case counts in eastern Ontario, Canada (2010-2017).

VARIABLE	Estimate	Standard Error	95% CI lower	95% CI upper	P-value
Total *I. scapularis* ticks	0.0059	0.0072	-0.0083	0.0201	0.4185
Total *I. scapularis* nymphs	0.1760	0.0907	-0.0017	0.3537	0.0523
Total *B. burgdorferi*-positive ticks	0.0833	0.0395	0.0059	0.1607	0.0350
Proportion of treed land	0.0327	0.0034	0.0261	0.0394	<.0001
Proportion of infrastructure	-0.0118	0.0012	-0.0142	-0.0094	<.0001
Neighbourhood walk score 1 (low)	reference	—	—	—	<.0001
Neighbourhood walk score 2	-0.7177	0.1655	-1.0421	-0.3933	
Neighbourhood walk score 3	-1.8718	0.2892	-2.4386	-1.3050	
Neighbourhood walk score 4	-2.6570	0.4969	-3.6308	-1.6831	
Neighbourhood walk score 5 (high)	-2.6350	0.2596	-3.1439	-2.1261	
Commute to work (minutes)	0.1854	0.0843	0.0202	0.3507	0.0279
Population density per km2	-0.0003	0.0001	-0.0004	-0.0002	<.0001
Residential instability	-0.1577	0.0235	-0.2038	-0.1116	<.0001
Material deprivation	-0.0573	0.0771	-0.2083	0.0938	0.4575
Dependency	0.3378	0.0408	0.2578	0.4177	<.0001
Ethnic concentration	-1.2309	0.2495	-1.7199	-0.7418	<.0001