The impact of abiotic and biotic factors on the tick-host-pathogen disease systems in Canada

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December 2023

A thesis submitted to the University of Ottawa and McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy – Cotutelle in Biology

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At the start of my degree, I lost a pivotal person in my life. Your life lessons on tenacity, ambition, and empathy carried me through to the end.

I dedicate this to you.

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Abstract

Emerging or re-emerging tick-borne pathogens are expected to increase in prevalence and become more geographically widespread in Canada. *Borrelia burgdorferi*, the bacterium causing Lyme disease, is the most common vector-borne pathogen in North America, but additional tick-borne pathogens have started to be detected more frequently through surveillance efforts in Canada. The spread and transmission of these tick-borne pathogens are modulated by changes in the abundance and distribution of tick and host populations. Abiotic factors, such as temperature, precipitation, and snow, may affect tick and host abundances as well as host dispersal. Furthermore, biotic factors, such as the abundance and diversity of hosts, may alter tick abundance and consequent tick-borne disease risk. In this dissertation, I assess the historical associations and spatiotemporal changes of the tick vectors, hosts, and pathogens in Canada as well as the impact of abiotic and biotic factors on these key players.

In Chapter 1, I present the first systematic assessment of the literature that identifies historical associations and spatiotemporal changes in the tick-host-pathogen disease systems in Canada over broad spatial and temporal scales. *Borrelia burgdorferi* was the most detected tick-borne pathogen and *Ixodes scapularis* harboured the greatest number of tick-borne pathogens. Several spatial outliers of high pathogen presence in ticks in addition to five spatiotemporal clusters were identified, which were located in areas of southern Canada with long-established tick populations. In addition, six spatiotemporal clusters of high pathogen presence were also identified, with four clusters associated with passive surveillance and two clusters related to active surveillance.

In chapter 2, I concurrently evaluated high-resolution environmental and host-related factors to determine the relative impacts of abiotic and biotic factors on questing *I. scapularis* abundance in Ontario and Quebec. High-resolution abiotic factors were derived from remote sensing satellite imagery and meteorological towers, while biotic factors related to mammal hosts were derived from active surveillance data that I collected in the field. Important abiotic and biotic drivers of questing *I. scapularis* abundance were identified, which included monthly mean precipitation, accumulated snow, and mammal species richness. These results demonstrate the need to incorporate host active surveillance data with high-resolution environmental factors

when trying to determine the key drivers impacting the abundance and distribution of tick populations and tick-borne pathogens.

In Chapter 3, I analyzed the presence and prevalence of multiple tick-borne pathogens extracted from tick and small mammal specimens collected during field surveys in Ontario and Quebec. Three pathogen species were detected in ticks, which included *Babesia odocoilei* and *B. burgdorferi* in *I. scapularis* as well as *Rickettsia rickettsii* in *Haemaphysalis leporispalustris*. In small mammal hosts, three pathogen species were detected including *B. odocoilei* in one shrew, *B. microti* in one deer mouse, and *Hepatozoon* in one deer mouse and one white-footed mouse. My findings provide evidence that emerging or re-emerging tick-borne pathogens may be present outside currently defined risk areas identified by surveillance efforts in Canada.

Finally, in chapter 4, I examined the effect of biotic factors related to *I. scapularis* and mammal hosts on the presence, prevalence, and diversity of pathogens in Ontario and Quebec using data from field surveys. Local infection prevalence ranged from 0% to 25.4% in questing ticks and from 0% to 16.7% in small mammal hosts. Local pathogen presence and prevalence were not impacted by *I. scapularis* abundance nor the abundance and diversity of mammal hosts. However, mammal species richness was a key predictor of the number of pathogen species.

Collectively, my dissertation provides insight into the historical and contemporary relationships between ticks, hosts, and pathogens in Canada. My results demonstrate that additional tick species such as *H. leporispalustris* may be of public health importance due to their ability to maintain pathogens within the environment without needing a host. In addition, certain emerging or re-emerging tick-borne pathogens, such as *B. odocoilei* and *R. rickettsii*, were detected outside of currently defined risk areas in southeastern Quebec, which may impact future surveillance efforts in these regions. Furthermore, this work highlights the need for proactive and comprehensive surveillance efforts that test questing and feeding ticks of all life stages and species, as well as their hosts in areas outside currently defined risk areas or those targeted by sentinel surveillance to better determine the spread, transmission, and co-occurrence of tick-borne pathogens in Canada.

Résumé

Les agents pathogènes émergents ou ré-émergents transmis par les tiques devraient voir leur incidence augmenter et se répandre géographiquement au Canada. *Borrelia burgdorferi*, la bactérie responsable pour la maladie de Lyme, est l'agent pathogène à transmission vectorielle le plus courant en Amérique du Nord, mais d'autres agents pathogènes transmis par les tiques ont commencé à être détectés plus fréquemment dans le cadre de la surveillance au Canada. La propagation et la transmission de ces agents pathogènes transmis par les tiques sont influencées par les changements de l'abondance et la distribution des populations de tiques et d'hôtes. Les facteurs abiotiques, tels que la température, les précipitations et la neige, affectent directement les abondances des tiques et des hôtes ainsi que la dispersion des hôtes. En outre, les facteurs biotiques, tels que l'abondance et la diversité des hôtes, peuvent modifier l'abondance des tiques et, par conséquent, le risque de maladies transmises par les tiques. Dans cette thèse, j'évalue les associations historiques et les tendances spatio-temporelles des tiques vectrices, des hôtes et des agents pathogènes au Canada, ainsi que l'impact des facteurs abiotiques et biotiques sur ces acteurs clés.

Dans chapitre 1, je présente la première évaluation systématique de la littérature qui identifie les associations historiques et les tendances spatio-temporelles dans les systèmes de tiques-hôtes-pathogènes au Canada sur de larges échelles spatiales et temporelles. *Borrelia burgdorferi* est l'agent pathogène le plus détecté et *Ixodes scapularis* hébergent le plus grand nombre d'agents pathogènes. Plusieurs zones spatiales avec une forte présence d'agents pathogènes dans les tiques ont été identifiés, ainsi que cinq regroupements spatio-temporels qui se trouvaient dans des régions du sud du Canada où les populations de tiques sont établies de longue date. De plus, six regroupements spatio-temporels avec une forte présence d'agents pathogènes ont également été identifiés, dont quatre sont associés à la surveillance passive et deux à la surveillance active.

Dans chapitre 2, j'ai évalué simultanément des facteurs environnementaux à haute résolution et des facteurs liés aux hôtes afin de déterminer les impacts relatifs des facteurs abiotiques et biotiques sur l'abondance d'*I. scapularis* en quête d'hôte en Ontario et au Québec. Les facteurs abiotiques à haute résolution ont été dérivés de l'imagerie satellite de télédétection et des tours météorologiques, tandis que les facteurs biotiques liés aux mammifères hôtes ont été

dérivés des données de surveillance active que j'ai recueillies sur le terrain. D'importants facteurs abiotiques et biotiques de l'abondance d'*I. scapularis* en quête d'hôte ont été identifiés, notamment les précipitations moyennes mensuelles, la neige accumulée et la richesse des espèces de mammifères. Ces résultats démontrent la nécessité d'incorporer les données de surveillance active des hôtes avec des facteurs environnementaux à haute résolution lorsqu'on essaie de déterminer les facteurs clés ayant un impact sur l'abondance et la distribution des populations de tiques et des agents pathogènes transmis par les tiques.

Dans chapitre 3, j'ai analysé la présence et la prévalence de plusieurs agents pathogènes transmis par les tiques provenant de spécimens de tiques et de petits mammifères recueillis lors de notre étude sur le terrain en Ontario et au Québec. Trois espèces d'agents pathogènes ont été détectées chez les tiques, dont *Babesia odocoilei* et *B. burgdorferi* chez *I. scapularis*, ainsi que *Rickettsia rickettsii* chez *Haemaphysalis leporispalustris*. Chez les petits mammifères, trois espèces d'agents pathogènes ont été détectées incluant *B. odocoilei* chez une musaraigne, *B. microti* chez une souris sylvestre et *Hepatozoon* chez une souris sylvestre et une souris à pattes blanches. Mes résultats démontrent que des agents pathogènes émergents ou ré-émergents transmis par les tiques peuvent être présents en dehors des zones à risques actuellement définies par les efforts de surveillance au Canada.

Enfin, dans chapitre 4, j'ai examiné l'effet des facteurs biotiques liés à *I. scapularis* et aux mammifères hôtes sur la présence, la prévalence et la diversité des agents pathogènes en Ontario et au Québec à l'aide de données provenant de recensement sur le terrain. La prévalence des infections locales variait de 0% à 25,4% chez les tiques en quête d'hôte et de 0% à 16,7% chez les petits mammifères hôtes. La présence et la prévalence des agents pathogènes locaux n'ont pas été influencées par l'abondance d'*I. scapularis* ni par l'abondance et la diversité des mammifères hôtes. Cependant, la richesse en espèces de mammifères était un facteur prédictif clé du nombre d'espèces de pathogènes.

Collectivement, ma thèse synthétise les connaissances sur les relations historiques et contemporaines entre les tiques, les hôtes et les agents pathogènes au Canada. Mes résultats démontrent que la prise en compte d'autres espèces de tiques, telles que *H. leporispalustris*, peut être importante pour la santé publique en raison de leur capacité à maintenir des agents pathogènes dans l'environnement sans avoir besoin d'un hôte. En outre, certains pathogènes transmis par les tiques, tels que *B. odocoilei* et *R. rickettsii*, ont été détectés en dehors des zones à

risque définies dans le sud-est du Québec, ce qui pourrait avoir un impact sur les efforts de surveillance futurs dans ces régions. De plus, ce travail met en évidence la nécessité d'efforts de surveillance proactifs et complets qui testent les tiques en quête d'hôte et les tiques nourricières de tous les stades de vie et de toutes les espèces, ainsi que leurs hôtes dans des régions situées en dehors des zones à risques définies ou de celles ciblées par la surveillance sentinelle, afin de mieux déterminer la propagation, la transmission et la cooccurrence des agents pathogènes transmis par les tiques au Canada.

Traditional Indigenous Territory Acknowledgement

As a settler and visitor on Turtle Island, I have the responsibility to acknowledge the lands where I conducted my research as well as those of my educational institutions. This acknowledgement is only a small step towards reconciliation with Indigenous peoples who continue to struggle for equity and justice in a land that was taken from them.

I would like to honour and respect the unceded traditional lands of several Indigenous peoples that I accessed during my research, which include the lands of the Abenaki, Anishinaabeg, Attiwonderonk, Haudenosaunee, Mississauga, Kanien'kehá:ka, Nanrantsouak, Wabanaki, and Huron-Wendat. I recognize that these peoples are the custodians and stewards of these traditional lands and waters. I would also like to pay my respects to the non-human beings that I removed from these lands as part of my research.

In addition, I acknowledge the unceded traditional lands that are currently occupied by each university. McGill University (Tiohtiá:ke/Montreal) is situated on the traditional territory of the Kanien'kehá:ka, which has served as a site of meeting and exchange among Indigenous peoples including the Haudenosaunee and Anishinaabeg. The University of Ottawa (Odawaa/Ottawa) is situated on the traditional territory of the Anishinaabe Algonquin Nation.

Today, these lands are home to generations of diverse populations of Indigenous and non-Indigenous peoples. I respect the ongoing connections with the past, present, and future in our ongoing relationships with Indigenous and non-Indigenous peoples within these communities. Settlers, including myself, should continue to work towards educating ourselves to decolonize our individual ideological frameworks, as a step towards dismantling our currently oppressive systems.

Acknowledgements

I still can't believe I've made it to the end of this PhD. It took a lot of help and support throughout the years to get here.

To my thesis co-supervisors: Dr. Jeremy Kerr and Dr. Virginie Millien. Thank you both for not falling off this roller coaster ride with me, even through all the twists, turns, and loop-the-loops that either me or this project threw your way. I am extremely grateful for both of you providing me with this experience. Dr. Kerr: Your knowledge and experience related to science communication was an inspiration for me. It triggered a cascade of cool opportunities and fulfilling experiences throughout my PhD that allowed me to express my passion for the fun and fascinating world of ticks. Dr. Millien: You have helped me develop from a young and apprehensive undergraduate student to an independent and confident disease ecologist. By providing me with the space to pursue various research interests, you allowed me to discover my passions, explore some unusual research ideas, and learn from my inevitable scientific failures throughout the years.

Thank you to my thesis committee members, Dr. Vincent Careau and Dr. Gregor Fussmann, for their advice and great feedback throughout the years. I would also like to thank my examiners, Dr. Vincent Careau, Dr. Vett Lloyd, Dr. Jessica Gillung, and Dr. Albrecht Schulte-Hostedde, for their time and involvement in the completion of this thesis.

Thanks to the past and present members of the Millien lab (Fred, Jihane, Jonathan, Erica, Kari, and Kelsey) and the Kerr lab (Peter, Sarah, Olga, Cat, Anouk, Susan, Matt, Alexa, Gabby, and Tiffany)! Your conversations, feedback, and insights helped me get through this PhD. I also learned some awesome things along the way about the animals that were pivotal in your projects.

My PhD would literally not have been completed without this next set of individuals. To the landowners and stakeholders: I express my upmost appreciation for allowing me access to your properties for field surveys. To my field assistants (Daniella Cross, Connia Ren, Dania Shaban, Ellen Bidulka and Jihane Benbahtane): You all stuck through intense fieldwork even when the weather, environment, or fatigue were making things difficult. To Anthony Howell: Thank you

for showing me how to efficiently dissect my specimens for fieldwork as well as providing them with safe housing at the Redpath Museum making them accessible to future researchers. I'd also like to thank Robbin Lindsay and Antonia Dibernardo for sharing the protocols used by the National Microbiology Lab that were used for the DNA extractions and PCR testing of my field specimens. A very special shout out to Justin Wood and the Geneticks team! You helped my research goals come to fruition and discussed my inquiries every step of the way through genetic and pathogen testing – it was greatly appreciated.

I was able to complete this PhD with funding from a lot of different sources including McGill University, the University of Ottawa, Hydro-Québec, the Natural Sciences and Engineering Research Council of Canada, les Fonds de Recherche du Québec – Nature et Technologies, and the Anne Vallée Ecological Fund.

Thank you to the teams at STEMM Diversity @ McGill and the BioMatters Magazine! I had an absolute blast working with all of you. You gave me the space to slowly develop and train skills that have become essential to the way I pursue and communicate science endeavours.

Three inspiring female scientists helped me get through the mentally tough parts of grad school. Nat, Jess, and Victoria – you are each true beacons of light that are also amazing scientists! You each allowed me to exchange research ideas, vent when things were frustrating me, laugh when I really needed it, play games together on the Switch, and gush about our pets. I predict only great things for each of you as you finish your PhDs!

Special shout out to Sarah who was the ultimate cheerleader who helped pump me up through all the years of school and grad school! Your positive messages got me through the panic attacks and overwhelming situations. I imagine little kindergarteners Kirsten and Sarah would be so proud of where we are today!

My dream team of Carole, Andrew, and Marjorie: Thank you for keeping my mind and body in working condition throughout my years in grad school. I wouldn't want to imagine what it would be like without you.

À l’équipe zoonoses de l’INSPQ (surtout Anne, Ariane et Alejandra) : Merci pour les encouragements et le temps supplémentaire à la fin de mon doctorat pour bien le compléter – c’est extrêmement apprécié !

To my family: I cannot thank you enough for how you’ve helped me during this degree.
Aux minous, Cassini et Caprie – Travailler dans la présence de deux chatons est un peu compliqué, mais on a survécu après quelques mois ! Maintenant, j’ai des superviseurs chez nous pour s’assurer que les projets avancent et qui miaulent quand ce n’est pas à leurs standards. Jokes aside – these two cats have helped enormously with my mental health throughout this degree, and I don’t know what I’d do without your shenanigans.

Seb – Durant mon doctorat, tu m’as aidé à travers des moments les plus difficiles avec de l’encouragement et tu étais là pour célébrer les bons moments. Tes capacités de réflexion critique et de résolution de problèmes m’ont aidé énormément quand que j’étais bloqué dans mes pensées. Ton amour, motivation et patience au fil du temps m’ont réconforté jusqu’à la fin. Merci pour ton support constant pour moi !

Mom – You were a pillar of support that was present during the wildest adventures of this degree from the really lows to the highs. Of all the people I talk to about ticks, you’ve probably heard me discuss them for the longest amount of time, but you never changed subjects and always listened to me with a smile and patience. I also greatly appreciated all your hours of help during fieldwork, especially for the long car drives. We had some hilarious moments along the way like understanding how important it is to remember your shoes for fieldwork!

And finally, thank you to the ticks and small mammals that were a crucial part of this research! You fascinate me so much, and your pivotal presence is acknowledged and respected.

Preface

Contribution to original knowledge

In this dissertation, a variety of methods such as a meta-analysis, spatial analyses, field surveys, and genetic analyses were used to evaluate the impacts of abiotic and biotic factors on the tick-host-pathogen disease systems in Canada. My research investigated the historical associations and spatiotemporal changes of the tick vectors, hosts, and pathogens in Canada as well as the local abiotic and biotic mechanisms influencing these key players. My findings identified specific abiotic and biotic factors that influenced questing *Ixodes scapularis* abundances as well as the spread and transmission of pathogens in tick and host populations in Canada.

Chapter 1 | Since the early 20th century, ticks and their pathogens have been detected during field surveys in Canada. Current sentinel surveillance efforts in Canada primarily focus on tracking the spread and transmission of *Borrelia burgdorferi* sensu stricto, one of the genospecies causing Lyme disease, in *I. scapularis* populations. As a result, it is challenging to determine the geographic extent and temporal progression of additional tick-borne pathogens of concern that may not be reportable to public health agencies. Here, we assessed the historical tick-host-pathogen associations and identified spatiotemporal changes of high pathogen presence in ticks in Canada using data extracted from a systematic review of the literature. Differences in tick-pathogen associations may be related to the feeding habits of ticks, with generalist tick species, such as *I. scapularis*, harbouring a greater number of tick-borne pathogens compared to specialist tick species. In addition, several spatial outliers and five spatiotemporal clusters of high pathogen presence in ticks were found in Canada, which primarily coincide with areas with long-established tick populations. Finally, six spatiotemporal clusters of pathogen presence were identified, with a greater number of clusters found via passive surveillance than active surveillance. This study represents the first systematic assessment of the literature that identifies historical associations and spatiotemporal changes in the tick-host-pathogen disease systems in Canada over broad spatial and temporal scales.

Chapter 2 | Recently, an Earth observation-informed framework was designed that combines high-resolution environment data with traditional vector surveillance data for climate-related risk assessments and mapping of disease vectors, such as *I. scapularis*. However, this current framework does not incorporate information related to vertebrate hosts, which are important predictors of tick populations. In this chapter, we incorporated high-resolution environmental and host-related factors to evaluate the combined impact of abiotic and biotic factors on questing *I. scapularis* abundance along the northward edge of their range in Ontario and Quebec. Combinations of abiotic and biotic factors were identified as important drivers of abundances of questing *I. scapularis*, which included monthly mean precipitation, accumulated snow, and mammal species richness. Our study is the first to combine high-resolution environmental and host-related factors to determine the mechanisms driving questing *I. scapularis* abundance to better anticipate the spread of tick populations and their pathogens in Canada.

Chapter 3 | Current surveillance efforts in Canada focus on a subset of emerging tick-borne pathogens such as *Anaplasma phagocytophilum*, *Babesia* species, and *Borrelia* species, which are found at a higher prevalence in tick and host populations. Yet, certain emerging or re-emerging tick-borne pathogens may be rare, but present in Canada, with limited knowledge of their geographic range and degree of establishment. Using pathogen testing, the presence of pathogens was assessed in tick and small mammal specimens that were collected during field surveys in Ontario and Quebec. Three different pathogen species were detected in tick specimens, which included *Babesia odocoilei*, *Borrelia burgdorferi*, and *Rickettsia rickettsii*. In small mammal hosts, three pathogens were also detected, namely *Babesia odocoilei*, *B. microti*, and *Hepatozoon*. Of note, *B. odocoilei* and *R. rickettsii* were found at localities outside their known geographic range limits in southeastern Quebec. We also provide evidence of transovarial transmission of *R. rickettsii* from adult female to larval *Haemaphysalis leporispalustris*. To our knowledge, the detection of *R. rickettsii* is the first time it has been reported in Quebec. Finally, *B. odocoilei* was also detected for the first time in a shrew in Canada, which may be indicative of this host contributing to the cryptid transmission of this pathogen.

Chapter 4 | Pathogen spread and transmission may be modulated by changes in the abundance and distribution of tick and host populations in Canada. In this chapter, we assessed the

relationships between local pathogen presence, prevalence, and diversity with *I. scapularis* abundance and the abundance and diversity of mammal hosts at sites of distinct levels of disease risk in Ontario and Quebec. Neither the abundance of *I. scapularis* nor the abundance and diversity of mammal hosts altered local pathogen presence and prevalence. However, greater mammal species richness within study locations was associated with a greater diversity of pathogens, with up to three pathogen species being detected locally. As a result, the co-occurrence of multiple tick-borne pathogens may lead to an increased risk of co-infections in local tick, wildlife, and human populations. This study demonstrates the need for future surveillance efforts that test questing and feeding *I. scapularis* of all life stages, as well as their hosts to better determine the spread, transmission, and co-occurrence of tick-borne pathogens in Canada.

Contribution of authors

This dissertation is presented in a manuscript-based format and composed of my original work, where each chapter consists of an individual manuscript that has been published or is intended for publication in a peer-reviewed, academic journal. This research was approved by the McGill University Animal Care Committee (AUP #2019-8086), the Ministère des Forêts, de la Faune et des Parcs (SEG permit No. 2019-06-04-008-00-S-F), and the Ontario Ministry of Natural Resources and Forestry (WSCA No. 1093495). To be concise, I refer to co-authors through their initials: Kirsten E. Crandall (KEC), Dr. Jeremy T. Kerr (JTK), and Dr. Virginie Millien (VM).

Chapter 1 | This manuscript was published in *Zoonoses and Public Health* in November 2023, with the following citation:

> Crandall KE, Millien V, Kerr JT. Historical associations and spatiotemporal changes of pathogen presence in ticks in Canada: A systematic review. *Zoonoses and Public Health*, 00, 1-16. doi: 10.1111/zph.13093

As stated in the publication, KEC was responsible for the literature review, data collection, formal analysis, visualizations, and writing of the manuscript. VM and JTK jointly supervised the research, helped design the analyses, and contributed to the writing.

Chapter 2 | This manuscript is currently under review at *Ecology and Evolution*.

> Crandall KE, Millien V, Kerr JT. High-resolution environmental and host-related factors impacting questing *Ixodes scapularis* at their northern range edge.

KEC was the lead investigator responsible for data collection, formal analysis, visualizations, and writing the manuscript. VM and JTK jointly supervised the research, helped design the analyses, and contributed to the writing.

Chapter 3 | This manuscript was published in *Vector-Borne and Zoonotic Diseases* in November 2022, with the following citation:

Crandall KE, Kerr JT, Millien V. 2022. Emerging tick-borne pathogens in Central Canada: Recent detections of *Babesia odocoilei* and *Rickettsia rickettsii*. *Vector-Borne and Zoonotic Diseases* 11(22):535-544. doi: 10.1089/vbz.2022.0036.

As stated in the publication, KEC was the lead investigator responsible for the data collection, formal analysis, visualizations, and writing the manuscript. JTK and VM jointly supervised the research, helped design analyses, and contributed to writing. Robbin Lindsay and Antonia Dibernardo shared the testing protocols used by the National Microbiology Lab for DNA extraction and PCR testing. Genetic analyses were performed by Geneticks, Inc. The publishers (Mary Ann Liebert Inc.) have granted permission to use the publication in this dissertation under license ID 1349934-1.

Chapter 4 | This manuscript is currently under review at *Frontiers in Parasitology – Epidemiology and Ecology*.

Crandall KE, Kerr JT, Millien V. Pathogen presence, prevalence, and diversity in *Ixodes scapularis* and mammal hosts at their expanding northern range limits.

KEC was the lead investigator responsible for data collection, formal analysis, visualizations, and writing the manuscript. JTK and VM jointly supervised the research, helped design the analyses, and contributed to the writing. Robbin Lindsay and Antonia Dibernardo shared the testing protocols used by the National Microbiology Lab for DNA extraction and PCR testing. Genetic analyses were performed by Geneticks, Inc.

Finally, I am grateful to have had the opportunity to work with inspiring peers to help write additional publications during my PhD related to equity, diversity, and inclusion issues in the STEMM fields:

Dei-Sharpe J, Crandall K*, Ford J, Jreidini N, Ozyonum E, Sidibé H. Under Review. Addressing biases through decoloniality and belonging in STEM: A cross-disciplinary dialogue. In *Handbook on Equity-Oriented, Discipline-Based STEM Education Research*. Springer Nature.

Ford J, Jreidini N, Crandall KE*, Sanderson S, Xu CCY. 2021. Promoting equity and inclusion with student-driven initiatives. *Trends in Ecology and Evolution* 36(12):1063-1066. doi: 10.1016/j.tree.2021.08.013.

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Figure 1.1. The reviewed studies encompassed wide spatial and temporal ranges. Studies were conducted in all the Canadian provinces: British Columbia (n = 15), Alberta (n = 10), Saskatchewan (n = 10), Manitoba (n = 10), Ontario (n = 44), Quebec (n = 10), New Brunswick (n = 8), Nova Scotia (n = 9), Prince Edward Island (n = 2), and Newfoundland and Labrador (n = 5). No studies were conducted in the Canadian territories. Studies ranged from 1960 to 2020, which were categorized into temporal bins 1–13: 1960–1964 (n = 4), 1965–1969 (n = 4), 1970–1974 (n = 0), 1975–1979 (n = 0), 1980–1984 (n = 1), 1985–1989 (n = 1), 1990–1994 (n = 7), 1995–1999 (n = 5), 2000–2004 (n = 7), 2005–2009 (n = 15), 2010–2014 (n = 22), 2015–2019 (n = 23), and 2020 (n = 1).

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detected based on different types of surveillance (active, passive, or combined) while accounting for temporal period. Significant clusters of pathogen presence were detected by active surveillance in eastern Ontario from 2005 to 2019 (Cluster 1) as well as in southeastern Alberta and southwestern Saskatchewan from 2005 to 2014 (Cluster 4). Passive surveillance detected clusters of pathogen presence in ticks in southeastern Ontario from 1995 to 2020 (Cluster 2), in southern British Columbia from 2000 to 2009 (Cluster 3), in parts of the Atlantic provinces from 1990 to 2019 (Cluster 5), and in Kenora from 2010 to 2019 (Cluster 6).

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List of Abbreviations

EVI: Enhanced Vegetation Index

GAM: Generalized Additive Model

GLM: Generalized Linear Model

GLMM: Generalized Linear Mixed Model

LST: Land Surface Temperature

MODIS: Moderate Resolution Imaging Spectroradiometer

OLR: Ordinal Logistic Regression

PCR: Polymerase Chain Reaction

PRECIP: Precipitation

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RR: Relative risk

SNOW: Snow on the Ground

TE: Total Evapotranspiration

General introduction

0.1. Emerging vector-borne zoonotic diseases

Zoonotic diseases can be transmitted from non-human hosts to humans by the bite of an infected vector, such as a tick or mosquito (Kulkarni et al. 2015). Over 90% of the documented instances of emerging vector-borne zoonotic diseases are transmitted by Ixodidae ticks and Culicidae mosquitoes (Swei et al. 2020). More specifically, Ixodidae transmit up to 40% of documented emerging vector-borne zoonotic diseases globally (Swei et al. 2020). Generally, tick-borne illnesses are predominantly located in temperate regions, with the greatest disease risk in North America (Swei et al. 2020). As a result, infected tick vectors in these regions can more readily transmit a wide diversity of pathogens including bacteria, protozoans, and viruses (Kulkarni et al. 2015, Bouchard et al. 2019, Swei et al. 2020).

0.2. The rise of tick-borne illnesses in humans in Canada

In the early 20th century, several human cases of tularemia and Rocky Mountain spotted fever were observed in western Canada, which are caused by *Francisella tularensis* and *Rickettsia rickettsii*, respectively (Humphreys and Campbell 1947, Wood and Artsob 2012, Bouchard et al. 2019). Field surveys conducted in regions nearby these human cases detected several tick vectors that harboured each pathogen (Humphreys and Campbell 1947, Wood and Artsob 2012). In 1930, tularemia was added to the Canadian list of notifiable diseases (Government of Canada 2023). *F. tularensis* may be spread through contact with infected mammals such as hunted animals, infective aerosols, or arthropod bites including ticks (Petersen et al. 2009, Gabriele-Rivet et al. 2016). Since the 1980s, limited detections of these pathogens have been identified in tick vectors or humans in Canada (Dergousoff et al. 2009, Teng et al. 2011, Wood and Artsob 2012, Yunik et al. 2015, Wood et al. 2016).

Over the past few decades, the number of reported cases of tick-borne diseases in humans, an incidental host of ticks, have been steadily increasing in Canada. Lyme disease is the most reported tick-borne illness in North America and may be caused by the species complex *Borrelia burgdorferi* sensu lato, which includes several different *Borrelia* genospecies (Gasmi et al. 2022). Since 2009, Lyme disease has been listed as a nationally notifiable disease in Canada

(Bouchard et al. 2019). The annual number of reported cases of Lyme disease in Canada have increased from 144 cases in 2009 to over 2500 cases in 2019 (Gasmi et al. 2022). The majority of Lyme disease cases were reported from three provinces: Ontario, Quebec, and Nova Scotia (Gasmi et al. 2022). However, the reported number of Lyme disease cases is an underestimate of the actual incidence in Canada due to an elevated number of false negative results in serology testing (Lloyd and Hawkins 2018).

In 2009, the first detected case of human granulocytic anaplasmosis was reported in Alberta, which is caused by the bacteria *Anaplasma phagocytophilum* (Parkins et al. 2009). Additional cases have since been detected in Alberta, Manitoba, Ontario, and Quebec (Uminski et al. 2018, Edginton et al. 2018, Nelder et al. 2019b, Stokes et al. 2020, Campeau et al. 2022). In 2021, the largest known cluster of 25 human cases of anaplasmosis was identified in the Estrie region of Quebec (Campeau et al. 2022). However, anaplasmosis in humans is only reportable in Manitoba, Ontario, and Quebec, as a result of the comparatively higher incidences in these provinces (Uminski et al. 2018).

More recently, several distinct *Babesia* species have been isolated from humans that have caused babesiosis (Bullard et al. 2014, Scott 2017, Scott et al. 2021). In 2013, the first detected case of human babesiosis in Canada occurred in a child in Manitoba (Bullard et al. 2014). A retroactive study then found that cases of human babesiosis were found across a wide geographic distribution in Canada from 2011 to 2017 (Scott and Scott 2018). An increased detection of *Babesia* species in blood donors in Canada may be especially problematic, as uninfected individuals may become infected via blood transfusions (Tonnetti et al. 2019).

Currently, only two tick-borne diseases are listed as nationally notifiable infectious diseases in Canada: tularemia and Lyme disease (Gasmi et al. 2022, Government of Canada 2023). Yet, many tick-borne diseases, such as anaplasmosis or babesiosis, are only reportable to public health agencies in certain Canadian provinces. Therefore, the geographic extent and temporal progression of several emerging tick-borne illnesses may be difficult to assess in Canada. As a result, the concurrent use of passive and active surveillance efforts assessing tick and host populations of significant public health concern must continue to be conducted in Canada to better detect the emergence or re-emergence of tick-borne pathogens.

0.3. The tick-host-pathogen disease systems in Canada

The presence of tick-borne diseases in Canada requires the simultaneous co-occurrence of three key players: tick vectors, vertebrate hosts, and pathogens. If one of these key players is missing, then tick-borne pathogen spread and transmission cannot occur.

0.3.1. Tick vectors

In Canada, blacklegged ticks (*Ixodes scapularis*) and western blacklegged ticks (*I. pacificus*) have been detected at increased abundances through passive and active surveillance efforts. These two tick species represent disease vectors of significant public health concern due to their rapid geographic range expansion in Canada. *I. pacificus* can be detected in coastal British Columbia, while *I. scapularis* have a widely distributed geographic range across most provinces in Canada (Lindquist et al. 2016, Wilson et al. 2022). Both tick vectors have generalist feeding habits, where they may feed on a wide variety of vertebrate hosts including humans (Lindquist et al. 2016). Currently, *I. scapularis* represents the tick vector that is most commonly found across Canada (Ogden et al. 2014, Nelder et al. 2014, Guillot et al. 2020, Wilson et al. 2022).

There are three additional *Ixodes* species of public health concern: *I. cookei*, *I. marxi*, and *I. muris*. *I. cookei* feed on rodents and medium-sized mammals such as groundhogs, raccoons, foxes, skunks, porcupines, and domestic pets (Lindquist et al. 2016, Gasmi et al. 2018). This tick species has a wide geographic range from Newfoundland to Manitoba, with the greatest densities in Ontario and Quebec (Nelder et al. 2014, Lindquist et al. 2016, Gasmi et al. 2018). Tree squirrels are the preferred host of *I. marxi*, but this tick vector may also feed on other small to medium-sized mammals such as rodents and domestic pets (Lindquist et al. 2016). *I. marxi* have been mainly detected in Ontario, Quebec, and parts of the Atlantic provinces (Nelder et al. 2014, Lindquist et al. 2016, Gasmi et al. 2018). Although *I. muris* predominantly feeds on mice and other small mammals, the immature stages have been reported on ground-foraging birds (Scott et al. 2001, Morshed et al. 2005, Lindquist et al. 2016). This tick species has been recorded across a wide geographic range from Manitoba to the Atlantic provinces (Nelder et al. 2014, Lindquist et al. 2016, Gasmi et al. 2018).

Three species of *Dermacentor* ticks have been detected in Canada: *D. andersoni*, *D. albipictus*, and *D. variabilis*. Populations of *D. andersoni* ticks occur in British Columbia,

Alberta, and Saskatchewan, with limited observations in eastern Canada (Dergousoff et al. 2013, Lindquist et al. 2016, Lysyk et al. 2021). Immature life stages infest small mammals, such as mice, sciurids, and voles, while adults feed on wild mammals, livestock, and domestic pets (Lindquist et al. 2016). This tick vector has also been found to readily attach to humans (Lindquist et al. 2016, Kanji et al. 2022). *D. albipictus* only feed on one host, such as a moose or elk, but this tick vector has been found on other mammals (Lindquist et al. 2016, Chenery et al. 2022). The northernmost records for *D. albipictus* are in the Yukon Territory and the Northwest Territories, with additional records found across Canada (Lindquist et al. 2016, Chenery et al. 2023, Kirby et al. 2023). In Canada, moose populations are especially impacted by their hypersensitivity to salivary antigens in *D. albipictus*, which has resulted in severe losses of moose in Alberta and eastern Canada since the 1930s (Lindquist et al. 2016). The geographic range of *D. variabilis* is widely distributed across Canada, with greater densities found in Saskatchewan, Manitoba, Ontario, Quebec, and Nova Scotia (Dergousoff et al. 2013, Nelder et al. 2014, Lindquist et al. 2016, Gasmi et al. 2018, Kirby et al. 2023). Immature ticks are found feeding on small mammals, especially rodents, and domestic pets. Adult *D. variabilis* infest medium to large-sized mammals including dogs, cervids, livestock, and humans (Lindquist et al. 2016, Kanji et al. 2022)

Populations of *Haemaphysalis leporispalustris* have been observed in surveys in Canada since the early 20th century. Individuals of this tick species are present in every province in Canada (Humphreys and Campbell 1947, Nelder et al. 2014, Clow et al. 2016, Lindquist et al. 2016, Gasmi et al. 2018). Although rabbits and hares are the most important hosts of this tick vector, they also infest ground-foraging birds and small mammals and, in rarer instances, large mammals and humans (Lindquist et al. 2016).

Populations of *Amblyomma* and *Rhipicephalus* ticks are not yet established in Canada, but numerous records have occurred in many provinces (Nelder et al. 2014, Lindquist et al. 2016, Gasmi et al. 2018). One species, *Amblyomma americanum*, is a tick vector of significant public health concern due to its ability to transmit numerous tick-borne pathogens (Bouchard et al. 2019). As an aggressive, generalist feeder, *A. americanum* actively pursues a wide variety of bird and mammal hosts, which can include humans (Goddard and Varela-Stokes 2009, Lindquist et al. 2016). Recent observations of this tick species in central and eastern Canada are most likely adventitious ticks that are dispersed and deposited annually by migratory birds from the southern

United States (Nelder et al. 2014, 2019a, Lindquist et al. 2016, Gasmi et al. 2018). Similarly, *Rhipicephalus sanguineus* is a tick vector that is found globally and typically parasitizes dogs. This tick vector is usually transported to Canada by travellers, as well as travelling or imported animals (Nelder et al. 2014, Lindquist et al. 2016, Gasmi et al. 2018).

0.3.2. Vertebrate hosts

Vertebrate hosts of tick populations in Canada fall under two main categories: reservoir hosts or reproductive hosts (Mather et al. 1989, LoGiudice et al. 2003, Brunner et al. 2008, Brisson et al. 2008).

Generally, small mammals are the most important reservoir hosts for tick vectors and pathogens. These hosts can successfully feed larger burdens of immature ticks and may more readily transmit tick-borne pathogens (Mather et al. 1989, LoGiudice et al. 2003, Brunner et al. 2008, Brisson et al. 2008). White-footed mice (*Peromyscus leucopus*) are the most competent reservoir host for ticks, where they are estimated to have a realized reservoir competence of over 90% (Mather et al. 1989, LoGiudice et al. 2003, Brunner et al. 2008). Previous studies in southern Quebec have found that adult male *Peromyscus* mice carry larger burdens of ticks and may be more likely to transmit infections than adult females or juveniles (Bouchard et al. 2011, Dumas et al. 2022). However, chipmunks (*Tamias striatus*) and shrews (*Blarina brevicauda* and *Sorex cinereus*) are also highly efficient reservoir hosts, with realized reservoir competencies of 55-57% and 33-51%, respectively (Brunner et al. 2008, Brisson et al. 2008). Shrews are also thought to feed up to 55% of infected ticks compared to 25% of infected ticks in mice (Brisson et al. 2008).

Certain species of ground-foraging birds may also be competent reservoir hosts (LoGiudice et al. 2003, Ginsberg et al. 2005). Generally, a reservoir competence of ~12% was found across specimens of American robins (*Turdus migratorius*), ovenbirds (*Seiurus aurocapilla*), veeries (*Catharus fuscescens*), and wood thrushes (*Hylocichla mustelina*) (LoGiudice et al. 2003). More specifically, American robins and song sparrows (*Melospiza melodia*) were found to have higher levels of reservoir competence, while northern cardinals (*Cardinalis cardinalis*) and gray catbirds (*Dumetella carolinensis*) have moderate levels of reservoir competence (Ginsberg et al. 2005). A study in southern Quebec found that the relative reservoir potential ranged from 1 to 11% for five species of birds including chipping sparrows

(*Spizella passerina*), dark-eyed juncos (*Junco hyemalis*), hermit thrushes (*Catharus guttatus*), house wrens (*Troglodytes aedon*), and song sparrows (Dumas et al. 2022). They also demonstrated that female birds may be more likely to infect ticks than male birds (Dumas et al. 2022).

Several host species with lower reservoir competence may be important for maintaining tick populations, but not for pathogen transmission (Mather et al. 1989, LoGiudice et al. 2003). Many mid-size and large mammals act as reproductive hosts in Canada including white-tailed deer (*Odocoileus virginianus*), raccoons (*Procyon lotor*), opossums (*Didelphis virginiana*), and skunks (*Mephitis mephitis*) (LoGiudice et al. 2003, Brunner et al. 2008). The reservoir competence of these reproductive hosts ranges from 1.0% to 24.0% depending on the examined tick-borne pathogen (LoGiudice et al. 2003, Brunner et al. 2008, Ostfeld et al. 2018). Opossums may even act as ecological traps, where they may efficiently kill up to 96% of their tick burdens (Keesing et al. 2009). However, these results were obtained from wildlife unconditioned to laboratory conditions that may exhibit non-typical behaviours (Keesing et al. 2009). In contrast, a recent review found that ticks were not found in the stomach contents of opossums across several studies, thereby questioning their ability to remove large burdens of ticks (Hennessy and Hild 2021).

0.3.3. Tick-borne pathogens

The predominant bacteria isolated from ticks in Canada are from the genus *Borrelia* (Bouchard et al. 2019). The most common tick-borne bacteria detected during sentinel surveillance efforts in Canada is *Borrelia burgdorferi* sensu stricto, which is one of the genospecies that causes Lyme disease (Guillot et al. 2020, Wilson et al. 2022). This pathogen is primarily transmitted by *I. scapularis* in central and eastern Canada and *I. pacificus* in western Canada, with greater infection prevalence occurring in Ontario, Quebec, New Brunswick, and Nova Scotia (Bouchard et al. 2019, Guillot et al. 2020, Wilson et al. 2022). In contrast, *Borrelia miyamotoi*, the bacterium causing tick-borne relapsing fever, was only identified in *I. scapularis* in Canada in 2012. Since its first detection, this pathogen has been found in several Canadian provinces (Dibernardo et al. 2014, Bouchard et al. 2019, Zinck and Lloyd 2022, Dumas et al. 2022, Wilson et al. 2022).

Additional bacteria, such as the genera of *Anaplasma*, *Bartonella*, *Coxiella*, and *Ehrlichia*, have also been detected at a lower prevalence in ticks in Canada (Nelder et al. 2016, Bouchard et al. 2019). Anaplasmosis in wildlife and humans can be caused by several distinct bacteria species. *Anaplasma phagocytophilum* has been detected across a large geographic range in Canada and is predominantly transmitted by *I. scapularis* and *I. pacificus* (Guillot et al. 2020). In addition, isolated cases of *A. marginale* and *A. bovis* have been detected in *Dermacentor* and livestock in the Prairies, Ontario, and Quebec (Howden et al. 2010, Dergousoff and Chilton 2011, Lindquist et al. 2016, Chilton et al. 2018). Fleas, lice, and, in rarer instances, ticks may transmit *Bartonella* species to wildlife and humans causing bartonellosis (Lindquist et al. 2016). In Canada, this bacterium has been found in *I. scapularis*, wildlife, and humans from British Columbia to Quebec (Leighton et al. 2001, Jardine et al. 2005, Gary et al. 2006, André et al. 2017, Breitschwerdt et al. 2019, Kho et al. 2021, Boodman et al. 2022). *Coxiella burnetii*, the bacteria causing Q fever, has been detected in each Canadian province, but it was first isolated from a human in Quebec in 1952 (Pavilanis et al. 1952). *C. burnetii* has been detected in ticks as well as livestock, rodents, and domestic pets in Canada (Lang 1989, Hatchette et al. 2001, Marrie et al. 2008, Angelakis and Raoult 2010, Celina and Cerný 2022). *Ehrlichia* bacteria were first detected in a horse in Canada in the 1990s (Berrington et al. 1996). These bacteria are typically transmitted by *A. americanum* and *R. sanguineus* feeding on wildlife such as livestock, domestic pets, and white-tailed deer (Berrington et al. 1996, Gary et al. 2006, Villeneuve et al. 2011, Lobanov et al. 2012, Lindquist et al. 2016, Evason et al. 2019).

Two bacteria, *Francisella tularensis* and *Rickettsia rickettsii*, were the earliest tick-borne pathogens isolated from ticks and humans in Canada (Bow and Brown 1943, 1952, Humphreys and Campbell 1947). Detections of *F. tularensis* in wildlife and human populations have been found in all the Canadian provinces and territories, yet it has not been found recently in ticks (Banfield 1954, Ditchfield and Julian 1960, Gordon et al. 1983, Artsob et al. 1984, Leighton et al. 2001, Zarnke et al. 2004, Wobeser et al. 2009, Gabriele-Rivet et al. 2016, Buhler et al. 2022). In contrast, limited detections of *R. rickettsii* have been found in *Dermacentor* and *Haemaphysalis* in western and central Canada as well as Nova Scotia (McKiel 1960, Leighton et al. 2001, Wood and Artsob 2012, Nelder et al. 2020, Crandall et al. 2022).

Ticks are also capable of transmitting protozoans, which are small parasites that infect the red blood cells of wildlife and human hosts (Lindquist et al. 2016). *Babesia* protozoans, such as

B. duncani, *B. microti*, and *B. odocoilei*, have only recently been detected in Canada. *Babesia microti* was the first *Babesia* species detected in Canada in 2010, and was found in *I. scapularis* in Manitoba. Instances of *B. microti* have since been identified in ticks, wildlife, and humans across Canada (Dibernardo et al. 2014, Bullard et al. 2014, O'Brien et al. 2016, Wilson et al. 2022). The next *Babesia* species found in Canada was *B. odocoilei*, which was isolated from an elk in 2012 in Saskatchewan. This pathogen has much more isolated cases than other *Babesia* species, as it has only been detected in the Prairies, Ontario, and Quebec (Pattullo et al. 2013, Mathieu et al. 2018, Milnes et al. 2019, Crandall et al. 2022). Finally, *B. duncani* was isolated from a human in Ontario in 2017 and later identified as occurring in humans across Canada from coast to coast (Scott 2017, Scott and Scott 2018). Starting in 2021, an additional protozoan genus, *Hepatozoon*, was identified in *I. scapularis* in Ontario (Scott and Pesapane 2021).

Powassan virus is the only known tick-borne *Flavivirus* that currently circulates in Canada (Lindquist et al. 2016, Pierson and Diamond 2020). This virus was first isolated from a child in 1958 in Powassan, Ontario (McLean and Donohue 1959). Since then, two genetic lineages of the virus have been identified, with lineage II also known as deer-tick virus (Ebel et al. 2001). Several tick vectors are capable of transmitting Powassan virus, with each lineage predominantly maintained by specific tick species. Powassan virus lineage I is predominantly maintained by *I. cookei*, while lineage II is more commonly associated with *I. scapularis* (Lindquist et al. 2016, Pierson and Diamond 2020). In Canada, several cases of the virus have been isolated from ticks and humans across the country (McLean et al. 1960, 1970, Kettyls et al. 1972, Fitch and Artsob 1990, Smith et al. 2018, Bogaty and Drebot 2018).

0.3.4. The tick-host-pathogen transmission cycle

The tick-host-pathogen transmission cycle in Canada typically lasts one to three years depending on the location and climate, where most tick vectors feed on a blood meal on three different hosts that may be infected with a pathogen (Ostfeld 2011**).** In the spring, adult female ticks will lay their eggs in leaf litter. In the summer, the eggs then hatch into larvae, which will begin to search their environment (i.e., quest) for a vertebrate host. Larvae feed on their first host, which is when they may acquire a pathogen (Ostfeld 2011, Bouchard et al. 2019). This first host tends to be ground-foraging birds or small mammals (Keirans et al. 1996). After their blood meal, the ticks will either overwinter as larvae or molt into a nymph and then overwinter (Ostfeld

2011, Bouchard et al. 2019). Transstadial pathogen transmission from one life stage to another may first occur at this point in the life cycle, where pathogens are carried from the larval life stage through the molt to the nymphal life stage (Bouchard et al. 2019). The next spring or early summer, the nymph will feed on another host. This host may be domestic pets (cats or dogs), livestock (cattle, horses, or sheep), ground-foraging birds, small mammals, or humans (Keirans et al. 1996). If the nymph is infectious, it may transmit the pathogen to the host that it is feeding on (Ostfeld 2011, Bouchard et al. 2019). During the fall, the nymph will then molt into the final adult life stage. After reproduction, adult ticks typically feed on larger mammals, such as white-tailed deer, in the late fall before overwintering or early spring after overwintering. Adult female ticks will then lay their eggs in the spring, allowing the cycle to begin once more (Ostfeld 2011, Bouchard et al. 2019).

Although transstadial transmission is the primary mode of pathogen transmission, alternatives modes may occur in tick populations. Transovarial transmission occurs when pathogens are spread from adult females to offspring without the need for an infected host (Freitas et al. 2009, Zembsch et al. 2021). Certain pathogens, such as *Babesia* or *Rickettsia*, have been identified as capable of being transmitted transovarially in *Ixodes* and *Haemaphysalis* (Freitas et al. 2009, Zembsch et al. 2021). Yet, only a portion of filial ticks may become infected due to partial pathogen transmission, resulting in low infection prevalence (Freitas et al. 2009, Zembsch et al. 2021). Pathogens may also be transmitted when infected and uninfected tick vectors are feeding at the same time in close proximity to each other on a reservoir host (Voordouw 2015, Cutler et al. 2021). However, co-feeding transmission relies heavily on a localized pathogen infection in the host's skin (Voordouw 2015).

0.4. Abiotic factors impacting tick populations and tick-borne pathogens

Several abiotic factors associated with changes in climate and land use have been identified as potential drivers in the establishment and distribution of tick populations and their pathogens in Canada.

0.4.1. Temperature

Temperature variability and extremes affect the survival and distribution of tick populations. With climate warming, higher temperatures are expected to increase tick

abundances through faster development rates and longer seasonal activity periods (Ogden et al. 2004, 2021, Eisen et al. 2016, Ogden and Lindsay 2016). Laboratory studies on *I. scapularis*, *D. andersoni*, *D. variabilis*, and *H. leporispalustris* have found that extreme cold or hot temperatures may lead to decreased development, disturbed physiological processes, and limited survival (Campbell and Glines 1979, Ogden et al. 2004, Eisen et al. 2016, Ogden and Lindsay 2016, Fieler et al. 2021). In addition, 50% larval mortality was found to occur between 38°C to 42°C for several of these tick species, demonstrating the potential impacts of future climate warming on the survival of immature ticks (Fieler et al. 2021). However, the use of microclimate refuges under the leaf litter in nature may help ticks avoid adverse weather conditions and maintain their optimal thermal thresholds (Linske et al. 2019, Volk et al. 2022). As a result, climate warming may affect tick population in distinct ways depending on temperature variability and extremes experienced locally at their range edges, with increased extirpation risk at the southern range edge with high temperatures and facilitated establishment at the northern range edge with warming temperatures (Ogden et al. 2013).

Temperature changes may also affect pathogen replication and transmission. Transmission cycles are most efficient when there is seasonal synchrony between the activity periods of immature ticks. Nymphs that feed before larvae may infect hosts, which then remain virulent throughout the feeding period (Eisen et al. 2016, Ogden et al. 2021). As a result, co-feeding between immature ticks on a host may permit additional pathogen transmission to occur (Voordouw 2015, Ogden et al. 2021). With climate warming, fitter, generalist tick-borne pathogens are also more likely to emerge in Canada, which are expected to be short-lived, less efficiently transmitted, and more pathogenic to their hosts (Ogden et al. 2008a). In addition, *Borrelia* bacteria exhibited a greater amount of *in vitro* growth with higher temperatures, demonstrating that greater pathogen replication may occur with warming temperatures (Veinović et al. 2016).

0.4.2. Humidity and precipitation

Sufficient humidity within the microclimate is required to maintain tick populations. Laboratory studies have found that sustained humidity levels above 85% are necessary for tick survival, with mortality risk increasing exponentially below 75% (Stafford 1994, Ginsberg et al. 2017). In nature, higher humidity levels may be sustained through moderate levels of

precipitation (Burtis et al. 2016). In addition, extended periods of low moisture may reduce tick survival and densities (Berger et al. 2014a, 2014b, Ginsberg et al. 2017, Dumas et al. 2022). However, behavioural changes in ticks may help mitigate their desiccation through modifications in their questing activity (Vail and Smith 2002).

In contrast, precipitation extremes, such as drought or heavy rainfall, may lead to limited questing activity and tick survival, subsequently impacting tick-borne disease risk (Eisen et al. 2016, Ogden and Lindsay 2016, Burtis et al. 2016). Hot, dry summer days are especially harmful to questing *I. scapularis* nymphs, resulting in increased mortality risk due to greater water loss and desiccation (Eisen et al. 2016, Burtis et al. 2016). Consequently, significantly reduced human incidences of Lyme disease in the northeast United States were correlated with hot, dry summer weather conditions, especially in areas with long-established tick populations (McCabe and Bunnell 2004, Burtis et al. 2016). In the northeastern United States, Lyme disease incidence was also found to be strongly related to 2-year lagged moisture levels (Subak 2003). However, the relationship between climatic conditions and disease incidence was not detected in certain recently endemic areas of Lyme disease (Burtis et al. 2016).

0.4.3. Winter conditions

Abundant snow cover may increase tick survival by providing an additional insulative layer from subzero temperatures. Lower lethal temperature limits of larval *D. andersoni*, *D. variabilis*, and *I. scapularis* are thought to occur between -8.5°C to -24°C (Fieler et al. 2021). However, this laboratory study was conducted on larval ticks from reared colonies, which may not be consistent with the low thermal thresholds of immature ticks in a natural setting. To avoid these lethal temperatures, ticks will experience their diapause in microclimate refuges under the leaf litter and snow, which will help them maintain their optimal thermal and humidity thresholds (Linske et al. 2019, Volk et al. 2022). Snow cover alone or in combination with leaf litter has been found to increase overwintering tick survival by providing the necessary insulation to prevent inoculative freezing or desiccation (Linske et al. 2019, Volk et al. 2022). In addition, tick abundances may also increase the subsequent summer after winters with greater snowfall (Hayes et al. 2015).

Milder winters due to climate warming may affect tick-borne disease risk due to variability in the survival and abundance of ticks. Positive associations between mild winter days

and summer *I. scapularis* encounters have been found (Lin et al. 2019). Milder prior-year winter temperatures are also associated with decreased incidences of Lyme disease in the northeastern United States, but this relationship has been reported to be weak and inconsistent (Subak 2003, Schauber et al. 2005). In contrast, winters with intermittent snow cover may lead to decreased tick abundances and lower disease incidence, as vectors exposed to harsher frozen soil may experience inoculative freezing or death (Eisen et al. 2016, Linske et al. 2019, Volk et al. 2022).

0.4.4. Land cover and vegetation

Different tick vectors may exhibit distinct habitat and vegetation preferences. Greater *I. scapularis* abundances are found in forested areas compared to urban or agricultural areas (Ferrell and Brinkerhoff 2018, Talbot et al. 2019, Burrows et al. 2021). However, higher *I. scapularis* densities have been found in deciduous or mixed forests compared to coniferous forests (Burrows et al. 2021, Mathisson et al. 2021). Distinct seasonal patterns of litter deposition in each forest type may explain this difference, as coniferous litter has been linked to lower tick survival (Mathisson et al. 2021). Positive associations have also been found for questing *I. scapularis* abundances with canopy cover, as well as shrub and vegetation density (Schulze and Jordan 2001, Werden et al. 2014, Clow et al. 2017b, Ginsberg et al. 2020, Mathisson et al. 2021). In contrast, *D. variabilis* are more likely to be found in open canopy environments dominated by grass or underbrush compared to forested areas with high vegetation densities (Stein et al. 2008, Trout Fryxell et al. 2015, Mathisson et al. 2021). This habitat preference is likely due to their ability to better survive xeric conditions (Trout Fryxell et al. 2015, Mathisson et al. 2021).

Land use changes and habitat fragmentation may also impact tick populations and tick-borne pathogens. Tick-borne disease risk may be affected non-linearly along an urbanization gradient, with low disease risk in urbanized areas with no exposure to tick habitat compared to greater disease risk in suburban or rural areas with forest fragmentation (Faust et al. 2018, Diuk-Wasser et al. 2021). However, urban green spaces may also facilitate the invasion of infected ticks and hosts, leading to variable human exposure to tick-borne pathogens based on their contact with these natural areas (Diuk-Wasser et al. 2021). Yet, the highest spillover risk is expected to occur in transitional zones with intermediate levels of anthropogenic modifications and forest cover (Faust et al. 2018). This relationship is thought to be affected by habitat or patch connectivity in both urban and rural settings, which may modulate tick and pathogen densities

(Estrada-Peña 2005, VanAcker et al. 2019, Diuk-Wasser et al. 2021). As a result, highly fragmented areas may encompass isolated patches that experience frequent extinctions of tick populations, leading to temporal variability of disease risk (LoGiudice et al. 2008, Diuk-Wasser et al. 2021).

0.5. Biotic factors impacting tick populations and tick-borne pathogens

The survival and distribution of tick vectors and their pathogens rely on the abundance, diversity, and movements of vertebrate host populations.

0.5.1. Host abundance and diversity

The abundances of tick and host populations may be impacted by their degree of local establishment. In areas with long-established tick populations, an equilibrium may exist between the abundances of ticks and their hosts (Dobson 2014). In these areas, higher densities of hosts may lead to increased tick-host contact rates, resulting in increased tick abundances due to reduced questing time and lower mortality risk (Estrada-Peña and De La Fuente 2014). In contrast, tick-host interactions in areas with emergent tick populations, such as those near the poleward range edge, may be dynamic and lead to variable tick abundances due to limited host availability (Dobson 2014, Millien et al. 2023). This relationship may be especially impacted by asynchronous seasonal activity of tick and host populations, where greater tick activity may take place when host abundances are low (Estrada-Peña and De La Fuente 2014).

The diversity of mammal species may also affect the survival and abundance of ticks due to the quality of blood meal hosts present within the community. The addition or loss of a host species locally due to predation or interspecific competition may have differential impacts on tick abundance, especially if tick populations have not yet established (Levi et al. 2016). However, the quality of hosts as blood meals may differ based on the probability of ticks feeding successfully as well as the ability of hosts to transmit pathogens (Mather et al. 1989, LoGiudice et al. 2003, Keesing et al. 2009, Levi et al. 2016). Small mammals, such as mice, shrews, and chipmunks, are important reservoir hosts for the maintenance of tick populations and tick-borne pathogens (Mather et al. 1989, LoGiudice et al. 2003, Brisson et al. 2008). Yet, certain mid-size and large mammals, such as raccoons and white-tailed deer, are especially important for feeding large burdens of ticks and act as key reproductive hosts for adult ticks (LoGiudice et al. 2003). In

addition, host-specific behaviours, such as grooming or increased physiological immune responses, may either kill ticks or limit them from successfully feeding to completion, respectively (Levin and Fish 1998, Keesing et al. 2009, Jones et al. 2015).

Furthermore, host abundance and diversity may concurrently dilute and amplify pathogen transmission and prevalence, especially in areas with emergent tick populations (Luis et al. 2018, Millien et al. 2023). The composition and frequency of host species within the community are important in determining local tick-borne disease risk (LoGiudice et al. 2003, 2008, Brunner et al. 2008, Keesing et al. 2009). For example, mammal communities that predominantly contain reservoir hosts, such as white-footed mice or shrews, may have greater densities of infected ticks and greater tick-borne disease risk (LoGiudice et al. 2003, Bouchard et al. 2011, 2013, Werden et al. 2014, Levi et al. 2016). As a result, an amplification effect may occur with increased host diversity, where increased densities of reservoirs hosts may lead to greater tick-host contact rates and greater pathogen transmission (Levi et al. 2016, Luis et al. 2018). In contrast, a decrease in disease transmission and prevalence (or dilution effect) may also take place with increased host diversity. In this scenario, lower densities of reservoir hosts may lead to less contact with ticks, thereby lowering pathogen transmission and resulting in lower infection prevalence among ticks (Wood and Lafferty 2013, Levi et al. 2016, Luis et al. 2018). Yet, dilution and amplification effects may not be mutually exclusive within the same community, as it depends on the composition and population dynamics of hosts locally (Ogden and Tsao 2009, Occhibove et al. 2022, Millien et al. 2023).

0.5.2. Host movements

Migratory birds as well as travelling humans and pets act as key contributors to the long-distance dispersal of ticks and tick-borne pathogens into new locations in North America (Scott et al. 2001, Ogden et al. 2008b, 2015). Earlier spring onset with climate warming has resulted in phenological shifts in both bird migration and seasonal tick activity, facilitating their chance of contact (Ogden et al. 2008b, Levi et al. 2015). Bird migrants resting at stopover sites in the United States provide opportunities for neotropical ticks from South America to detach and native ticks to attach (Mukherjee et al. 2014, Cohen et al. 2015). Each spring, an estimated 50 to 175 million ticks are predicted to be transported to Canada from the United States via long-distance bird migration (Ogden et al. 2008b, 2015, Scott et al. 2012). Similarly, travelling

humans and pets may contribute to the long-distance dispersals of adventitious ticks, such as the travel-related submissions of *A. americanum* in Ontario from the southern United States (Nelder et al. 2019a). As a result, adventitious ticks may be dispersed to new poleward locations in Canada, which may allow the establishment of reproducing tick populations and the circulation of novel tick-borne pathogens (Ogden et al. 2008b, 2013, Milnes et al. 2019).

Locally, tick vectors are dispersed by the movements of hosts that are shifting their geographic ranges poleward in response to land use and climate changes (Roy-Dufresne et al. 2013, Diuk-Wasser et al. 2021). Hosts may disperse ticks and their pathogens over short distances into nearby forest patches while searching for food resources (Borgmann-Winter et al. 2021). Larger and more connected forest patches have been associated with a greater degree of host movement and diversity (LoGiudice et al. 2008, Simon et al. 2014, Diuk-Wasser et al. 2021). In contrast, fragmented forests, and geographic barriers, such as water bodies and roadways, may reduce host movements, resulting in increased tick abundances and the establishment of tick-borne pathogens in small forest patches (Simon et al. 2014, Talbot et al. 2019, Diuk-Wasser et al. 2021). Certain habitat generalists, such as mice and shrews, may also become dominant in these isolated forest patches, allowing them to feed larger burdens of immature ticks and more readily transmit pathogens (Allan et al. 2003, Marrotte et al. Roy-Dufresne et al. 2013). In addition, decreased winter severity may lead to greater movements and habitat use of mammal hosts, especially for white-tailed deer (Dawe and Boutin 2016). With climate warming, milder winters have been associated with greater poleward range expansions in white-footed mice and white-tailed deer, which have assisted in the range expansions of tick populations (Roy-Dufresne et al. 2013, Simon et al. 2014, Dawe and Boutin 2016, Kennedy-Slaney et al. 2018, Fisher et al. 2020). As a result, tick vectors, such as *I. scapularis*, may expand their established ranges by 7 to 46 km per year (Leighton et al. 2012, Simon et al. 2014, Clow et al. 2017a, Ripoche et al. 2022).

0.6. Surveillance methods for detecting ticks, hosts, and pathogens

Active tick surveillance uses field surveys to collect ticks from their natural habitat through tick dragging or from animals during small mammal trapping (Wilson et al. 2022). This type of surveillance may be used to identify areas with emergent or long-established tick and host populations and to determine tick-borne disease risk through pathogen testing (Teng et al.

2011, Yunik et al. 2015, Chilton et al. 2019, Guillot et al. 2020, Wilson et al. 2022). These tick and pathogen surveillance programs are typically conducted by federal or provincial public health agencies and academics, which may help track the spatiotemporal progression of tick vectors and tick-borne pathogens across varying scales (Clow et al. 2019, Guillot et al. 2020).

Passive tick surveillance uses ticks collected from the environment or from hosts that are submitted voluntarily by doctors, veterinarians, wildlife researchers, or the public (Wilson et al. 2022). This type of surveillance provides an early signal for tick vectors and tick-borne pathogens across broad spatial scales and may be used to form a baseline for the introduction and establishment of newly detected tick vectors and tick-borne pathogens (Ogden et al. 2014, Ripoche et al. 2018, Gasmi et al. 2018, Chilton et al. 2019, Nelder et al. 2021, Morshed et al. 2021). Yet, the breadth of passive tick surveillance has been reduced or discontinued by certain public health agencies in Canada due to time and resource limitations (Clow et al. 2019, Guillot et al. 2020, Wilson et al. 2022). More recently, community-science initiatives related to passive tick and pathogen surveillance, such as eTick and Geneticks, has resulted in better assessments of tick vectors and their pathogens across broader geographic regions with the help of members of the public (Wilson et al. 2022). In addition, these community-science initiatives are increasingly being used by public health agencies, as a way to supplement their limited passive tick surveillance programs.

0.7. Scope of the dissertation

Generally, studies on ticks and tick-borne pathogens in Canada focus primarily on *I. scapularis* and *B. burgdorferi* sensu stricto, which are the most common tick vector and tick-borne pathogen in North America. In my dissertation, I wanted to provide a more generalized point of view, which encompassed a wide variety of tick vectors, hosts, and tick-borne pathogens to allow me to disentangle the relationships of these complex disease systems. By conducting surveys at sites of distinct levels of disease risk, I was also able to assess the impact of abiotic and biotic factors on the gradient of establishment of tick and host populations, which subsequently may impact tick-borne disease risk.

I had two primary research objectives in my dissertation, which were divided across four chapters. My first objective was to explore the historical associations and spatiotemporal changes between tick vectors, hosts, and pathogens in Canada, which was associated with Chapter 1. This

standardized approach helped me determine the spatial and temporal progression of the tick-host-pathogen disease systems in Canada. My second objective focused on the determination of key abiotic and biotic drivers that may impact ticks, mammal hosts, and emerging or re-emerging tick-borne pathogens in Ontario and Quebec, where tick populations have been established for decades and multiple tick-borne pathogens are circulating. This second objective was associated with Chapters 2, 3, and 4.

In Chapter 1, I performed a systematic review of the literature to determine the historical tick-host-pathogen associations as well as the spatiotemporal changes of high pathogen presence in ticks across broad spatial and temporal ranges in Canada. This information helped me identify the potential key players of the tick-host-pathogen disease systems that I may encounter at my sites during field surveys in Ontario and Quebec. Chapter 2 incorporated host active surveillance data with high-resolution environmental data to determine their relative contributions on questing *I. scapularis* abundance, as I saw the importance of hosts and, more specifically, mammal hosts in my previous chapter. This framework allowed me to concurrently assess the effect of high-resolution abiotic factors derived from remote sensing satellites and meteorological towers as well as biotic factors related to host active surveillance that were obtained during field surveys on questing *I. scapularis* abundance in Ontario and Quebec. Both environmental and host-related factors were found to be key predictors of questing *I. scapularis* abundance across my broad study region. In Chapter 3, pathogen testing was conducted to determine which tick-borne pathogens were present in tick and small mammal specimens collected during field surveys in Ontario and Quebec. Building on the observations made in Chapter 2 and 3, Chapter 4 was used to determine how biotic factors related to *I. scapularis* abundance and the abundance and diversity mammal hosts were influencing pathogen presence, prevalence, and diversity across my sites in Ontario and Quebec.

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Chapter 1 | Historical associations and spatiotemporal changes of pathogen presence in ticks in Canada: A systematic review

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1.1. Abstract

Starting in the early 20th century, ticks and their pathogens have been detected during surveillance efforts in Canada. Since then, the geographic spread of tick vectors and tick-borne pathogens has steadily increased in Canada with the establishment of tick and host populations. Sentinel surveillance in Canada primarily focuses on *Ixodes scapularis*, which is the main vector of *Borrelia burgdorferi*, the bacterium causing Lyme disease. Other tick-borne pathogens, such as *Anaplasma*, *Babesia*, and *Rickettsia* species, have lower prevalence in Canada, but they are emerging or re-emerging in tick and host populations. Here, we assessed the historical associations between tick vectors, hosts and pathogens and identified spatiotemporal clusters of pathogen presence in ticks in Canada using data extracted from the literature. Approximately one-third of ticks were infected with a pathogen, and these ticks were feeding primarily on bird and mammal hosts. *B. burgdorferi* was the most detected pathogen and *I. scapularis* harboured the greatest number of pathogens. We identified several spatial outliers of high pathogen presence in ticks in addition to five spatiotemporal clusters in southern Canada, all of which have long-established tick populations. Six spatiotemporal clusters of high pathogen presence in ticks were also identified based on surveillance method, with four clusters associated with passive surveillance and two clusters associated with active surveillance. Our review represents the first systematic assessment of the literature that identifies historical associations and spatiotemporal changes in tick-host-pathogen disease systems in Canada over broad spatial and temporal scales. As distinct spatiotemporal clusters were identified based on surveillance method, it is imperative that surveillance efforts employ standardized methods and data reporting to comprehensively assess the presence, spread and risk of tick-borne pathogens in tick and host populations.

Keywords: Canada, host, pathogen, spatiotemporal, systematic review, tick

1.2. Introduction

Since the early 20th century, tick populations and their pathogens have been detected through surveillance efforts in Canada (Barker et al., 1992; Humphreys & Campbell, 1947). In the 1930s and 1940s, several cases of tularemia and Rocky Mountain spotted fever were observed in humans in western Canada (Humphreys & Campbell, 1947; Wood & Artsob, 2012). Field surveys conducted near these human cases detected *Dermacentor* and *Haemaphysalis* ticks that tested positive for *Francisella tularensis* and *Rickettsia rickettsii*, the bacteria causing tularemia and Rocky Mountain spotted fever, respectively (Humphreys & Campbell, 1947; Wood & Artsob, 2012). By the 1980s, *F. tularensis* and *R. rickettsii* were detected in *Haemaphysalis leporispalustris* and *D. variabilis* ticks in Saskatchewan, Ontario, and Nova Scotia (Artsob et al., 1984; Ditchfield & Julian, 1960; Gordon et al., 1983; Wood & Artsob, 2012).

More recently, *Ixodes* ticks (including blacklegged ticks, *Ixodes scapularis*) have increased exponentially in Canada, with their distributions rapidly spreading. In the early 1970s, the sole endemic population of *I. scapularis* ticks was at Long Point, Ontario (Watson & Anderson, 1976). By the 2000s, *I. scapularis* ticks were detected at locations across Canada, with endemic populations in Ontario, Quebec, and Nova Scotia (Artsob et al., 1992; Barker et al., 1992; Ogden et al., 2005, 2006). Starting in the 1990s, *I. scapularis* ticks in Canada began testing positive for *Borrelia burgdorferi*, the bacterium causing Lyme disease (Barker et al., 1992; Ogden et al., 2006). By the 2010s, *I. scapularis* ticks with *B. burgdorferi* were established in Manitoba, Ontario, Quebec, New Brunswick, and Nova Scotia (Ogden et al., 2014). Today, *I. scapularis* ticks are widely distributed across southern Canada (Guillot et al., 2020; Wilson et al., 2022). This tick vector is of growing public health concerns due to its ability to transmit multiple tick-borne pathogens, including *Anaplasma phagocytophilum*, *Babesia* species, *Borrelia* species, *Ehrlichia* species, and Powassan virus (Table 1.1; Dibernardo et al., 2014; Nelder et al., 2021; Wilson et al., 2022). *Ixodes cookei*, a vector of Powassan virus, has also been identified as a tick species of public health concern due to its increasing abundance and range expansion, especially in Ontario and Quebec (Gasmi et al., 2018; Nelder et al., 2014).

In Canada, range expansions and increased abundances of ticks and their pathogens over time occurred alongside northward dispersals of host populations (Roy-Dufresne et al., 2013; Simon et al., 2014). Migratory birds disperse adventitious ticks over long distances, allowing

them to establish in new locations (Ogden et al., 2015; Scott, Clark, Foley, Bierman & Durden, 2018). In addition, several bird species in Canada are competent reservoirs for *Anaplasma*, *Babesia*, and *Borrelia* species (Dumas et al., 2022; Munro et al., 2019; Scott et al., 2020). Regionally, tick vectors are dispersed short distances by the range shifts of vertebrate hosts (Diuk-Wasser et al., 2021; Roy-Dufresne et al., 2013). Certain small mammals, such as mice, chipmunks, and shrews, also act as important reservoir hosts and blood meal sources (Brisson et al., 2008; Brunner et al., 2008; LoGiudice et al., 2003). As infected ticks and reservoir hosts become more abundant within communities, tick-borne disease risk for wildlife and human populations is likely to rise (Alkishe et al., 2021).

Current surveillance programmes in Canada have detected several emerging tick-borne pathogens in tick and host populations (Table 1.1 and Table A1). The most commonly detected pathogen is *B. burgdorferi*, which is predominantly transmitted by *I. pacificus* ticks in British Columbia and *I. scapularis* ticks in the rest of Canada (Guillot et al., 2020, 2022; Wilson et al., 2022). This pathogen was first detected in ticks and white-footed mice in the late 1980s in Ontario (Barker et al., 1992; Lindsay et al., 1991). In 2012, *Borrelia miyamotoi*, the bacterium causing tick-borne relapsing fever, was first identified in *I. scapularis* ticks, but it has now been found in several Canadian provinces (Dibernardo et al., 2014; Dumas et al., 2022; Wilson et al., 2022; Zinck & Lloyd, 2022). *Anaplasma* bacteria cause anaplasmosis in wildlife and humans with species such as *A. phagocytophilum* geographically widespread across Canada and isolated cases of *Anaplasma marginale* and *A. bovis* occurring in Alberta and Saskatchewan (Chilton et al., 2018; Dergousoff & Chilton, 2011; Howden et al., 2010; Wilson et al., 2022). Since 2010, cases of babesiosis caused by *Babesia* protozoans (*Babesia microti*, *B. duncani*, and *B. odocoilei*) have been reported across Canada in humans, wildlife, and *I. scapularis* ticks (Bullard et al., 2014; Crandall et al., 2022; Guillot et al., 2020; Milnes et al., 2019; Scott & Pesapane, 2021; Tonnetti et al., 2019; Werden et al., 2015).

Certain tick-borne pathogens may be rarer in prevalence, especially with their emergence or re-emergence in tick and host populations in Canada (Table 1.1 and Table A1). Limited detections of *Bartonella*, the bacterium causing bartonellosis, have been reported in *I. scapularis* ticks, wildlife, and humans in Canada (André et al., 2017; Breitschwerdt et al., 2019; Kho et al., 2021; Leighton et al., 2001). Ehrlichiosis is caused by *Ehrlichia* bacteria, which have been detected at low prevalence in Canada in ticks and wildlife since the 1990s (Berrington et al.,

1996; Morshed et al., 2020; Villeneuve et al., 2011). Since 1952, *Coxiella burnetii*, the bacterium causing Q fever, has been detected in ticks, rodents, and humans across Canada (Duron et al., 2014; Thompson et al., 2012). Powassan virus (lineage I) was first identified in 1958 in Powassan, Ontario, but several cases of lineages I and II have since been reported in ticks and humans across Canada (Artsob et al., 1984; Gholam et al., 1999; Guillot et al., 2020; Smith et al., 2018). Although *F. tularensis* has not recently been found in ticks, it has become geographically widespread across all Canadian provinces and territories with cases in domestic pets, wild animals, and humans (Gabriele-Rivet et al., 2016; Leighton et al., 2001). More recently, *R. rickettsii* was detected in *H. leporispalustris* ticks in Quebec (Crandall et al., 2022). Additional *Rickettsia* species have been detected in *Dermacentor* and *Amblyomma* ticks in other regions of Canada (Dergousoff et al., 2009; Teng et al., 2011; Wood et al., 2016; Yunik et al., 2015).

Current surveillance in Canada is tracking emerging tick-borne pathogens that are primarily found in *I. scapularis* ticks. However, it is challenging to determine the geographic and temporal extent of other emerging or re-emerging tick-borne pathogens in tick and host populations that are not reportable to public health agencies. Here, we assessed the historical tick–host–pathogen associations and identified spatial outliers in addition to spatiotemporal clusters of high pathogen presence in ticks in Canada using data extracted from a systematic review of the literature. We first examined the rate of publications focusing on tick-borne pathogens since 1960 as well as the frequency of distinct ticks, host groups, and pathogens being reported. We then examined spatial and temporal changes in the presence of ticks and their pathogens by detecting isolated and clustered regions of high pathogen presence across southern Canada. Finally, we evaluated the congruence between passive and active surveillance methods for detecting spatiotemporal clusters of pathogen presence. Our review is the first systematic assessment of the literature that identifies historical associations and spatiotemporal changes in the tick–host–pathogen disease systems in Canada over broad spatial and temporal scales.

1.3. Materials and Methods

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines to conduct a systematic review of the literature (Rethlefsen et al., 2021; Figure A1). We searched for peer-reviewed articles with no language or time restrictions up to 26 October 2021 in Web of Science. Our search terms included “tick*” AND (“Lyme disease” OR

“Borrelia” OR “babesiosis” OR “Babesia” OR “anaplasmosis” OR “Anaplasma” OR “bartonellosis” OR “Bartonella” OR “ehrlichiosis” OR “Ehrlichia” OR “Q fever” OR “Coxiella” OR “tularemia” OR “Francisella” OR “Rocky Mountain spotted fever” OR “Rickettsia” OR “Powassan virus” OR “flavivirus”) AND “Canada.” Reference lists of included articles were manually screened to identify potentially relevant studies (Figure A1).

1.3.1. Relevance screening and inclusion criteria

The full text of each article was screened to determine whether the study met our following inclusion criteria: (i) the study was conducted in Canada, (ii) ticks were tested for tick-borne pathogens of interest, (iii) the abundance or prevalence of infected individual ticks or tick pools were reported, (iv) ticks were naturally infected (e.g., not experimental transmission), (v) localities or coordinates of tested ticks were listed, and (vi) the temporal resolution of tested ticks did not exceed 5 years. Duplicate studies or studies that did not fit our inclusion criteria were excluded (Figure A1). Several additional studies were removed that only tested hosts or where ticks were not collected and/or tested for pathogens (e.g., reviews, modelling, molecular experiments). One study was retained where ticks tested positive for *Hepatozoon canis* after testing for *Babesia* species due to the genetic similarities between these two pathogens.

1.3.2. Data extraction

All studies that met the inclusion criteria were catalogued and data extracted. A data extraction table was populated with the study's authors, title, and year of publication, the province, locality name or coordinates, collection date, tick abundances, tick species, life stage, sex, and activity, pathogen genus and species, pathogen absence or presence, host species and group, testing method, and surveillance method. Tick abundances consisted of either individual nymphs, individual adults, pools of larvae, or pooled life stages. As these data are freely available in the public domain, ethics approval is not required.

Furthermore, classifications were made for variables with distinct categories. Tick activity was classified as questing if ticks were collected in the environment, and feeding if the tick was found actively feeding on a host. The surveillance method was designated as either active, passive, or a combined approach. Active surveillance uses field surveys to collect ticks from their natural habitat or from animals (Wilson et al., 2022). Passive surveillance uses ticks

collected from hosts that are submitted voluntarily by doctors, veterinarians, wildlife researchers, or the public (Wilson et al., 2022). We included the distinct testing methods of each study. Before 1985, studies would use suspensions made with ground ticks to inoculate hosts through intracerebral injections to identify if the collected ticks harboured pathogens. After 1985, studies used various testing methods on ticks including dark-field or immunofluorescence microscopy, monoclonal antibody tests, different polymerase chain reactions (PCR), and single-strand conformation polymorphism.

1.3.3. Data preparation

Extracted data were further prepared for our analyses. Prior to 1993, *I. scapularis* and *Ixodes dammini* were considered two distinct species; however, *I. dammini* is now considered to be a junior synonym to *I. scapularis* (Wesson et al., 1993). Therefore, specimens identified *as I. dammini* in studies published before 1993 were instead listed as *I. scapularis*. Ticks tested for *Rickettsia peacockii* were removed from the dataset, as this species is non-pathogenic (Wood et al., 2016). We further categorized the hosts of feeding ticks into groups of non-human animal (bird, mammal, or unknown) or human origins. When no spatial coordinates were supplied, we identified the latitude and longitude of a specimen based on its locality using Google Earth (Google Inc., 2022). Collection year was grouped into 5-year temporal bins starting from 1960 until the final 1-year interval in 2020. If ticks were collected over several years and did not indicate a precise year, we calculated the median year of collection and rounded down to determine the specimen's temporal bin. We also removed known duplicated results found at the same locality and time. Infection prevalence was calculated by dividing the infected tick abundance by the total tick abundance. The pathogen presence was coded as “absent” (0) if the infected tick prevalence was zero and “present” (1) if the infected tick prevalence was greater than zero.

1.3.4. Publications per year

To capture if there was a relationship between the number of publications and publication year, a generalized linear model with a Poisson distribution was performed using the *glm* function in the *stats* package in R version 4.2.2. (R Core Team, 2022).

1.3.5. Tick-host-pathogen associations

We created heat maps to evaluate the frequencies of the associations between tick vectors, hosts, and pathogens in Canada. The first pathogen/vector heat map was used to determine whether certain pathogens were more often detected in specific tick species. The second pathogen/host heat map was used to identify associations between pathogen species and host groups on which infected ticks were feeding. Finally, with a third vector/host heat map, we assessed the associations between feeding tick species and host groups.

1.3.6. Spatiotemporal clusters of infected ticks

Using ArcMap version 10.8.1. (Esri Inc., 2020), we first analysed the spatial autocorrelation of neighbouring dissemination areas across Canada to determine spatial outliers and clusters of high pathogen presence in ticks. A dissemination area is the smallest standard geographic area that census data are circulated, which represents relatively small and stable geographic units (Statistics Canada, 2021). Our response variable was the binary outcome of pathogen presence (1) or absence (0) in ticks. We then calculated Anselin Local Moran's I statistic (Anselin, 1995) for 999 replications using row standardization and contiguity along edges and corners. This analysis assessed the level of pathogen presence in a dissemination area compared to its neighbours to determine whether significant clusters or outliers are present. Here, we focused on dissemination areas with high levels of pathogen presence that had similar or lower levels of pathogen presence in neighbouring dissemination areas. A positive I value indicates that a dissemination area has neighbours with similar high counts of pathogen presence (i.e., high–high cluster). In contrast, a negative I value indicates that a dissemination area has neighbours with a low count of pathogen presence (i.e., high–low outlier).

Next, we used SaTScan™ version 10.1 (Kulldorff & Information Management Services, Inc., 2022) to detect spatiotemporal clusters of high pathogen presence in ticks in Canada, which accounted for the temporal intervals of our data. This method uses a cylindrical scanning window to determine whether spatiotemporal clusters are present, where the observed counts of pathogen presence are higher than the expected counts outside this window under the null hypothesis of spatial randomness (Kulldorff, 1997). The first spatiotemporal model used a Bernoulli-based probability model with the binary variable of pathogen presence (1) or absence (0) in ticks. A second multinomial model focused specifically on the spatiotemporal changes of

pathogen presence that were significantly different based on surveillance methods (passive, active, or a combination). For both models, we did not allow spatial overlap between spatiotemporal clusters. A minimum and maximum temporal time window of one time interval and 11 time intervals were used, respectively. Due to the large geographic scale of our analysis, we used a maximum spatial cluster size equal to 30% of cases of pathogen presence within a 250-km radius. For each cluster, at least two cases of pathogen presence were required. Most likely spatiotemporal clusters of high rates were detected using likelihood ratio tests, with p-values calculated based on 999 Monte Carlo replications. Spatiotemporal clusters were significant when $p < 0.05$.

1.4. Results

A total of 586 papers were retrieved using the search terms in Web of Science (Figure A1). After applying the inclusion criteria, 529 studies were no longer eligible for analysis due to duplicate studies or data (n = 10), the study being conducted outside Canada (n = 135), a temporal resolution greater than 5 years (n = 2), ticks not being tested for pathogens of interest (n = 47), ticks not collected (n = 278), ticks not naturally infected (n = 17), inability to find a full-text publication (n = 4), unknown collection year (n = 2), and unknown locality and/or tick abundances (n = 34). Our review retained 74 studies, including 17 studies found by scanning the references of the extracted studies.

1.4.1. Descriptive analysis

The reviewed studies encompassed wide spatial and temporal ranges, with studies conducted in all the Canadian provinces and spanning from 1960 to 2020 (Figure 1.1). We found 27 distinct tick species that were tested for tick-borne pathogens in Canada. Overall, ticks were more often uninfected (67.0%; n = 931) than infected (33.0%; n = 459) (Figure 1.2). Ticks were more often feeding than questing in uninfected (56.5%; n = 527 versus 21.2%; n = 197) and infected ticks (54.3%; n = 249 versus 35.1%; n = 161). However, 22.3% (n = 207) of uninfected ticks and 10.6% (n = 49) of infected ticks had unknown activity (Figure 1.2). The life stages of ticks consisted of 50.4% adults, 26.8% nymphs, 12.6% larval pools, 3.4% pooled life stages, and 6.8% unknown life stages (Figure 1.2). Ticks were collected with different surveillance methods

(Figure 1.2): passive surveillance (59.4%; n = 825), active surveillance (38.1%; n = 530), or a combined approach (2.5%; n = 35).

Collected ticks were tested for a wide array of pathogens from 10 genera. The three most frequently tested genera were *Borrelia* (50.6%; n = 704), *Rickettsia* (13.7%; n = 190), and *Anaplasma* (10.6%; n = 148). In addition, the three pathogen genera that were most often detected in infected ticks were *Borrelia* (61.4%; n = 282), *Rickettsia* (12.4%; n = 57), and *Babesia* (12.2%; n = 56). Pathogen testing was conducted in various ways, with the three most frequent testing techniques being single PCR (70.0%; n = 973), combined testing techniques (23.1%; n = 322), and the inoculation of ground tick suspensions in hosts through intracerebral injections (2.9%; n = 40) (Figure 1.2).

1.4.2. Publications per year

We found a significant positive relationship between the number of publications and publication year, with a greater number of publications over time ($z = 4.021$, $p < 0.001$; Figure A2).

1.4.3. Tick-host-pathogen associations

We first explored the associations between different pathogen species detected in infected ticks (Figure 1.3A). The most common association that we found was *B. burgdorferi* harboured in *I. scapularis* ticks. The two most frequent tick-borne pathogens detected in infected ticks were *B. burgdorferi* sensu stricto (38.1%; n = 175) and *B. burgdorferi* sensu lato (17.6%; n = 81). These pathogens were found in several tick species including *Amblyomma longistre*, *Dermacentor albipictus*, *D. variabilis*, *H. leporispalustris*, and multiple *Ixodes* spp. The tick species that were most frequently infected with multiple tick-borne pathogens were *I. scapularis* (56.9%; n = 261), *D. variabilis* (10.7%; n = 49), and *Ixodes auritulus* (6.1%; n = 28).

Next, we examined the associations between pathogens found in ticks feeding on different host groups (Figure 1.3B). The most frequent association was for *B. burgdorferi* found in ticks feeding on birds. All host groups harboured ticks that were infected with *B. burgdorferi* sensu stricto (34.1%; n = 85) and *B. burgdorferi* sensu lato (29.3%; n = 73). In addition, tick-borne pathogens were primarily found in ticks feeding on birds (48.2%; n = 120) and mammals (47.4%; n = 118) and were less often feeding on humans (3.2%; n = 8).

Finally, we investigated the combinations of distinct feeding tick species on different host groups (Figure 1.3C). *I. scapularis* and *I. cookei* were the only two species associated with all host groups. Although one-third of feeding ticks were *I. scapularis* (34.3%; n = 266), other tick species commonly found feeding on hosts included *H. leporispalustris* (10.6%; n = 82), *I. auritulus* (9.0%; n = 70), *I. pacificus* (8.5%; n = 66), and *D. variabilis* (8.0%; n = 62). Here again, most ticks were found feeding on birds (51.3%; n = 398) and mammals (39.9%; n = 310) rather than humans (7.7%; n = 60). A small number of feeding ticks were of unknown origin (0.3%; n = 2) or were listed as feeding on either a non-human mammal or human (0.8%; n = 6).

1.4.4. Spatiotemporal clusters of infected ticks

We found spatial clustering of high pathogen presence in ticks in most Canadian provinces (Figure 1.4). In British Columbia, clusters of high pathogen presence were located in Burnaby, Port Moody, and the Metchosin and Thompson-Nicola municipalities (Figure A3). In southeastern Alberta, we found spatial clusters in the Cypress, Forty Mile, Warner, and Lethbridge counties. Spatial clusters were also present in southern Saskatchewan near Lacadena, Saskatoon, and Carnduff and in southern Manitoba near Ethelbert, Birch River, and east of Winnipeg (Figure A4). In Ontario, we found spatial clusters near Kenora; the Haldimand, Norfolk, and Wellington counties; Ottawa; and the Peterborough and Leeds and Grenville counties (Figure A5). In the Atlantic provinces, few spatial clusters were identified, including areas in Division No. 1 and near Gros Morne National Park in Newfoundland, Three Rivers in Prince Edward Island, and Grand Manan in New Brunswick (Figure A6). No spatial clusters of high pathogen presence were identified in Quebec or Nova Scotia. In addition, we found 246 spatial outliers, which represented areas of high pathogen presence surrounded by areas of low pathogen presence. These spatial outliers were found across the southern portions of all the Canadian provinces.

When accounting for the year, we detected five spatiotemporal clusters of high pathogen presence in ticks in southern Canada (Figure 1.4 and Table A2). A first cluster was detected near Kenora in northeastern Ontario and the east of Winnipeg in Manitoba from 2005 to 2019 [Cluster 1: 125 km, relative risk (RR) = 2.78, $p < 0.001$; Figure A4]. Another cluster was found in southern Manitoba and southeastern Saskatchewan from 2005 to 2019 [Cluster 3: 231 km, RR = 2.12, $p < 0.01$; Figure A4]. In southeastern Ontario, one cluster was found from 2010 to 2019

[Cluster 2: 214 km, RR = 1.66, $p < 0.001$; Figure A5]. A smaller cluster was detected in southern Alberta from 2005 to 2019 [Cluster 5: 96 km, RR = 3.07, $p < 0.05$; Figure A3]. Finally, a recent cluster was found across parts of the Atlantic provinces from 2015 to 2019 [Cluster 4: 245 km, RR = 2.84, $p < 0.05$; Figure A6]. No spatiotemporal cluster of high pathogen presence was detected in Quebec or Nova Scotia.

We also found differences in spatiotemporal clusters of high pathogen presence in ticks when accounting for the surveillance method used to collect ticks (Figure 1.5 and Table A3). Two clusters were predominantly associated with active surveillance. The first cluster was located near eastern Ontario from 2005 to 2019 [Cluster 1: 131 km, RR = 2.79 (active) and 0.04 (passive), $p < 0.01$; Figure A8]. An additional cluster was identified in southeastern Alberta and southwestern Saskatchewan from 2005 to 2014 [Cluster 4: 213 km, RR = 2.30 (active), 0.07 (passive), $p < 0.01$; Figure A7]. Several clusters of high pathogen presence in ticks were found based on passive surveillance activities. A cluster was detected in southeastern Ontario from 1995 to 2020 [Cluster 2: 188 km, RR = 0.06 (active), 1.89 (passive), 11.91 (combined), $p < 0.01$; Figure A8]. An additional cluster was identified in southern British Columbia from 2000 to 2009 [Cluster 3: 126 km, RR = 1.94 (passive), $p < 0.01$; Figure A7]. A large cluster was found among the Atlantic provinces from 1990 to 2019 [Cluster 5: 248 km, RR = 1.92 (passive), $p < 0.01$; Figure A9]. Finally, a small cluster was identified in Kenora from 2010 to 2019 [Cluster 6: 40 km, RR = 1.91 (passive), $p < 0.05$; Figure A8].

1.5. Discussion

Our systematic review documented nine genera of pathogens present in 20 tick species across broad spatial and temporal ranges in Canada. We found a significant increase in the number of publications through time, likely a result of the greater spread and establishment of tick populations throughout Canada. Approximately one-third of ticks were infected with a pathogen and were primarily found feeding on a host (bird, mammal, or human). Certain common pathogen and tick species were often associated with each other, including *B. burgdorferi* being most frequently isolated in *I. scapularis* ticks. This tick vector was found feeding on various host groups and harboured the greatest number of tick-borne pathogens. We also detected several spatial outliers, which represent isolated regions of high pathogen presence in ticks found across southern Canada. In addition, five spatiotemporal clusters of high pathogen

presence were identified, which coincided with areas of southern Canada with long-established tick populations. Finally, distinct spatiotemporal clusters of high pathogen presence were identified based on the surveillance methods used to collect ticks, with two clusters associated with active surveillance and four clusters related to passive surveillance.

1.5.1. Spatial and temporal range of studies

Pathogens were present in ticks over a wide temporal range in Canada. In our review, the first case of pathogen presence was for Powassan virus in 1962, which was detected in a *Ixodes marxi* tick feeding on a red squirrel (*Tamiasciurus hudsonicus*) in Ontario (McLean & Larke, 1963). Between 1960 and 2004, few studies tested ticks for our pathogens of interest ($n < 10$ per time interval). It was only in the early 1990s that passive surveillance, especially for *I. scapularis* ticks, began to increase in Canada (Ogden et al., 2006). By the 2000s, *I. scapularis* ticks were identified across southern Canada, with greater numbers of established populations in Central and Atlantic Canada (Barker et al., 1992; Ogden et al., 2006). As a result, more studies began testing for pathogens in *I. scapularis* ticks in Canada by 2005, coinciding with the start of large-scale surveillance (Ogden et al., 2008; Scott et al., 2012). Since then, continuous reports of increased tick abundances, higher prevalence of tick-borne pathogens, and increased human cases of tick-borne illnesses have been reported across Canada (Gasmi et al., 2022; Guillot et al., 2020; Parkins et al., 2009; Wilson et al., 2022).

However, this increased prevalence of tick-borne pathogens through time may be impacted by testing method, as pathogens are more likely to be detected with technological advancements. Early studies used ground tick specimens in suspensions that would be used to inoculate hosts through intracerebral injections to identify neutralizing antibodies of pathogens, which were difficult to interpret due to variability in host immune responses (Humphreys & Campbell, 1947; McLean & Larke, 1963). Therefore, tick-borne pathogens were easier to isolate from humans with active infections than ticks or hosts at this time (Humphreys & Campbell, 1947). In comparison, newer PCR techniques and combined testing methods have improved specificity and sensitivity for detecting various tick-borne pathogens (Wills et al., 2018).

The geographic extent of our review encompassed all Canadian provinces. Overall, the greatest number of studies was conducted in Ontario compared to the other provinces. This centralized study of ticks and their pathogens in Ontario coincides with the occurrence of the

first endemic *I. scapularis* tick population in Canada (Watson & Anderson, 1976). The greatest abundances of *Ixodes* ticks found during ongoing sentinel surveillance are located in Ontario, Quebec, and certain Atlantic provinces (Guillot et al., 2020; Wilson et al., 2022). In contrast, we found few eligible studies for our review from the Atlantic provinces, especially in Prince Edward Island and Newfoundland and Labrador.

1.5.2. Historical tick-host-pathogen associations

Over one-third of ticks were infected with a pathogen, and *B. burgdorferi* was the most frequently detected pathogen. Similarly, over 60% of feeding ticks were infected with *B. burgdorferi* sensu stricto or sensu lato, which was not associated with ticks feeding on a particular host group. Since its first discovery in tick populations in Ontario (Barker et al., 1992), *B. burgdorferi* has become the most common tick-borne pathogen in Canada, which may be impacted by a bias in predominantly testing for this pathogen in *I. scapularis* and *I. pacificus* (Guillot et al., 2020; Wilson et al., 2022). However, *B. burgdorferi* was also isolated in *D. variabilis*, *H. leporispalustris*, and other *Ixodes* spp. (Banerjee et al., 1995; Barker et al., 1992; Morshed et al., 2005; Ogden et al., 2008; Scott et al., 2010, 2012; Scott & Durden, 2015).

The presence and prevalence of tick-borne pathogens may be dependent on the host groups of feeding ticks. Over 90% of feeding ticks were found parasitizing birds or mammals at a similar rate, with only a small percentage found feeding on humans. Certain generalist tick species, such as *I. scapularis*, feed on various hosts including mammals, birds, and humans (Keirans et al., 1996; Lindquist et al., 2016). *I. scapularis* ticks were found feeding on a diverse set of hosts and were infected with six distinct pathogens from the genera *Anaplasma*, *Babesia*, *Borrelia*, and *Hepatozoon* (Cockwill et al., 2009; Lewis & Lloyd, 2019; Scott et al., 2012; Scott & Pesapane, 2021; Smith et al., 2019; Stokes et al., 2020). However, certain specialist tick vectors, such as *I. auritulus* and *I. cookei*, fed exclusively on specific host groups such as birds and mammals, respectively (Lindquist et al., 2016). We found that *I. auritulus* ticks feeding on birds were only infected with *Borrelia* species, including *B. americana* and *B. burgdorferi* (Morshed et al., 2005; Scott, Clark, Foley, Anderson et al., 2018; Scott & Foley, 2016). Similarly, *I. cookei* ticks were found feeding on groundhogs, skunks, weasels, and cats and were infected with *B. burgdorferi* sensu lato, *B. microti*, and Powassan virus (Artsob et al., 1984; McLean et al., 1964; Scott, Clark, Foley, Anderson et al., 2018; Scott et al., 2019).

1.5.3. Emerging spatiotemporal clusters of pathogen presence

A spatial sampling bias may be present in our review, resulting from certain areas being highly sampled for ticks by researchers. This potential bias is especially apparent when examining the spatial outliers of high pathogen presence, which are typically found in isolated areas located further away from hotspots (Figure 1.4 and Figures A3– A6). Historical surveys were generally conducted in areas with greater tick-borne disease incidence in humans to determine which factors may contribute to illness (Humphreys & Campbell, 1947; McLean et al., 1961). More recently, sentinel surveillance has been used to track the environmental risk of pathogens in tick populations at key locations across Canada (Guillot et al., 2020, 2022). Due to distinct surveillance objectives through time, we document a historical snapshot of surveyed ticks and pathogens found in clustered and isolated areas in Canada.

We detected spatiotemporal clusters of high pathogen presence in ticks that coincide with regions of long-established tick populations in Alberta, Manitoba, Ontario, and certain Atlantic provinces (Wilson et al., 2022). Four spatiotemporal clusters are located in areas with endemic *I. scapularis* tick populations, which may harbour species of *Anaplasma*, *Babesia*, and *Borrelia* (Dibernardo et al., 2014; Guillot et al., 2020; Lewis & Lloyd, 2019; Nelder et al., 2021; Smith et al., 2018; Wilson et al., 2022). In contrast, the spatiotemporal cluster located in Alberta was associated with *Dermacentor* ticks. From 2000 to 2019, *Dermacentor andersoni* and *D. variabilis* ticks were the most frequently submitted vectors found feeding on humans in this area in Alberta (Kanji et al., 2022). These ticks are primarily found in western Canada and may transmit pathogens including *F. tularensis*, *Anaplasma* spp., and *Rickettsia* spp. (Bouchard et al., 2019; Chilton et al., 2018; Humphreys & Campbell, 1947; Wood & Artsob, 2012). Hotspots of tick-borne pathogens may be more likely to appear within these broad regions. Yet, the low-risk areas identified within these clusters may exhibit a faster conversion to high-risk areas. As a result, active surveillance in these low-risk areas should be prioritized to track this anticipated shift. Surprisingly, we did not detect a spatiotemporal cluster in southern Quebec or Nova Scotia (Wilson et al., 2022). This inconsistency is likely due to our conservative inclusion criteria, as many studies in Quebec and Nova Scotia did not specify the localities or coordinates of collected ticks. In addition, the retained data from Quebec were predominantly from one time interval (2015–2019). Similarly, a limited number of observations in Nova Scotia detected tick-borne

pathogens in collected ticks, where half of these observations occurred during one time interval (2015–2019).

We also demonstrated that the detection of spatiotemporal clusters of pathogen presence may depend on the surveillance method used to collect ticks (Figure 1.5 and Figures A7–A9). Active surveillance aims to identify areas with establishing tick populations to follow the spread of tick-borne pathogens (Wilson et al., 2022). Here, we identified two spatiotemporal clusters where ticks were primarily collected after 2005 through active surveillance. These spatial clusters are in regions with targeted research efforts focusing on populations of *I. scapularis* ticks in eastern Ontario and *Dermacentor* ticks in Alberta and Saskatchewan. Although active surveillance requires more time and resources, it provides a more accurate estimate of the progression and establishment of tick populations and their pathogens (Clow et al., 2019; Guillot et al., 2020). In contrast, passive surveillance requires less time and funds, but is difficult to maintain uniformly across Canada due to limited resources (Clow et al., 2019; Guillot et al., 2020). This type of surveillance can be used to identify geographic regions that may be at risk for the introduction of tick populations and tick-borne pathogens (Morshed et al., 2021; Ripoche et al., 2018; Wilson et al., 2022). We found that clusters of pathogen presence detected by passive surveillance had a wide geographic extent across Canada, with clusters located from British Columbia to the Atlantic provinces. In addition, these clusters were detected earlier, starting in the 1990s and 2000s. Therefore, our spatiotemporal clusters of pathogen presence may be related to provincial differences in surveillance efforts (Guillot et al., 2020; Wilson et al., 2022).

Furthermore, each type of surveillance method may be used in distinct ways for the detection and documentation of tick-borne pathogen emergence and subsequent disease risk. Passive surveillance may be used to monitor the early detection of emerging tick-borne pathogens at macro-ecological scales. This kind of surveillance may also be employed to develop a baseline for the presence and distribution of newly detected tick-borne pathogens in Canada (Morshed et al., 2021; Nelder et al., 2021). In contrast, active surveillance may be more sensitive for determining the geographic extents and range expansions of tick vectors and subsequent risk for tick-borne diseases, such as Lyme disease (Guillot et al., 2022; Ripoche et al., 2022). However, the concurrent use of both surveillance strategies will provide the most detailed and comprehensive representation of the geographic extent of tick vectors and their pathogens, which may not be captured when using each surveillance strategy alone (Lyons et al., 2021).

1.5.4. Limitations

While comprehensive, our systematic review was limited due to the nature and sparsity of the reported data. We found that there was a significant publication bias, with a greater number of studies testing ticks for pathogens through time (Figure S2). Studies with negative results may not be easily published and therefore missing from our systematic review (Uttley et al., 2023). In addition, we may also be missing studies from grey literature, as they are not typically included in large databases (Uttley et al., 2023). The heterogeneous nature of the studies including the testing methods as well as the tick activity and life stage likely impacted pathogen detection. However, the use of conservative inclusion criteria in our review allowed us to standardize our data across studies to limit this heterogeneity. In many cases, the host species of feeding ticks were not specified requiring us to classify vertebrate hosts to larger host groups, thereby limiting the analyses we could conduct.

When reporting data on ticks, hosts, and pathogens in Canada, we suggest that simple measures should be used to facilitate spatiotemporal comparisons across studies. For example, supplying the spatial coordinates, collection year, and the infected and total tick abundances would provide essential information allowing greater standardization across space and through time. A consistent record of which host species are feeding and transmitting tick-borne pathogens at local scales would provide additional knowledge regarding their contributions and reservoir competence in these disease systems. As a result, greater clarity, transparency, and standardization in the reported data would allow better assessment of the associations and spatiotemporal changes of the tick–host–pathogen disease systems. Therefore, comprehensive surveillance that uses these simple measures will be an imperative tool in the future for detecting the presence, spread, and risk of tick-borne pathogens in tick, wildlife, and human populations throughout Canada.

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1.7. Tables

Table 1.1. Current and historical tick-borne pathogens that have been detected in Canada. The spatial and temporal information of each first detection in Canada has been noted as well as if this detection was first found in a tick, animal, or human. See Table A1 for detailed list of references for each pathogen.

Pathogen	Primary tick vector(s)	Human disease	Geographic extent (tick, wildlife, or human cases)
Anaplasma marginale	*Dermacentor andersoni, D. variabilis*	N/A	SK, MB, ON, QC
Anaplasma bovis	*D. andersoni*	N/A	AB, SK
Anaplasma phagocytophilum	*Ixodes scapularis, I. pacificus*	Anaplasmosis	BC, AB, SK, MB, ON, QC, NB, NL, NS, PE
Babesia duncani	*I. scapularis*	Babesiosis	BC, AB, SK, MB, ON, QC, NB, NL, NS, PE
Babesia microti	*I. scapularis*	Babesiosis	AB, MB, ON, QC, NB, NS, PE
Babesia odocoilei	*I. scapularis*	Babesiosis	SK, MB, ON, QC
Bartonella spp.	*I. scapularis, I. pacificus*	Bartonellosis	BC, AB, SK, MB, ON, QC
Borrelia burgdorferi	*I. scapularis, I. pacificus*	Lyme disease	BC, AB, SK, MB, ON, QC, NB, NL, NS, PE
Borrelia miyamotoi	*I. scapularis, I. pacificus*	Tick-borne relapsing fever	AB, MB, ON, QC, NB, NS, PE
Coxiella burnetii	*D. andersoni*	Q fever	BC, AB, SK, MB, ON, QC, NB, NL, NS, PE
Ehrlichia spp.	*Amblyomma americanum, Rhipicephalus sanguineus*	Ehrlichiosis	BC, AB, MB, ON, QC, NB, NL, NS, PE
Francisella tularensis	*D. andersoni, D. variabilis, Haemaphysalis leporispalustris*	Tularemia	BC, AB, SK, MB, ON, QC, NB, NL, NS, PE, NT, NU, YT
Powassan virus – Lineage I	*I. cookei, I. marxi, I. spinipalpis*	Powassan virus	BC, AB, MB, ON, QC, NB, NS, PE

Powassan virus – Lineage II	*I. scapularis, D. andersoni*	Deer tick virus	MB, ON, NS
Rickettsia rickettsii	*D. andersoni, D. variabilis, H. leporispalustris*	Rocky Mountain spotted fever	BC, AB, SK, ON, QC, NS

Province and locality of first detection	Year of first detection	Host or vector of first detection in Canada	References
MB	1968	*Bos taurus*	[1-2]
AB	2005	*D. andersoni*	[3-4]
ON (Long Point)	2005	*I. scapularis* and *I. dentatus*	[1, 5-10]
ON	2017	Human	[11-12]
MB	2010	*I. scapularis*	[1, 10, 13-16]
SK	2012	*Cervus canadensis*	[17-24]
AB and SK	1995	*Felis catus*	[25-31]
ON (Long Point)	1987	*Peromyscus leucopus*	[1, 9-10, 32-35]
		I. scapularis and *D. variabilis*	
Canada-wide	2012	*I. scapularis*	[9-10, 15, 36-38]
QC	1952	Human	[1, 39-44]
BC	1996	*Equus caballus*	[1, 27, 45-48]
ON (Timmins)	1929	Human	[1, 25, 49-58]
ON (Powassan)	1958	Human	[1, 59-65]
ON	1997	Human	[1, 36, 66-67]
AB	1935	Human	[1, 23, 25, 50, 68-71]

1.8. Figures

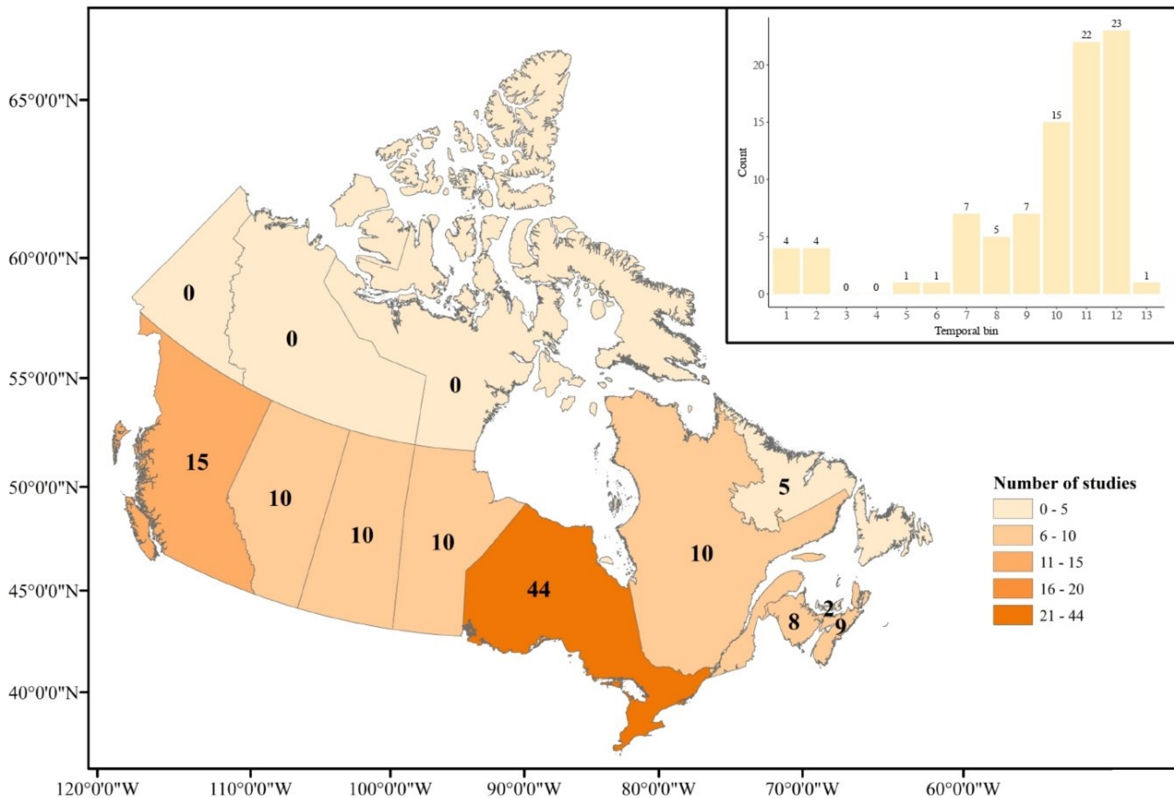

Figure 1.1. The reviewed studies encompassed wide spatial and temporal ranges. Studies were conducted in all the Canadian provinces: British Columbia (n = 15), Alberta (n = 10), Saskatchewan (n = 10), Manitoba (n = 10), Ontario (n = 44), Quebec (n = 10), New Brunswick (n = 8), Nova Scotia (n = 9), Prince Edward Island (n = 2), and Newfoundland and Labrador (n = 5). No studies were conducted in the Canadian territories. Studies ranged from 1960 to 2020, which were categorized into temporal bins 1–13: 1960–1964 (n = 4), 1965–1969 (n = 4), 1970–1974 (n = 0), 1975–1979 (n = 0), 1980–1984 (n = 1), 1985–1989 (n = 1), 1990–1994 (n = 7), 1995–1999 (n = 5), 2000–2004 (n = 7), 2005–2009 (n = 15), 2010–2014 (n = 22), 2015–2019 (n = 23), and 2020 (n = 1).

Figure 1.2. The infection status, type of activity (questing versus feeding), life stage, surveillance method, and testing methods varied considerably across the studies encompassed in our review.

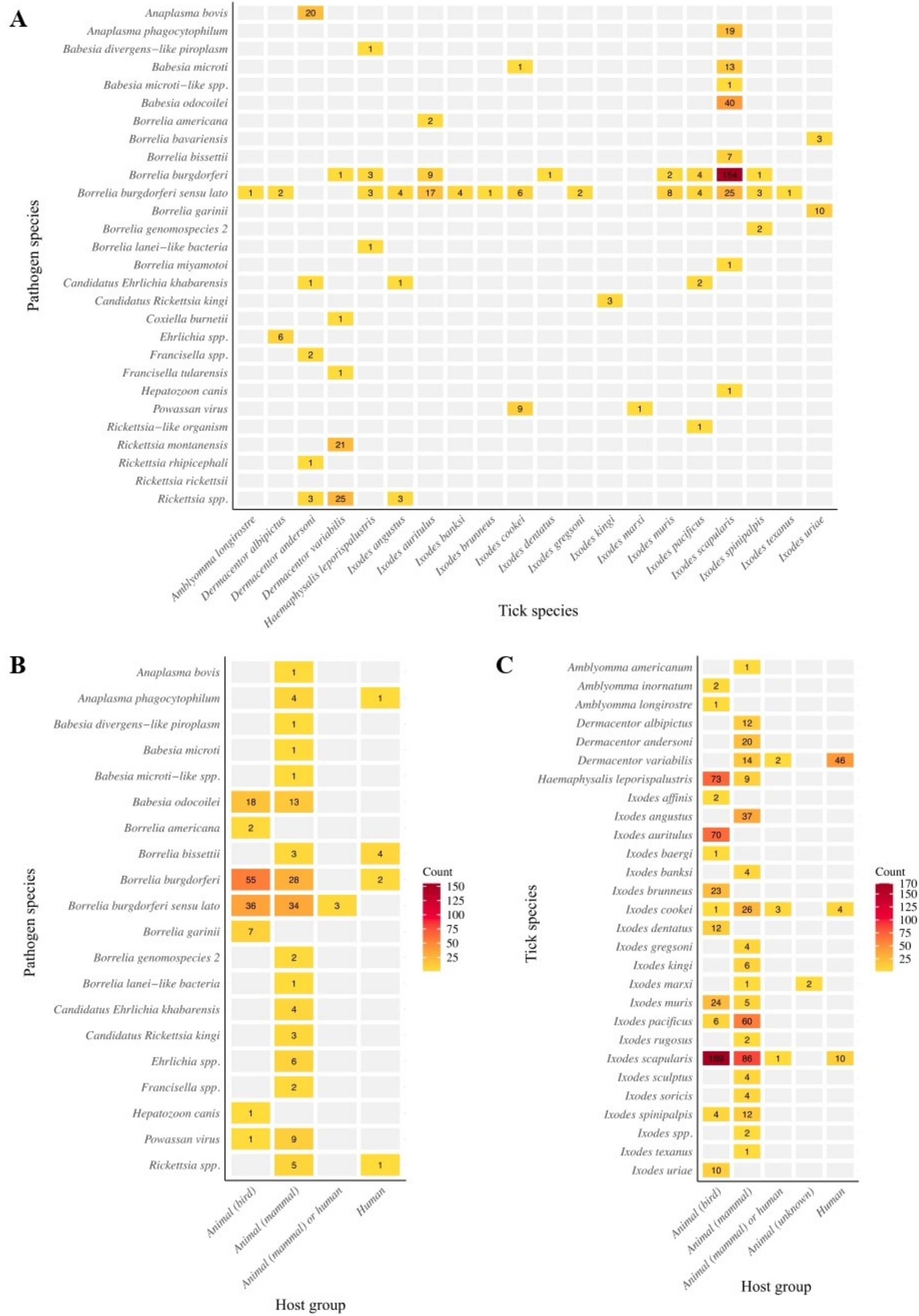

Figure 1.3. Heat maps of the distinct associations between historical observations of (A) pathogen species and infected tick species, (B) pathogen species and host groups, and (C) feeding tick species and host groups in the tick-borne disease system in Canada.

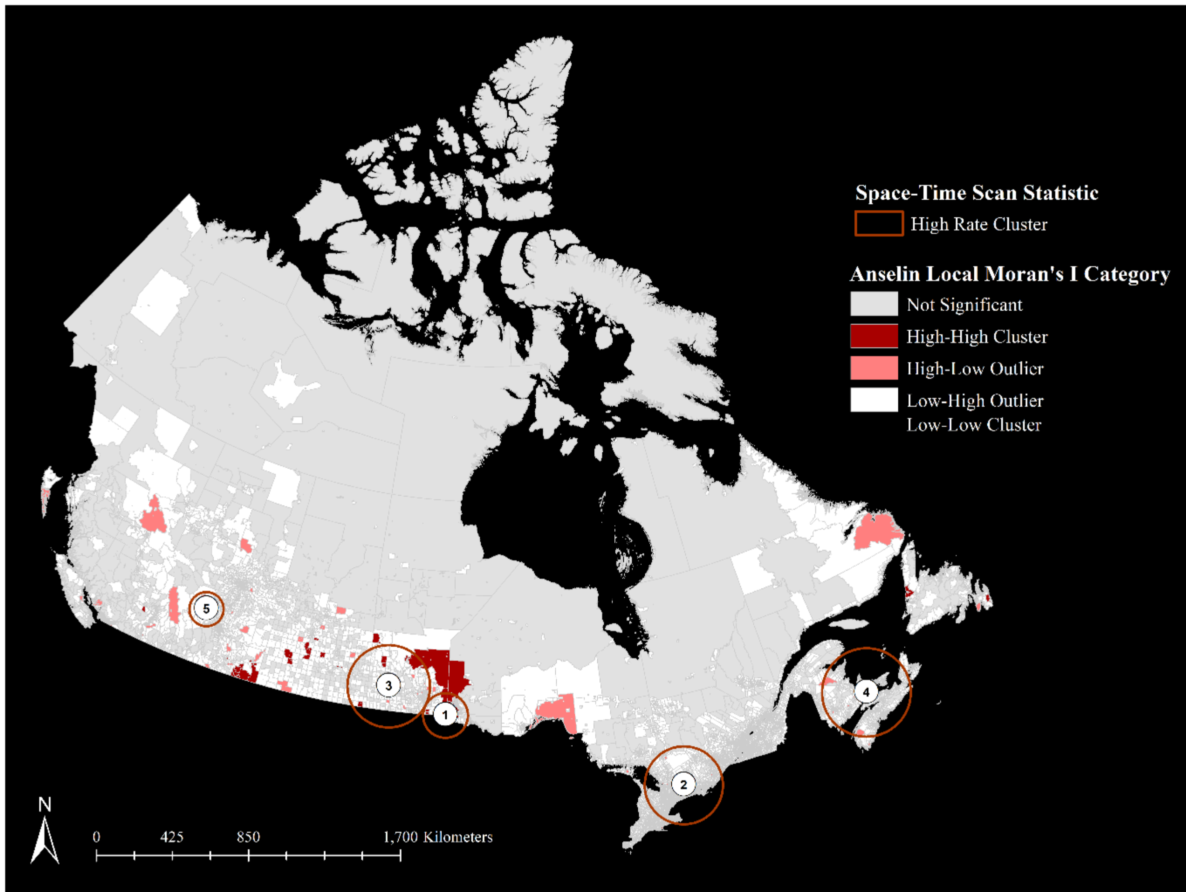

Figure 1.4. Historical spatiotemporal clusters of high pathogen presence in ticks in Canada from 1960 to 2020. By calculating Anselin Local Moran's I statistics, we found high pathogen presence in dissemination areas across southern Canada as spatial clusters (red) and outliers (salmon). Using SaTScan™, spatiotemporal clusters of high rates of pathogen presence in ticks were also detected while accounting for temporal period. Significant spatiotemporal clusters were found in southern Alberta, southeastern Saskatchewan, southern Manitoba, and northwestern Ontario from 2005 to 2019 (Clusters 1, 3, and 5), in southeastern Ontario from 2010 to 2019 (Cluster 2), and parts of the Atlantic provinces from 2015 to 2019 (Cluster 4).

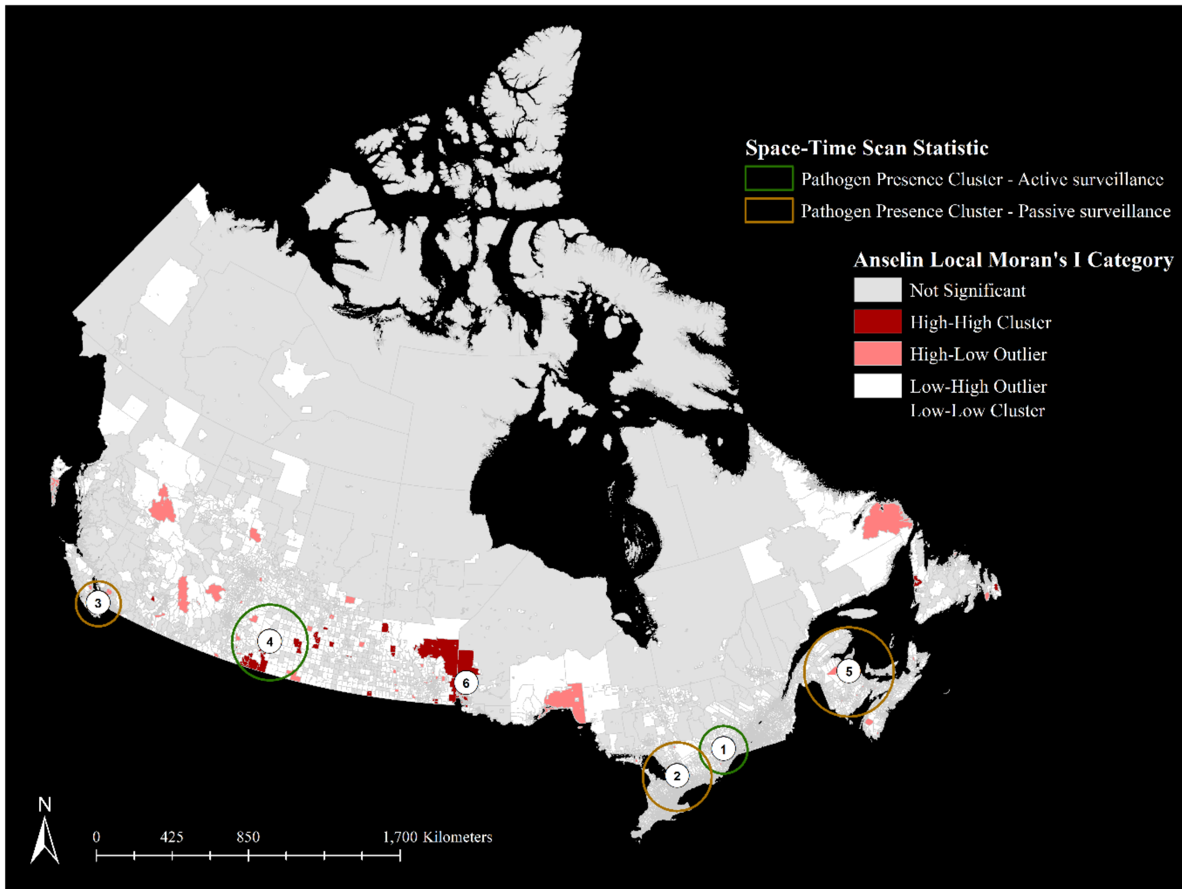

Figure 1.5. Historical spatiotemporal clusters of pathogen presence in ticks in Canada from 1960 to 2020 based on the types of surveillance. By calculating Anselin Local Moran's I statistics, we found high pathogen presence in dissemination areas across southern Canada as spatial clusters (red) and outliers (salmon). Using SaTScan™, spatiotemporal clusters of pathogen presence in ticks were also detected based on different types of surveillance (active, passive, or combined) while accounting for temporal period. Significant clusters of pathogen presence were detected by active surveillance in eastern Ontario from 2005 to 2019 (Cluster 1) as well as in southeastern Alberta and southwestern Saskatchewan from 2005 to 2014 (Cluster 4). Passive surveillance detected clusters of pathogen presence in ticks in southeastern Ontario from 1995 to 2020 (Cluster 2), in southern British Columbia from 2000 to 2009 (Cluster 3), in parts of the Atlantic provinces from 1990 to 2019 (Cluster 5), and in Kenora from 2010 to 2019 (Cluster 6).

Connecting statement between Chapter 1 and 2

Emerging or re-emerging tick-borne pathogens have steadily increased in prevalence in Canada due to the establishment and range expansion of tick and host populations. In Chapter 1, I distinguished the historical associations of the tick-host-pathogen disease systems and identified spatiotemporal clusters of high tick-borne pathogen presence in Canada using data extracted from the literature. Although surveillance efforts in Canada primarily target *I. scapularis* and *B. burgdorferi*, this analysis demonstrated that additional tick vectors and pathogens of public health concern may be present depending on the geographic location. In addition, we found that infected ticks were most often found feeding on wildlife (birds and mammals), demonstrating the pivotal role of these hosts in pathogen transmission.

Based on Chapter 1, I identified which contributors of the tick-host-pathogen disease systems may be present at my study sites in Ontario and Quebec. Small mammals that were identified as important reservoir hosts were sampled through small mammal trapping. Similarly, trail cameras were used to determine which mid-size and large mammals were present locally. Tick specimens were actively collected by dragging a flannel through the environment or removed from trapped small mammals. Chapter 2 incorporates host active surveillance data with high-resolution environmental data to simultaneously evaluate the combined impact of abiotic factors, such as temperature, precipitation, and snow, and biotic factors, such as the abundance and diversity of mammal hosts, on questing *I. scapularis* abundance in Ontario and Quebec. This study uses high-resolution abiotic factors derived from remote sensing satellites and meteorological towers and biotic factors derived from host active surveillance data collected in the field.

Chapter 2 | High-resolution environmental and host-related factors impacting questing *Ixodes scapularis* at their northern range edge

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Kirsten E. Crandall, Virginie Millien, and Jeremy T. Kerr

2.1. Abstract

The geographic range of tick populations have expanded in Canada due to climate warming and the associated poleward range shifts of their vertebrate hosts. Abiotic factors, such as temperature, precipitation, and snow, are known to directly affect tick abundance. Yet, biotic factors, such as the abundance and diversity of mammal hosts, may also alter tick abundance and consequent tick-borne disease risk. Here, we incorporated host surveillance data with high-resolution environmental data to evaluate the combined impact of abiotic and biotic factors on questing *Ixodes scapularis* abundance in Ontario and Quebec, Canada. High-resolution abiotic factors were derived from remote sensing satellites and meteorological towers, while biotic factors related to mammal hosts were derived from active surveillance data that we collected in the field. Generalized additive models were used to determine the relative importance of abiotic and biotic factors on questing *I. scapularis* abundance. Combinations of abiotic and biotic factors were identified as important drivers of abundances of questing *I. scapularis*. Positive and negative linear relationships were found for questing *I. scapularis* abundance with precipitation and accumulated snow, but no effect was found for the relative abundance of white-footed mice. Positive relationships were also identified between questing *I. scapularis* abundance with monthly mean precipitation and mammal species richness. Therefore, future studies that assess *I. scapularis* should incorporate host active surveillance data with high-resolution environmental factors to determine the key drivers impacting the abundance and geographic spread of tick populations and tick-borne pathogens.

Keywords: climate, host diversity and abundance, *Ixodes scapularis*, mammal, *Peromyscus leucopus*

2.2. Introduction

Blacklegged ticks (*Ixodes scapularis*) are a disease vector of significant public health concern in the temperate regions of North America. Currently, this tick vector has a wide geographic range across Canada, with long-established populations in Manitoba, Ontario, Quebec, New Brunswick, and Nova Scotia (Ogden et al. 2014, Guillot et al. 2020, Wilson et al. 2022, Crandall et al. 2022). In addition, *I. scapularis* is capable of transmitting various tick-borne pathogens that cause tick-borne diseases in humans, including anaplasmosis, babesiosis, and Lyme disease (Dibernardo et al. 2014, Nelder et al. 2021, Wilson et al. 2022). In response to changes in climate and land use, several environmental and host-related factors may act as potential drivers in the establishment and poleward expansion of *I. scapularis* in Canada (Table B1; Ogden and Lindsay 2016, Bouchard et al. 2019, Alkishe et al. 2021).

With climate warming, higher temperatures are expected to increase tick abundances through faster development rates and longer seasonal activity periods (Ogden et al. 2004, 2021, Eisen et al. 2016, Ogden and Lindsay 2016). Laboratory studies on *I. scapularis* have found that extreme cold or hot temperatures have been associated with increased mortality rates and disturbed physiological processes, such as oviposition and egg mass development (Ogden et al. 2004, Eisen et al. 2016, Fieler et al. 2021). However, *I. scapularis* in nature may use microclimate refuges under leaf litter to avoid adverse weather conditions and maintain their optimal thermal thresholds (Linske et al. 2019, Volk et al. 2022). As a result, climate warming may affect *I. scapularis* populations in distinct ways depending on the temperature variability and extremes experienced locally at their range edges, with increased extirpation risk at the southern edge with very high temperatures and facilitated establishment at the northern range edge with warming temperatures (Ogden et al. 2013).

Abundant snow cover may also increase tick survival by providing an additional insulative layer from cold subzero temperatures. Snow cover alone or in combination with leaf litter have been found to increase overwintering survival by providing sufficient insulation to prevent inoculative freezing or desiccation (Linske et al. 2019, Volk et al. 2022). Summer nymphal *I. scapularis* densities have been found to increase after greater winter precipitation including snowfall (Hayes et al. 2015). In contrast, milder winters with intermittent snow cover may increase the risk of inoculative freezing in ticks, thereby limiting tick survival and densities the subsequent summer (Eisen et al. 2016, Linske et al. 2019, Volk et al. 2022).

Greater precipitation may maintain sufficient humidity within the microclimate leading to greater tick densities, while extended periods of low moisture may reduce tick survival and densities (Berger et al. 2014a, 2014b, Dumas et al. 2022). Hot, dry summer days have been found to increase mortality in *I. scapularis* due to greater water loss and desiccation stress (Eisen et al. 2016, Burtis et al. 2016). However, behavioural changes in ticks may mitigate their desiccation through modifications in their questing activity (Vail and Smith 2002). Yet, increased levels of hydric stress may result in higher desiccation risk and lower *I. scapularis* abundances (Diuk-Wasser et al. 2006, Berger et al. 2014b, Burtis et al. 2016).

Denser vegetation, especially in forested areas, may provide more suitable habitats for *I. scapularis* survival and development (Schulze and Jordan 2001, Clow et al. 2017b, Ginsberg et al. 2020, Mathisson et al. 2021). Several studies have found a positive association between *I. scapularis* and dense shrub vegetation (Mathisson et al. 2021). More specifically, the density of understory and shrubs were found to impact the risk of *I. scapularis* present in Ontario, with greater risk with low understory density and a medium to high relative abundance of shrubs due to its suitability for ticks and small mammals (Clow et al. 2017b). In contrast, lower *I. scapularis* densities have been found in grasslands and other open canopy environments, where humidity conditions may be unsuitable for their survival (Ginsberg et al. 2020, Mathisson et al. 2021).

Greater host abundances may variably influence tick populations locally. In regions with long-established tick populations, higher densities of mammal hosts, especially white-tailed deer (*Odocoileus virginianus*), are expected to increase tick abundances due to additional contact opportunities (Dobson 2014, Estrada-Peña and De La Fuente 2014, Levi et al. 2016). In addition, the relative abundance of white-footed mice (*Peromyscus leucopus*) may also impact the immature *I. scapularis* abundance feeding on small mammals (Bouchard et al. 2011, 2013, Werden et al. 2014). In contrast, tick-host interactions in areas with emergent tick populations, such as those near the poleward range edge, may be dynamic and lead to variable *I. scapularis* abundances because of limited host availability (Dobson 2014, Millien et al. 2023). Yet, these relationships may be unclear if only tick dragging were conducted (Dobson 2014). Therefore, the complementary use of small mammal trapping with tick dragging during active surveillance may provide a better metric to assess the relationships between *I. scapularis* and small mammal communities.

The diversity of mammal species may also affect tick abundances due to the quality of blood meal hosts present within the community. Different mammal species may vary in their ability to successfully feed ticks (Mather et al. 1989, LoGiudice et al. 2003). Small mammal hosts, such as white-footed mice, chipmunks (*Tamias striatus*), and shrews (*Blarina brevicauda* and *Sorex cinereus*), play an important role in tick survival by successfully feeding a greater abundance of immature ticks (Mather et al. 1989, LoGiudice et al. 2003). Certain mid-size and large mammals, such as raccoons (*Procyon lotor*) and white-tailed deer, may also impact tick abundance by feeding large burdens of ticks, and act as key reproductive hosts for adult *I. scapularis* (LoGiudice et al. 2003). However, *I. scapularis* abundance may also be restricted by host-specific behaviours, where lower quality hosts may kill ticks while grooming or exhibit physiological immune responses that result in ticks unsuccessfully feeding (Levin and Fish 1998, Keesing et al. 2009, Jones et al. 2015).

Recently, an Earth observation-informed framework was designed that combines high-resolution environment data with traditional vector surveillance data for climate-related risk assessments and mapping of disease vectors, such as *I. scapularis*, at varying geographic scales (Kotchi et al. 2019, 2021). These high-resolution environmental factors may be derived from satellite-based remote sensing imagery and direct-contact sensors from meteorological towers (Kotchi et al. 2019, 2021). However, this framework does not incorporate information related to hosts, which are important predictors of the abundance and distribution of *I. scapularis*. Here, we incorporated high-resolution environmental and host surveillance data to provide a better understanding of the dynamics between the abiotic and biotic factors impacting questing *I. scapularis* abundance. More specifically, we evaluated the concurrent impact of abiotic factors derived from remote sensing imagery and meteorological towers in addition to biotic factors obtained through host active surveillance in the field on questing *I. scapularis* abundance along the northward edge of their range in Ontario and Quebec, Canada. We predicted that the questing *I. scapularis* abundance may be variably influenced by combinations of abiotic and biotic factors. We expected increased *I. scapularis* abundances at localities with more suitable environmental habitats, including those with warmer temperatures, greater precipitation or lower evapotranspiration, greater snow accumulation, and greater vegetation greenness. We also expected variable relationships between questing *I. scapularis* with small mammal abundance, the relative abundance of *P. leucopus*, and mammal species richness, which may relate to the

degree of establishment of tick and host populations locally. Our study is the first to combine high-resolution environmental and host-related factors to determine the mechanisms driving local *I. scapularis* abundance, as a means to better anticipate the spread of tick populations and tick-borne pathogens in Canada.

2.3. Materials and Methods

2.3.1. Field sampling

Sixteen forested sites were visited for sampling in July and August 2019 in Ontario and Quebec, Canada (Figure 2.1). These sites were selected based on distinct levels of estimated Lyme disease risk related to the abundances and life stages of *I. scapularis* present locally as defined by the Institut national de santé publique du Québec (2018) and Public Health Ontario (2018), which ranged from possible to significant risk (Table B2). At each site, three grids of 40 m by 70 m were delineated in contiguous forest areas suitable for tick dragging, small mammal trapping, and the placement of trail cameras. These grids were maximally separated by 100 metres due to geographic barriers (e.g., streams or park trails).

Within each grid, a one m^2 cotton flannel was dragged over the low-lying vegetation along four 70-metre long transects to collect questing ticks. Every 10 metres, flannels were checked, and ticks were removed and placed into microvials with 95% ethanol. Larvae were pooled, while nymphs and adults were kept individually. Tick specimens were identified to the species with dichotomous keys (Lindquist et al. 2016, Egizi et al. 2019).

At each site, a total of 84 Sherman live traps (H.B. Sherman Traps, Inc., Florida, United States) were placed along four parallel transects within each grid for 3 consecutive nights. Targeted small mammal species included mice (*Peromyscus leucopus* and *P. maniculatus*), shrews (*Blarina brevicauda* and *Sorex cinereus*), voles (*Microtus pennsylvanicus* and *Myodes gapperi*), and jumping mice (*Napaeozapus insignis* and *Zapus hudsonius*). In the afternoon, we placed bait (peanut butter and oatmeal), a water source (apple piece), and nesting material (cotton ball) in each trap, which were checked the following morning. Non-targeted species and juveniles were immediately released on site. Individuals of targeted species were euthanized by isoflurane inhalation followed by cervical dislocation. One red squirrel (*Tamiasciurus hudsonicus*) and two hairy-tailed moles (*Parascalops breweri*) were also euthanized due to severe injuries. Small mammals were screened for feeding ticks, and liver tissues were dissected

from collected specimens and placed into microvials with 95% ethanol. Using molecular methods based on a modified protocol of Tessier et al. (2004), each *Peromyscus* specimen was identified to the species using their liver tissues (Supplementary Methods). All samples were accessioned at the Redpath Museum, McGill University (Table B3). Ethical approval and permits were issued by McGill University (AUP No. 2019-8086), the Ministère des Forêts, de la Faune et des Parcs (SEG permit No. 2019-06-04-008-00-S-F), and the Ministry of Natural Resources and Forestry (WSCA No. 1093495).

Nine trail cameras (Force-10, SpyPoint Inc., Quebec, Canada) were concurrently placed on trees one metre above the ground inside our sampling area and set to take three consecutive photos without delay for each detection. We identified each mammal host species seen in the photographs taken by the camera traps. Birds, domestic pets, humans, and unidentified individuals were not included in the dataset.

At each site, questing *I. scapularis* abundance was calculated as the sum of questing ticks collected along transects while tick dragging. The total number of collected small mammals was used as a proxy for the abundance of small mammals locally. The relative abundance of *P. leucopus* was estimated as the number of collected *P. leucopus* individuals at each site divided by the local abundance of collected small mammals. The number of mammal host species was quantified as the number of the distinct species found via small mammal trapping and detected in camera photographs.

2.3.2. Meteorological data

We extracted historical data from December 2018 to December 2019 for precipitation (PRECIP) in mm and snow on the ground (SNOW) in cm using nearby local meteorological towers (Environment and Climate Change Canada 2021). The distance from our sites to nearby meteorological towers ranged from 7.13 km to 36.21 km (Table B4). We removed estimated and flagged values from the dataset. We calculated monthly mean PRECIP (July or August) dependent on when field surveys were conducted at each locality. We determined the accumulated SNOW by calculating the difference in snow on the ground between two days (i.e., (n+1) – n) from December 2018 to June 2019. If the difference was greater than 0, then this value was used; otherwise, the value was set to 0. These calculated values were then summed from the start of winter to the end of spring.

2.3.3. Remote sensing data

Broad-scale remote sensing data were used to extract historical values for temperature, evapotranspiration, and vegetation greenness across our sites. All GIS analyses were conducted in ArcMap version 10.7.1. (Esri Inc. 2019). Shapefiles and rasters were re-projected into the NAD 83 Canada Atlas Lambert projection.

We extracted three version 6 data products for two adjacent tiles (12,4 and 13,4) from December 2018 to December 2019 using the Moderate Resolution Imaging Spectroradiometer (MODIS) on board NASA's Terra satellite. MOD11A2 is an average 8-day land surface temperature (LST) at a 1 km spatial resolution (Wan et al. 2015). MOD13A3 provided a monthly average of the enhanced vegetation index (EVI) at a 1 km spatial resolution (Didan 2015). MOD16A2 is an 8-day composite of total evapotranspiration (TE) at a 500 m spatial resolution (Running et al. 2017).

Raster processing of MODIS data included format conversion, re-projection, clipping, resampling, mosaicking, applying scale factors, converting measurements (if applicable), creating masks based on pixel quality control, and calculating zonal statistics for each of our sites. HDF-EOS files were converted to TIFF files using NASA's HEG Conversion Tool (2019). Rasters were resampled with cubic convolution to 500 m by 500 m cells using the *Resample* tool. The two tiles were then mosaicked together by Julian date using the *Mosaic to New Raster* tool. Using the *Raster Calculator* tool, scale factors and measurement conversions were applied. For LST, day and night values were converted to °C from K by multiplying the values by a scale factor of 0.02 and subtracting 273.15. Values were multiplied by a 0.0001 scale factor for EVI and a 0.1 scale factor for TE.

Quality assessment layers were decoded using the *MODIS Decode Quality* tool from the ArcGIS MODIS Python toolbox. Valid pixels were extracted using the *Extract by Attributes* tool. For LST, valid pixels included a low LST error ($<=$ 2K), a low emissivity error ($<=$ 0.02), and good or other quality data (Wan et al. 2015). For EVI, valid pixels included a VI usefulness of the two highest quality categories (0000 and 0001) and pixel reliability with good or marginal data (Didan 2015). For TE, valid pixels included those of good quality using the main algorithm, detectors fine for up to 50% of channels, and a cloud state that was clear or not defined, but

appeared to be clear (Running et al. 2017). Finally, we clipped valid pixels to a 1 km buffer around each site in Central Canada using the *Clip* tool.

Using the *Extract by Attributes* tool, masks of the valid pixels were applied to remove outlier values. Day and night LST values less than -50°C and greater than 100°C and TE values below 0 or greater than 3276.1 (fill values) were discarded. We then calculated zonal statistics (mean, maximum, and minimum) of LST, EVI, and TE from December 2018 to December 2019 within the 1 km buffer using the *Zonal Statistics as Table* tool. The daily, weekly, and monthly mean LST as well as the winter minimum LST and summer maximum LST were calculated for all our sites. Summer mean TE and summer mean EVI were calculated from June 2019 to September 2019.

2.3.4. Statistical analyses

All statistical analyses were performed in R version 4.1.1. (R Core Team 2021). Using the *rcorr* function in the *Hmisc* package (Harrell Jr 2021), Spearman correlations were conducted to assess if correlation coefficients were above 0.50 and if significant collinearity ($p < 0.05$) was present between abiotic factors (monthly mean PRECIP, accumulated SNOW, monthly mean LST, minimum winter LST, maximum summer LST, summer mean TE, and summer mean EVI) and biotic factors (small mammal abundance, relative abundance of *P. leucopus*, and mammal species richness). This type of correlation was selected due to its non-parametric nature, which could assess potential non-linear relationships between abiotic and biotic factors. Due to high collinearity, minimum winter LST and maximum summer LST were dropped from further analyses. Similarly, correlated biotic variables were to be run separately in further analyses to not violate statistical assumptions. Due to data limitations, summer mean TE and summer mean EVI were removed as independent variables, as values could only be calculated for 13 of our 16 sites. The remaining abiotic and biotic factors were each centred and standardized with the *scale* function.

Spatial autocorrelation among abiotic and biotic variables was assessed with Moran's I with an inverse distance weights matrix using the *moran.test* function in the *spdep* package (Bivand and Wong 2018).

Finally, we conducted count regression generalized additive models (GAM) using the *gam* function in the *mgcv* package with a negative binomial family (Wood 2017) to investigate

the concurrent impact of abiotic and biotic factors on questing *I. scapularis* abundance. GAMs were chosen for our analyses as we expected that the biotic factors may exhibit variable relationships with *I. scapularis* abundance. A negative binomial family rather than a Poisson family was selected based on AIC, rootograms, and Pearson dispersion parameters. All GAMs were fitted using penalized thin plate spline regressions (bs = "tp") and a double penalty approach to account for sparse data and to allow variable selection (Marra and Wood 2011). We used a REML method for our GAMs, as this method is more robust to under-smoothing and small sample sizes (Wood 2017). Using the argument select = TRUE in the *gam* function, a double penalty approach can penalize function components in both the range and null space, which can then be shrunk to zero (Marra and Wood 2011). As a result, this approach allows for model selection without requiring a stepwise selection procedure and uses fewer effective degrees of freedom (Marra and Wood 2011). A smoothed interaction of latitude and longitude was used in all models to account for spatial autocorrelation (Marra and Wood 2011). All models were inspected for goodness of fit using the *gam.check* function, AIC, adjusted R^2, and deviance explained. We also assessed if collinearity was present between the model's smooth terms using the *concurvity* function (Wood 2017). If concurvity was high, we then ran simplified models that only included the significant terms to determine if any predictors should be removed.

Three GAMs were conducted to determine how abiotic and biotic factors were concurrently affecting questing *I. scapularis* abundance across our sites. Model 1 assessed the impact of the small mammal community on questing *I. scapularis* abundance by including small mammal abundance, monthly mean LST, monthly mean PRECIP, accumulated SNOW, and spatial autocorrelation as independent variables. A high-leverage outlier (Site 9: Saint-Polycarpe) was detected after running this model. As a result, we removed this outlier and ran a subsequent Model 1 using the remainder of the data. Model 2 assessed the relative contribution of *P. leucopus* within the small mammal community by including the relative abundance of *P. leucopus*, monthly mean LST, monthly mean PRECIP, accumulated SNOW, and spatial autocorrelation as independent variables. Model 3 assessed the mammal community as a whole using mammal species richness, monthly mean LST, monthly mean PRECIP, and spatial autocorrelation as independent variables. Accumulated SNOW was not used in Model 3 due to high collinearity with mammal species richness.

2.4. Results

2.4.1. Field sampled I. scapularis and mammal hosts

We collected 382 questing *I. scapularis* from 8 of our 16 sites ranging from 2 to 164 ticks (Figure 2.1; Table B5). These questing *I. scapularis* included 255 larvae (29 pools), 126 nymphs, and one adult male. We found that questing *I. scapularis* abundance increased with decreasing longitude, likely due to the majority of *I. scapularis* being collected during field surveys in eastern Ontario and southern Quebec at sites 8 to 11 (Figure 2.2).

We collected a total of 105 individuals with live traps, which belonged to twelve small mammal species (Table B5). *Peromyscus leucopus* was the most abundant species (31.4% of collected individuals; $n = 33$) and was present at 9 of our 16 sites. Other small mammals collected, in decreasing order of abundance, included *N. insignis* (22.8%; $n = 24$), *B. brevicauda* (16.2%; $n = 17$), *M. gapperi* (15.2%; $n = 16$), *P. maniculatus* (10.5%; $n = 11$), *P. breweri* (1.9%; $n = 2$), *S. cinereus* (1.0 %; $n = 1$), and *M. pennsylvanicus* (1.0%; $n = 1$). Small mammal abundance ranged from 1 to 18 individuals, and the relative abundance of *P. leucopus* at each site varied from 0.00 to 1.00 (Table B5). Five mammal host species were identified from camera photographs, which included squirrels (*Sciurus carolinensis*), chipmunks (*T. striatus*), white-tailed deer (*O. virginianus*), raccoons (*P. lotor*), and coyotes (*Canis latrans*). Between 2 and 8 mammal species were detected locally via small mammal trapping and in camera photographs (Table B5).

2.4.2. Collinearity of abiotic and biotic factors

Several factors were found to be significantly correlated with each other (Figure B1). Mammal species richness was significantly correlated with small mammal abundance ($r = 0.54$, $p < 0.05$), accumulated SNOW ($r = -0.56$, $p < 0.05$), and minimum winter LST ($r = 0.59$, $p < 0.05$). Maximum summer LST was significantly correlated with the relative abundance of *P. leucopus* ($r = 0.65$, $p < 0.01$) and accumulated SNOW ($r = -0.50$, $p < 0.05$). Finally, summer mean TE and summer mean EVI were significantly correlated ($r = -0.71$, $p < 0.05$). As a result of significant collinearity or data limitations, minimum winter LST, maximum summer LST, summer mean TE, and summer mean EVI were removed from further analyses. To limit multicollinearity, separate analyses were conducted for each biotic factor with the remaining abiotic factors.

2.4.3. Spatial autocorrelation

We detected spatial autocorrelation for several variables, which included questing *I. scapularis* abundance (Moran's I = 0.206, $p < 0.05$), monthly mean PRECIP (Moran's I = 0.389, $p < 0.01$), accumulated SNOW (Moran's I = 0.226, $p < 0.05$), and monthly mean LST (Moran's I = 0.385, $p < 0.01$). However, we did not detect any spatial autocorrelation for the biotic factors: small mammal abundance (Moran's I = -0.199, $p = 0.797$), relative abundance of *P. leucopus* (Moran's I = 0.072, $p = 0.207$), and mammal species richness (Moran's I = 0.138, $p = 0.115$).

2.4.4. Effect of abiotic and biotic factors on questing I. scapularis abundance

We found that the questing *I. scapularis* abundance was modulated in different ways depending on the abiotic and biotic factors that were assessed. A high-leverage outlier (Site 9: Saint-Polycarpe) was detected in Model 1, which incited our removal of this outlier to re-assess Model 1 with the remainder of the data. In Model 1, small mammal abundance and monthly mean PRECIP had a significant effect on questing *I. scapularis* abundance (Figure 2.2A; Table B6). However, even with the removal of this outlier, we obtained a negative adjusted R-squared value, indicating that this model did not have predictive power (Table B6). In Model 2, we found a linear positive relationship for monthly mean PRECIP and a linear negative relationship for accumulated SNOW with questing *I. scapularis* abundance, respectively (Figure 2.2B; Table B7). However, we did not find that a relationship between the relative abundance of *P. leucopus* and questing *I. scapularis* abundance in this model (Figure 2.2B; Table B7). In Model 3, both mammal species richness and monthly mean PRECIP were found to have positive effects on questing *I. scapularis* abundance (Figure 2.2C; Table B8). The concurvity of all our models were assessed through simplified models using only significant factors and the spatial autocorrelation term. These simplified models indicated that we did not need to remove any predictor variables when fitting our data.

2.5. Discussion

Using a combination of high-resolution environmental data and field-based sampling, we provide evidence that combinations of abiotic and biotic factors drive questing *I. scapularis* abundance across our sites in Central Canada. We first found that greater precipitation and less accumulated snow were associated with increased questing *I. scapularis* abundance, but no effect

was found for the relative abundance of white-footed mice. We also found that questing *I. scapularis* abundance was most positively influenced by monthly mean precipitation and mammal species richness, where tick abundances increased with greater precipitation and greater numbers of mammal species locally. These results highlight the importance of incorporating host active surveillance data with high-resolution environmental data when assessing which abiotic and biotic factors are impacting questing *I. scapularis* abundance.

2.5.1. Influence of biotic factors on I. scapularis abundance

We found that larger abundances of questing *I. scapularis* were associated with greater mammal species richness locally (Figure 2.2C). Areas with more diverse mammal communities may have increased *I. scapularis* abundances, but only if host abundances increase with species richness allowing for greater tick-host contact rates (Ogden and Tsao 2009, Luis et al. 2018). Here, a significant positive correlation between small mammal abundance and mammal species richness was found, which may provide additional contact and feeding opportunities for *I. scapularis*. This relationship may be especially impacted by mid-size and large mammals, such as raccoons and white-tailed deer, that can successfully feed large burdens of ticks including immature and adult *I. scapularis* (LoGiudice et al. 2003, Werden et al. 2014). These mammal hosts may also be important for the local dispersal and establishment of *I. scapularis* to locations further north. Moreover, the addition or loss of mammal host species locally due to predation or interspecific competition may have variable impacts on tick abundances, especially for *I. scapularis* populations that have not yet established at their northward range edge (Levi et al. 2016). In addition, *I. scapularis* may be variably affected if lower quality mammal hosts are present that may kill or unsuccessfully feed ticks due to host-specific behaviours including grooming or physiological immune responses (Levin and Fish 1998, Keesing et al. 2009, Jones et al. 2015).

In contrast, we did not find relationships between questing *I. scapularis* abundance with small mammal abundance nor the relative abundance of *P. leucopus* in Central Canada. Areas with long-established populations of *I. scapularis* were associated with higher abundances of small mammal hosts. In southern Quebec, increased abundances of infected *I. scapularis* have also been associated with increased abundances of small mammals across the same geographic extent as our study (Millien et al. 2023). However, it may be that avian hosts or larger mammals,

such as white-tailed deer, play a larger role in the maintenance of tick populations across our sites, where increasing densities of these hosts may lead to increased abundances of questing *I. scapularis* locally (Mather et al. 1989, LoGiudice et al. 2003, Brisson et al. 2008, Bouchard et al. 2011, 2013). In addition, fluctuating host densities within the small mammal community may result in variable *I. scapularis* abundances due to limited tick-host contacts (Dobson 2014, Linske et al. 2018, Luis et al. 2018). This variability in tick-host interactions may be especially discernible in areas where *I. scapularis* may not have fully established, such as those populations located near the northward range edge. For example, several of our sites in northeastern Ontario and southeastern Quebec did not harbour any *P. leucopus* or *I. scapularis*, which likely impacted this relationship. It may be that populations of *P. leucopus* and *I. scapularis* are not yet present in these areas or remain scarce, but they are expected to become established in the near future with their northward geographic range expansion (Roy-Dufresne et al. 2013, Simon et al. 2014, Clow et al. 2017a, Ripoche et al. 2022).

2.5.2. Impacts of abiotic factors on tick and host populations

Greater amounts of precipitation were associated with increased abundances of questing *I. scapularis* (Figure 2.2B and 2.2C). Sufficient levels of moisture and precipitation may sustain suitable humidity levels within the microclimate for the survival of *I. scapularis* (Berger et al. 2014a, 2014b, Dumas et al. 2022). At localities with greater amounts of precipitation, *I. scapularis* may not be required to mitigate their desiccation through behavioural changes (Vail and Smith 2002). As a result, these tick vectors are more likely to be actively questing within their environment for a suitable host rather than remaining close to the leaf litter with limited activity (Vail and Smith 2002, Burtis et al. 2016). In addition, greater amounts of precipitation may also lead to increased vegetation greenness, yet the spatial heterogeneity of precipitation may variably impact this relationship locally (Jiang et al. 2016). Here, we found a weak negative relationship between monthly mean PRECIP and summer mean EVI ($r = -0.38$), which may be primarily due to several sites in Ontario with no *I. scapularis* present that had low to moderate levels of vegetation greenness. Therefore, we may find a positive association between precipitation, vegetation, and questing *I. scapularis* abundance in the future as tick populations become established at these localities.

Surprisingly, we found that increased levels of accumulated snow were associated with decreased abundances of questing *I. scapularis* (Figure 2.2B). Snow cover alone or in combination with leaf litter typically leads to increased overwintering survival, resulting in increased abundances of questing *I. scapularis* in the subsequent summer (Hayes et al. 2015, Linske et al. 2019, Volk et al. 2022). However, the localities with the highest questing *I. scapularis* abundances were at our southernmost sites in Ontario and Quebec, which were associated with the lowest amounts of accumulated snow. Although these areas may have experienced milder winters, it does not seem that an increased mortality risk due to inoculative freezing impacted subsequent summer *I. scapularis* abundances (Eisen et al. 2016, Linske et al. 2019, Volk et al. 2022). In contrast, localities at the northern range edge experienced large snow accumulations, yet *I. scapularis* populations were absent or were present in very low abundances the following summer. Therefore, it is possible that an additional metric other than accumulated snow may be beneficial to completely capture the microclimatic winter conditions.

Changes in climatic conditions, such as precipitation, snow, or vegetation, may also impact the movements and poleward range expansions of mammal hosts, altering the abundance and distribution of *I. scapularis* (Ogden and Lindsay 2016, Diuk-Wasser et al. 2021). Small mammal hosts may disperse short distances searching for food resources, such as acorns or seed crops, in nearby forested areas, which may result in fluctuations of local tick populations (Marrotte et al. 2017, Borgmann-Winter et al. 2021, Sullivan et al. 2023). The summer following high abundances of acorns or other seed crops have been associated with higher abundances and greater overwintering survival in *Peromyscus* mice, resulting in a lagged increase in *I. scapularis* abundance (Falls et al. 2007, Ostfeld et al. 2018, Sullivan et al. 2023). In addition, decreased winter severity may lead to greater movements and habitat use of mammal hosts, especially for white-tailed deer (Dawe and Boutin 2016, Fisher et al. 2020). With climate warming, milder winters have been associated with greater poleward range expansions in white-tailed deer and white-footed mice, which have assisted in the range expansion of *I. scapularis* to new poleward locations (Roy-Dufresne et al. 2013, Simon et al. 2014, Dawe and Boutin 2016, Kennedy-Slaney et al. 2018, Fisher et al. 2020).

Finally, unexplored factors, such as microclimatic conditions or avian host communities, may be affecting questing *I. scapularis* abundances across our sites in Central Canada. In addition, *I. scapularis* abundance as well as abiotic and biotic factors may change through time,

which will require further studies to explore the interannual dynamics of this system. Therefore, future analyses should be conducted over several consecutive years to capture this interannual variability, where both high-resolution abiotic and biotic factors can be assessed simultaneously to determine their relative impact on questing *I. scapularis*.

2.5.3. Implications for surveillance efforts

Our study demonstrates that host active surveillance data should be incorporated with high-resolution abiotic variables to comprehensively assess the relationships and dynamics between questing *I. scapularis* and host populations. The current design of the Earth observation-informed framework (Kotchi et al. 2019) excludes vertebrate hosts, which are a key player in tick-host-pathogen disease systems, especially at the poleward range edge. We propose the incorporation of host active surveillance data in this framework or future studies that rely on high-resolution environmental data, as it may provide greater knowledge of the driving mechanisms of increased *I. scapularis* abundances across large geographic areas or time frames at varying scales (Kotchi et al. 2019). Public health agencies may then be better informed as to which areas may have increased abundances of *I. scapularis* and hosts and should therefore be targeted by active surveillance or control efforts (Kotchi et al. 2019). We encourage future studies to use a combination of biotic factors obtained during field-based surveys, such as the abundance and diversity of mammal hosts, in addition to high-resolution abiotic factors derived from remote sensing imagery and meteorological tower data to better assess the spread of tick populations and tick-borne pathogens in Canada.

2.6. Conclusions

We incorporated host active surveillance data obtained through field-based sampling with high-resolution, multitemporal environmental data derived from remote sensing imagery and meteorological towers to evaluate the concurrent effects of abiotic and biotic factors on questing *Ixodes scapularis* abundance. Combinations of abiotic and biotic factors were identified as important drivers of abundances of questing *I. scapularis*. Positive and negative linear relationships were found for questing *I. scapularis* abundance with monthly mean precipitation and accumulated snow, respectively, but no effect was found for the relative abundance of white-footed mice. Positive relationships were found between questing *I. scapularis* abundance with

monthly mean precipitation and mammal species richness, where increased tick abundances occurred with greater precipitation and greater mammal species richness locally. Therefore, future studies assessing *I. scapularis* should incorporate host active surveillance data to enable more sensitive tests of the relative importance of the abiotic environmental conditions on the abundance and diversity of hosts and disease vectors, such as *I. scapularis*. Such relationships may prove especially important in areas with emerging populations of medically important tick vectors. Disentangling the influence of abiotic and biotic factors remains critical to understanding how the environment and host community affect tick populations and tick-borne disease risk in Canada and elsewhere.

2.7. Acknowledgements

We wish to express our utmost appreciation to the landowners and stakeholders that allowed us to access our study sites. We want to thank all our devoted assistants for their help in the field: Daniella Cross, Connia Ren, Dania Shaban, Ellen Bidulka, Jihane Benbahtane, Christina Provost, and Sébastien Cyr. K.E.C. was funded by Hydro-Québec and Natural Sciences and Engineering Research Council of Canada fellowships. V.M. was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant (RGPIN-2017-03839). J.T.K. was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant (RGPIN-2017-147544), Natural Sciences and Engineering Research Council of Canada Discovery Accelerator Supplement, and the University Research Chair in Macroecology and Conservation at the University of Ottawa.

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2.9. Figures

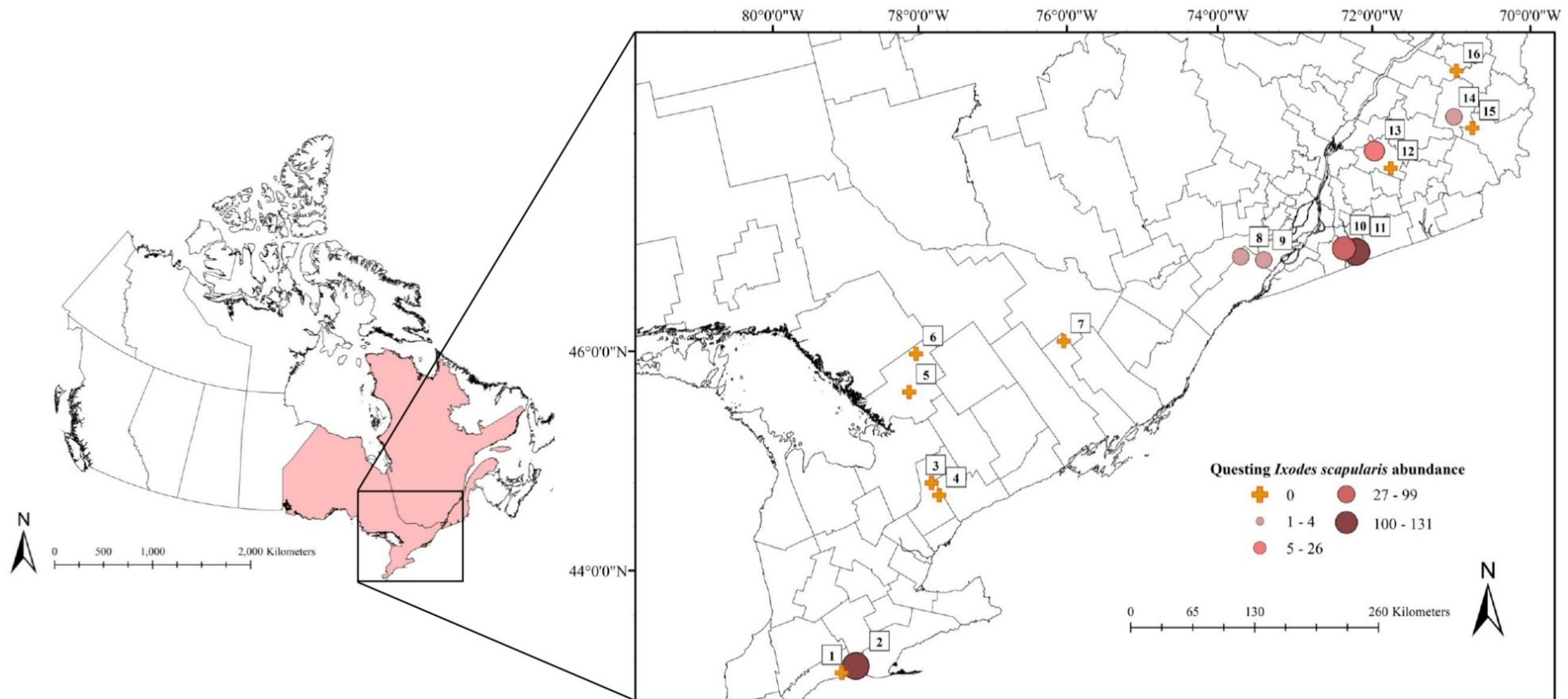

Figure 2.1. Questing *Ixodes scapularis* abundance across our study sites in Central Canada. Circle size and colour represent the questing abundance of *I. scapularis*, with larger circles in darker colours denoting greater tick abundance. Sites with no *I. scapularis* are represented by orange crosses. (1) 3 Ridges Farm, (2) New New Age Farm, (3) North Tract, (4) Brown Hill Tract, (5) Upjohn Nature Reserve, (6) Dyer Memorial Nature Reserve, (7) Rose Hill Nature Reserve, (8) Kirkview Farm, (9) Saint-Polycarpe, (10), Saint-Valentin, (11) Henryville, (12) Lefebvre, (13) Parc du Sanctuaire Saint-Majorique, (14) Serpentine-de-Coleraine Ecological Reserve, (15) Frontenac National Park, and (16) Saint-Sylvestre.

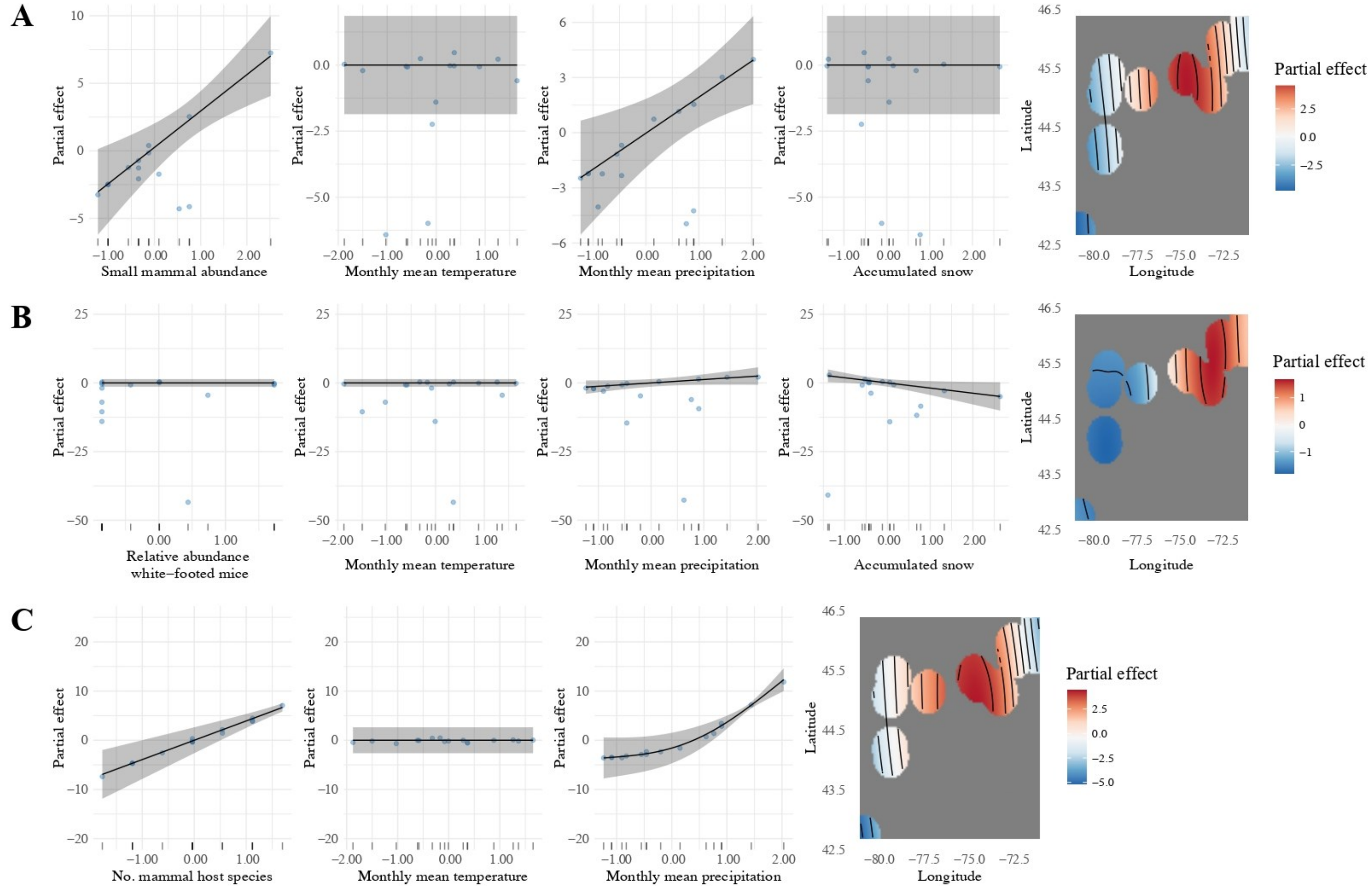

Figure 2.2. Partial effects of generalized additive models (Model 1-3) with questing *Ixodes scapularis* abundance as a response variable with a smoothed interaction of latitude and longitude to account for spatial autocorrelation. All abiotic and biotic factors were centred and standardized. Smoothed abiotic factors include monthly mean land surface temperature (LST) ranging from 10.30°C to

26.41°C, monthly mean precipitation (PRECIP) ranging from 0.77 mm to 5.19 mm, and accumulated snow on the ground (SNOW) ranging from 43 cm to 299 cm. Smoothed biotic factors include small mammal abundance ranging from 1 to 18, relative abundance of *P. leucopus* ranging from 0 to 1, and mammal species richness ranging from 2 to 8. Shaded areas represent the 95% confidence intervals of the model and points represent the residual values. (A) Although significant relationships for questing *I. scapularis* abundance were found for small mammal abundance and monthly mean PRECIP in Model 1, this model does not have predictive power based on graphical visualizations and the adjusted R-squared value. R-sq (adj.) = -31.300, deviance explained = 82.20%. (B) Positive and negative relationships were found between questing *I. scapularis* abundance with monthly mean PRECIP and accumulated SNOW, respectively, but no effect was found for the relative abundance of *P. leucopus*. R-sq (adj.) = 0.445, deviance explained = 57.60%. (C) Mammal species richness and monthly mean PRECIP both significantly impacted questing *I. scapularis* abundance. R-sq (adj.) = 0.994, deviance explained = 99.70%.

Connecting statement between Chapter 2 and 3

Abiotic factors, such as temperature, precipitation, and snow, may affect the abundance and survival of ticks. Similarly, biotic factors, such as the abundance and diversity of mammal hosts, may increase tick abundances due to variability in contact opportunities and host quality, subsequently affecting tick-borne disease risk. In Chapter 2, I incorporated host active surveillance data with high-resolution environmental data to concurrently evaluate the relative contributions of abiotic and biotic factors on questing *I. scapularis* abundance in Ontario and Quebec. Positive and negative linear relationships were found for questing *I. scapularis* abundance with monthly mean precipitation and accumulated snow, but no effect was found for the relative abundance of white-footed mice. Positive relationships were also identified between questing *I. scapularis* abundance with monthly mean precipitation and mammal species richness.

In Chapter 1, I identified potential tick-borne pathogens that may be present in different tick vectors located across our study region. In Chapter 2, I demonstrated the importance of mammal hosts for modulating tick abundances, which may consequently affect pathogen spread and transmission. Guided by this information, Chapter 3 assessed the presence and prevalence of several tick-borne pathogens in ticks and small mammal hosts that were collected during field surveys in Ontario and Quebec. Comprehensive pathogen testing was conducted in this chapter, which included testing all tick life stages and species as well as the inclusion of certain tick-borne pathogens that are not typically targeted in surveillance efforts. This study helps identify the geographic extent of several pathogen species and potential hotspots of tick-borne disease risk that are present outside currently defined risk areas identified by surveillance activities in Canada.

Chapter 3 | Emerging tick-borne pathogens in Central Canada: Recent detections of *Babesia odocoilei* and *Rickettsia rickettsii*

Kirsten E. Crandall, Jeremy T. Kerr, and Virginie Millien

3.1. Abstract

The spread of emerging tick-borne pathogens has steadily increased in Canada with the widespread establishment of tick vectors and vertebrate hosts. At present, *Borrelia burgdorferi*, the bacterium causing Lyme disease, is the most common tick-borne pathogen in Canada and primarily transmitted by *Ixodes scapularis*. A low prevalence of other emerging tick-borne pathogens, such as *Anaplasma phagocytophilum*, *Babesia* species, *Borrelia miyamotoi*, and *Francisella tularensis* have also been detected through surveillance efforts in Canada. Although *Rickettsia rickettsii* has been historically detected in *Haemaphysalis leporispalustris* in Canada, the current prevalence and geographic extent of this pathogen is unknown. In this study, we assessed the presence and prevalence of several emerging tick-borne pathogens in ticks and hosts collected through tick dragging and small mammal trapping in Central Canada. Nested PCR testing detected three pathogen species in ticks, with *Babesia odocoilei* and *B. burgdorferi* in *I. scapularis* in addition to *R. rickettsii* in *H. leporispalustris*. Three pathogen species were detected in small mammals by nested PCR including *B. odocoilei* in *Blarina brevicauda*, *Babesia microti* in *Peromyscus leucopus*, and a *Hepatozoon* species in *P. leucopus* and *Peromyscus maniculatus*. *B. burgdorferi* and *Babesia* species were the pathogens most often detected in our samples, suggesting they are widely distributed across Central Canada. We also detected *B. odocoilei* and *R. rickettsii* beyond their known geographic distribution. Our results provide evidence that emerging tick-borne pathogens may be present outside defined risk areas identified by current surveillance efforts in Canada. As a result, emerging tick-borne pathogens introduced by the dispersal of infected ticks by migratory birds or maintained by hosts and vectors through cryptic transmission cycles may go undetected. More comprehensive testing including all tick life stages and additional tick-borne pathogens will help detect the spread and potential risk of emerging or re-emerging tick-borne pathogens for human and wildlife populations throughout Canada.

Key words: Pathogen, tick vectors, mammal hosts, *Peromyscus leucopus*, abundance, diversity

3.2. Introduction

Emerging tick-borne pathogens have increased in prevalence in Canada with climate warming, habitat fragmentation, and changes in the abundances and distributions of tick and host populations (Bouchard et al, 2019; Leo et al, 2016; Ogden and Lindsay, 2016). Pathogen transmission cycles are sustained by contact between ticks and their hosts (Radolf et al, 2012). Generalist tick species feed on a wide variety of hosts, including *Ixodes scapularis* feeding on mammals, birds, and humans and *Haemaphysalis leporispalustris* feeding on lagomorphs and birds (Keirans et al, 1996; Kollars and Oliver, 2003; Lindquist and Wu, 2016). Although some host specificity may occur depending on local host availability (McCoy et al, 2013), specialist tick species feed on select hosts such as *Ixodes banksi* and beavers (Lindquist and Wu, 2016).

Birds and small mammals are competent reservoir hosts that successfully feed large numbers of ticks and have greater probabilities of infecting feeding ticks with pathogens (Bouchard et al, 2011; LoGiudice et al, 2003; Ogden et al, 2008; Scott et al, 2019; Zinck and Lloyd, 2022). As a result, emerging tick-borne pathogens may be detected in areas beyond currently defined risk areas in Canada owing to the dispersal of infected ticks by migratory birds and the distributional shifts of host populations (Fiset et al, 2015; Garcia-Elfring et al, 2017; Roy-Dufresne et al, 2013; Simon et al, 2014).

The predominant tick-borne pathogen found in sentinel surveillance efforts in Canada is *Borrelia burgdorferi*, the bacterium causing Lyme disease (Guillot et al, 2020). Lyme disease prevalence has grown rapidly to become the most common tick-borne disease in North America (Gasmi et al, 2017; Nelder et al, 2018). The first case of another *Borrelia* species in Canada, *Borrelia miyamotoi*, was reported in 2013 (Bouchard et al, 2019). These spirochetes are primarily transmitted by *I. scapularis* and *Ixodes pacificus* ticks as well as rodents, especially *Peromyscus* mice (Kulkarni et al, 2015).

Other emerging tick-borne pathogens targeted by current surveillance efforts in Canada are expected to increase in the future, as Ixodidae transmit 40% of documented emerging vector-borne zoonotic diseases globally (Swei et al, 2020). Many pathogens including *Anaplasma phagocytophilum* and *Babesia microti* are primarily transmitted by *Ixodes* ticks and small mammals in Canada (Bouchard et al, 2019; Kulkarni et al, 2015). More recently, *Babesia odocoilei* has been found in cervids and *I. scapularis* in Canada (Mathieu et al, 2018; Milnes et al, 2019; Pattullo et al, 2013). In contrast, *Francisella tularensis*, the bacterium causing

tularemia, is transmitted by *Dermacentor, Amblyomma*, and *Haemaphysalis* ticks. Tularemia can also spread through contact with infected mammals, infective aerosols, or arthropod bites (Gabriele-Rivet et al, 2016; Petersen et al, 2009).

Rickettsia rickettsii, the bacterium causing Rocky Mountain spotted fever, is vectored by *Dermacentor, Haemaphysalis*, and *Rhipicephalus* ticks. In Canada, the historical extent of *R. rickettsii* has relied on surveys and human cases owing to its rare occurrence (Bouchard et al, 2019; Humphreys and Campbell, 1947; Kulkarni et al, 2015). This bacterium has been found previously in *H. leporispalustris* ticks and dogs in Ontario, Nova Scotia, and Western Canada (Gary et al, 2006; Leighton et al, 2001; Wood and Artsob, 2012). Although *H. leporispalustris* does not typically bite humans, this tick species is important for the maintenance of *R. rickettsii* strains of variable virulence in ecosystems (Freitas et al, 2009; Parker et al, 1951). The current prevalence and geographic extent of *R. rickettsii* strains in *H. leporispalustris* ticks in Canada are unknown.

Only two of these emerging tick-borne pathogens are listed as nationally notifiable diseases in Canada: Lyme disease and tularemia (Bouchard et al, 2019). However, current sentinel surveillance efforts in Canada primarily target *A. phagocytophilum*, *Borrelia* species, and *Babesia* species (Guillot et al, 2020; Wilson et al, 2022). As certain emerging tick-borne pathogens are not reportable to public health agencies, it is challenging to assess their degree of establishment and spread across Canada. In this study, we assess the presence and prevalence of several emerging tick-borne pathogens along the northward edge of their range in Ontario and Quebec, Canada. We report recent detections of *B. odocoilei* and *R. rickettsii* in southeastern Quebec outside of their known geographic extent based on ongoing surveillance efforts. Our results highlight the need for comprehensive pathogen testing to detect the presence and monitor the spread of emerging tick-borne pathogens throughout Canada.

3.3. Materials and Methods

Sixteen forested sites of varying risk for Lyme disease were sampled in July and August 2019 in Central Canada [Institut national de santé publique du Québec, 2018; Ontario Agency for Health Protection and Promotion (Public Health Ontario), 2018]. At each site, tick dragging and small mammal trapping were conducted across three 40 meters by 70 meters grids. Within each grid, a 1 m^2 cotton flannel was dragged along four transects over low-lying vegetation and

checked every 10 meters. Questing ticks were removed and placed into microvials with 95% ethanol and were later identified to the species using standard taxonomic keys (Egizi et al, 2019; Lindquist and Wu, 2016).

Small mammal trapping was conducted over 3 consecutive nights using 84 Sherman live traps (H.B. Sherman Traps, Inc.). Targeted species included mice (*Peromyscus leucopus* and *Peromyscus maniculatus*), shrews (*Blarina brevicauda* and *Sorex cinereus*), voles (*Microtus pennsylvanicus* and *Myodes gapperi*), and jumping mice (*Napaeozapus insignis* and *Zapus hudsonius*). Traps were baited with peanut butter and oatmeal, a water source (apple), and nesting material (cotton ball) in the late afternoon and checked the following morning. Nontargeted species and juveniles of targeted species were immediately released. Target species were killed in the field with isoflurane inhalation overdose followed by cervical dislocation. Owing to serious injuries, one red squirrel (*Tamiasciurus hudsonicus*) and two hairy-tailed moles (*Parascalops breweri*) were killed.

The liver of each specimen was dissected and placed into microvials with 95% ethanol. Feeding ticks were removed from the host and placed into microvials with 95% ethanol. All tick and small mammal specimens were accessioned in the collections of the Redpath Museum at McGill University (Montreal, Canada). All procedures were approved by McGill University (AUP No. 2019-8086), the Quebec Ministère des Forêts, de la Faune et des Parcs (SEG permit No. 2019-06-04-008-00-S-F), and the Ontario Ministry of Natural Resources and Forestry (WSCA No. 1093495).

Questing larval ticks were pooled together for testing by grid, with 2–10 larvae per pool. Feeding larval ticks found on the same individual host were pooled together, ranging from 1 to 10 larvae per pool. Questing and feeding nymphs and adults were tested individually. All DNA extractions and PCR were performed by Geneticks, Inc. As in Wills et al (2018), cleaned ticks were cut and homogenized using a microtube pestle in AquaGenomic solution. Samples were incubated in a heat block at 60°C for 45 min, vortexed briefly, and centrifuged for 4 min at 13,300 rpm. The supernatant was transferred to a microvial with 50 µL isopropanol, inverted, and centrifuged as before. After decanting the supernatant, the remaining DNA pellet was rinsed with 50 µL of 70% ethanol and left to air dry for 15 min at room temperature. This pellet was resuspended with 50 µL of 1 mM Tris pH 8.0 and incubated in a heat block at 60°C for 1 h.

DNA from mammal livers was extracted using the Thermo Scientific GeneJET Genomic

Purification Kit (Thermo Fisher Scientific). The following modifications were made to the Mammalian Tissue Genomic DNA Purification protocol (Protocol A, 2016): 10 mg of liver was used, the extra centrifugation step 9 was included to remove residual solution, and no additional elution buffer was required after sitting for 5 min before centrifugation. DNA was used directly for PCR and then stored at -20°C.

Nested PCRs to identify *Peromyscus* specimens to species level were run using species-specific COIII primers following Tessier et al (2004). An initial denaturation time of 5 min was used. PCR products were run on 3% agarose gel, stained with Eco-Stain (Bio Basic, Markham, Canada), and visualized using a blue light transilluminator.

Nested PCRs were performed to target several pathogens in our tick and mammal specimens. The primers and conditions used are described in detail in Table C1. All specimens were tested for *A. phagocytophilum*, *Babesia* species, and *Borrelia* species. *B. burgdorferi* and *B. miyamotoi* were tested once using the 5S–23S intergenic space (IGS) and 18S rRNA region, respectively (Dibernardo et al. 2014; Zinck et al 2021). If a band was visible (*i.e.*, positive PCR), two more replicates were conducted to identify false positives. An additional test using the *flaB* gene was performed to confirm the presence of *B. burgdorferi* sensu lato (Figure C1; Wodecka 2011). *B. microti* and *B. odocoilei* were targeted once with the 18S ribosomal RNA (rRNA) gene using the *mic 494* and *odo563* inner primers, respectively (Figure C2). If a band was visible, *mic494* and/or *odo563* primers were then tested twice more to distinguish false positives. An additional primer set targeting the 18S rRNA of each *Babesia* species was used to confirm positive testing (Persing et al. 1992). The *p44* gene was targeted to test for *A. phagocytophilum* (Holden et al. 2003).

Targeted testing of *F. tularensis* (*fdx* gene; Fulop et al. 1996) and *R. rickettsii* (*RRi6 hypothetical protein* gene; Kato et al. 2013) was conducted for *H. leporispalustris* specimens, as these pathogens are typically found in this tick species, but not in *Ixodes* species. Amplified DNA was visualized on a 1.8% agarose gel stained with Eco-Stain (Bio Basic) using a blue light transilluminator. If no band was visualized, the sample was considered negative for that pathogen.

Amplified products were purified before sequencing using a cotton cushion following Sun et al. (2012). The fragments were spun for 7 min at 5000 rpm, which were then reamplified using the corresponding inner primers to concentrate the amplicons for sequencing. Sanger DNA

sequencing was performed at Bio Basic DNA Sequencing with the forward inner PCR primers.

All sequences were assessed for ambiguous base calls, end-reading errors, and quality scores using the 4Peaks software (https://nucleobytes.com/4peaks). Sequences with quality scores < 20 were not included in our analyses. A MEGABLAST search using the nucleotide BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi#_blank) in GenBank was used to determine the pathogen species of a sequence.

3.4. Results

A total of 644 ticks were collected, with 506 larvae, 136 nymphs, and 2 adults (Table 3.1). For *I. scapularis*, we found 382 questing ticks including 255 larvae (29 pools), 126 nymphs, and 1 adult male in addition to 65 feeding ticks comprising 57 larvae (17 pools) and 8 nymphs. For *H. leporispalustris*, we only found 195 questing ticks at 4 sites in Quebec, which consisted of 194 larvae (22 pools) and 1 nymph. One *Ixodes auritulus* nymph feeding on one *M. gapperi* at Rose Hill and one *Ixodes marxi* adult female feeding on one *T. hudsonicus* at Saint-Majorique were found.

For questing *I. scapularis*, 29 larval pools and an adult male tested negative for all pathogens. However, 27 of 126 questing nymphs (21.42%) tested positive for a pathogen (Figure 3.1 and Table 3.1). Five nymphs were infected with *B. odocoilei* (3.97%), including one nymph at New New Age Farm, Henryville, and Saint-Valentin, and two nymphs at Saint-Majorique. Similarly, 22 nymphs were infected with *B. burgdorferi* (17.46%), with 1, 3, and 18 nymphs at New New Age Farm, Henryville, and Saint-Valentin, respectively. For *H. leporispalustris*, 5 of 22 questing larval pools were infected with *R. rickettsii* (22.72%), with 4 pools at Coleraine and 1 pool at Frontenac. One uninfected *H. leporispalustris* nymph was also found at Coleraine.

Feeding ticks were found on 22 small mammals (Table 3.1). For feeding *I. scapularis* ticks, 2 of 17 larval pools (11.76%) and 1 of 8 nymphs (12.50%) were infected (Figure 3.1 and Table 3.1). One feeding larva from Henryville was infected with *B. odocoilei*, whereas its *P. leucopus* host was infected with *B. microti*. A larva feeding on an uninfected *P. maniculatus* from Saint-Majorique and a nymph feeding on an uninfected *P. leucopus* at Kirkview Farm were infected with *B. burgdorferi*. The feeding *I. auritulus* and *I. marxi* tested negative for all pathogens.

We did not detect similar infection rates in small mammals (Figure 3.2 and Table 3.2). Only 4 of 105 small mammal hosts tested positive for a pathogen (3.8%). One *B. brevicauda* from Saint-Sylvestre tested positive for *B. odocoilei* and one *P. leucopus* from Henryville tested positive for *B. microti*. Two *Peromyscus* individuals tested positive for a *Hepatozoon* species, with one *P. leucopus* from New New Age Farm and one *P. maniculatus* from Henryville. We found a feeding larva on both infected *P. leucopus*, with one uninfected larva from New New Age Farm and one larva infected with *B. odocoilei* from Henryville.

3.5. Discussion

We detected *B. odocoilei* and *R. rickettsii* in ticks and small mammals beyond their distributional limits based on current surveillance efforts in southeastern Quebec. No recent detections have been reported for *R. rickettsii* in *Haemaphysalis* or *Dermacentor* ticks in Canada (Dergousoff et al, 2009; Teng et al, 2011; Wood and Artsob, 2012; Wood et al, 2016; Yunik et al, 2015). Historically, *R. rickettsii* has been identified in *H. leporispalustris* ticks in Alberta, British Columbia, Ontario, and Nova Scotia, but has not been detected in Quebec (Humphreys and Campbell, 1947; Wood and Artsob, 2012). In Canada, *B. odocoilei* was first detected through cervid infections (Mathieu et al, 2018; Pattullo et al, 2013). More recently, our study as well as others have detected *B. odocoilei* in *I. scapularis* ticks in Central Canada (Milnes et al, 2019; Robinson et al, 2022; Scott and Pesapane, 2021; Scott et al, 2021; Scott et al, 2020). We also report for the first time *B. odocoilei* being detected in a shrew in Canada.

These pathogens use two modes of transmission in ticks including transovarial transmission from female to larvae and transstadial transmission between immature and adult stages—the latter relying on a bloodmeal taken from a competent host (Freitas et al, 2009; Moore et al, 2018; Roth et al, 2017; Zembsch et al, 2021). The dispersal of ticks infected with *Babesia* species and *R. rickettsii* by migratory songbirds has allowed the spread of these pathogens in Canada (Scott et al, 2021; Scott et al, 2020; Scott et al, 2019). Local competent hosts can maintain these pathogens in the environment, but feeding ticks may not become infected owing to low levels of pathogen circulation and variable strain virulence (Freitas et al, 2009; McDade and Newhouse, 1986).

Transovarial transmission can help spread these pathogens without requiring an infected

host, although not all offspring may become infected owing to partial transovarial transmission, leading to low larval infection rates (Freitas et al, 2009; Zembsch et al, 2021). Therefore, it is unknown how quickly these emerging or re-emerging pathogens will spread in Canada owing to biotic barriers affecting pathogen transmission.

We also detected a *Hepatozoon* species in two white-footed mice. This pathogen is vectored by many arthropods including mosquitoes, ticks, mites, fleas, and flies, which are its definitive hosts (Smith, 1996). In Canada, competent intermediate hosts of this parasite include snakes, frogs, and small mammals (Boulianne et al, 2007; Léveillé et al, 2021; Léveillé et al, 2020). This parasite is transmitted to a mouse or another intermediate host through the accidental or intentional ingestion of an arthropod with infective oocytes. Arthropods consist of a large portion of the diet of *Peromyscus* mice, thereby providing greater opportunities for parasite transmission (Wolff et al, 1985). The parasite will then develop in its intermediate host, where it will be ready to infect future feeding arthropods (Smith, 1996).

The most prevalent pathogen among our samples was *B. burgdorferi*. Current surveillance efforts in Canada detect a high incidence of *B. burgdorferi* in ticks and vertebrate hosts, with an increase in positive *I. scapularis* ticks from 5.9% to 23% in the past decade in Central Canada (Gasmi et al, 2017; Gasmi et al, 2016; Guillot et al, 2020; Kulkarni et al, 2019; Milnes et al, 2019; Nelder et al, 2014; Slatculescu et al, 2020). Lower prevalence of *A. phagocytophilum*, *B. miyamotoi*, and *B. microti* have also been found in *I. scapularis* ticks through surveillance efforts in Canada (Guillot et al, 2020; O'Brien et al, 2016; Wilson et al, 2022). We only found *B. microti* in one *P. leucopus* individual in an endemic region of Quebec.

Surprisingly, no small mammals tested positive for *B. burgdorferi*. We tested liver tissues, which have been used for *B. burgdorferi* detection in wild rodents (Zinck and Lloyd, 2022). Higher *B. burgdorferi* detection rates might have been found if tail, tongue, ear samples (Zawada et al, 2020), or lung tissues (André et al, 2017) were used. A study analyzing liver microbiomes in *P. leucopus* found a low number of *Borrelia* sequences with next-generation sequencing, which were undetectable by classic endpoint PCR (André et al, 2017). However, we used longer *B. burgdorferi* fragments here (~340 or 605 bp) than in André et al (2017) (142 bp). Therefore, *B. burgdorferi* may not be detected in some samples because of low concentrations or longer amplicons.

The primary tick-borne pathogens of interest for sentinel surveillance efforts in Canada

are *A. phagocytophilum*, *Borrelia* species, and *Babesia* species (Guillot et al, 2020). Typically, these pathogens are tested in nymphs and adults. Larvae are rarely tested for pathogens, as transovarial transmission would be required. However, tick-borne pathogens are expected to increase in the coming decades, as tick and host populations continue to expand into new areas in Canada. Therefore, future surveillance efforts in Canada should focus on two key aspects for more comprehensive emerging tick-borne pathogen testing. First, pathogen testing should be performed on all tick life stages to detect possible transovarial transmission. Second, testing of nontargeted pathogens such as *Rickettsia* species can help detect pathogens that may be introduced by the dispersal of infected ticks by migratory birds or maintained through cryptic transmission cycles (Hamer et al, 2011). As a result, surveillance efforts would better detect the spread and potential risk of emerging or re-emerging tick-borne pathogens for human and wildlife populations throughout Canada.

3.6. Conclusions

Our study identified two emerging tick-borne pathogens, *B. odocoilei* and *R. rickettsii*, outside of their known range in Quebec. We also detected cases of *B. burgdorferi*, *B. microti*, and *Hepatozoon* spp. in tick and small mammal specimens in Central Canada. We demonstrate that all tick life stages should be tested comprehensively for pathogens, especially with the increased presence and spread of emerging or re-emerging tick-borne pathogens in Canada.

3.7. Acknowledgements

The authors express their appreciation to the landowners and stakeholders who allowed access to their property for field sampling. The authors gratefully thank all their assistants for their invaluable help in the field: Daniella Cross, Connia Ren, Dania Shaban, Ellen Bidulka, Jihane Benbahtane, Christina Provost, and Sébastien Cyr. The authors also thank Robbin Lindsay and Antonia Dibernardo for sharing the protocols used by the National Microbiology Lab for DNA extraction and PCR testing of tick samples.

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3.9. Tables

Table 3.1. Total number and number of infected ticks at our study sites in Ontario and Quebec, Canada. Larvae were tested together in pools, which were separated by grid if questing and by host if feeding. Nymphs and adults were tested individually. Questing ticks are indicated by a "Q" and ticks found feeding on small mammals are indicated by an "F". Numbers in parentheses represent the number of infected and total ticks, which consisted of larval pools between 1 to 10 larvae, individual nymphs, or individual adults. An asterisk denotes an infected small mammal host. These two infected small mammal individuals harbored different pathogen species than feeding larvae, with one *P. leucopus* with a *Hepatozoon* spp. from New New Age Farm and one *P. leucopus* with *Babesia microti* from Henryville.

Site	Latitude	Longitude	*H. leporispalustris*		*I. auritulus*	*I. marxi*
			Larval pools	Nymph	Nymph	Adult
(1) 3 Ridges Farm	42.70	-81.03	0	0	0	0
(2) New New Age Farm	42.73	-80.84	0	0	0	0
(3) North Tract	44.08	-79.31	0	0	0	0
(4) Brown Hill Tract	44.21	-79.36	0	0	0	0
(5) Upjohn Nature Reserve	45.08	-79.36	0	0	0	0
(6) Dyer Memorial Nature Reserve	45.40	-79.15	0	0	0	0
(7) Rose Hill Nature Reserve	45.16	-77.22	0	0	F: 1 (0/1)	0
(8) Kirkview Farm	45.42	-74.67	0	0	0	0
(9) Saint-Polycarpe	45.33	-74.39	0	0	0	0

(10) Saint-Valentin	45.18	-73.35	0	0	0	0
(11) Henryville	45.12	-73.21	0	0	0	0
(12) Lefebvre	45.74	-72.41	Q: 1 (0/1)	0	0	0
(13) Parc Sanctuaire de Saint-Majorique	45.94	-72.53	0	0	0	F: 1 (0/1)
(14) Serpentine-de-Coleraine Ecological Reserve	45.98	-71.37	Q: 19 (4/19)	Q: 1 (0/1)	0	0
(15) Frontenac National Park	45.82	-71.20	Q: 1 (1/1)	0	0	0
(16) Saint-Sylvestre	46.37	-71.12	Q: 1 (0/1)	0	0	0

I. scapularis			Host of feeding ticks	Pathogen species found in ticks
Larval pools	Nymph	Adult		
0	0	0	None	None
Q: 12 (0/12) F: 7 (0/7)	Q: 15 (2/15) F: 2 (0/2)	0	4 *Peromyscus leucopus**, 2 *Napaeozapus insignis*	*Borrelia burgdorferi*, *Babesia odocoilei*
0	F: 2 (0/2)	0	2 *N. insignis*	None
0	0	0	None	None
0	0	0	None	None
0	0	0	None	None
0	0	0	1 *Myodes gapperi*	None
0	Q: 4 (0/4) F: 1 (1/1)	0	1 *P. leucopus*	*B. burgdorferi*
F: 1 (0/1)	Q: 1 (0/1)	0	1 *P. leucopus*	None

Q: 4 (0/4) F: 4 (0/4)	Q: 67 (19/67) F: 1 (0/1)	0	3 *P. leucopus*, 1 *M. gapperi*	*B. burgdorferi*, *B. odocoilei*
Q: 10 (0/10) F: 3 (1/3)	Q: 26 (4/26) F: 2 (0/2)	Q: 1 (0/1)	2 *P. leucopus**, 1 *Peromyscus maniculatus*, 1 *M. gapperi*, 1 human (not tested)	*B. burgdorferi*, *B. odocoilei*
0	0	0	None	None
Q: 2 (0/2) F: 2 (1/2)	Q: 12 (2/12)	0	1 *B. brevicauda*, 1 *P. maniculatus,* 1 *T. hudsonicus* (not tested)	*B. burgdorferi*, *B. odocoilei*
Q: 1 (0/1)	Q: 1 (0/1)	0	None	*R. rickettsii*
0	0	0	None	*R. rickettsii*
0	0	0	None	None

Table 3.2. Total number and number of infected small mammal individuals at our study sites in Ontario and Quebec, Canada. Numbers in parentheses represent the number of infected and total small mammal individuals. Only four small mammal specimens were infected across our sites. Two *Peromyscus* individuals were infected with a *Hepatozoon* spp., with one *P. leucopus* from New New Age Farm and one *P. maniculatus* from Henryville. One *P. leucopus* individual was also infected with *B. microti* from Henryville. One *B. brevicauda* individual from Saint-Sylvestre was infected with *Babesia odocoilei*.

Site	Latitude	Longitude	*P. leucopus*	*P. maniculatus*	*B. brevicauda*	*S. cinereus*
(1) 3 Ridges Farm	42.70	-81.03	1 (0/1)	0	0	0
(2) New New Age Farm	42.73	-80.84	6 (1/6)	0	0	0
(3) North Tract	44.08	-79.31	1 (0/1)	0	0	0
(4) Brown Hill Tract	44.21	-79.36	5 (0/5)	0	0	0
(5) Upjohn Nature Reserve	45.08	-79.36	2 (0/2)	0	0	0
(6) Dyer Memorial Nature Reserve	45.40	-79.15	0	1 (0/1)	0	0
(7) Rose Hill Nature Reserve	45.16	-77.22	0	3 (0/3)	0	0
(8) Kirkview Farm	45.42	-74.67	5 (0/5)	0	0	0
(9) Saint-Polycarpe	45.33	-74.39	8 (0/8)	0	5 (0/5)	0
(10) Saint-Valentin	45.18	-73.35	3 (0/3)	0	0	0
(11) Henryville	45.12	-73.21	2 (1/2)	3 (1/3)	0	0
(12) Lefebvre	45.74	-72.41	0	0	3 (0/3)	0

(13) Parc du Sanctuaire Saint-Majorique	45.94	-72.53	0	1 (0/1)	1 (0/1)	0
(14) Serpentine-de-Coleraine Ecological Reserve	45.98	-71.37	0	3 (0/3)	0	0
(15) Frontenac National Park	45.82	-71.20	0	0	0	1 (0/1)
(16) Saint-Sylvestre	46.37	-71.12	0	0	8 (1/8)	0

M. pennsylvanicus	*M. gapperi*	*P. breweri*	*N. insignis*	Pathogen species found in small mammals
0	0	1 (0/1)	0	None
0	0	1 (0/1)	11 (0/11)	*Hepatozoon* spp. (*P. leucopus*)
1 (0/1)	0	0	4 (0/4)	None
0	0	0	0	None
0	0	0	0	None
0	0	0	1 (0/1)	None
0	2 (0/2)	0	2 (0/2)	None
0	0	0	0	None
0	0	0	0	None
0	5 (0/5)	0	1 (0/1)	None
0	1 (0/1)	0	0	*Hepatozoon* spp. (*P. maniculatus*) *Babesia microti* (*P. leucopus*)
0	2 (0/2)	0	0	None

0	4 (0/4)	0	4 (0/4)	None
0	1 (0/1)	0	0	None
0	0	0	0	None
0	1 (0/1)	0	1 (0/1)	*Babesia odocoilei* (*B. brevicauda*)

3.10. Figures

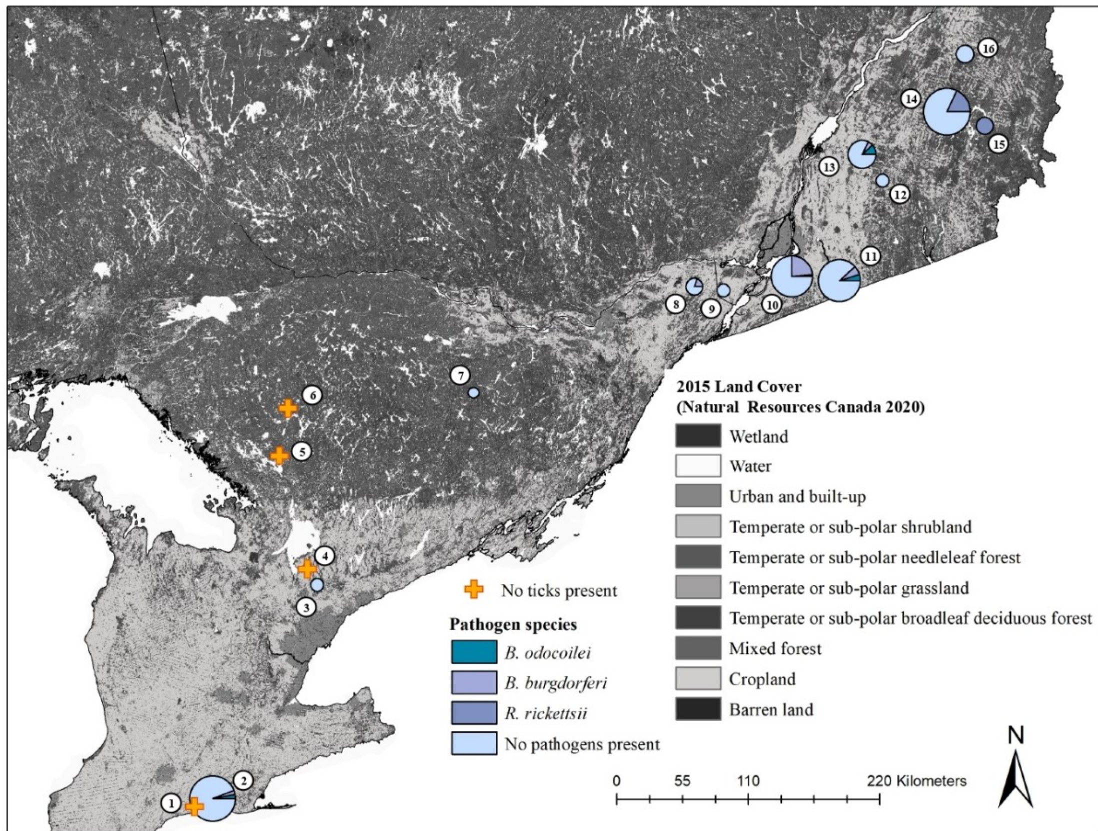

Figure 3.1. Pathogens detected in ticks at our study sites in Ontario and Quebec, Canada. Circle size is representative of the abundance of local ticks, where larger circles denote greater tick abundances. No ticks were detected at sites with orange crosses. Circle coloration demonstrates the proportional results of pathogen testing for ticks, which consisted of larval pools, individual nymphs, and individual adults. The proportion of the circle in light blue represents the ticks that were negative for pathogen testing. Proportions of ticks that were positive and harboring *Babesia odocoilei* (turquoise), *Borrelia burgdorferi* (light purple), or *Rickettsia rickettsii* (dark purple) are noted on the map. Study sites include (1) 3 Ridges Farm, (2) New New Age Farm, (3) North Tract—York Regional Forest, (4) Brown Hill Tract—York Regional Forest, (5) Upjohn Nature Reserve, (6) Dyer Memorial Nature Reserve, (7) Rose Hill Nature Reserve, (8) Kirkview Farm, (9) Saint-Polycarpe, (10) Saint-Valentin, (11) Henryville, (12) Lefebvre, (13) Parc du Sanctuaire Saint-Majorique, (14) Serpentine-de-Coleraine Ecological Reserve, (15) Frontenac National Park, (16) Saint-Sylvestre. The land cover map was extracted from the 2015 Land Cover of Canada raster by Natural Resources of Canada (2020).

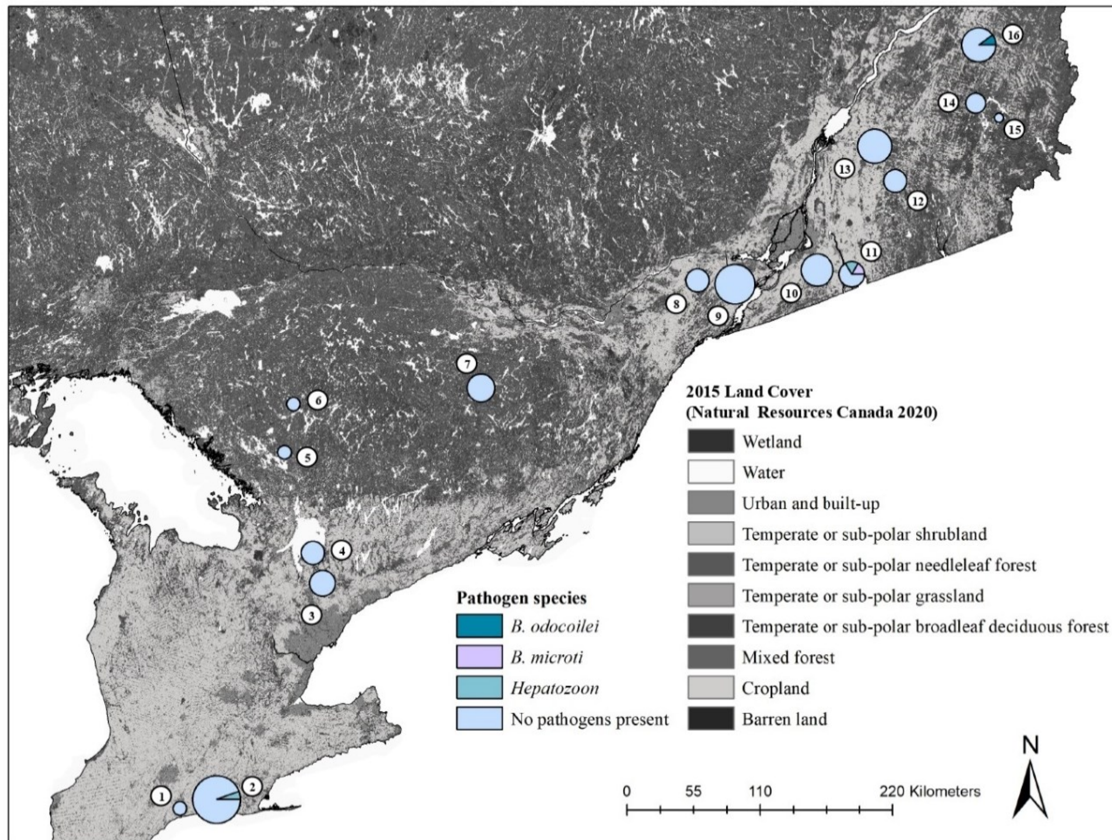

Figure 3.2. Pathogens detected in small mammals in Ontario and Quebec, Canada. Circle size is representative of the abundance of collected small mammals, where larger circles denote greater abundances of collected small mammals. Circle coloration demonstrates the proportional results of pathogen testing for individual small mammals. The proportion of the circle in light blue represents the collected small mammals that were negative for pathogen testing. Individual small mammals that were positive and harboring *Babesia odocoilei* (turquoise), *Babesia microti* (pink), and a *Hepatozoon* species (teal) are noted on the map. Study sites include (1) 3 Ridges Farm, (2) New New Age Farm, (3) North Tract—York Regional Forest, (4) Brown Hill Tract—York Regional Forest, (5) Upjohn Nature Reserve, (6) Dyer Memorial Nature Reserve, (7) Rose Hill Nature Reserve, (8) Kirkview Farm, (9) Saint-Polycarpe, (10) Saint-Valentin, (11) Henryville, (12) Lefebvre, (13) Parc du Sanctuaire Saint-Majorique, (14) Serpentine-de-Coleraine Ecological Reserve, (15) Frontenac National Park, (16) Saint-Sylvestre. The land cover map was extracted from the 2015 Land Cover of Canada raster by Natural Resources of Canada (2020).

Connecting statement between Chapter 3 and 4

Sentinel surveillance in Canada focuses on testing *I. scapularis* for more prevalent tick-borne pathogens, such as *A. phagocytophilum*, *B. microti*, and *B. burgdorferi*. However, certain tick-borne pathogens, such as *F. tularensis* and *R. rickettsii*, that were more prevalent in the 20th century may begin to re-emerge with time. In Chapter 3, comprehensive pathogen testing was conducted on tick and small mammal specimens that were collected at sites of distinct degrees of *B. burgdorferi* risk in Ontario and Quebec. Three pathogen species (*B. odocoilei*, *B. burgdorferi*, and *R. rickettsii*) were detected in *I. scapularis* and *H. leporispalustris*. Three pathogen species were identified in small mammal hosts, including *B. odocoilei*, *B. microti*, and a *Hepatozoon* species. Both *B. odocoilei* and *R. rickettsii* were found outside their known geographic distribution in southeastern Quebec based on current surveillance efforts.

Pathogen spread and transmission may be modulated by changes in the abundance and distribution of tick and host populations. Building on the information obtained from each previous chapter, Chapter 4 focuses on the relationships between pathogens with *I. scapularis* and mammal hosts. More specifically, this chapter assesses the relationship between local pathogen presence, prevalence, and diversity with the abundance of *I. scapularis* as well as the abundance and diversity of mammal hosts at sites of distinct levels of disease risk in Ontario and Quebec. This chapter adds knowledge of biotic factors that may help explain tick-borne pathogen spread and transmission at their frontier of range expansion in Canada.

Chapter 4 | Pathogen presence, prevalence, and diversity in *Ixodes scapularis* and mammal hosts at their expanding northern range limits

Kirsten E. Crandall, Jeremy T. Kerr, and Virginie Millien

4.1. Abstract

With climate and land use changes, tick-borne pathogens are expected to become more widely distributed in Canada. Pathogen spread and transmission in this region is modulated by changes in the abundance and distribution of tick and host populations. Here, we assessed the relationships between pathogens detected in *Ixodes scapularis* and mammal hosts at sites of different levels of disease risk using data from summer field surveys in Ontario and Quebec, Canada. Generalized linear mixed models and ordinal logistic regressions were used to determine the influence of the abundance of *I. scapularis* and the abundance and diversity of mammal hosts on pathogen presence, prevalence, and diversity. We detected three pathogen species in *I. scapularis* and small mammals using nested PCRs, namely *Borrelia burgdorferi* sensu stricto, *Babesia odocoilei*, and *Babesia microti*. Depending on the analyzed pathogen, local infection prevalence ranged from 0% to 25.4% in questing ticks and from 0% to 16.7% in small mammal hosts. We detected *B. odocoilei* in localities beyond its known range limits in southeastern Quebec suggesting ongoing range expansion of this pathogen. Neither the abundance of *I. scapularis* nor the abundance and diversity of mammal hosts altered local pathogen presence and prevalence, contrary to expectations. However, mammal species richness was a key predictor of the number of pathogen species. Our study demonstrates the need for future surveillance efforts that test questing and feeding *I. scapularis* of all life stages, as well as their hosts to better determine the spread, transmission, and co-occurrence of tick-borne pathogens in Canada.

Keywords: pathogen, *Ixodes scapularis*, mammal hosts, *Peromyscus leucopus*, abundance, diversity

4.2. Introduction

Tick-borne pathogens have increased in prevalence and geographic range in Canada due to changes in the abundance and distribution of tick and host populations (Ogden and Lindsay, 2016; Bouchard *et al.*, 2019). Host populations are expanding their geographic ranges poleward in response to changes in climate and land use, thereby dispersing tick vectors to new poleward locations in Canada (Diuk-Wasser, VanAcker and Fernandez, 2021). As a result, reproducing tick populations may become established, which may subsequently increase tick-borne pathogen spread and transmission locally (Milnes *et al.*, 2019). Consequently, increased tick abundances as well as increased prevalences and co-occurrences of tick-borne pathogens may lead to a greater number of cases of tick-borne diseases and co-infections in human populations (Cutler *et al.*, 2021).

The predominant tick-borne pathogen detected via sentinel surveillance in Canada is *Borrelia burgdorferi* sensu stricto, one of the *Borrelia* genospecies that causes Lyme disease (Guillot *et al.*, 2020; Wilson *et al.*, 2022). This pathogen is transmitted by blacklegged ticks (*Ixodes scapularis*) in central and eastern Canada, as well as western blacklegged ticks (*I. pacificus*) in British Columbia (Guillot *et al.*, 2020; Wilson *et al.*, 2022). In these regions, the prevalence of *B. burgdorferi* in nymph and adult *Ixodes* ticks ranges from 0% to 56.0%, with the highest infection prevalences documented in Ontario, Quebec, New Brunswick, and Nova Scotia (Guillot *et al.*, 2020; Dumas *et al.*, 2022).

However, additional emerging tick-borne pathogens have been detected at a lower prevalence in *Ixodes* ticks through surveillance efforts in Canada (Dibernardo *et al.*, 2014; Guillot *et al.*, 2020; Wilson *et al.*, 2022). *Anaplasma phagocytophilum*, the bacterium causing anaplasmosis, has been found in *I. scapularis* in Ontario, Quebec, New Brunswick, and Nova Scotia (Guillot *et al.*, 2020). *Babesia microti*, a protozoan causing babesiosis, has also been identified in localities in British Columbia, Ontario, Quebec, New Brunswick, and Nova Scotia (Guillot *et al.*, 2020; Wilson *et al.*, 2022). Similarly, *Babesia odocoilei* has been found more recently in *I. scapularis* in Ontario and Quebec (Milnes *et al.*, 2019; Scott and Pesapane, 2021; Crandall, Kerr and Millien, 2022). *Borrelia miyamotoi*, a bacterium causing tick-borne relapsing fever, has been found at a low prevalence in *I. scapularis* in Ontario and Quebec (Guillot *et al.*, 2020; Dumas *et al.*, 2022).

The prevalence and transmission of tick-borne pathogens may be modulated by the abundance and composition of mammal communities (Levi *et al.*, 2016; Luis, Kuenzi and Mills, 2018). Small mammal hosts, such as white-footed mice (*Peromyscus leucopus*), chipmunks (*Tamias striatus*), and shrews (*Blarina brevicauda* and *Sorex cinereus*), can successfully feed a greater number of ticks and more readily transmit pathogens including *B. burgdorferi* (Mather *et al.*, 1989; LoGiudice *et al.*, 2003). In addition, mid-size or larger mammals, such as raccoons (*Procyon lotor*) and white-tailed deer (*Odocoileus virginianus*), feed large burdens of ticks resulting in increased tick abundances, yet these hosts may not be as efficient in transmitting pathogens (LoGiudice *et al.*, 2003). In Ontario and Quebec, mammal species richness and the relative abundance of *P. leucopus* were both identified as significant contributors to increased *I. scapularis* abundance and *B. burgdorferi* prevalence, demonstrating the importance of host community composition for pathogen spread and transmission (Simon *et al.*, 2014; Werden *et al.*, 2014; Dumas *et al.*, 2022; Millien *et al.*, 2023).

Tick and mammal host populations have been identified as key contributors to the spread and transmission of emerging tick-borne pathogens in Canada (Ogden, Mechai and Margos, 2013; Bouchard *et al.*, 2019). However, the degree that *I. scapularis* and mammal hosts impact tick-borne pathogen spread and transmission remains uncertain relative to their time since establishment (Millien *et al.*, 2023). Here, we assessed the relationships between local pathogen presence, prevalence, and diversity with the abundance of *I. scapularis* as well as the abundance and diversity of mammal hosts at sites of distinct levels of disease risk in Ontario and Quebec, Canada. These results add knowledge of biotic factors that may help explain tick-borne pathogen spread and transmission at their frontier of range expansion in Canada.

4.3. Materials and Methods

4.3.1. *Field sampling*

Field surveys were conducted at 16 sites with contiguous forest in Ontario and Quebec, Canada in July and August 2019 (Figure 4.1). Sites were selected based on their different degrees of *B. burgdorferi* risk related to the abundances and life stages of *I. scapularis* present locally as defined by the Institut national de santé publique du Québec (2018) and Public Health Ontario (2018), which ranged from possible to significant risk (Table D1). At each site, three

grids of 40 m by 70 m were set up for sampling ticks and mammal hosts, which were maximally separated by 100 metres due to geographic barriers (e.g., streams or park trails).

Within each grid, four 70-metre long transects were used to sample ticks one time by dragging a 1 m^2 white flannel over low-lying vegetation. Flannels were checked every 10 metres, and questing ticks were removed. All ticks were kept in microvials with 95% ethanol, and larvae were pooled while nymphs and adults were kept individually. Tick specimens were identified to the species using dichotomous keys (Lindquist *et al.*, 2016).

At each site, 84 Sherman live traps (H.B. Sherman Traps, Inc., Florida, United States) were placed along four parallel transects within each grid for three consecutive nights, representing a total of 4032 trap nights in our study. We targeted mouse (*P. leucopus* and *P. maniculatus*), shrew (*B. brevicauda* and *S. cinereus*), vole (*Microtus pennsylvanicus* and *Myodes gapperi*), and jumping mouse (*Napaeozapus insignis* and *Zapus hudsonius*) species. In the afternoon, a bait mixture of peanut butter and oatmeal, an apple piece, and a cotton ball were placed in each trap. Traps were checked the following morning. Juveniles and non-targeted rodent species were immediately released at the site of capture. Individuals of targeted species were euthanized via isoflurane inhalation followed by cervical dislocation. One red squirrel (*Tamiasciurus hudsonicus*) and two hairy-tailed moles (*Parascalops breweri*) were also euthanized due to severe injuries. Small mammals were searched for feeding ticks, and mammalian liver tissues were dissected and placed into microvials with 95% ethanol. Liver tissues were selected for pathogen testing, as they have been used for *B. burgdorferi* detection in wild rodents (Zinck and Lloyd, 2022). As in Tessier et al. (2004), a nested PCR using species-specific COIII primers was used to identify *Peromyscus* species (Supplementary Methods). All samples were accessioned in the collections of the Redpath Museum, McGill University (Table D2). Ethical approval and permits were issued by McGill University (AUP No. 2019-8086), the Ministère des Forêts, de la Faune et des Parcs (SEG permit No. 2019-06-04-008-00-S-F), and the Ministry of Natural Resources and Forestry (WSCA No. 1093495).

Concurrently, nine trail cameras (Force-10, SpyPoint Inc., Quebec, Canada) were placed 1 metre above the ground facing inside our grids and set to take three consecutive photos without delay for each detection. Host species were identified from photographs taken by camera traps. Birds, domestic pets, humans, and unidentified individuals were not included in our dataset.

For each site, total *I. scapularis* abundance was estimated by the sum of questing and feeding ticks collected from tick dragging and small mammal trapping, respectively. We used the total number of collected mammal individuals as a proxy for the abundance of small mammals locally. The relative abundance of *P. leucopus* was quantified as the number of collected *P. leucopus* individuals divided by the local abundance of collected small mammals. The number of mammal host species was estimated as the number of distinct species collected via small mammal trapping and detected in camera photographs.

4.3.2. *Pathogen testing*

DNA extractions and nested PCRs conducted by Geneticks, Inc. targeted five pathogens in our tick and small mammal specimens (Supplementary Methods; Table D3). Adults and nymphs were tested individually, while larvae were pooled by grid if questing (2-10 larvae per pool) and by host if feeding (1-10 larvae per pool). All *I. scapularis* and small mammal specimens were tested for *Anaplasma phagocytophilum*, *Babesia* species, and *Borrelia* species. If a band was visible (i.e., positive PCR), we then tested twice more for false positives. The *p44* gene was targeted to test for *A. phagocytophilum* (Holden *et al.*, 2003). *Babesia odocoilei* and *B. microti* were targeted with the 18S rRNA region using the *mic494* and *odo563* inner primers, respectively. An additional primer set targeting the 18S rRNA of each *Babesia* species was used for confirmation (Persing *et al.*, 1992). We also tested for *B. burgdorferi* sensu stricto and *B. miyamotoi* using the 5S-23S intergenic space region and the 18S rRNA region, respectively (Dibernardo *et al.*, 2014; Zinck *et al.*, 2021). An additional test using the *flaB* gene confirmed the presence of *B. burgdorferi* sensu lato (Wodecka, 2011). Bio Basic DNA Sequencing (Ontario, Canada) completed Sanger DNA sequencing of positive samples, with sequences assessed for quality control, ambiguous base calls, and end-reading errors using 4Peaks software. Pathogen species were confirmed with GenBank using a MEGABLAST search in the nucleotide BLAST database.

For each site, we calculated pathogen presence, prevalence, and diversity in *I. scapularis* and small mammal hosts. Pathogen presence indicated whether pathogens were present (1) or absent (0) locally in *I. scapularis* or in small mammal hosts. Pathogen prevalence was calculated as a proportion for questing *I. scapularis* by dividing the number of infected individuals and larval pools of *I. scapularis* by the total number of *I. scapularis* (individuals and larval pools).

Feeding *I. scapularis* were excluded from this calculation, as they better represent the pathogens circulating in hosts and may artificially increase local infection prevalence. Pathogen diversity was defined as the total number of pathogen species found in *I. scapularis* and small mammal hosts.

4.3.3. *Statistical analyses*

All statistical analyses were performed in R v4.2.2. (R Core Team, 2022). We assessed the effect of *I. scapularis* and mammal hosts on pathogen presence, prevalence, and diversity across our sites in Central Canada. Using the *rcorr* function in the *Hmisc* package (Harrell Jr, 2021), we first calculated the correlation coefficients between small mammal abundance, the relative abundance of *P. leucopus*, mammal species richness, questing *I. scapularis* abundance, and total *I. scapularis* abundance. Small mammal abundance was highly correlated with mammal diversity ($r = 0.54$, $p < 0.05$), and was not included in further analyses. Using the *scale* function, biotic factors were centred by subtracting the variable average from each value and standardized. Spatial autocorrelation among biotic factors was assessed with Moran's I with an inverse distance weights matrix using the *moran.test* function in the *spdep* package (Bivand and Wong, 2018).

We first evaluated the effect of *I. scapularis* abundance and mammal hosts on pathogen presence with two binomial generalized linear mixed models with a cloglog link function using the *glmer* function in the *lme4* package (Bates *et al.*, 2015). Our binary response variable was pathogen presence (1) or absence (0) in *I. scapularis* and small mammal hosts. Two separate models were run to determine the independent impacts that *I. scapularis* and mammal hosts have on pathogen presence. The first model used total *I. scapularis* abundance as an independent variable. The independent variables of a second model included the relative abundance of *P. leucopus* and mammal species richness. We subsequently analyzed the impact of mammals hosts on pathogen prevalence in questing *I. scapularis* with a binomial generalized linear mixed model and a cloglog link function using the *glmer* function. A binomial model was used, as pathogen prevalence was calculated as a proportion. The independent variables in this third model were the relative abundance of *P. leucopus* and mammal species richness. Site was included as a random factor in all three models to account for site-specific associations that are not due to our

fixed factors. Model selection was based on AIC values, with a smaller AIC indicating a better model fit, and the variance and standard deviation of Site.

Finally, we ran an ordinal logistic regression with the *polr* function in the MASS package (Venables and Ripley, 2002) to assess if pathogen diversity across our sites was affected by mammal hosts. Our independent variables included the relative abundance of *P. leucopus* and the number of mammal host species. We used the *stepAIC* function in the *cAIC4* package (Säfken *et al.*, 2021) to determine if additional models should be assessed.

4.4. Results

4.4.1. *Field sampled ticks and mammal hosts*

The abundance of questing and feeding *I. scapularis* ranged from 0 to 164 individuals across our sites (Table 4.1 and 4.2). We collected a total of 382 questing *I. scapularis* including 255 larvae (29 pools), 126 nymphs, and one adult male, as well as 65 feeding *I. scapularis* including 57 larvae (17 pools) and 8 nymphs.

We collected a total of 105 small mammal individuals (Table 4.2; Table D4 and D5). The most abundant species was *P. leucopus* (31.4% of collected individuals; $n = 33$), which was present at 9 of 16 sites. Other collected small mammals, in decreasing order of abundance, were *N. insignis* (22.8%; $n = 24$), *B. brevicauda* (16.2%; $n = 17$), *M. gapperi* (15.2%; $n = 16$), *P. breweri* (1.9%; $n = 2$), *S. cinereus* (1.0%; $n = 1$), and *M. pennsylvanicus* (1.0%; $n = 1$). At each site, small mammal abundance ranged from 1 to 18 individuals, while the relative abundance of *P. leucopus* ranged from 0 to 1 (Table 4.2). We identified 5 mammal species in photographs taken by cameras, which included squirrels (*Sciurus carolinensis*), chipmunks (*T. striatus*), white-tailed deer (*O. virginianus*), raccoons (*P. lotor*), and coyotes (*Canis latrans*). Between 2 and 8 mammal species were detected at each site via small mammal trapping and in camera photographs.

4.4.2. *Pathogen diversity*

For questing *I. scapularis*, only nymphs tested positive for our pathogens of interest, while the 255 questing larvae (29 pools) and one adult male that we collected all tested negative. Of 126 *I. scapularis* nymphs, five tested positive for *B. odocoilei*, with one nymph at Site 2, Site 10, and Site 11 and two nymphs at Site 13 (Table 4.1 and 4.2). Similarly, *I. scapularis* nymphs

infected with *B. burgdorferi* were found at Site 2 (one nymph), Site 10 (18 nymphs), and Site 11 (three nymphs). Local infection prevalence in questing *I. scapularis* ranged from 0% to 14.3% for *B. odocoilei* and from 0% to 25.4% for *B. burgdorferi* (Table D6). No questing ticks tested positive for *A. phagocytophilum*, *B. microti*, or *B. miyamotoi*.

For feeding *I. scapularis*, 2 of 17 larval pools and 1 of 8 nymphs were infected (Table 4.1 and 4.2; Table D6). One larva at Site 11 was infected with *B. odocoilei*, but it was found feeding on a *P. leucopus* infected with *B. microti*. A larva from Site 13 feeding on an uninfected *P. maniculatus* and a nymph from Site 8 feeding on an uninfected *P. leucopus* were both infected with *B. burgdorferi*. No feeding ticks tested positive for *A. phagocytophilum*, *B. microti*, or *B. miyamotoi*.

Only two of the 105 small mammals that we collected were infected (Table D4 and D5). At Site 11, one *P. leucopus* was infected with *B. microti* and at Site 16, one *B. brevicauda* also tested positive for *B. odocoilei*. Local infection prevalence of small mammal hosts ranged from 0% to 10% for *B. odocoilei* and from 0% to 16.7% for *B. microti* (Table D7). No small mammals tested positive for *A. phagocytophilum*, *B. burgdorferi*, or *B. miyamotoi*.

We detected pathogens in *I. scapularis* and small mammals hosts at 6 of our 16 sites, where up to 3 different pathogen species were present locally (Figure 4.1; Table 4.1 and 4.2). Local pathogen prevalence in questing *I. scapularis* adults, nymphs, and pools of larvae ranged from 0% to 26.8% (Table 4.2). Pathogen diversity was found to be highest in areas with long-established populations of *I. scapularis* in southern Ontario and Quebec (Sites 2, 10, and 11). These sites had over 100 *I. scapularis* from at least two different life stages, where 2 or 3 pathogen species were detected locally (Figure 4.1; Table 4.1 and 4.2).

4.4.3. *Effect of biotic factors on pathogen presence, prevalence, and diversity*

Small mammal abundance was significantly correlated to mammal species richness ($r = 0.54$, $p < 0.05$), questing *I. scapularis* abundance ($r = 0.54$, $p < 0.05$), and total *I. scapularis* abundance ($r = 0.55$, $p < 0.05$). Therefore, we excluded small mammal abundance from further analyses. We detected spatial autocorrelation in questing *I. scapularis* abundance (Moran's I = 0.206, $p < 0.05$), but none was detected for the relative abundance of *P. leucopus* (Moran's I = 0.072, $p = 0.207$) and mammal species richness (Moran's I = 0.138, $p = 0.115$).

There was no effect of pathogen presence with total *I. scapularis* abundance (p = 0.144; Table D8), the relative abundance of *P. leucopus* (p = 0.874; Table D9), or mammal species richness (p = 0.192; Table D9). Similarly, pathogen prevalence in questing *I. scapularis* was not affected by the relative abundance of *P. leucopus* (p = 0.824) or mammal species richness (p = 0.767; Table D10). Feeding ticks were not included in this model, as to not artificially increase the local pathogen prevalence. Finally, the relative abundance of *P. leucopus* (p = 0.822) did not significantly predict pathogen diversity and was subsequently removed from our model (Table D11). Only mammal species richness (OR = 11.826, $p < 0.05$) was found to significantly predict pathogen diversity, with higher odd ratios of pathogen detection with greater mammal species richness (Figure 4.2; Table D11).

4.5. Discussion

We provide evidence that mammal host populations contribute to the local diversity of emerging tick-borne pathogens in Central Canada. Pathogen diversity was highest in areas with long-established populations of *I. scapularis* in southern Ontario and Quebec. Greater mammal species richness within study locations was associated with a greater diversity of pathogens. This relationship was detected using molecular techniques across this broad region, where pathogen presence and prevalence have been increasing. However, we did not find an effect of the abundance of *I. scapularis* nor the abundance and diversity of mammal hosts on local pathogen presence and prevalence. These results demonstrate the complex mechanism driving the poleward expansion and transmission of these tick-borne pathogens.

Local infection prevalence in questing ticks varied depending on pathogen species. In line with surveillance data, we found that the local infection prevalence of *B. odocoilei* was up to 14.3% in questing ticks (Milnes *et al.*, 2019; Scott *et al.*, 2020, 2021; Scott and Pesapane, 2021). Of note, we detected *B. odocoilei* in questing ticks in Saint-Majorique-de-Grantham and a shrew in Saint-Sylvestre, which are outside of its known range limit in Sainte-Anne-de-Bellevue, Quebec (Figure D1; Scott and Pesapane 2021). This ongoing range expansion may be facilitated by the dispersal of infected ticks by bird hosts that are known to be reservoirs for *B. odocoilei* (Scott *et al.*, 2022). *B. burgdorferi* had the broadest geographic range amongst our tick samples, with infection prevalence highest in areas with long-established *I. scapularis* populations where

the pathogen has been circulating for decades (Ogden *et al.*, 2014). These results also parallel the infection rates reported at sentinel sites in Ontario and Quebec (Guillot *et al.*, 2020).

4.5.1. *The impact of biotic factors on pathogens*

Abundances of key hosts, such as white-tailed deer and white-footed mice, have been associated with greater *I. scapularis* abundance and greater *B. burgdorferi* prevalence in Ontario and Quebec (Bouchard *et al.*, 2011, 2013; Simon *et al.*, 2014; Werden *et al.*, 2014). However, here, pathogen presence and prevalence were not affected by the abundance of *I. scapularis* nor the abundance and diversity of mammal hosts across our sites. Pathogen spread and transmission may have been affected by bird hosts, an unexplored factor in our study, due to their ability to feed immature ticks and harbour several tick-borne pathogens (Scott *et al.*, 2022). Migratory birds may facilitate range expansions of tick-borne pathogens by introducing infected adventitious ticks to new locations in Canada (Scott *et al.*, 2020; Scott and Pesapane, 2021). In addition, ground foraging birds were found to be significant contributors to the spread and transmission of tick-borne pathogens at the most northern parts of the distribution range of *I. scapularis* (Leo, Gonzalez and Millien, 2017; Dumas *et al.*, 2022).

We observed that mammal species richness significantly predicted pathogen diversity, with up to 3 pathogen species being detected locally (Figure 4.1 and 4.2). Sites with greater pathogen diversity were associated with more diverse mammal communities. Locally, small mammals play an important role in feeding immature ticks and pathogen maintenance (Mather *et al.*, 1989; LoGiudice *et al.*, 2003). As these small mammals search for food resources, they may disperse ticks and their pathogens over short distances into nearby forest patches (Marrotte, Gonzalez and Millien, 2017; Borgmann-Winter, Oggenfuss and Ostfeld, 2021). Larger mammal hosts, such as white-tailed deer, can feed large burdens of ticks and act as key reproductive hosts for adult *I. scapularis* (LoGiudice *et al.*, 2003; Werden *et al.*, 2014). These mammal hosts may act as important facilitators for the long-range dispersal and establishment of tick populations and tick-borne pathogens, as they expand their ranges poleward in response to climate and land use changes (Dawe and Boutin, 2016; Diuk-Wasser, VanAcker and Fernandez, 2021). However, the spread and transmission of tick-borne pathogens may be limited in areas where *I. scapularis* or reservoir hosts, such as *P. leucopus*, have not yet established (Roy-Dufresne *et al.*, 2013; Simon *et al.*, 2014; Ripoche *et al.*, 2022; Millien *et al.*, 2023). In these areas, other medically

significant tick vectors (e.g., *Ixodes cookei* with Powassan virus; Gasmi et al. 2018) and reservoir hosts (e.g. chipmunks or shrews) may contribute more strongly to the spread and transmission of tick-borne pathogens (Levi *et al.*, 2016; Gasmi *et al.*, 2018; Dumas *et al.*, 2022).

The co-occurrence of multiple tick-borne pathogens may increase the risk of co-infection in tick and host populations locally (Cutler *et al.*, 2021). Co-infections can occur in adult ticks after feeding on different infected reservoir hosts or when co-feeding with infected ticks on the same host (Voordouw, 2015; Cutler *et al.*, 2021). Although we did not detect any co-infections in our tick and small mammal specimens, co-infections have been detected at varying levels of infection prevalence in *I. scapularis* in Canada (Dibernardo *et al.*, 2014; Dumas *et al.*, 2022; Wilson *et al.*, 2022). The majority of these co-infections occurred in areas near our sites in southern Ontario and Quebec, where long-established *I. scapularis* populations are located (Dumas *et al.*, 2022; Wilson *et al.*, 2022). If more tick-borne pathogens are co-occurring locally, it may lead to an increased risk of co-infections of tick-borne diseases in human populations. As a result, humans co-infected with multiple tick-borne pathogens may display complex clinical manifestations that present diagnostic challenges (Cutler *et al.*, 2021). Although we detected no pathogen species at some sites, there does not appear to be any environmental or host suitability limitations in these areas that will prevent those tick-borne pathogens from spreading there in the future.

4.5.2. *Future surveillance of tick-borne pathogens*

Our study demonstrates that comprehensive surveillance efforts targeting questing and feeding *I. scapularis* of all life stages and small mammal hosts is required to detect the geographic extent and co-occurrence of tick-borne pathogens in Canada. Concurrent testing of multiple tick-borne pathogens is necessary to better detect the risk of co-infections, especially as the co-occurrence of pathogens become more prevalent in areas with increased tick abundances and more diverse host communities. These results show expanding ranges of certain tick-borne pathogens transmitted by *I. scapularis*, especially in areas where *B. burgdorferi* has not yet established. It would also be relevant to test questing larval *I. scapularis* for tick-borne pathogens with known transovarial transmission, such as *B. odocoilei* (Zembsch, Bron and Paskewitz, 2021). Emerging tick-borne pathogens are advancing poleward in Canada with the expanding ranges of tick and host populations, where risks for pathogen transmission will rise. Proactive

surveillance efforts outside the known distributions of pathogens of concern for wildlife and human health will improve our ability to better anticipate the risk for tick-borne diseases in these regions.

4.6. Acknowledgements

We wish to express our appreciation to the landowners and stakeholders that gave us permission to access our study sites. We also want to thank our field assistants: Daniella Cross, Connia Ren, Dania Shaban, Ellen Bidulka, Jihane Benbahtane, Christina Provost, and Sébastien Cyr. K.E.C. was funded by Hydro-Québec and Natural Sciences and Engineering Research Council of Canada fellowships. J.T.K. was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant (RGPIN-2017-147544), Natural Sciences and Engineering Research Council of Canada Discovery Accelerator Supplement, and the University Research Chair in Macroecology and Conservation at the University of Ottawa. V.M. was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant (RGPIN-2017-03839).

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4.8. Tables

Table 4.1. The number of infected and total *Ixodes scapularis* at our study sites in Ontario and Quebec, Canada. Tick abundances consisted of larval pools between 1 to 10 larvae, individual nymphs, or individual adults. Questing ticks are denoted by a "Q" and ticks feeding on small mammals are indicated by an "F". Pathogens detected in ticks included *Babesia odocoilei* and *Borrelia burgdorferi*. See Table D6 for details regarding infection prevalence by tick activity and pathogen species. (1) 3 Ridges Farm, (2) New New Age Farm, (3) North Tract, (4) Brown Hill Tract, (5) Upjohn Nature Reserve, (6) Dyer Memorial Nature Reserve, (7) Rose Hill Nature Reserve, (8) Kirkview Farm, (9) Saint-Polycarpe, (10) Saint-Valentin, (11) Henryville, (12) Lefebvre, (13) Parc du Sanctuaire Saint-Majorique, (14) Serpentine-de-Coleraine Ecological Reserve, (15) Frontenac National Park, (16) Saint-Sylvestre.

Site ID	Larval pools	Nymphs	Adults	Pathogen species identified in ticks
1	0	0	0	None
2	Q: 0/12 F: 0/7	Q: 2/15 F: 0/2	0	*B. burgdorferi* (1 nymph), *B. odocoilei* (1 nymph)
3	0	F: 0/2	0	None
4	0	0	0	None
5	0	0	0	None
6	0	0	0	None
7	0	0	0	None
8	0	Q: 0/4 F: 1/1	0	*B. burgdorferi* (1 nymph)
9	F: 0/1	Q: 0/1	0	None

10	Q: 0/4 F: 0/4	Q: 19/67 F: 0/1	0	*B. burgdorferi* (18 nymphs), *B. odocoilei* (1 nymph)
11	Q: 0/10 F: 1/3	Q: 4/26 F: 0/2	Q: 0/1	*B. burgdorferi* (3 nymphs), *B. odocoilei* (1 nymph, 1 larva)
12	0	0	0	None
13	Q: 0/2 F: 1/2	Q: 2/12	0	*B. burgdorferi* (1 larva), *B. odocoilei* (2 nymphs)
14	Q: 0/1	Q: 0/1	0	None
15	0	0	0	None
16	0	0	0	None

Table 4.2. Summary of pathogen presence, prevalence, diversity in addition to the abundance and diversity of *Ixodes scapularis* and mammal hosts found at each site in Ontario and Quebec, Canada (listed as increasing latitudes and decreasing longitudes). Total *I. scapularis* abundance represents the abundance of questing and feeding ticks at each site found via tick dragging and small mammal trapping, respectively. Pathogen presence indicated whether pathogens were present (1) or absent (0) in *I. scapularis* or in small mammal hosts at a locality. Pathogen diversity is the number of tick-borne pathogen species found at each site in *I. scapularis* and small mammals. Pathogen prevalence was calculated as a proportion with questing *I. scapularis* by dividing the number of infected individual *I. scapularis* and larval pools by the total *I. scapularis* (individuals and larval pools). The relative abundance of *Peromyscus leucopus* was estimated by dividing the number of collected *P. leucopus* individuals by the local abundance of small mammals that were collected. The number of mammal host species was quantified as the sum of the different host species found via small mammal trapping and in trail camera photographs. (1) 3 Ridges Farm, (2) New New Age Farm, (3) North Tract, (4) Brown Hill Tract, (5) Upjohn Nature Reserve, (6) Dyer Memorial Nature Reserve, (7) Rose Hill Nature Reserve, (8) Kirkview Farm, (9) Saint-Polycarpe, (10) Saint-Valentin, (11) Henryville, (12) Lefebvre, (13) Parc du Sanctuaire Saint-Majorique, (14) Serpentine-de-Coleraine Ecological Reserve, (15) Frontenac National Park, (16) Saint-Sylvestre.

Site ID	Latitude (ºN)	Longitude (ºW)	Abundance *I. scapularis*	Pathogen presence	Pathogen diversity	Pathogen prevalence
1	42.70	-81.03	0	0	0	0.000
2	42.73	-80.84	164	1	2	0.074
3	44.08	-79.31	2	0	0	0.000
4	44.21	-79.36	0	0	0	0.000
5	45.08	-79.36	0	0	0	0.000
6	45.40	-79.15	0	0	0	0.000
7	45.16	-77.22	0	0	0	0.000
8	45.42	-74.67	5	1	1	0.000
9	45.33	-74.39	2	0	0	0.000
10	45.18	-73.35	117	1	2	0.268
11	45.12	-73.21	125	1	3	0.108
12	45.74	-72.41	0	0	0	0.000
13	45.94	-72.53	29	1	2	0.143
14	45.98	-71.37	3	0	0	0.000
15	45.82	-71.20	0	0	0	0.000
16	46.37	-71.12	0	1	1	0.000

Abundance small mammals	Relative abundance *P. leucopus*	No. host species (trapping and camera)
2	0.500	7
18	0.333	7
6	0.167	5
5	1.000	3
2	1.000	3
2	0.000	3
7	0.000	5
5	1.000	6
13	0.615	5
9	0.333	5
6	0.333	7
5	0.000	5
10	0.000	8
4	0.000	2
1	0.000	4
10	0.000	6

4.9. Figures

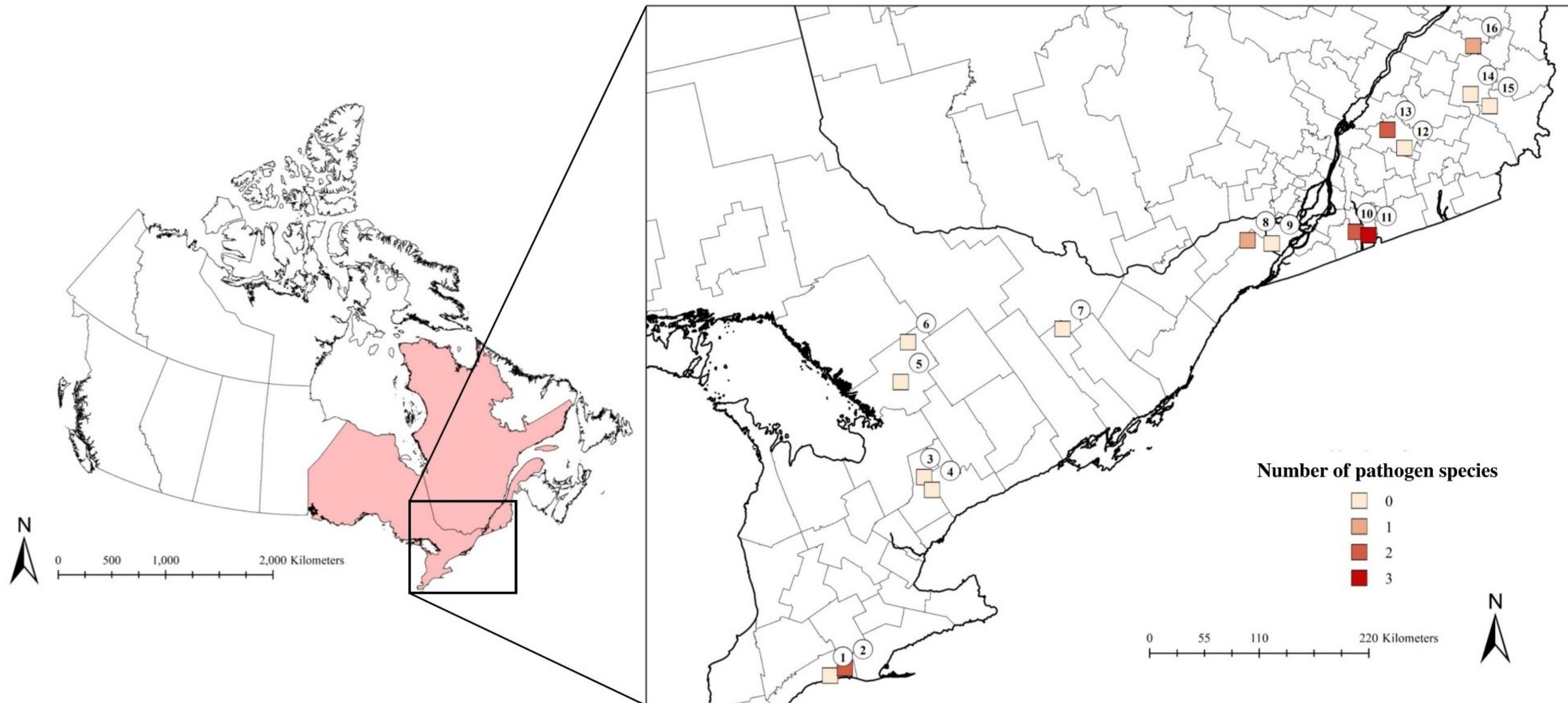

Figure 4.1. The number of pathogen species present at our sites in Ontario and Quebec, Canada that were detected in *Ixodes scapularis* and small mammals collected in July and August 2019. Lighter shades correspond to low numbers of pathogen species, while darker shades indicate a higher number of pathogen species. (1) 3 Ridges Farm, (2) New New Age Farm, (3) North Tract, (4) Brown Hill Tract, (5) Upjohn Nature Reserve, (6) Dyer Memorial Nature Reserve, (7) Rose Hill Nature Reserve, (8) Kirkview Farm, (9) Saint-Polycarpe, (10) Saint-Valentin, (11) Henryville, (12) Lefebvre, (13) Parc du Sanctuaire Saint-Majorique, (14) Serpentine-de-Coleraine Ecological Reserve, (15) Frontenac National Park, (16) Saint-Sylvestre.

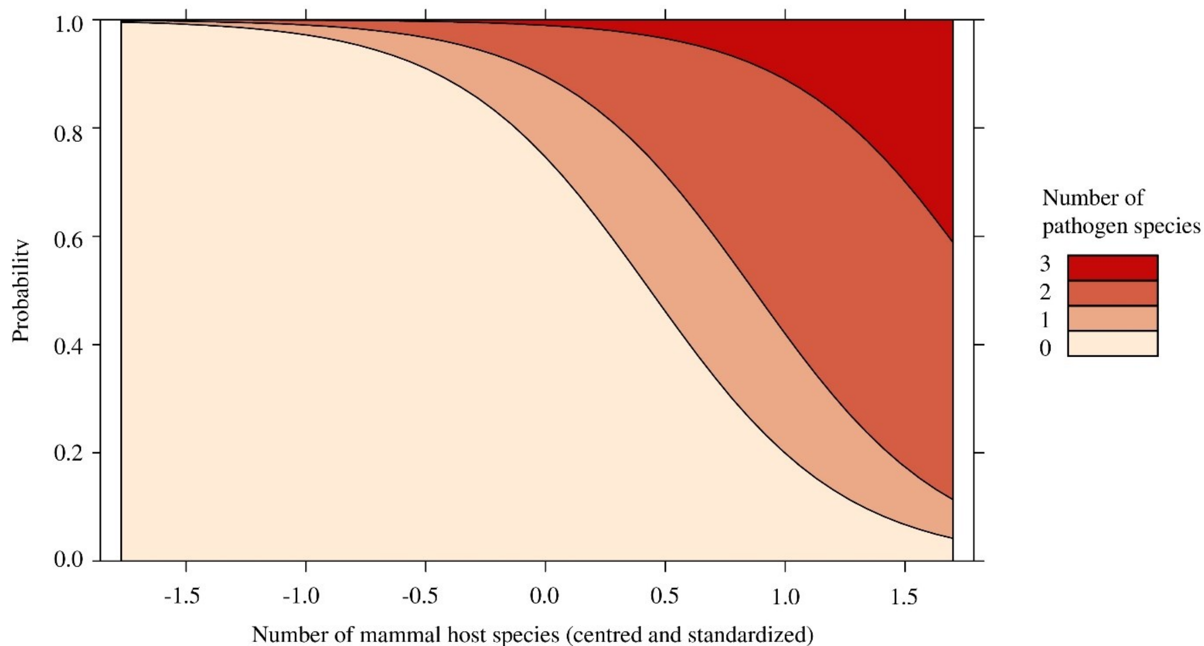

Figure 4.2. Stacked effect plot of the ordinal logistic regression predicting the number of pathogen species found in *Ixodes scapularis* and small mammals in Ontario and Quebec, Canada. The y-axis represents the probability that a certain number of pathogen species are present locally and the x-axis represents the centred and standardized values of mammal species richness, which ranged from 2 to 8 species. Local pathogen diversity is significantly predicted by the number of mammal host species found via small mammal trapping and trail cameras. As the number of mammal host species increased, the probability of presence and diversity of pathogens increased.

General discussion and concluding remarks

With changes in climate and land use, tick-borne pathogens are expected to increase in prevalence and become more widely distributed globally due to changes in the abundance and distribution of tick and host populations (Jones et al. 2008, Swei et al. 2020). In this thesis, I evaluated the relationships between tick vectors, hosts, and pathogens in Canada by determining the relative impacts of abiotic and biotic factors on each of these key players. In the first chapter, I explored the historical tick-host-pathogen associations, and identified spatial outliers and spatiotemporal clusters of high pathogen presence in ticks in Canada. In the three subsequent chapters, I evaluated the influence of abiotic and biotic factors on the presence, abundance, and diversity of tick populations and their pathogens. Each of my four chapters improves on the knowledge associated with the tick-host-pathogen disease systems in Quebec and Ontario and, more generally, in Canada. In addition, I identified important ways to standardize data reporting and to conduct more proactive surveillance efforts and comprehensive pathogen testing, which aim to improve risk assessment and distribution mapping of current and future disease vectors and their pathogens.

5.1. Identification of key tick-pathogen associations in Canada

Certain associations between tick vectors and pathogen species were identified at a higher prevalence both historically and in contemporary times (Chapter 1, 3, and 4). The most frequently detected tick-borne pathogen in historical studies and contemporary field surveys, including my own, was *Borrelia burgdorferi* sensu stricto (Guillot et al. 2020, Wilson et al. 2022, Crandall et al. 2022). This pathogen has been reported across a large geographic extent and it is predominantly associated with populations of *Ixodes scapularis* and *I. pacificus* (Guillot et al. 2020, Wilson et al. 2022). I also reported that *B. burgdorferi* s.s. was the pathogen species found at the highest prevalence during my field surveys in Ontario and Quebec, which aligns with the results obtained by recent surveillance efforts in these two provinces (Chapter 3 and 4; Guillot et al. 2020, Wilson et al. 2022, Crandall et al. 2022). However, previous studies have also reported lower incidences of *B. burgdorferi* s.s. and s.l. in *Dermacentor variabilis*, *Haemaphysalis leporispalustris*, and other *Ixodes* ticks (Chapter 1; Barker et al. 1992, Banerjee

et al. 1995, Morshed et al. 2005, Ogden et al. 2008, Scott et al. 2010, 2012, Scott and Durden 2015).

Significant associations were also identified for *I. scapularis* and *H. leporispalustris* with *Babesia odocoilei* and *Rickettsia rickettsii*, respectively, which may both be transmitted without the need of an infected host (Chapter 3). In Ontario and Quebec, *B. odocoilei* has recently been reported in *I. scapularis*, which includes my detections of this pathogen species in southeastern Quebec past its current range limits (Chapter 3 and 4; Milnes et al. 2019, Scott et al. 2021, Scott and Pesapane 2021, Crandall et al. 2022). The maintenance of *R. rickettsii* in nature is more difficult due to biological barriers such as partial transovarial transmission, but *H. leporispalustris* have been identified as important tick vectors for the replication of this pathogen (Parker et al. 1951). Of note, both of these tick-borne pathogens may be acquired through transovarial transmission from adult females to immature ticks, or transstadial transmission where the pathogen persists in the vector for multiple life stages after becoming infected from a host (Freitas et al. 2009, Zembsch et al. 2021). I provided evidence for the transovarial transmission of *R. rickettsii* in *H. leporispalustris*, as this pathogen species was detected in larvae in nature (Chapter 3). However, biotic barriers, such as partial pathogen transmission and variability in pathogen concentrations in hosts, may limit the ability of immature ticks to become infected in nature (Freitas et al. 2009, Paddock et al. 2014, Zembsch et al. 2021). As a result, it still remains unknown how quickly emerging or re-emerging tick-borne pathogens that use additional modes of transmission, such as transovarial transmission, will spread in Canada.

5.2. Key abiotic factors impacting questing *I. scapularis*

Greater amounts of precipitation and limited snow accumulations were associated with increased questing *I. scapularis* abundances, which are likely due to the maintenance of optimal microclimate conditions (Chapter 2). Sufficient precipitation and moisture are required to enable ticks to survive within the microclimate (Berger et al. 2014a, 2014b, Dumas et al. 2022b). If high humidity levels are sustained due to precipitation, then ticks may not be required to mitigate their desiccation through behavioural changes leading to increased activity times (Vail and Smith 2002, Burtis et al. 2016). Similarly, snow cover alone or in combination with leaf litter typically leads to increased overwintering survival and increased tick abundances in the subsequent summer (Hayes et al. 2015, Linske et al. 2019, Volk et al. 2022). Although the highest *I.*

scapularis abundances were in areas with the lowest snow accumulations and milder winter conditions, there did not appear to be an increased mortality risk due to inoculative freezing, which may indicate adequate humidity and thermal conditions in the microclimate (Eisen et al. 2016, Linske et al. 2019, Volk et al. 2022). In contrast, localities at northern range edge experienced large snow accumulations, where *I. scapularis* populations were absent or were present in low abundances the following summer. Therefore, it is possible that an additional metric other than accumulated snow, such as leaf litter presence and depth, may be beneficial to completely capture the microclimate winter conditions.

5.3. Importance of vertebrate hosts for tick vectors and tick-borne pathogens

The type of host may modulate the presence and prevalence of tick-borne pathogens in feeding ticks (Chapter 1). Using data extracted from the literature, I found that over 90% of feeding ticks in Canada were found parasitizing either a bird or a mammal rather than humans (7.7%) or hosts of unknown origin (0.3%) (Chapter 1). Generalist tick species may feed on a wide variety of vertebrate hosts, such as the ability of *I. scapularis* to feed on at least 125 different hosts including mammals, birds, and humans (Keirans et al. 1996, Lindquist et al. 2016). Furthermore, generalist tick vectors may be able to acquire a greater number of tick-borne pathogens, as a result of them feeding on diverse kinds of hosts (Cockwill et al. 2009, Scott et al. 2012, Smith et al. 2019, Lewis and Lloyd 2019, Stokes et al. 2020, Scott and Pesapane 2021). Tick vectors may also prefer to feed on specific host groups, such as the preference of *I. cookei* for mammal hosts (Lindquist et al. 2016). Although this tick vector may exhibit host preferences, it can still become infected with multiple tick-borne pathogens including Powassan virus, *B. burgdorferi* sensu lato, and *B. microti* (Chapter 1; McLean et al. 1964, Artsob et al. 1984, Scott et al. 2019). Specialist tick species may exclusively feed on certain hosts, such as *I. auritulus* feeding solely on birds. This tick vector was found to only be infected with *Borrelia* species including *B. americana*, and *B. burgdorferi*.

Mammal species richness may be an important predictor for tick populations (Chapter 2). Areas with more diverse mammal communities may have increased *I. scapularis* abundance, but only if host abundances increase with species richness, allowing for greater tick-host contact rates (Ogden and Tsao 2009, Luis et al. 2018). Here, I found that there was a significant positive correlation between small mammal abundance and mammal species richness, which may provide

additional feeding opportunities. Therefore, it may be that mid-size and large mammals, such as raccoons and white-tailed deer, are contributing to a greater degree to *I. scapularis* abundances, where they act as sites of tick reproduction and can successfully feed larger burdens of ticks (LoGiudice et al. 2003, Bouchard et al. 2013b, Werden et al. 2014). Moreover, the addition or loss of a species within the mammal community due to predation or interspecific competition may variably impact *I. scapularis* abundances, especially where tick populations have not yet established at the northward range edge (Levi et al. 2016).

Furthermore, mammal species richness also played a significant role in predicting local pathogen diversity (Chapter 3 and 4). Areas with more diverse mammal communities were associated with a greater number of pathogen species, with the highest pathogen diversity in regions with long-established tick populations and a higher *B. burgdorferi* prevalence. This relationship may be due to the fact that a wider variety of host species that are present may lead more diverse pathogen circulation. In addition, reproduction hosts, such as raccoons and white-tailed deer, may act as important facilitators for the long-range dispersal and establishment of ticks and tick-borne pathogens, as they expand their ranges poleward in response to climate and land use changes (Dawe and Boutin 2016, Diuk-Wasser et al. 2021). However, the relationship between mammal species richness and pathogen diversity varies to a greater extent in regions where *I. scapularis* or important reservoir hosts, such as white-footed mice (*Peromyscus leucopus*), have not yet established (Roy-Dufresne et al. 2013, Simon et al. 2014, Ripoche et al. 2022, Millien et al. 2023). Instead, other medically significant tick vectors, such as *I. cookei*, and reservoir hosts, such as chipmunks and shrews, may play a larger role for pathogen spread and transmission in these areas (Levi et al. 2016, Gasmi et al. 2018, Dumas et al. 2022a).

Surprisingly, small mammal abundance and the relative abundance of *P. leucopus* were not found to be important predictors of *I. scapularis* abundance (Chapter 2). Areas with long-established *I. scapularis* populations were associated with higher abundances of small mammal hosts. In southern Quebec, increased abundances of infected *I. scapularis* have also been associated with increased small mammal abundances and variable densities of *P. leucopus* across the same geographic extent as our study (Millien et al. 2023). In our study, avian hosts or larger mammals, such as white-tailed deer, may have played a larger role in the maintenance of *I. scapularis* populations across our sites, where increased densities of these hosts may result in increased *I. scapularis* abundances locally (LoGiudice et al. 2003, Bouchard et al. 2013b, Dumas

et al. 2022a). In addition, the density and distribution of small mammal hosts across our sites may have been impacted by food availability or environmental conditions, resulting in variable *I. scapularis* abundances due to limited tick-host contacts (Dobson 2014, Luis et al. 2018). This variability in tick-host interactions may be more prominent in areas where *I. scapularis* populations have not yet established, such as those populations located near the northward range edge (Dobson 2014). For example, sites that did not harbour any *P. leucopus* or *I. scapularis* were more likely to be associated with other tick vectors such as *H. leporispalustris* (Chapter 3) or reservoir hosts such as chipmunks and shrews (Chapter 3 and 4). Yet, populations of *P. leucopus* and *I. scapularis* are expected to become established in these areas in the near future with their northward range expansion (Roy-Dufresne et al. 2013, Simon et al. 2014, Clow et al. 2017, Ripoche et al. 2022).

Small mammals have been identified as key reservoir hosts for tick populations, as they that can more readily transmit tick-borne pathogens such as *B. burgdorferi* (Mather et al. 1989, LoGiudice et al. 2003, Brunner et al. 2008, Brisson et al. 2008). In Ontario and Quebec, the relative abundance of *P. leucopus* has previously been identified as a significant predictor of *B. burgdorferi* prevalence (Bouchard et al. 2013a, Werden et al. 2014, Dumas et al. 2022a). However, I did not find that the relative abundance of *P. leucopus* affected pathogen presence, prevalence, and diversity across my study sites (Chapter 4). It should be noted that the small mammal hosts that I found that were infected with distinct tick-borne pathogens included a northern short-tailed shrew and *Peromycus* mice (two white-footed mice and one deer mouse), which were identified to species using genetic testing (Chapter 3). This disparity between my results and those of previous studies may be due to additional unexplored biotic factors such as avian host communities or interannual fluctuations of small mammal populations. Furthermore, local ground foraging birds have been found to be significant contributors for greater tick-borne pathogen spread and transmission, which has been previously demonstrated for *B. burgdorferi* in southern Quebec (Dumas et al. 2022a).

5.4. The implications of type of surveillance method

Passive tick surveillance and pathogen testing may provide an early signal for establishing tick populations and emerging or re-emerging tick-borne pathogens across broad spatial scales (Ripoche et al. 2018). This surveillance method may help identify the first

detections of tick vectors and tick-borne pathogens in isolated or clustered geographic regions (Ogden et al. 2014, Ripoche et al. 2018, Gasmi et al. 2018, Chilton et al. 2019, Morshed et al. 2021). Public health agencies also use this form of surveillance to create a baseline for the introduction and establishment of tick populations and their pathogens, where this method generally requires less collection time and labour investments (Nelder et al. 2021, Morshed et al. 2021). Spatiotemporal clusters of pathogen presence in ticks associated with passive surveillance were detected much earlier starting in 1990s, which demonstrates the greater sensitivity of this method (Chapter 1). However, current passive tick surveillance programs have been limited or discontinued by certain public health agencies in Canada (Clow et al. 2019, Guillot et al. 2020, Wilson et al. 2022). More recently, community-science passive tick surveillance initiatives, such as eTick, have allowed the assessment of broader geographic regions and more detailed representations of animal hosts for tick populations, but not for tick-borne pathogens (Wilson et al. 2022). Community-science passive tick surveillance and pathogen testing programs, such as Geneticks and the Lloyd Tick Lab, allow members of the public to submit their ticks for comprehensive testing for a wide variety of pathogens. This information is then made publicly available online, which can be used to track the progression of multiple tick-borne pathogens from several tick vectors across Canada through time. These community-science initiatives are increasingly being used by public health agencies, as a way to supplement their limited passive tick surveillance programs.

In contrast, active surveillance through drag sampling or host trapping may be used to identify areas with high tick-borne disease risk based on the establishment of ticks, hosts, and pathogens (Teng et al. 2011, Yunik et al. 2015, Chilton et al. 2019, Guillot et al. 2020, Wilson et al. 2022). Active tick and host surveillance programs and pathogen testing are typically funded by research programs or public health agencies, allowing researchers to track the spatiotemporal progression of each key player of the tick-host-pathogen disease systems across varying scales in Canada (Clow et al. 2019, Guillot et al. 2020). Typically, the information collected from active surveillance efforts are used to create detailed risk maps of tick vectors and tick-borne disease risk (Guillot et al. 2022, Ripoche et al. 2022). Yet, the start of large-scale tick and pathogen surveillance efforts in Canada only started around 2005, as demonstrated by the spatiotemporal clusters of pathogen presence in ticks related to active surveillance (Chapter 1; Ogden et al. 2008, Scott et al. 2012). These clustered regions focused on populations of predominant tick

vectors (*I. scapularis* and *Dermacentor*), which were related to targeted research efforts by academics and public health agencies. However, proactive tick surveillance and comprehensive pathogen testing should be employed outside currently defined risk areas or sentinel sites to better anticipate the spread and establishment of tick vectors and tick-borne pathogens beyond their northward range limits. Consequently, active tick surveillance and comprehensive pathogen testing helped detect two emerging or re-emerging tick-borne pathogens, *B. odocoilei* and *R. rickettsii*, outside of their known range limits in southeastern Quebec (Chapter 3 and 4).

5.5. Thesis limitations

Certain limitations are present in each chapter of this thesis related to data collection and unexplored predictor variables.

Although my systematic review was comprehensive, it was limited by the nature and sparsity of the data reported in the literature (Chapter 1). A significant publication bias was present because of a greater number of studies assessing tick vectors and tick-borne pathogens in Canada through time. The nature of the studies varied considerably with marked differences between testing methods, as well as tick activity and life stages. As a result, certain tick-borne pathogens, such as *B. burgdorferi*, may have been more likely to be detected in ticks, especially across broad temporal scales (Gasmi et al. 2017). Many studies also did not specify the host species of feeding ticks, which required my generalization of hosts into host groups. More detailed analyses on the role of specific hosts in contributing to tick populations and their pathogens would have been possible had the host information been more transparently reported.

The major limitation related to my field surveys was the temporal study period (Chapter 2, 3, and 4). Due to the COVID-19 pandemic, I was unable to access my study sites for more than one field season, where field surveys were conducted in Ontario in July and in Quebec in August. As a result, the associations that were found as part of this thesis would require further studies to explore the interannual and intraseasonal dynamics of the tick-host-pathogen disease systems across my study sites in Ontario and Quebec.

Unexplored abiotic factors could be affecting our assessments of the predictors modulating tick populations and tick-borne pathogens (Chapter 2). I focused on high-resolution environmental data obtained from remote sensing satellites and meteorological towers, which were assumed to capture microclimate conditions based on my selected abiotic factors.

Microclimate refuges are used by ticks to maintain their optimal thermal and humidity conditions, thereby increasing their survival (Ginsberg et al. 2017, Linske et al. 2019, Volk et al. 2022). In southern Quebec, microclimatic conditions were demonstrated to influence immature tick densities, where adverse moisture events were particularly detrimental to larvae and nymphs (Dumas et al. 2022b). Furthermore, climatic extremes and variability may change through time due to interannual fluctuations (Ostfeld et al. 2006, Ginsberg et al. 2020). The use of high-resolution data loggers (e.g., iButtons) across my study sites may have been able to capture these fine-scale environmental determinants. In addition, forest fragmentation was not assessed as part of this thesis. Habitat fragmentation and forest patch connectivity have been shown to affect tick densities and subsequent tick-borne disease risk at varying scales (Diuk-Wasser et al. 2021). All study sites used during my field surveys were in contiguous forested areas (Chapter 2). However, the distance to urban centers and the degree of habitat fragmentation located around my study sites were not assessed.

Finally, local avian host communities were not explored as part of this thesis (Chapter 2, 3, and 4). Migratory birds are important hosts for the long-distance dispersal of ticks and tick-borne pathogens into new poleward locations in Canada (Scott et al. 2001, Ogden et al. 2008). Furthermore, several bird species, such as American robins and song sparrows, have been identified as reservoir hosts for *B. burgdorferi* (LoGiudice et al. 2003, Ginsberg et al. 2005). Songbirds in Quebec have also been found to harbour *A. phagocytophilum*, *Babesia* spp., and *B. burgdorferi* (Dumas et al. 2022a, Scott et al. 2022). Although the reservoir competence of avian hosts is generally lower than that of key small mammals, they still may contribute significantly to local pathogen spread and transmission (LoGiudice et al. 2003, Dumas et al. 2022a).

5.6. Future research and concluding remarks

Simple measures should be employed to facilitate studying the spread and establishment of ticks, hosts, and pathogens at varying scales. Reporting spatial coordinates, collection years, and total and infected tick abundances would provide standardized data that could be used to assess the tick-host-pathogen disease systems across broad spatial and temporal scales. In addition, the consistent record of specific host species acting as blood meals for tick vectors and transmitting tick-borne pathogens at local scales may provide precise information regarding their reservoir competence (Mather et al. 1989). These measures aim to provide greater clarity,

transparency, and standardization in the reported data, as this variability constitutes an additional avoidable obstacle that may be limiting our knowledge of tick-host-pathogen disease systems.

Future studies should also incorporate host active surveillance data with high-resolution environmental data in risk assessments and mapping distributions of disease vectors and pathogens. The inclusion of host active surveillance data may provide a more comprehensive examination of the relationships and dynamics of tick-host-pathogen disease systems. Public health agencies may then be better informed as to which human populations are exposed to greater tick-borne disease risk currently or in the future (Kotchi et al. 2019). In addition, these future analyses should be conducted over several consecutive years to capture the interannual variability in climate conditions as well as the abundances and distributions of tick and host populations, which subsequently affect tick-borne disease risk.

In the future, proactive tick and host surveillance efforts as well as comprehensive pathogen testing in Canada should focus on four key aspects (Crandall et al. 2022). First, active tick and host surveillance programs should be conducted in regions outside of currently defined risk areas and sentinel sites to better determine the northward geographic extent of tick populations. Second, comprehensive pathogen testing should be performed on all tick life stages and species in addition to small mammal hosts to detect the geographic extent and co-occurrence of pathogens. In addition, testing questing larval ticks for tick-borne pathogens with known transovarial transmission, such as *B. odocoilei* and *R. rickettsii*, may help determine hotspot regions for these pathogens outside currently defined risk areas. Third, concurrent testing of multiple tick-borne pathogens that may not typically be targeted may help detect areas with an increased risk of co-infections in human populations due greater pathogen co-occurrence (Cutler et al. 2021). Areas with greater tick abundances and host communities with a greater number of distinct host species present should be targeted first, as they are more likely to have a greater number of distinct tick-borne pathogens. Finally, the concurrent use of active and passive tick surveillance should be used to provide the most detailed and comprehensive representation of the geographic extent of tick vectors and their pathogens, which may not be captured when using each surveillance strategy alone (Lyons et al. 2021).

With climate warming and land use changes, emerging vector-borne pathogens, such as those transmitted by ticks and mosquitos, are advancing poleward into new locations, with vector-borne disease risk likely rising in the future for humans, domestic animals, or wildlife in

Canada (Ogden and Lindsay 2016, Swei et al. 2020, Diuk-Wasser et al. 2021). This thesis provides actionable suggestions that can be employed by researchers and public health agencies to better track vector-borne zoonotic diseases in Canada and, more specifically, in Ontario and Quebec. The use of systematic and standardized data reporting, concurrent active and passive tick surveillance, and comprehensive pathogen testing can allow thorough risk assessments and distribution mapping of vector populations and their pathogens. In Canada, we should continue to strive for broader surveillance efforts that are proactive rather than reactive to limit the number of human lives affected by vector-borne zoonotic diseases, such as Lyme disease and West Nile Virus. While the findings of this thesis focus on the tick-borne disease systems in Canada, continued research efforts are critical to further disentangle the complex interactions and impacts of climatic conditions and host communities affecting vector populations and vector-borne disease risk in Canada and elsewhere.

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Werden, L., I. Barker, J. Bowman, E. Gonzales, P. Leighton, L. Lindsay, and C. Jardine. 2014. Geography, deer, and host biodiversity shape the pattern of Lyme disease emergence in the Thousand Islands archipelago of Ontario, Canada. PLoS One 9.

Wilson, C., S. Gasmi, A.-C. Bourgeois, J. Badcock, N. Chahil, M. Kulkarni, M.-K. Lee, R. Lindsay, P. Leighton, M. Morshed, C. Smolarchuk, and J. Koffi. 2022. Surveillance for *Ixodes scapularis* and *Ixodes pacificus* ticks and their associated pathogens in Canada, 2019. Canada Communicable Disease Report 48:208–218.

Yunik, M. E. M., T. D. Galloway, and L. R. Lindsay. 2015. Assessment of prevalence and distribution of spotted fever group rickettsiae in Manitoba, Canada, in the American Dog

Tick, *Dermacentor variabilis* (Acari: Ixodidae). Vector-Borne and Zoonotic Diseases 15:103–108.

Appendices

Appendix A: Supplementary information for Chapter 1

Appendix B: Supplementary information for Chapter 2

Appendix C: Supplementary information for Chapter 3

Appendix D: Supplementary information for Chapter 4

Appendix E: *Environmental factors limiting the overwintering survival of Ixodes scapularis nymphs in Quebec, Canada* (field-based translocation experiment)

Appendix A

Supplementary information for Chapter 1

Historical associations and spatiotemporal changes of pathogen presence in ticks in Canada: A systematic review

Content

3 tables

9 figures

Table A1. The detailed list of references used in Table 1.1. to identify the current and historical pathogens that have been detected in Canada.

Ref. No.	Reference	Pathogen
1	Lindquist, E., T. Galloway, H. Artsob, R. Lindsay, M. Drebot, H. Wood, and R. Robbins. 2016. A Handbook to the Ticks (Ixodida: Ixodidae, Argasidae) of Canada. 1st edition. Biological Survey of Canada.	*Anaplasma marginale*, *A. phagocytophilum*, *Babesia microti*, *Borrelia burgdorferi*, *Coxiella burnetii*, *Ehrlichia* spp., *Francisella tularensis*, Powassan virus, *Rickettsia rickettsii*
2	Howden, K. J., D. W. Geale, J. Paré, E. J. Golsteyn-Thomas, and A. A. Gajadhar. 2010. An update on bovine anaplasmosis (*Anaplasma marginale*) in Canada. The Canadian Veterinary Journal 51:837–840.	*Anaplasma marginale*
3	Dergousoff, S. J., and N. B. Chilton. 2011. Novel genotypes of *Anaplasma bovis*, "*Candidatus* Midichloria" sp. and *Ignatzschineria* sp. in the Rocky Mountain wood tick, *Dermacentor andersoni*. Veterinary Microbiology 150:100–106.	*Anaplasma bovis*
4	Chilton, N. B., S. J. Dergousoff, and T. J. Lysyk. 2018. Prevalence of *Anaplasma bovis* in Canadian populations of the Rocky Mountain wood tick, *Dermacentor andersoni*. Ticks and Tick-borne Diseases 9:1528–1531.	*Anaplasma bovis*
5	Lester, S. J., E. B. Breitschwerdt, C. D. Collis, and B. C. Hegarty. 2005. *Anaplasma phagocytophilum* infection (granulocytic anaplasmosis) in a dog from Vancouver Island. The Canadian Veterinary Journal 46:825–827.	*Anaplasma phagocytophilum*
6	Ogden, N., L. Lindsay, K. Hanincova, I. Barker, M. Bigras-Poulin, D. Charron, A. Heagy, C. Francis, C. O'Callaghan, I. Schwartz, and R. Thompson. 2008. Role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. Applied and Environmental Microbiology 74:1780–1790.	*Anaplasma phagocytophilum*

7	Cockwill, K. R., S. M. Taylor, E. C. R. Snead, R. Dickinson, K. Cosford, S. Malek, L. R. Lindsay, and P. P. V. de Paiva Diniz. 2009. Granulocytic anaplasmosis in three dogs from Saskatoon, Saskatchewan. The Canadian Veterinary Journal 50:835–840.	*Anaplasma phagocytophilum*
8	Burgess, H., N. B. Chilton, C. N. Krakowetz, C. Williams, and K. Lohmann. 2012. Granulocytic anaplasmosis in a horse from Saskatchewan. The Canadian Veterinary Journal 53:886–888.	*Anaplasma phagocytophilum*
9	Jacob, A. E., J. S. Weese, J. Rosseau, and K. M. Clow. 2022. Spatial patterns of *Borrelia burgdorferi*, *Borrelia miyamotoi* and *Anaplasma phagocytophilum* detected in *Ixodes* spp. ticks from Canadian companion animals, 2019–2020. Zoonoses and Public Health 69:944–955.	*Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *B. miyamotoi*
10	Wilson, C., S. Gasmi, A.-C. Bourgeois, J. Badcock, N. Chahil, M. Kulkarni, M.-K. Lee, R. Lindsay, P. Leighton, M. Morshed, C. Smolarchuk, and J. Koffi. 2022. Surveillance for *Ixodes scapularis* and *Ixodes pacificus* ticks and their associated pathogens in Canada, 2019. Canada Communicable Disease Report 48:208–218.	*Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia burgdorferi*, *B. miyamotoi*
11	Scott, J. 2017. First record of locally acquired human babesiosis in Canada caused by *Babesia duncani*: A case report. SAGE Open Medical Case Reports 5.	*Babesia duncani*
12	Scott, J., and C. Scott. 2018. Human babesiosis caused by *Babesia duncani* has widespread distribution across Canada. Healthcare 6.	*Babesia duncani*
13	Hersh, M. H., M. Tibbetts, M. Strauss, R. S. Ostfeld, and F. Keesing. 2012. Reservoir competence of wildlife host species for *Babesia microti*. Emerging Infectious Diseases 18:1951–1957.	*Babesia microti*
14	Bullard, J., A. Ahsanuddin, A. Perry, L. Lindsay, M. Iranpour, A. Dibernardo, and P. Van Caeseele. 2014. The first case of locally acquired tick-borne *Babesia microti* infection in Canada. Canadian Journal of Infectious Disease and Medical Microbiology 25:E87–E89.	*Babesia microti*
15	Dibernardo, A., T. Cote, N. Ogden, and L. Lindsay. 2014. The prevalence of *Borrelia miyamotoi* infection, and co-infections with other *Borrelia* spp. in *Ixodes scapularis* ticks collected in Canada. Parasites & Vectors 7.	*Babesia microti*, *Borrelia miyamotoi*
16	O'Brien, S. F., G. Delage, V. Scalia, R. Lindsay, F. Bernier, S. Dubuc, M. Germain, G. Pilot, Q.-L. Yi, and M. A. Fearon. 2016. Seroprevalence of *Babesia microti* infection in Canadian blood donors. Transfusion 56:237–243.	*Babesia microti*
17	Pattullo, K. M., G. Wobeser, B. P. Lockerbie, and H. J. Burgess. 2013. *Babesia odocoilei* infection in a Saskatchewan elk (*Cervus elaphus canadensis*) herd. Journal of Veterinary Diagnostic Investigation 25:535–540.	*Babesia odocoilei*

18	Mathieu, A., A. R. Pastor, C. N. Berkvens, C. Gara-Boivin, M. Hébert, A. N. Léveillé, J. R. Barta, and D. A. Smith. 2018. *Babesia odocoilei* as a cause of mortality in captive cervids in Canada. The Canadian Veterinary Journal 59:52–58.	*Babesia odocoilei*
19	Milnes, E. L., G. Thornton, A. N. Léveillé, P. Delnatte, J. R. Barta, D. A. Smith, and N. Nemeth. 2019. *Babesia odocoilei* and zoonotic pathogens identified from *Ixodes scapularis* ticks in southern Ontario, Canada. Ticks and Tick-borne Diseases 10:670–676.	*Babesia odocoilei*
20	Scott, J., K. Clark, and L. Durden. 2019. Presence of *Babesia odocoilei* and *Borrelia burgdorferi* sensu stricto in a tick and dual parasitism of *Amblyomma inornatum* and *Ixodes scapularis* on a bird in Canada. Healthcare 7:46.	*Babesia odocoilei*
21	Scott, J., E. Pascoe, M. Sajid, and J. Foley. 2021. Detection of *Babesia odocoilei* in *Ixodes scapularis* ticks collected in Southern Ontario, Canada. Pathogens 10:327.	*Babesia odocoilei*
22	Scott, J. D., and R. R. Pesapane. 2021. Detection of *Anaplasma phagocytophilum*, *Babesia odocoilei*, *Babesia* sp., *Borrelia burgdorferi* sensu lato, and *Hepatozoon canis* in *Ixodes scapularis* ticks collected in Eastern Canada. Pathogens 10:1265.	*Babesia odocoilei*
23	Crandall, K. E., J. T. Kerr, and V. Millien. 2022. Emerging tick-borne pathogens in Central Canada: Recent detections of *Babesia odocoilei* and *Rickettsia rickettsii*. Vector-Borne and Zoonotic Diseases 22:535–544.	*Babesia odocoilei*, *Rickettsia rickettsii*
24	Scott, J. D., E. McGoey, A. Morales, and R. R. Pesapane. 2022. Molecular detection of *Anaplasma phagocytophilum*, *Babesia odocoilei*, *Babesia* species, and *Borrelia burgdorferi* sensu lato in songbirds. Journal of Biomedical Research & Environmental Sciences 3:1451–1459.	*Babesia odocoilei*
25	Leighton, F. A., H. A. Artsob, M. C. Chu, and J. G. Olson. 2001. A serological survey of rural dogs and cats on the southwestern Canadian prairie for zoonotic pathogens. Canadian Journal of Public Health 92:67–71.	*Bartonella* spp., *Francisella tularensis*, *Rickettsia rickettsii*
26	Jardine, C., G. Appleyard, M. Y. Kosoy, D. McColl, M. Chirino-Trejo, G. Wobeser, and F. A. Leighton. 2005. Rodent-associated *Bartonella* in Saskatchewan, Canada. Vector-Borne and Zoonotic Diseases 5:402–409.	*Bartonella* spp.
27	Gary, A. T., J. A. Webb, B. C. Hegarty, and E. B. Breitschwerdt. 2006. The low seroprevalence of tick-transmitted agents of disease in dogs from southern Ontario and Quebec. The Canadian Veterinary Journal 47:1194–1200.	*Bartonella* spp., *Ehrlichia* spp.
28	André, A., A. Mouton, V. Millien, and J. Michaux. 2017. Liver microbiome of *Peromyscus leucopus*, a key reservoir host species for emerging infectious diseases in North America. Infection, Genetics and Evolution 52:10–18.	*Bartonella* spp.

29	Breitschwerdt, E. B., R. G. Maggi, C. Quach, and J. M. Bradley. 2019. *Bartonella* spp. bloodstream infection in a Canadian Family. Vector-Borne and Zoonotic Diseases 19:234–241.	*Bartonella* spp.
30	Kho, J., C. Colbourne, E. Bent, A. E. Nabbout, and T. Rossolimo. 2021. Coinfection of *Bartonella* spp. and *Borrelia burgdorferi* in *Ixodes scapularis* using PCR assay, a case study in Nova Scotia. International Journal of Biology 13:57.	*Bartonella* spp.
31	Boodman, C., T. Wuerz, P. Lagacé-Wiens, R. Lindsay, A. Dibernardo, J. Bullard, D. R. Stein, and Y. Keynan. 2022. Serologic testing for *Bartonella* in Manitoba, Canada, 2010–2020: A retrospective case series. CMAJ Open 10:E476–E482.	*Bartonella* spp.
32	Barker, I. K., G. A. Surgeoner, H. Artsob, S. A. McEwen, L. A. Elliott, G. D. Campbell, and J. T. Robinson. 1992. Distribution of the Lyme disease vector, *Ixodes dammini* (Acari: Ixodidae) and isolation of *Borrelia burgdorferi* in Ontario, Canada. Journal of Medical Entomology 29:1011–1022.	*Borrelia burgdorferi*
33	Artsob, H., M. Garvie, R. J. Cawthorn, B. Horney, R. Maloney, D. Dick, and S. McBurney. 1992. Isolation of the Lyme disease spirochete, *Borrelia burgdorferi*, from *Ixodes dammini* (Acari: Ixodidae) collected on Prince Edward Island, Canada. Journal of Medical Entomology 29:1063–1066.	*Borrelia burgdorferi*
34	Scott, J. D., J. F. Anderson, and L. A. Durden. 2012. Widespread dispersal of *Borrelia burgdorferi*-infected ticks collected from songbirds across Canada. Journal of Parasitology 98:49–59.	*Borrelia burgdorferi*
35	Chilton, N. B., P. S. Curry, L. R. Lindsay, K. Rochon, T. J. Lysyk, and S. J. Dergousoff. 2019. Passive and active surveillance for *Ixodes scapularis* (Acari: Ixodidae) in Saskatchewan, Canada. Journal of Medical Entomology 57:156–163.	*Borrelia burgdorferi*
36	Bouchard, C., A. Dibernardo, J. Koffi, H. Wood, P. Leighton, and L. Lindsay. 2019. Increased risk of tick-borne diseases with climate and environmental changes. Canada Communicable Disease Report 45:83–89.	*Borrelia miyamotoi*
37	Dumas, A., C. Bouchard, A. Dibernardo, P. Drapeau, L. R. Lindsay, N. H. Ogden, and P. A. Leighton. 2022. Transmission patterns of tick-borne pathogens among birds and rodents in a forested park in southeastern Canada. PLoS One 17:e0266527.	*Borrelia miyamotoi*
38	Zinck, C. B., and V. K. Lloyd. 2022. *Borrelia burgdorferi* and *Borrelia miyamotoi* in Atlantic Canadian wildlife. PLoS One 17:e0262229.	*Borrelia miyamotoi*
39	Pavilanis, V., P. Lepine, and N. Morisset. 1952. Q fever complement fixing antibodies. Canadian Medical Association Journal 66:333–334.	*Coxiella burnetii*

40	Lang, G. H. 1989. Q fever: An emerging public health concern in Canada. Canadian Journal of Veterinary Research 53:1–6.	*Coxiella burnetii*
41	Hatchette, T. F., R. C. Hudson, W. F. Schlech, N. A. Campbell, J. E. Hatchette, S. Ratnam, D. Raoult, C. Donovan, and T. J. Marrie. 2001. Goat-associated Q fever: A new disease in Newfoundland. Emerging Infectious Diseases 7:413–419.	*Coxiella burnetii*
42	Marrie, T. J., N. Campbell, S. A. McNeil, D. Webster, and T. F. Hatchette. 2008. Q fever update, Maritime Canada. Emerging Infectious Diseases 14:67–69.	*Coxiella burnetii*
43	Angelakis, E., and D. Raoult. 2010. Q fever. Veterinary Microbiology 140:297–309.	*Coxiella burnetii*
44	Celina, S. S., and J. Cerný. 2022. *Coxiella burnetii* in ticks, livestock, pets and wildlife: A mini-review. Frontiers in Veterinary Science 9:1068129.	*Coxiella burnetii*
45	Berrington, A., R. Moats, and S. Lester. 1996. A case of *Ehrlichia equi* in an adult horse in British Columbia. The Canadian Veterinary Journal 37:174–175.	*Ehrlichia* spp.
46	Villeneuve, A., J. Goring, L. Marcotte, and S. Overvelde. 2011. Seroprevalence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Dirofilaria immitis* among dogs in Canada. The Canadian Veterinary Journal 52:527–530.	*Ehrlichia* spp.
47	Lobanov, V. A., A. A. Gajadhar, B. Al-Adhami, and H. M. Schwantje. 2012. Molecular study of free-ranging mule deer and white-tailed deer from British Columbia, Canada, for evidence of *Anaplasma* spp. and *Ehrlichia* spp. Transboundary and Emerging Diseases 59:233–243.	*Ehrlichia* spp.
48	Evason, M., J. Stull, D. Pearl, A. Peregrine, C. Jardine, J. Buch, Z. Lailer, T. O'Connor, R. Chandrashekar, and J. Weese. 2019. Prevalence of *Borrelia burgdorferi*, *Anaplasma* spp., *Ehrlichia* spp. and *Dirofilaria immitis* in Canadian dogs, 2008 to 2015: a repeat cross-sectional study. Parasites & Vectors 12.	*Ehrlichia* spp.
49	Bow, M. R., and J. H. Brown. 1943. Tularaemia in the "Seven Persons Coulee," Alberta. Canadian Journal of Public Health 34:5.	*Francisella tularensis*
50	Humphreys, F. A., and A. G. Campbell. 1947. Plague, Rocky Mountain spotted fever, and tularaemia surveys in Canada. Canadian Journal of Public Health 38:124–130.	*Francisella tularensis*, *Rickettsia rickettsii*
51	Banfield, A. W. F. 1954. Tularemia in beavers and muskrats, Waterton Lakes National Park, Alberta, 1952-53. Canadian Journal of Zoology 32:139–143.	*Francisella tularensis*
52	Ditchfield, J., and R. J. Julian. 1960. Tularemia of muskrats in Eastern Ontario. Canadian Journal of Public Health 51:474–478.	*Francisella tularensis*

53	Gordon, J. R., B. G. McLaughlin, and S. Nitiuthai. 1983. Tularaemia transmitted by ticks (*Dermacentor andersoni*) in Saskatchewan. Canadian Journal of Comparative Medicine 47:408–411.	*Francisella tularensis*
54	Artsob, H., L. Spence, G. Surgeoner, J. McCreadie, J. Thorsen, C. Th'ng, and V. Lampotang. 1984. Isolation of *Francisella tularensis* and Powassan virus from ticks (Acari: Ixodidae) in Ontario, Canada. Journal of Medical Entomology 21:165–168.	*Francisella tularensis*
55	Zarnke, R. L., J. M. Ver Hoef, and R. A. DeLong. 2004. Serologic survey for selected disease agents in wolves (*Canis lupus*) from Alaska and the Yukon territory, 1984–2000. Journal of Wildlife Diseases 40:632–638.	*Francisella tularensis*
56	Wobeser, G., G. D. Campbell, A. Dallaire, and S. McBurney. 2009. Tularemia, plague, yersiniosis, and Tyzzer's disease in wild rodents and lagomorphs in Canada: A review. The Canadian Veterinary Journal 50:1251–1256.	*Francisella tularensis*
57	Gabriele-Rivet, V., N. Ogden, A. Massé, K. Antonation, C. Corbett, A. Dibernardo, L. R. Lindsay, P. A. Leighton, and J. Arsenault. 2016. Eco-epizootiologic study of *Francisella tularensis*, the agent of tularemia, in Quebec wildlife. Journal of Wildlife Diseases 52:217.	*Francisella tularensis*
58	Buhler, K., É. Bouchard, S. Elmore, G. Samelius, J. Jackson, M. Tomaselli, H. Fenton, R. Alisauskas, and E. Jenkins. 2022. Tularemia above the treeline: Climate and rodent abundance influences exposure of a sentinel species, the Arctic fox (*Vulpes lagopus*), to *Francisella tularensis*. Pathogens 12:28.	*Francisella tularensis*
59	McLean, D. M., and W. L. Donohue. 1959. Powassan virus: Isolation of virus from a fatal case of encephalitis. Canadian Medical Association Journal 80:708–711.	Powassan virus
60	McLean, D. M., L. W. MacPherson, S. J. Walker, and G. Funk. 1960. Powassan virus: Surveys of human and animal sera. American Journal of Public Health and the Nations Health 50:1539–1544.	Powassan virus
61	McLean, D. M., M. A. Crawford, T. R. Ladyman, R. R. Peers, and K. W. Purvtn-Good. 1970. California encephalitis and Powassan virus activity in British Columbia, 1969. American Journal of Epidemiology:7.	Powassan virus
62	Kettyls, G. D., V. M. Verrall, L. D. Wilton, J. B. Clapp, D. A. Clarke, and J. D. Rublee. 1972. Arbovirus infections in man in British Columbia. Canadian Medical Association Journal 106:1175–1179.	Powassan virus
63	Fitch, W. M., and H. Artsob. 1990. Powassan encephalitis in New Brunswick. Canadian Family Physician 36:1289–1290.	Powassan virus

64	Smith, K., P. Oesterle, C. Jardine, A. Dibernardo, C. Huynh, R. Lindsay, D. Pearl, A. Bosco-Lauth, and N. Nemeth. 2018. Powassan virus and other arthropod-borne viruses in wildlife and ticks in Ontario, Canada. American Journal of Tropical Medicine and Hygiene 99:458–465.	Powassan virus
65	Bogaty, C., and M. Drebot. 2018. Powassan virus — an emerging public health concern. Canadian Medical Association Journal 190:E472–E472.	Powassan virus
66	Gholam, B. I. A., S. Puksa, and J. P. Provias. 1999. Powassan encephalitis: a case report with neuropathology and literature review. Canadian Medical Association Journal 161:1419-1422.	Powassan virus
67	Corrin, T., J. Greig, S. Harding, I. Young, M. Mascarenhas, and L. A. Waddell. 2018. Powassan virus, a scoping review of the global evidence. Zoonoses and Public Health 65:595-624.	Powassan virus
68	Bow, M. R., and J. H. Brown. 1952. Rocky Mountain spotted fever in Alberta, 1935-1950. Canadian Journal of Public Health 43:109–115.	*Rickettsia rickettsii*
69	McKiel, J. A. 1960. The rodent- and avian-borne diseases in Canada. Canadian Journal of Public Health 51:220–225.	*Rickettsia rickettsii*
70	Wood, H., and H. Artsob. 2012. Spotted fever group rickettsiae: A brief review and a Canadian perspective. Zoonoses and Public Health 59:65–79.	*Rickettsia rickettsii*
71	Nelder, M., C. Russell, S. Johnson, Y. Li, K. Cronin, B. Warshawsky, N. Brandon, and S. Patel. 2020. Assessing human exposure to spotted fever and typhus group rickettsiae in Ontario, Canada (2013-2018): A retrospective, cross-sectional study. BMC Infectious Diseases 20.	*Rickettsia rickettsii*

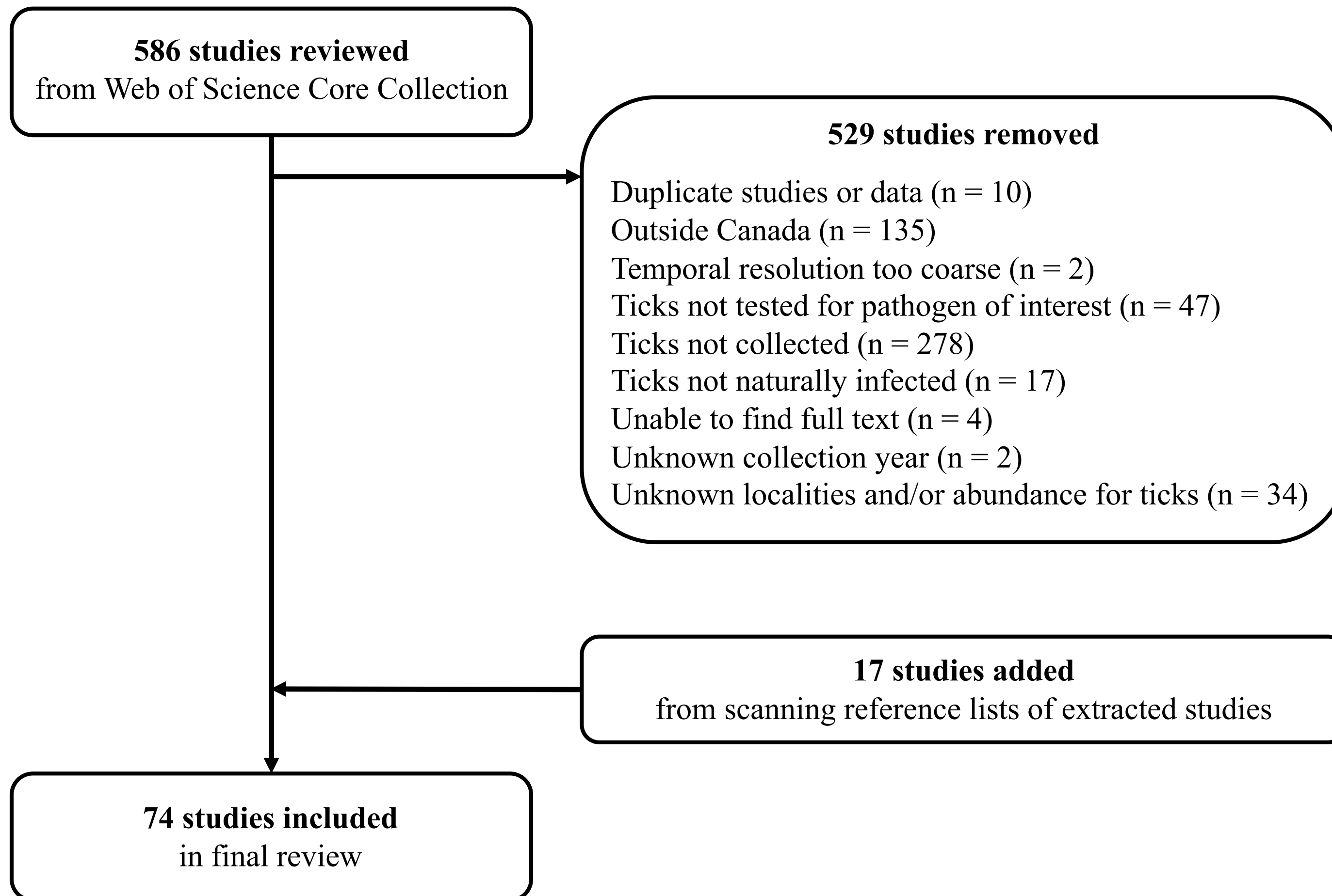

Figure A1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flowchart used with the primary search strategy and the selection of eligible studies.

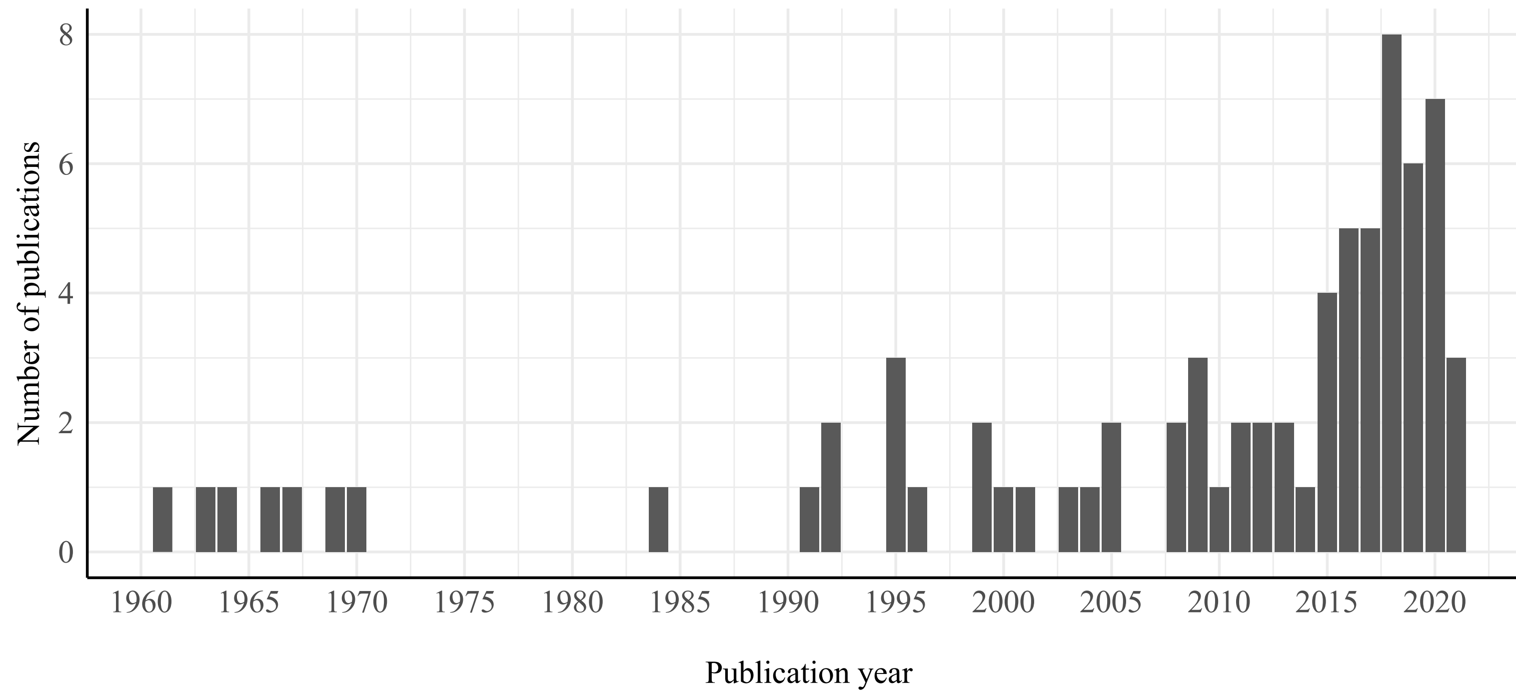

Figure A2. The number of published studies used in our review significantly increased over time.

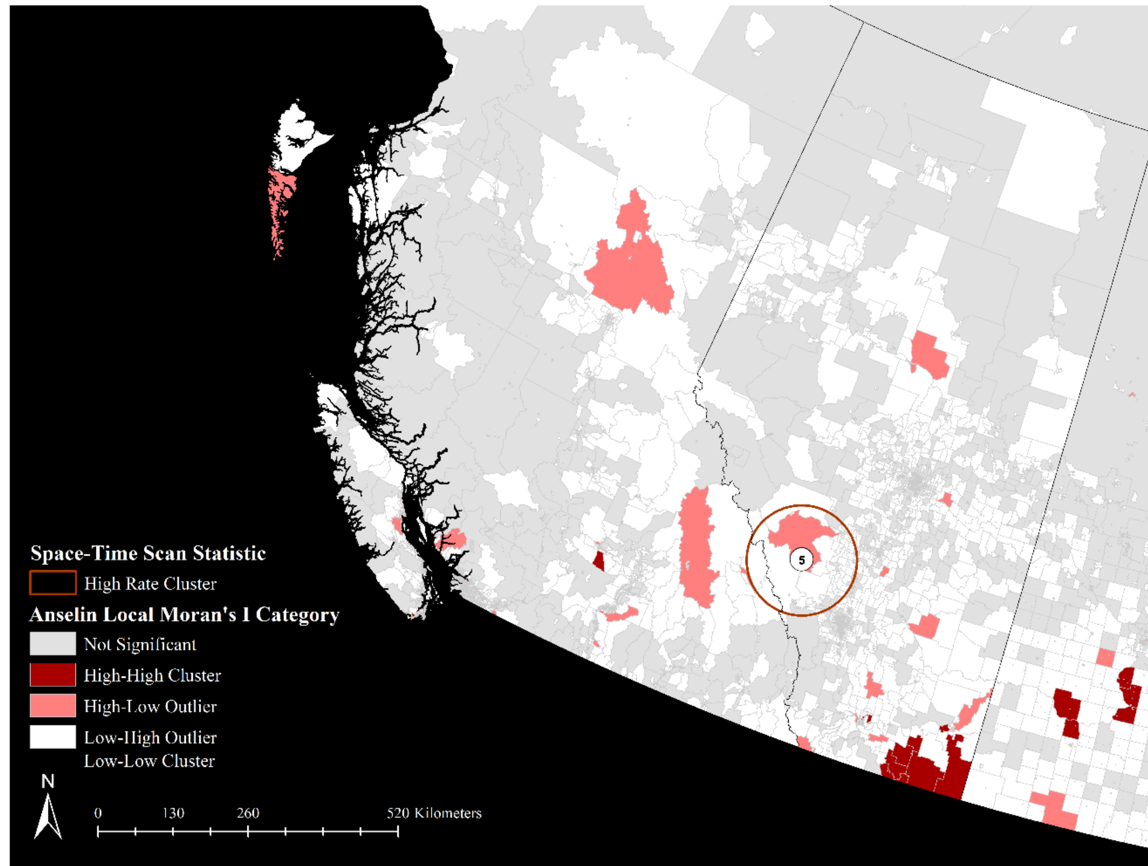

Figure A3. Historical spatiotemporal clusters of high pathogen presence in ticks in Western Canada. High pathogen presence based on Anselin Local Moran's I statistics in dissemination areas across Western Canada are represented as spatial clusters (red) and outliers (salmon). Using SaTScan^{TM}, a spatiotemporal cluster with a high rate of pathogen presence in ticks was also detected in southern Alberta from 2005 to 2019 ($p < 0.05$ for cluster 5).

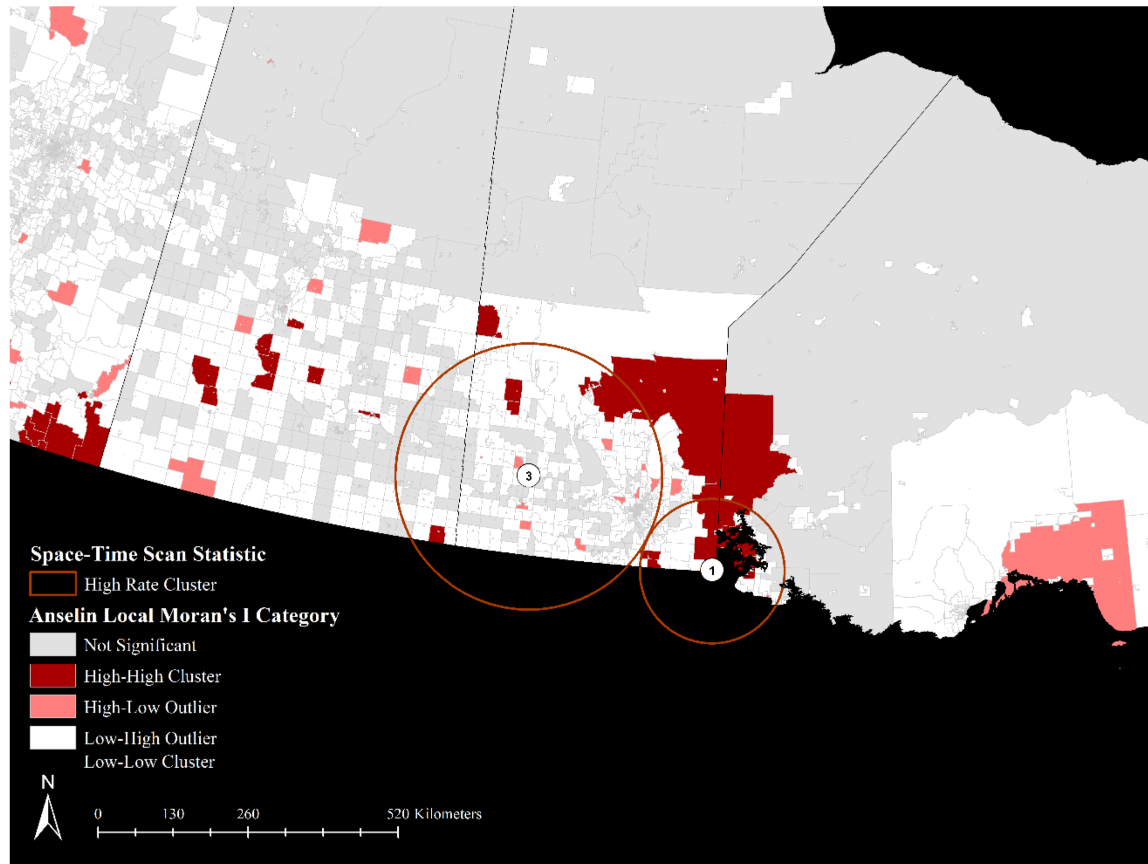

Figure A4. Historical spatiotemporal clusters of high pathogen presence in ticks in southern Saskatchewan and Manitoba in addition to northwestern Ontario. High pathogen presence based on Anselin Local Moran's I statistics in dissemination areas in Manitoba and northwestern Ontario are represented as spatial clusters (red) and outliers (salmon). Using SaTScan^{TM}, spatiotemporal clusters with high rates of pathogen presence in ticks were also detected in southern Manitoba and northwestern Ontario from 2005 to 2019 ($p < 0.01$ for cluster 3 and $p < 0.001$ for cluster 1).

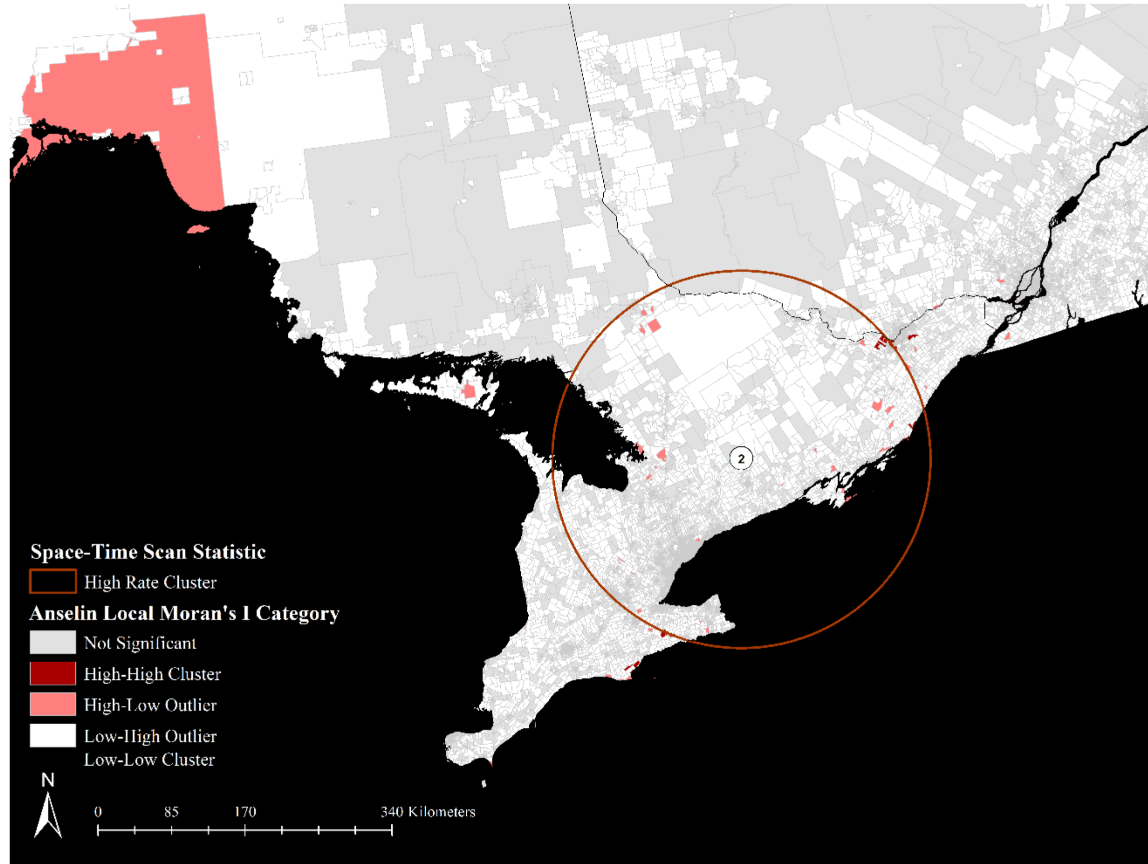

Figure A5. Historical spatiotemporal clusters of high pathogen presence in ticks in southeastern Ontario. High pathogen presence based on Anselin Local Moran's I statistics in dissemination areas in southeastern Ontario are represented as spatial clusters (red) and outliers (salmon). Using SaTScan™, a spatiotemporal cluster with a high rate of pathogen presence in ticks was also detected in southeastern Ontario from 2010 to 2019 ($p < 0.001$ for cluster 2).

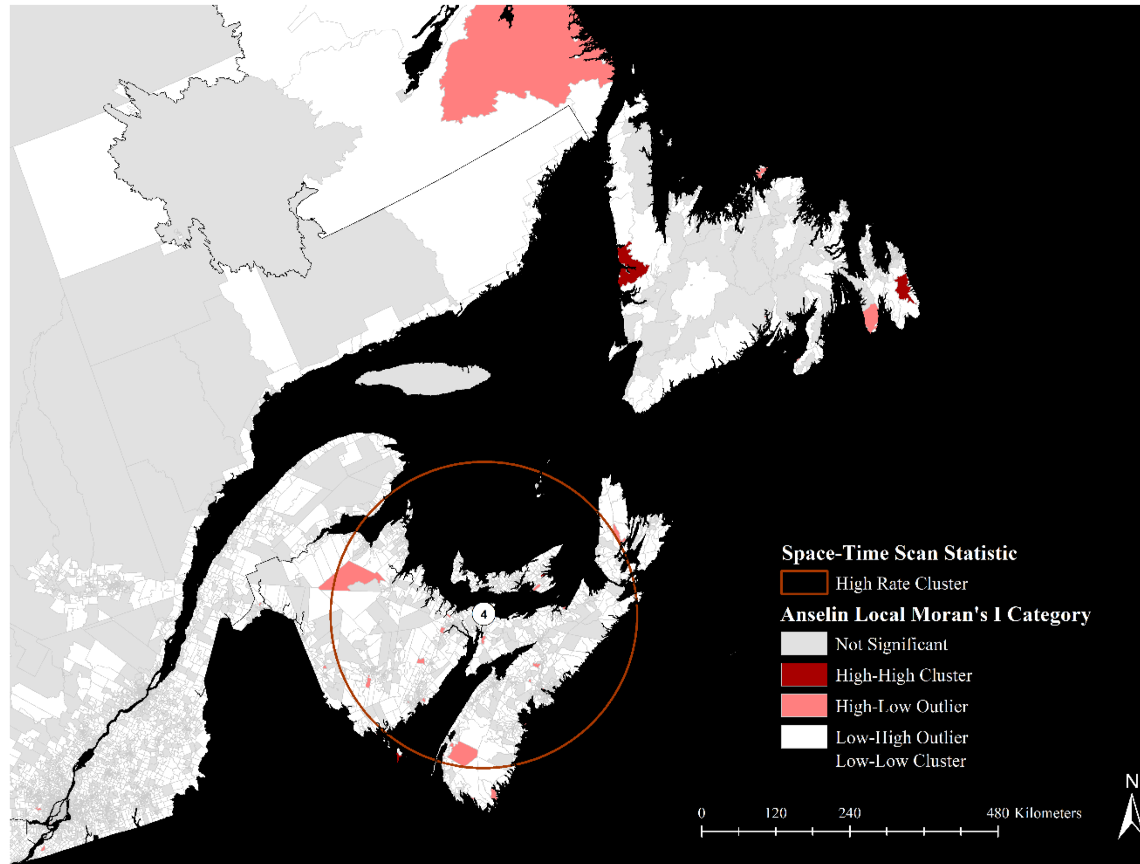

Figure A6. Historical spatiotemporal clusters of high pathogen presence in ticks in parts of Atlantic Canada. High pathogen presence based on Anselin Local Moran's I statistics in dissemination areas in Atlantic Canada are represented as spatial clusters (red) and outliers (salmon). Using SaTScan™, a spatiotemporal cluster with a high rate of pathogen presence in ticks was also detected in parts of Atlantic Canada from 2015 to 2019 ($p < 0.05$ for cluster 4).

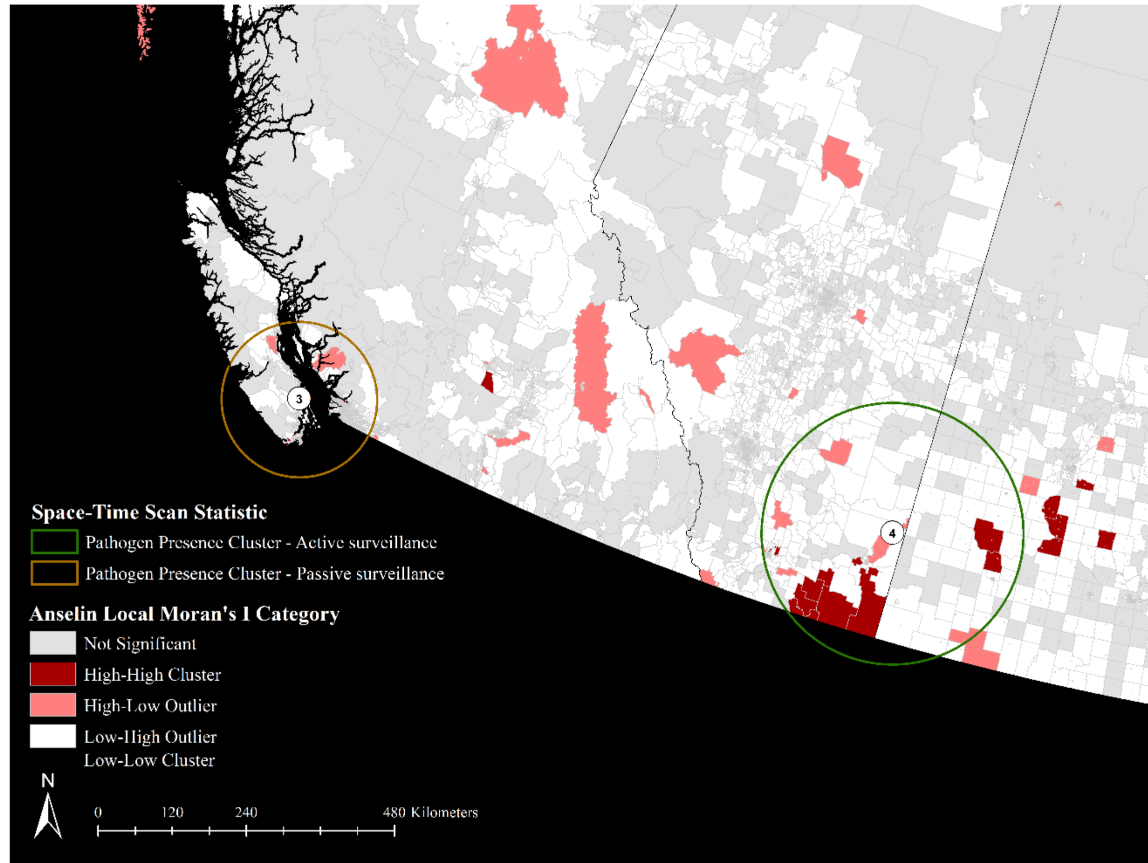

Figure A7. Historical spatiotemporal clusters of high pathogen presence in ticks in Western Canada based on surveillance method. High pathogen presence based on Anselin Local Moran's I statistics in dissemination areas across Western Canada are represented as spatial clusters (red) and outliers (salmon). Using SaTScan™, pathogen presence in ticks were predominantly detected by passive surveillance in southern British Columbia from 2000 to 2009 (p = 0.001 for cluster 3) and by active surveillance in southeastern Alberta and southwestern Saskatchewan from 2005 to 2014 (p = 0.001 for cluster 4).

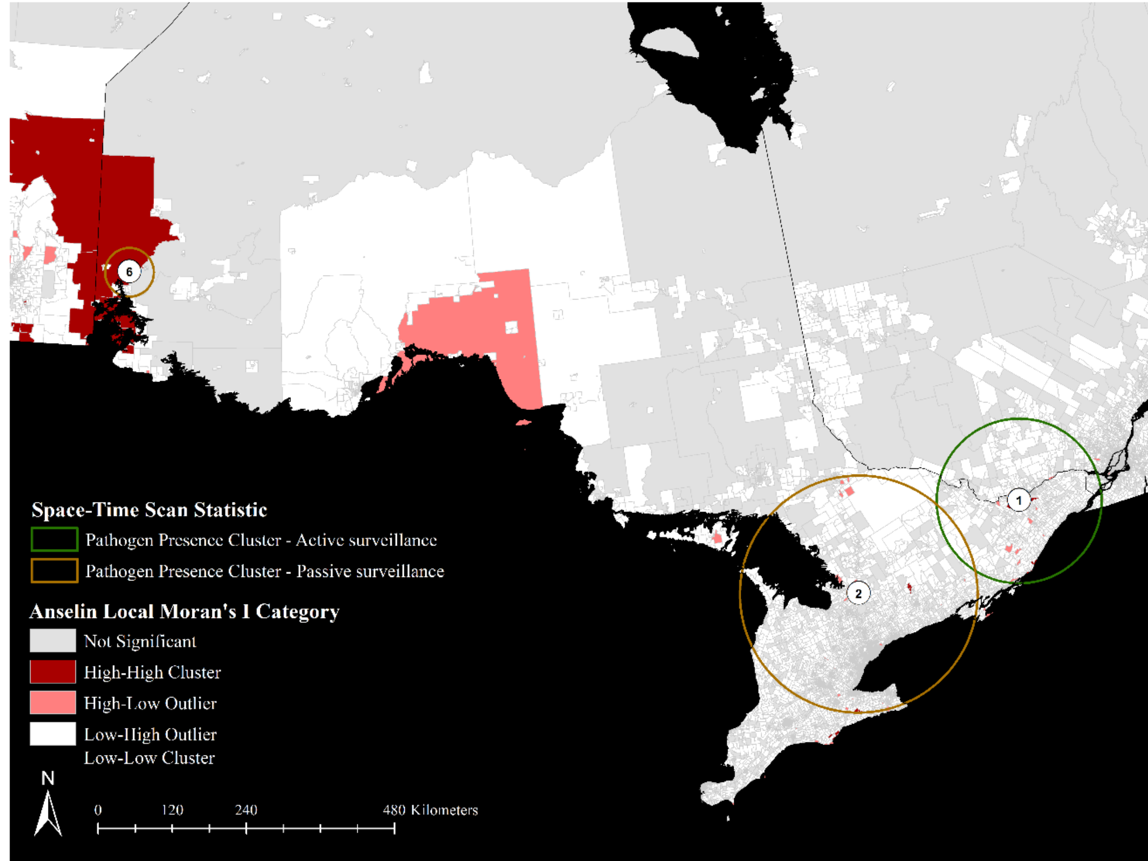

Figure A8. Historical spatiotemporal clusters of high pathogen presence in ticks in Western and Central Canada based on surveillance method. High pathogen presence based on Anselin Local Moran's I statistics in dissemination areas are represented as spatial clusters (red) and outliers (salmon). Using SaTScan™, pathogen presence in ticks were predominantly detected by active surveillance in eastern Ontario from 2005 to 2019 ($p = 0.001$ for cluster 1) and by passive surveillance in southeastern Ontario from 1995 to 2020 ($p = 0.001$ for cluster 2) and near Kenora from 2010 to 2019 ($p < 0.05$ for cluster 6).

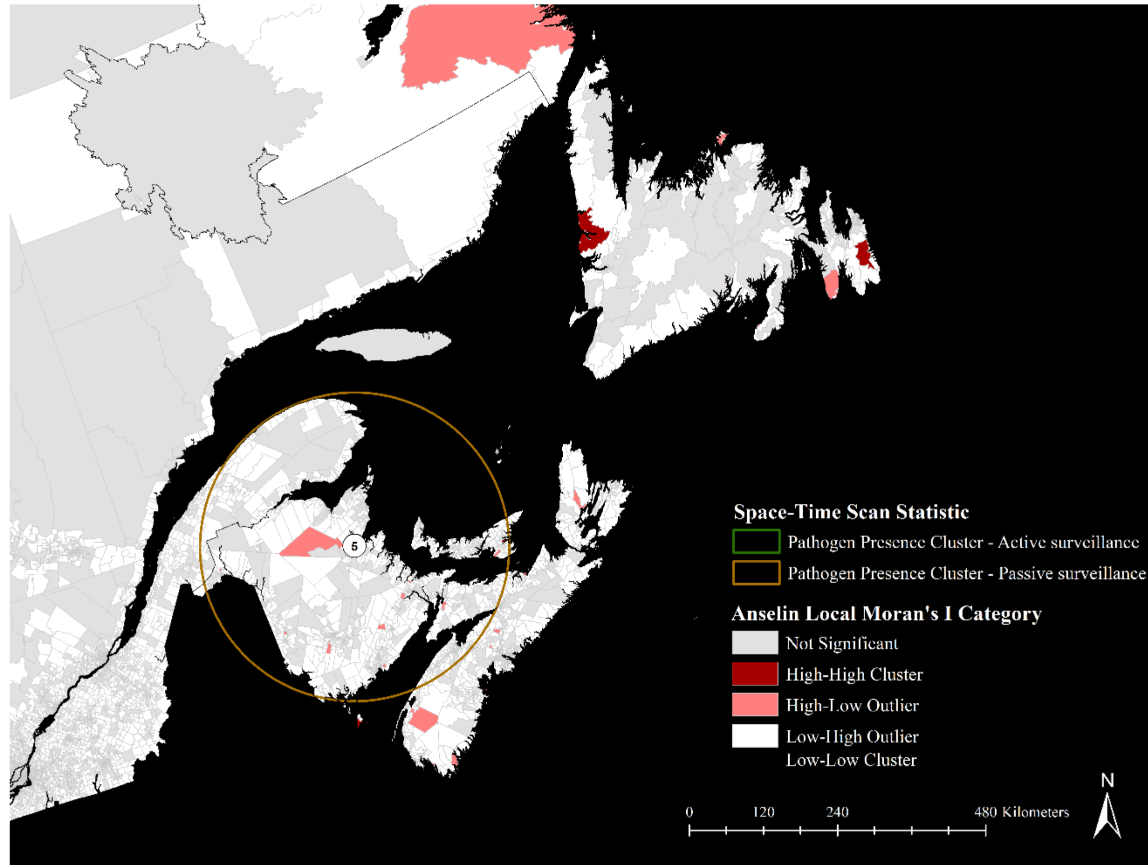

Figure A9. Historical spatiotemporal clusters of high pathogen presence in ticks in Atlantic Canada based on surveillance method. High pathogen presence based on Anselin Local Moran's I statistics in dissemination areas are represented as spatial clusters (red) and outliers (salmon). Using SaTScan™, pathogen presence in ticks were predominantly detected by passive surveillance in parts of the Atlantic provinces from 1990 to 2019 ($p < 0.01$ for cluster 5).

Table A2. Log likelihood ratios of historical spatiotemporal clusters of high pathogen presence in ticks across Canada. Significance of terms is denoted by *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Cluster	Lat (ºN), Long (ºW)	Radius (km)	Time frame	Observed cases	Expected cases	Log like. ratio	*P* value
1	49.012, -95.240	125.01	2005-2019	39	14.86	28.936	< 0.001***
2	44.557, -78.350	213.89	2010-2019	114	76.28	15.934	< 0.001***
3	50.233, -99.833	231.31	2005-2019	31	15.19	11.740	0.004**
4	46.050, -64.088	244.49	2015-2019	12	4.29	10.269	0.015*
5	51.754, -115.581	96.20	2005-2019	9	2.97	10.032	0.024*
6	45.430, -73.940	0	2015-2019	10	3.63	8.197	0.108
7	48.779, -123.708	83.81	1990-2009	22	11.23	7.306	0.223
8	50.883, -107.383	0	2010-2014	4	1.32	4.444	0.995
9	42.405, -82.191	77.17	1995-1999	4	1.32	4.444	0.995
10	49.029, -111.695	114.89	2005-2014	15	8.59	3.384	0.999

Table A3. Log likelihood ratios of historical spatiotemporal clusters of pathogen presence in ticks across Canada based on differences in surveillance types (1: active, 2: passive, 3: combined). Significance of terms is denoted by *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Cluster	Lat (ºN), Long (ºW)	Radius (km)	Time frame	Observed cases (1, 2, and 3)	Expected cases	Log like. ratio	*P* value
1	45.395, -75.844	131.13	2005-2019	69, 2, 0	31.56, 38.52, 0.93	55.542	0.001***
2	44.608, -79.420	188.21	1995-2020	2, 60, 4	29.33, 35.80, 0.86	36.456	0.001***
3	48.995, -123.816	125.66	2000-2009	0, 25, 0	11.11, 13.56, 0.33	15.896	0.001***
4	50.543, -110.285	212.61	2005-2014	23, 1, 0	10.67, 13.02, 0.31	15.818	0.001***
5	47.030, -65.506	247.61	1990-2019	0, 20, 0	8.89, 10.85, 0.26	12.615	0.007**
6	50.100, -94.400	39.56	2010-2019	0, 19, 0	8.44, 10.31, 0.25	11.966	0.014*
7	51.093, -118.04	221.59	2005-2019	12, 0, 0	5.33, 6.51, 0.16	9.933	0.073
8	49.666, -96.660	65.54	2000-2014	0, 14, 0	6.22, 7.59, 0.18	8.748	0.190
9	49.012, -95.240	90.30	2010-2019	14, 1, 0	6.67, 8.14, 0.20	8.537	0.219
10	41.775, -82.658	204.00	1985-1994	10, 0, 0	4.44, 5.42, 0.13	8.249	0.272
11	51.150, -100.05	193.03	2005-2014	10, 0, 0	4.44, 5.42, 0.13	8.249	0.272
12	47.262, -52.773	8.86	2005-2014	9, 0, 0	4.00, 4.88, 0.12	7.411	0.536
13	51.230, -105.442	106.85	2005-2009	6, 0, 0	2.67, 3.25, 0.08	4.915	0.993

Appendix B

Supplementary information for Chapter 2

High-resolution environmental and host-related factors impacting questing *Ixodes scapularis* at their northern range edge

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Supplementary Methods

Table B1. The expected directional relationships of abiotic and biotic factors on *Ixodes scapularis* abundance in Ontario and Quebec, Canada.

Factor	Expected directional change	Reasoning	References
Temperature	↑ Tick abundance	With climate warming, higher temperatures are expected to increase the interstadial development rates and the length of seasonal activity periods, resulting in increased tick abundances.	Ogden et al. 2004, Eisen et al. 2016, Ogden and Lindsay 2016
	↓ Tick abundance	Limited interstadial development and reduced activity is expected when temperatures within the microclimate are outside the optimal thermal thresholds of tick populations (e.g., too low or too high). With extreme hot and cold temperatures, increased mortality rates may occur due to water loss or inoculative freezing, respectively.	
Precipitation	↑ Tick abundance	The presence and activity of ticks may increase at moderate levels of precipitation due to sufficient humidity levels.	Eisen et al. 2016, Ogden and Lindsay 2016, Burtis et al. 2016
	↓ Tick abundance	Low precipitation may lead to desiccation stress in ticks, thereby limiting their questing activity and survival.	
Snow cover	↑ Tick abundance	Greater snow cover alone or in combination with leaf litter may increase the overwintering survival of tick populations.	Hayes et al. 2015, Eisen et al. 2016, Linske et al. 2019, Volk et al. 2022
	↓ Tick abundance	Milder winters may lead to reduced snow cover, which may expose ticks to inoculative freezing, even in the presence of leaf litter, resulting in decreased survival.	
Vegetation	↑ Tick abundance	Areas with dense vegetation may be more suitable habitats for tick populations, leading to greater tick densities.	Schulze and Jordan 2001, Clow et al. 2017, Ginsberg et al. 2020, Mathisson et al. 2021
	↓ Tick abundance	Areas with less dense vegetation may lead to decreased survival for tick populations due to low relative humidity conditions.	

Mammal host abundance	Variable tick abundance	Greater densities of mammal hosts, especially in areas with long-established tick populations, may lead to more contact opportunities between hosts and ticks, thus reducing the time for finding a suitable host and limiting mortality. Areas with tick populations that have dynamic tick-host interactions may have variable tick abundances. These areas may have limited abundances of key mammal hosts, such as white-footed mice or white-tailed deer, thus affecting tick survival and development.	Dobson 2014, Estrada-Peña and De La Fuente 2014
Mammal host diversity	Variable tick abundance	The composition of mammal communities may affect the ability of ticks to successfully feed and further develop because of their quality as blood meal hosts. Adding host species to the local community may increase the number of feeding opportunities for ticks, resulting in increased tick abundance. In addition, the presence of certain mammal hosts, such as white-footed mice and white-tailed deer, may increase tick abundances. However, host-specific differences, such as physiological immune responses, movements, and grooming behaviours, may impact tick burdens and lead to variable tick abundances locally.	Mather et al. 1989, LoGiudice et al. 2003, Jones et al. 2015

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Supplementary Methods

DNA extractions and polymerase chain reactions (PCR) were conducted by Geneticks Inc. to identify the species of *Peromyscus* specimens. Mammalian liver tissues were extracted using the Thermo Scientific GeneJET Genomic Purification Kit (Thermo Fisher Scientific, Massachusetts, United States). Modifications to the Mammalian Tissue and Rodent Tail Genomic DNA Purification protocol (Protocol A, 2016) were made, which included using 10 mg of liver, removing residual solution with extra centrifugation step 9, and not adding more elution buffer after sitting for 5 minutes prior to centrifugation. Nested PCRs were run using species-specific COIII primers with an initial denaturation time of 5 minutes (Tessier, Noël, and Lapointe 2004). PCR products were then run on 3% agarose gel, stained with Eco-Stain (Bio Basic, Markham, Canada), and visualized using a blue light transilluminator.

Reference

Tessier, N., S. Noël, and F.-J. Lapointe. 2004. A new method to discriminate the deer mouse (*Peromyscus maniculatus*) from the white-footed mouse (*Peromyscus leucopus*) using species-specific primers in multiplex PCR. Canadian Journal of Zoology 82:1832–1835.

Table B2. Estimated site-level Lyme disease risk based on the 2018 Lyme disease risk maps in Ontario and Quebec as well as the local abundances and life stages of questing and feeding *Ixodes scapularis* from our field surveys. In Ontario, estimated risk areas are calculated as a 20 km radius from the centre location of questing *I. scapularis* found through tick dragging (Public Health Ontario 2018). In Quebec, municipality risk levels were associated with human Lyme disease cases as well as the abundances and life stages of *I. scapularis* detected through passive and active surveillance (Institut national de santé publique du Québec 2018). Due to significant differences in the provincial classifications of risk areas, we provide two variables related to estimated local Lyme disease risk: (1) a binary variable (0 = possible risk, 1 = present risk) based on definitions by Public Health Ontario and (2) a 3-category variable (1 = possible risk, 2 = present risk, 3 = significant risk) based on definitions by Institut national de santé publique du Québec.

Site ID	Site	Estimated Lyme disease risk (possible or present)	Estimated Lyme disease risk (possible, present, or significant)
1	3 Ridges Farm	0	1
2	New New Age Farm	1	3
3	North Tract	1	2
4	Brown Hill Tract	1	2
5	Upjohn Nature Reserve	0	1
6	Dyer Memorial Nature Reserve	0	1
7	Rose Hill Nature Reserve	0	1
8	Kirkview Farm	1	2
9	Saint-Polycarpe	1	2
10	Saint-Valentin	1	3
11	Henryville	1	3

12	Lefebvre	1	2
13	Parc du Sanctuaire Saint-Majorique	1	3
14	Serpentine-de-Coleraine Ecological Reserve	1	2
15	Frontenac National Park	1	2
16	Saint-Sylvestre	1	2

Questing *I. scapularis* abundance	Feeding *I. scapularis* abundance	Life stage(s) present
0	0	None
131	33	Larvae and nymphs
0	2	Nymphs
0	0	None
0	0	None
0	0	None
0	0	None
4	1	Nymphs
1	1	Larvae and nymphs
99	18	Larvae and nymphs
118	7	Larvae, nymphs, and adults
0	0	None
26	3	Larvae and nymphs
3	0	Larvae and nymphs
0	0	None
0	0	None

References

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Public Health Ontario. 2018. Ontario Lyme disease map 2018: Estimated risk areas. Queen's Printer for Ontario.

Table B3. List of small mammal specimens collected during our field surveys and accessioned at the Redpath Museum, McGill University (Montreal, Quebec, Canada).

Accession number	Species	Site ID	Location	Latitude (ºN)	Longitude (ºW)
RMMA20210812	*Parascalops breweri*	1	3 Ridges Ecological Farm	42.697	-81.026
RMMA20210824	*Peromyscus leucopus*	1	3 Ridges Ecological Farm	42.697	-81.026
RMMA20210816	*Peromyscus leucopus*	2	New New Age Farm	42.73	-80.835
RMMA20210825	*Napaeozapus insignis*	2	New New Age Farm	42.73	-80.835
RMMA20210813	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210814	*Peromyscus leucopus*	2	New New Age Farm	42.731	-80.836
RMMA20210817	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210818	*Peromyscus leucopus*	2	New New Age Farm	42.731	-80.836
RMMA20210819	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210820	*Peromyscus leucopus*	2	New New Age Farm	42.731	-80.836

RMMA20210821	*Peromyscus leucopus*	2	New New Age Farm	42.731	-80.836
RMMA20210822	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.836
RMMA20210823	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210826	*Parascalops breweri*	2	New New Age Farm	42.731	-80.835
RMMA20210827	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210828	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210829	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210830	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210831	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210815	*Peromyscus leucopus*	2	New New Age Farm	42.771	-80.835
RMMA20210838	*Napaeozapus insignis*	3	North Tract	44.081	-79.311
RMMA20210840	*Microtus pennsylvanicus*	3	North Tract	44.081	-79.310
RMMA20210841	*Napaeozapus insignis*	3	North Tract	44.081	-79.311

RMMA20210839	*Napaeozapus insignis*	3	North Tract	44.090	-79.311
RMMA20210833	*Napaeozapus insignis*	3	North Tract	44.081	-79.310
RMMA20210832	*Peromyscus leucopus*	3	North Tract	44.082	-79.313
RMMA20210834	*Peromyscus leucopus*	4	Brown Hill Tract	44.209	-79.366
RMMA20210835	*Peromyscus leucopus*	4	Brown Hill Tract	44.209	-79.366
RMMA20210842	*Peromyscus leucopus*	4	Brown Hill Tract	44.209	-79.366
RMMA20210836	*Peromyscus leucopus*	4	Brown Hill Tract	44.210	-79.366
RMMA20210837	*Peromyscus leucopus*	4	Brown Hill Tract	44.210	-79.366
RMMA20210809	*Peromyscus leucopus*	5	Upjohn Nature Reserve	45.076	-79.36
RMMA20210810	*Peromyscus leucopus*	5	Upjohn Nature Reserve	45.076	-79.36
RMMA20210808	*Peromyscus maniculatus*	6	Dyer Memorial Nature Reserve	45.404	-79.149
RMMA20210811	*Napaeozapus insignis*	6	Dyer Memorial Nature Reserve	45.404	-79.149
RMMA20210803	*Myodes gapperi*	7	Rose Hill Nature Reserve	45.159	-77.226

RMMA20210806	*Myodes gapperi*	7	Rose Hill Nature Reserve	45.159	-77.227
RMMA20210807	*Peromyscus maniculatus*	7	Rose Hill Nature Reserve	45.159	-77.227
RMMA20210801	*Peromyscus maniculatus*	7	Rose Hill Nature Reserve	45.160	-77.227
RMMA20210802	*Napaeozapus insignis*	7	Rose Hill Nature Reserve	45.160	-77.226
RMMA20210804	*Peromyscus maniculatus*	7	Rose Hill Nature Reserve	45.160	-77.227
RMMA20210805	*Napaeozapus insignis*	7	Rose Hill Nature Reserve	45.160	-77.226
RMMA20210843	*Peromyscus leucopus*	8	Kirkview Farm	45.422	-74.670
RMMA20210844	*Peromyscus leucopus*	8	Kirkview Farm	45.422	-74.670
RMMA20210846	*Peromyscus leucopus*	8	Kirkview Farm	45.422	-74.670
RMMA20210847	*Peromyscus leucopus*	8	Kirkview Farm	45.422	-74.670
RMMA20210848	*Peromyscus leucopus*	8	Kirkview Farm	45.422	-74.670
RMMA20210852	*Blarina brevicauda*	9	Saint-Polycarpe	45.329	-74.394
RMMA20210855	*Peromyscus leucopus*	9	Saint-Polycarpe	45.329	-74.394

RMMA20210856	*Peromyscus leucopus*	9	Saint-Polycarpe	45.329	-74.394
RMMA20210845	*Peromyscus leucopus*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210849	*Peromyscus leucopus*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210850	*Peromyscus leucopus*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210851	*Peromyscus leucopus*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210853	*Peromyscus leucopus*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210854	*Blarina brevicauda*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210857	*Peromyscus leucopus*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210858	*Blarina brevicauda*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210859	*Blarina brevicauda*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210860	*Blarina brevicauda*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210866	*Peromyscus leucopus*	10	Saint-Valentin	45.185	-73.348
RMMA20210867	*Peromyscus leucopus*	10	Saint-Valentin	45.185	-73.348

RMMA20210868	*Peromyscus leucopus*	10	Saint-Valentin	45.185	-73.347
RMMA20210869	*Myodes gapperi*	10	Saint-Valentin	45.185	-73.347
RMMA20210870	*Napaeozapus insignis*	10	Saint-Valentin	45.185	-73.347
RMMA20210871	*Myodes gapperi*	10	Saint-Valentin	45.185	-73.347
RMMA20210872	*Myodes gapperi*	10	Saint-Valentin	45.185	-73.347
RMMA20210874	*Myodes gapperi*	10	Saint-Valentin	45.185	-73.348
RMMA20210875	*Myodes gapperi*	10	Saint-Valentin	45.185	-73.347
RMMA20210861	*Peromyscus maniculatus*	11	Henryville	45.117	-73.212
RMMA20210862	*Peromyscus maniculatus*	11	Henryville	45.117	-73.212
RMMA20210864	*Peromyscus maniculatus*	11	Henryville	45.117	-73.210
RMMA20210873	*Myodes gapperi*	11	Henryville	45.117	-73.211
RMMA20210863	*Peromyscus leucopus*	11	Henryville	45.118	-73.211
RMMA20210865	*Peromyscus leucopus*	11	Henryville	45.118	-73.211

RMMA20210883	*Myodes gapperi*	12	Lefebvre	45.738	-72.406
RMMA20210884	*Myodes gapperi*	12	Lefebvre	45.738	-72.406
RMMA20210885	*Blarina brevicauda*	12	Lefebvre	45.738	-72.406
RMMA20210886	*Blarina brevicauda*	12	Lefebvre	45.738	-72.406
RMMA20210892	*Blarina brevicauda*	12	Lefebvre	45.738	-72.406
RMMA20210888	*Myodes gapperi*	13	Parc du Sanctuaire Saint-Majorique	45.943	72.530
RMMA20210891	*Napaeozapus insignis*	13	Parc du Sanctuaire Saint-Majorique	45.943	-72.529
RMMA20210893	*Blarina brevicauda*	13	Parc du Sanctuaire Saint-Majorique	45.943	-72.530
RMMA20210894	*Myodes gapperi*	13	Parc du Sanctuaire Saint-Majorique	45.943	-72.531
RMMA20210881	*Peromyscus maniculatus*	13	Parc du Sanctuaire Saint-Majorique	45.944	-72.530
RMMA20210882	*Myodes gapperi*	13	Parc du Sanctuaire Saint-Majorique	45.944	-72.531
RMMA20210887	*Napaeozapus insignis*	13	Parc du Sanctuaire Saint-Majorique	45.944	-72.530
RMMA20210889	*Napaeozapus insignis*	13	Parc du Sanctuaire Saint-Majorique	45.944	-72.530

RMMA20210890	*Myodes gapperi*	13	Parc du Sanctuaire Saint-Majorique	45.944	-72.529
RMMA20210895	*Napaeozapus insignis*	13	Parc du Sanctuaire Saint-Majorique	45.944	-72.530
RMMA20210877	*Peromyscus maniculatus*	14	Serpentine-de-Coleraine Ecological Reserve	45.978	-71.370
RMMA20210878	*Peromyscus maniculatus*	14	Serpentine-de-Coleraine Ecological Reserve	45.978	-71.371
RMMA20210879	*Myodes gapperi*	14	Serpentine-de-Coleraine Ecological Reserve	45.978	-71.370
RMMA20210880	*Peromyscus maniculatus*	14	Serpentine-de-Coleraine Ecological Reserve	45.978	-71.370
RMMA20210876	*Sorex cinereus*	15	Frontenac National Park	45.815	-71.203
RMMA20210896	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.119
RMMA20210897	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.118
RMMA20210898	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.118
RMMA20210899	*Napaeozapus insignis*	16	Saint-Sylvestre	46.368	-71.118
RMMA202108100	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.118
RMMA202108101	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.119

RMMA202108102	*Myodes gapperi*	16	Saint-Sylvestre	46.368	-71.119
RMMA202108103	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.118
RMMA202108104	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.118
RMMA202108105	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.118

Table B4. Summarized local abiotic factors across our sites in Central Canada. High-resolution environmental factors included monthly mean precipitation (PRECIP), accumulated snow on the ground (SNOW), monthly mean land surface temperature (LST), winter minimum LST, summer maximum LST, summer mean total evapotranspiration (TE), and summer mean enhanced vegetation index (EVI), which were derived from Environment and Climate Change Canada weather towers and NASA's MODIS Terra remote sensing satellites. Latitudinal and longitudinal coordinates in degrees were used to account for spatial autocorrelation.

Site ID	Site	Latitude (ºN)	Longitude (ºW)	Weather tower ID	Distance of tower to site (km)
1	3 Ridges Farm	42.70	-81.03	6137362	16.19
2	New New Age Farm	42.73	-80.84	6138270	16.95
3	North Tract	44.08	-79.31	6110480	20.80
4	Brown Hill Tract	44.21	-79.36	6110480	7.13
5	Upjohn Nature Reserve	45.08	-79.36	6110607	7.50
6	Dyer Memorial Nature Reserve	45.40	-79.15	6117981	26.49
7	Rose Hill Nature Reserve	45.16	-77.22	6105762	36.21
8	Kirkview Farm	45.42	-74.67	7016470	25.27
9	Saint-Polycarpe	45.33	-74.39	7011947	17.52
10	Saint-Valentin	45.18	-73.35	7026916	11.06

11	Henryville	45.12	-73.21	7026734	10.78
12	Lefebvre	45.74	-72.41	7027470	13.67
13	Parc du Sanctuaire Saint-Majorique	45.94	-72.53	7027470	12.76
14	Serpentine-de-Coleraine Ecological Reserve	45.98	-71.37	7028442	11.11
15	Frontenac National Park	45.82	-71.20	7024320	24.46
16	Saint-Sylvestre	46.37	-71.12	7027656	15.27

Monthly mean PRECIP (mm)	Accumulated SNOW (cm)	Monthly mean LST (°C)	Winter minimum LST (°C)	Summer maximum LST (°C)
3.28	43.00	20.53	-26.35	28.53
4.39	45.00	24.63	-14.75	25.05
0.97	104.00	16.20	-21.21	26.21
0.97	104.00	16.07	-44.05	26.15
0.77	141.00	20.16	-29.91	23.99
1.70	299.00	22.90	-25.55	23.73
1.22	94.00	18.50	-22.61	22.99
1.33	104.00	26.41	-24.03	26.41
2.17	107.00	25.10	-30.39	34.83
3.66	124.00	18.10	-32.05	25.65

2.64	98.00	20.55	-24.49	29.95
1.82	135.00	18.84	-25.15	25.11
1.82	135.00	17.40	-20.71	21.87
5.19	216.00	10.30	-25.79	24.53
3.67	175.00	12.02	-26.83	23.37
3.47	181.00	14.18	-24.17	24.79

Summer mean TE (mm)	Summer mean EVI
599.94	0.59
528.69	0.60
522.19	0.47
93.93	0.53
49.49	0.64
312.29	0.59
1572.77	0.20
1791.37	0.19
NA	0.06
NA	0.15

1194.53	0.14
1319.40	NA
2215.20	0.16
936.20	0.10
1490.59	NA
NA	NA

Table B5. Summarized local biotic factors found via small mammal trapping and trail cameras across our sites in Central Canada. Biotic factors included small mammal abundance, the relative abundance of white-footed mice (*Peromycus leucopus*), and the number of mammal host species (or mammal species richness). Questing *I. scapularis* abundance was calculated as the sum of questing ticks collected along transects at each site while tick dragging. The total number of collected small mammals was used as a proxy for the abundance of small mammals locally. The relative abundance of *P. leucopus* was estimated as the number of collected *P. leucopus* individuals at each site divided by the local abundance of collected small mammals. Mammal species richness was quantified as the number of the distinct species found via small mammal trapping and detected in camera photographs. Latitudinal and longitudinal coordinates in degrees were used to account for spatial autocorrelation.

Site ID	Site	Latitude (ºN)	Longitude (ºW)	Questing *I. scapularis* abundance
1	3 Ridges Farm	42.70	-81.03	0
2	New New Age Farm	42.73	-80.84	131
3	North Tract	44.08	-79.31	0
4	Brown Hill Tract	44.21	-79.36	0
5	Upjohn Nature Reserve	45.08	-79.36	0
6	Dyer Memorial Nature Reserve	45.40	-79.15	0
7	Rose Hill Nature Reserve	45.16	-77.22	0

8	Kirkview Farm	45.42	-74.67	4
9	Saint-Polycarpe	45.33	-74.39	1
10	Saint-Valentin	45.18	-73.35	99
11	Henryville	45.12	-73.21	118
12	Lefebvre	45.74	-72.41	0
13	Parc du Sanctuaire Saint-Majorique	45.94	-72.53	26
14	Serpentine-de-Coleraine Ecological Reserve	45.98	-71.37	3
15	Frontenac National Park	45.82	-71.20	0
16	Saint-Sylvestre	46.37	-71.12	0

Small mammal abundance	Relative abundance *P. leucopus*	No. host species (small mammal trapping)	No. host species (camera)	No. host species (trapping and camera)
2	0.500	4	4	7
18	0.333	4	3	7
6	0.167	4	1	5
5	1.000	1	2	3
2	1.000	2	1	3
2	0.000	2	1	3
7	0.000	4	1	5
5	1.000	2	4	6
13	0.615	3	2	5
9	0.333	4	1	5
6	0.333	5	2	7
5	0.000	3	2	5
10	0.000	7	1	8
4	0.000	2	0	2

1	0.000	3	1	4
10	0.000	5	1	6

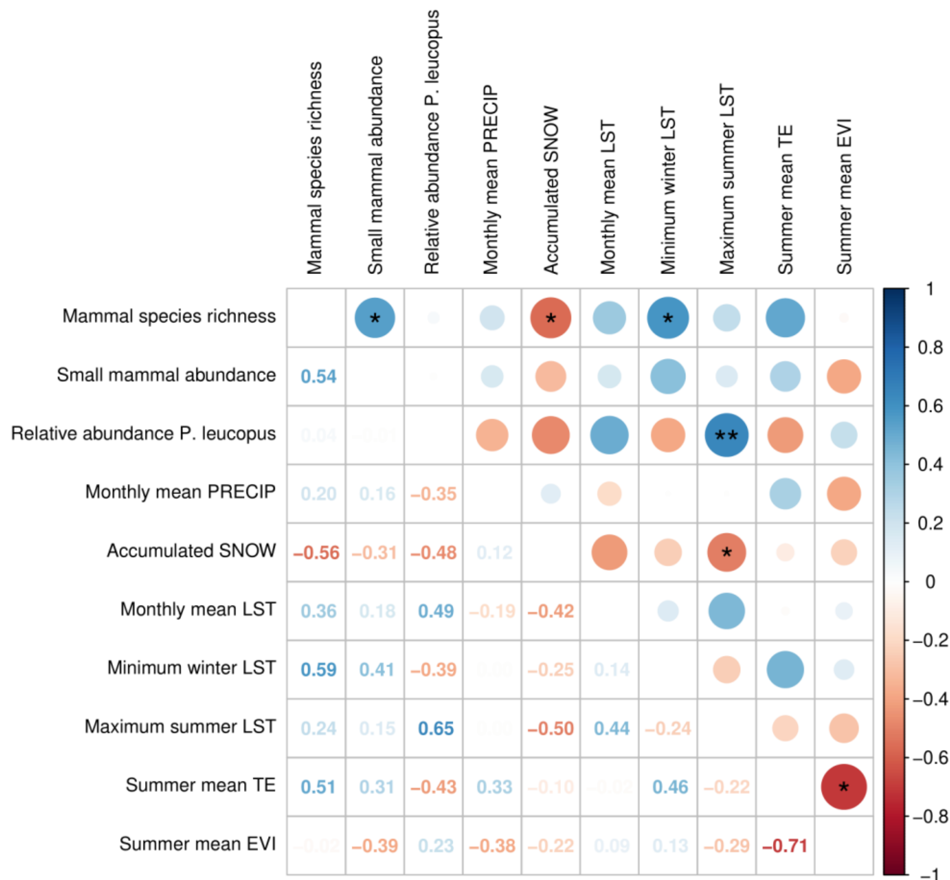

Figure B1. Correlogram showing Spearman correlations among abiotic and biotic factors across our sites in Ontario and Quebec, Canada. Spearman correlation coefficients are represented with the scale and below the diagonal (< 0 = negatively correlated, > 0 = positively correlated). Red circles and coefficients are related to negative correlations, while blue circles and coefficients represent positive correlations. Circles increase in size with larger coefficient values. Significance of terms is denoted by *** P < 0.001, ** P < 0.01, * P < 0.05. PRECIP: precipitation; SNOW: snow on the ground; LST: land surface temperature; TE: total evapotranspiration; EVI: enhanced vegetation index.

Table B6. Parameter estimates and fit measures for Model 1 assessing the effect of smoothed abiotic factors and small mammal abundance as well as the smooth interaction of spatial coordinates on the questing *Ixodes scapularis* abundance in Central Canada. The model formula is Questing *I. scapularis* abundance ~ s(Small mammal abundance) + s(Monthly mean LST) + s(Monthly mean PRECIP) + s(Accumulated SNOW) + s(Latitude, Longitude). Significance of terms is denoted by *** P < 0.001, ** P < 0.01, * P < 0.05. LST: land surface temperature; PRECIP: precipitation; SNOW: snow on the ground.

Parametric terms		Coefficient	Std. Error	*z* value	*P*-value
	Intercept	-0.803	0.949	-0.846	0.397
Smooth terms		**Estimated df**	**Ref. df.**	**Chi. Sq**	***P*-value**
	s(Small mammal abundance)	0.942	2	13.184	< 0.001***
	s(Monthly mean LST)	0.000	2	0.000	0.849
	s(Monthly mean PRECIP)	0.921	2	7.928	0.003**
	s(Accumulated SNOW)	0.000	2	0.000	0.365
	s(Latitude, Longitude)	0.946	3	13.981	< 0.001***
Fit measures		**AIC**	**Dev. Explained**	**Adj. R^2**	**REML**
		75.374	82.20%	-31.300	37.857

Table B7. Parameter estimates and fit measures for Model 2 assessing the effect of smoothed abiotic factors and the relative abundance of *Peromyscus leucopus* as well as the smooth interaction of spatial coordinates on the questing *Ixodes scapularis* abundance in Central Canada. The model formula is Questing *I. scapularis* abundance ~ s(Relative abundance *P. leucopus*) + s(Monthly mean LST) + s(Monthly mean PRECIP) + s(Accumulated SNOW) + s(Latitude, Longitude). Significance of terms is denoted by *** P < 0.001, ** P < 0.01, * P < 0.05. LST: land surface temperature; PRECIP: precipitation; SNOW: snow on the ground.

Parametric terms		Coefficient	Std. Error	*z* value	*P*-value
	Intercept	0.930	0.708	1.312	0.189
Smooth terms		Estimated df	Ref. df.	Chi. sq	*P*-value
	s(Relative abundance *P. leucopus*)	0.000	3.000	0.000	0.644
	s(Monthly mean LST)	0.000	2.000	0.000	0.469
	s(Monthly mean PRECIP)	0.788	2.000	2.612	0.043*
	s(Accumulated SNOW)	0.794	2.000	4.549	0.013*
	s(Latitude, Longitude)	1.452	5.000	4.665	0.024*
Fit measures		AIC	Dev. explained	Adj. R^2	REML
		90.065	57.60%	0.445	42.811

Table B8. Parameter estimates and fit measures for Model 3 assessing the effect of smoothed abiotic factors and mammal species richness as well as the smooth interaction of spatial coordinates on the questing *Ixodes scapularis* abundance in Central Canada. Accumulated snow was not included in this model due to high collinearity with mammal species richness. The model formula is Questing *I. scapularis* abundance ~ s(No. mammal species) + s(Monthly mean LST) + s(Monthly mean PRECIP) + s(Latitude, Longitude). Significance of terms is denoted by *** P < 0.001, ** P < 0.01, * P < 0.05. PRECIP: precipitation; LST: land surface temperature.

Parametric terms		Coefficient	Std. Error	*z* value	*P*-value
	Intercept	-1.813	1.344	-1.349	0.177
Smooth terms		Estimated df	Ref. df.	Chi. sq	*P*-value
	s(No. mammal species)	0.975	3.000	32.583	0.000***
	s(Monthly mean LST)	0.000	2.000	0.000	0.493
	s(Monthly mean PRECIP)	1.912	2.000	32.666	0.000***
	s(Latitude, Longitude)	2.363	5.000	45.582	0.000***
Fit measures		AIC	Dev. explained	Adj. R^2	REML
		53.597	99.70%	0.994	37.258

Appendix C

Supplementary information for Chapter 3

Emerging tick-borne pathogens in Central Canada: Recent detections of *Babesia odocoilei* and *Rickettsia rickettsii*

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Table C1. Primers and conditions used in genetic testing of tick and mammal samples with nested PCRs for *Anaplasma phagocytophilum*, *Babesia odocoilei*, *Babesia microti*, *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Francisella tularensis*, and *Rickettsia rickettsii*.

Species name	Primer name	Reference	Target gene
Anaplasma phagocytophilum	AnaP44OutL1-F AnaP44OutL1-R	In house	p44
	AnaP44InF AnaP44InR	Holden et al. 2003	
Babesia spp.	BabGenPCRF BabGenPCRR	Scott et al. 2021	18S rRNA
Babesia microti	Mic494 BabGenInR1	In house	
Babesia odocoilei	Odo563 BabGenInR1	In house	
Babesia microti	BabMicOutF BabMicOutR BabMicInF BabMicInR	Persing et al. 1992	18S rRNA
Babesia odocoilei	Bab1F Out Bab4R Out Bab2F In Bab3R In	National Microbiology Lab (Canada)	18S rRNA
Borrelia burgdorferi s.l.	MLP-0035 MLP-0036 MLP-0037 MLP-0038	Wodecka 2011	Flagellin B (*flaB*)
Borrelia spp.	VETTBOROUTF VETTBOROUTR	Dibernardo et al. 2014	5S-23S Intergenic Space Region

Borrelia burgdorferi s.s.	VETTBURGINF VETTBURGINR	Dibernardo et al. 2014	5S-23S Intergenic Space Region
Borrelia miyamotoi	VETTMIYINF VETTMIYINR	Zinck et al. 2021	18S rRNA
Francisella tularensis	TULfopAOutF TULfopAOutR TULfopAInF TULfopAInR	Fulop et al. 1996	Ferredoxin (*fdx*)
Rickettsia rickettsii	RRickettsiiOutFLong RRickettsiiOutRLong	In house	Hypothetical Protein Gene (RRi6)
	RRickettsiiInF RRickettsiiInR	Kato et al. 2013	

Sequence (5' > 3')	Amplicon size	Initial Denaturation		Denaturation		Annealing		Elongation	
		(s)	(°C)	(s)	(°C)	(s)	(°C)	(s)	(°C)
gtagaagaaaccgccctaat tctatgttggtttggattacag	850	300	95	30	95	30	53	60	72
gcccagtaacaacatcataagc ccagcgtttagcaagataagag	334	300	95	30	95	30	53	60	72
gtcttgtaattggaatgatgg tagtttatggttaggactacg	488	300	95	30	95	30	55	45	72
ccgtctcggctctttgcc tctgatcgtcttcgatcccc	308	300	95	30	95	15	63	20	72
ccgtattttgacttttgtcgactgt tctgatcgtcttcgatcccc	311	300	95	30	95	15	63	20	72
cttagtataagcttttatacagc ataggtcagaaacttgaatgataca	238	300	94	30	94	30	55	30	72
gttatagtttatttgatgttc aagccatgcgattcgctaat	155	300	94	30	94	30	55	30	72
ccgtcgtagtcctaacyataaac ccttgttacgacttctccttcc	767	300	95	30	95	60	52	60	72
ttcttgattctytgggtrgtgg ctaggcattcctcgttcawgat	343	300	95	30	95	60	55	60	72
tggtatgggagtttctgg tctgtcattgtagcatcttt	774	600	94	30	94	45	50	60	72
cagacaacagagggaaat tcaagtctattttggaaagcacc	605	600	94	30	94	45	54	60	72
gtatgtttagtgaggggggtg ggatcatagctcaggtggttag	1029 (*B. burgdorferi*); 588 (*B. miyamotoi*)	240	94	60	94	60	50	60	72

atgtattccattgttttaattacg gacaagtattgtagcgagc	340	300	95	30	95	30	51	30	72
ataaacctgaggtcggagg aaagtgtggctggatcacc	507	300	95	30	95	30	60	30	72
cgaggagtctcaatgtactaaggtttgccc caccattatcctggatattaccagtgtcat	900	180	95	15	95	15	55	30	72
cttgagtcttatgtttcggcatgtgaatag ccaactaattggttgtactgtacagcgaag	409	180	95	15	95	15	55	30	72
agcaatctgaaacaacacat aaaccccaacctttaacctt	416	300	95	45	95	45	50	30	72
aaatcaacggaagagcaaaac ccctccactacctgcatcat	153	300	95	45	95	30	50	30	72

Repetitions	Final Elongation (s)	(°C)
35	300	72
40	600	72
40	600	72
40	600	72
40	600	72
30	300	72
30	300	72
30	600	72
30	600	72
40	420	72
40	420	72
35	600	72

40	600	72
40	600	72
35	600	72
35	600	72
35	300	72
35	300	72

References

Dibernardo A, Cote T, Ogden NH, et al. The prevalence of *Borrelia miyamotoi* infection, and co-infections with other *Borrelia* spp. in *Ixodes scapularis* collected in Canada. Parasites Vectors 2014;7:183; doi: 10.1186/1756-3305-7-183

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Zinck C, Priest J, Shutler D, et al. Detection of *Borrelia* spp., *Ehrlichia canis*, *Anaplasma phagocytophilum*, and *Dirofilaria immitis* in eastern coyotes (*Canis latrans*) in Nova Scotia, Canada. J Wildl Dis 2021;57(3):678-682; doi: 10.7589/JWD-D-20-00188.

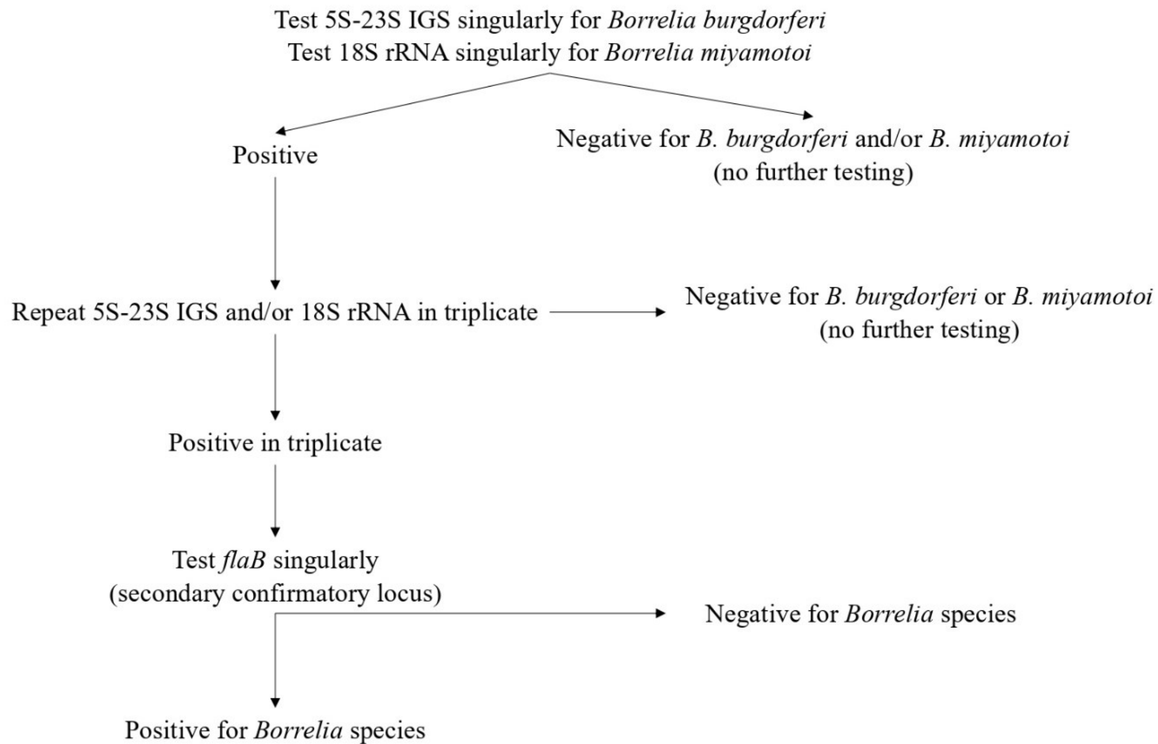

Figure C1. Workflow of pathogen testing for *Borrelia* species. The 5S-23S Intergenic Space Region (IGS) and 18S rRNA was first tested singularly for *Borrelia burgdorferi* and *Borrelia miyamotoi*, respectively. If the locus was positive, it was then tested two more times to identify false positives. If the locus was positive in triplicate, the *flaB* locus was tested singularly as a secondary confirmatory locus. If the sample was positive for IGS in triplicate and *flaB* singularly, then we considered the sample positive for *Borrelia burgdorferi* sensu stricto. If the sample was positive for 18S rRNA in triplicate and *flaB* singularly, then we considered the sample positive for *Borrelia miyamotoi*. If the sample failed to produce a visible band for any of the tests, it was considered a negative PCR, where no *Borrelia* species was present. Identification of *Borrelia* species was determined by sequencing and comparisons with samples in GenBank.

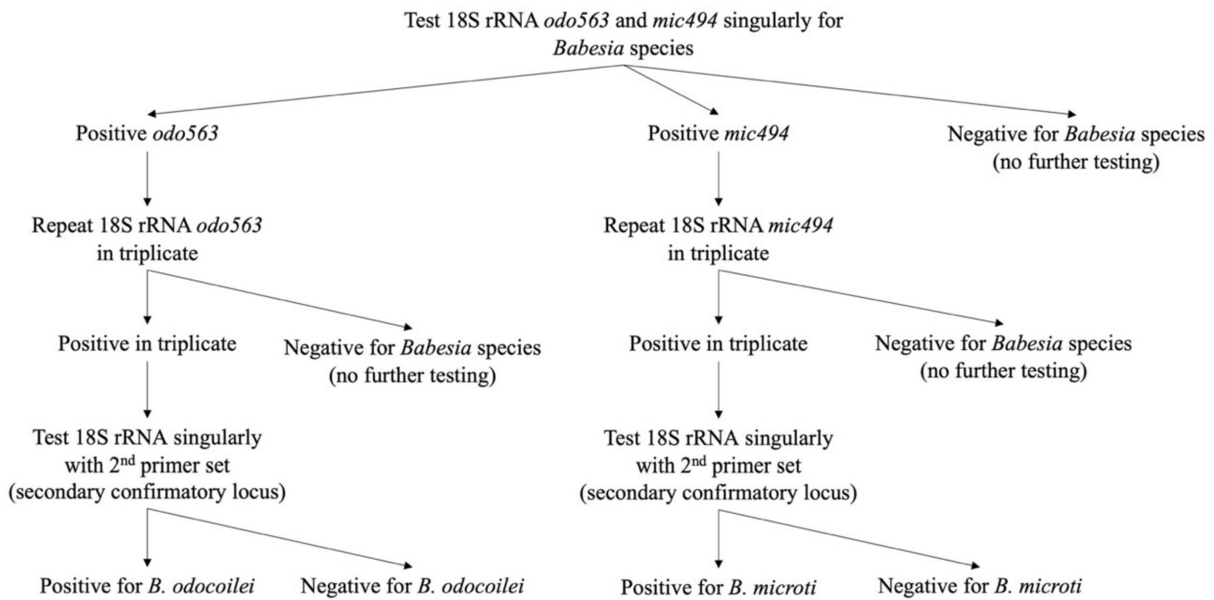

Figure C2. Workflow of pathogen testing for *Babesia odocoilei* and *Babesia microti*. The 18S rRNA was first tested singularly with both the *odo563* and *mic494* primers. If either locus was positive, it was then tested two more times to identify false positives. If a locus was positive in triplicate, an additional set of primers each targeting one *Babesia* species was tested once as a secondary confirmatory locus. If the samples were positive in triplicate for *odo563* or *mic494* and singularly for a second primer set, it was considered positive for *B. odocoilei* or *B. microti*, respectively. If the sample failed to produce a visible band for any of the tests, it was considered a negative PCR, where no *Babesia* species was present. Identification of *Borrelia* species was determined by sequencing and comparisons with samples in GenBank.

Appendix D

Supplementary information for Chapter 4

Pathogen presence, prevalence, and diversity in *Ixodes scapularis* and mammal hosts at their expanding northern range limits

<u>Content</u>

Supplementary Methods

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Supplementary Methods

Geneticks Inc. conducted all DNA extractions and polymerase chain reactions (PCR). Adults and nymphs were tested individually, while larvae were pooled. Larvae that were questing were pooled by grid (2-10 larvae per pool), while those feeding were pooled by their host (1-10 larvae per pool). Ticks were homogenized using a microtube pestle in AquaGenomic solution (Wills et al. 2018). Samples were incubated in a 60°C heat block for 45 minutes, vortexed briefly, and centrifuged at 13,300 rpm for four minutes. The supernatant was removed and placed into a microvial with 50 µL isopropanol, which was inverted and centrifuged as before. After decanting the supernatant, the DNA pellet was rinsed with 50 µL of 70% ethanol. The sample was left to air dry for 15 minutes at room temperature and then resuspended with 50 µL of 1mM Tris pH 8.0. Samples were incubated in a 60°C heat block for one hour.

The Thermo Scientific GeneJET Genomic Purification Kit (Protocol A, 2016) (Thermo Fisher Scientific, Massachusetts, United States) was used to extract DNA from mammalian liver tissues. A few modifications were made to the Mammalian Tissue and Rodent Tail Genomic DNA Purification protocol. These modifications included the use of 10 mg of liver, an additional centrifugation at step nine to remove the residual solution, and no additional elution buffer was used after sitting for five minutes prior to centrifugation.

Identification of Peromyscus species

As in Tessier et al. (2004), a nested PCR using species-specific COIII primers was run to identify *Peromyscus* species. An initial denaturation time of 5 minutes was used as a modification.

Pathogen screening in tick and mammal specimens

Five pathogens were targeted in *Ixodes scapularis* and small mammal specimens using nested PCRs (see Table D3 for a description of the primers and conditions for each pathogen). All tick and small mammal specimens were tested for *Anaplasma phagocytophilum*, *Babesia microti*, *Babesia odocoilei*, *Borrelia burgdorferi*, and *Borrelia miyamotoi*. If a band was visible (i.e., positive PCR), we then tested twice more to identify false positives. The *p44* gene was used to test for the presence of *A. phagocytophilum* (Holden et al. 2003). *Babesia odocoilei* and *B. microti* were targeted with the 18S rRNA region using the *mic494* and *odo563* inner primers,

respectively. An additional primer set targeting the 18S rRNA of each *Babesia* species was used for confirmation (Persing et al. 1992). We also tested for *B. burgdorferi* sensu stricto and *B. miyamotoi* using the 5S-23S intergenic space region and the 18S rRNA region, respectively (Dibernardo et al. 2014, Zinck et al. 2021). An additional test using the *flaB* gene confirmed the presence of *B. burgdorferi* sensu lato (Wodecka 2011).

DNA sequencing and quality control

Amplified products were purified for sequencing following Sun et al. (2012). A cotton cushion was placed in a 500 µL centrifuge tube after a hole was made in its bottom. An excised fragment of interest was removed from the agarose gel and laid on the cushion. The tube was capped and placed into an uncapped 1.7 mL Eppendorf tube, where samples were spun at 5000 rpm for 7 minutes. Purified DNA was then reamplified with the corresponding inner primers. Sanger DNA sequencing with forward inner primers was completed at Bio Basic DNA Sequencing (Markham, Ontario, Canada).

Using 4Peaks software (https://nucleobytes.com/4peaks/), sequences were assessed for quality control, ambiguous base calls, and end-reading errors. Our dataset consisted only of sequences with an average quality score of 20 or higher. We determined the pathogen species of our sequence with GenBank using a MEGABLAST search in the nucleotide BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi#_blank).

References

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Zinck, C., J. Priest, D. Shutler, M. Boudreau, and V. Lloyd. 2021. Detection of *Borrelia* spp., *Ehrlichia canis*, *Anaplasma phagocytophilum*, and *Dirofilaria immitis* in eastern coyotes (*Canis latrans*) in Nova Scotia, Canada. Journal of Wildlife Diseases 57:678–682.

Table D1. Estimated site-level Lyme disease risk based on the 2018 Lyme disease risk maps in Ontario and Quebec as well as the local abundances and life stages of questing and feeding *Ixodes scapularis* from our field surveys. In Ontario, estimated risk areas are calculated as a 20 km radius from the centre location of questing *I. scapularis* found through tick dragging (Public Health Ontario 2018). In Quebec, municipality risk levels were associated with human Lyme disease cases as well as the abundances and life stages of *I. scapularis* detected through passive and active surveillance (Institut national de santé publique du Québec 2018). Due to significant differences in the provincial classifications of risk areas, we provide two variables related to estimated local Lyme disease risk: (1) a binary variable (0 = possible risk, 1 = present risk) based on definitions by Public Health Ontario and (2) a 3-category variable (1 = possible risk, 2 = present risk, 3 = significant risk) based on definitions by Institut national de santé publique du Québec.

Site ID	Site	Estimated Lyme disease risk (possible or present)	Estimated Lyme disease risk (possible, present, or significant)
1	3 Ridges Farm	0	1
2	New New Age Farm	1	3
3	North Tract	1	2
4	Brown Hill Tract	1	2
5	Upjohn Nature Reserve	0	1
6	Dyer Memorial Nature Reserve	0	1
7	Rose Hill Nature Reserve	0	1
8	Kirkview Farm	1	2
9	Saint-Polycarpe	1	2
10	Saint-Valentin	1	3
11	Henryville	1	3

12	Lefebvre	1	2
13	Parc du Sanctuaire Saint-Majorique	1	3
14	Serpentine-de-Coleraine Ecological Reserve	1	2
15	Frontenac National Park	1	2
16	Saint-Sylvestre	1	2

Questing *I. scapularis* abundance	Feeding *I. scapularis* abundance	Life stage(s) present
0	0	None
131	33	Larvae and nymphs
0	2	Nymphs
0	0	None
0	0	None
0	0	None
0	0	None
4	1	Nymphs
1	1	Larvae and nymphs
99	18	Larvae and nymphs
118	7	Larvae, nymphs, and adults

0	0	None
26	3	Larvae and nymphs
3	0	Larvae and nymphs
0	0	None
0	0	None

References

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Table D2. List of small mammal specimens collected during our field surveys and accessioned at the Redpath Museum, McGill University (Montreal, Quebec, Canada).

Accession number	Species	Site ID	Location	Latitude (ºN)	Longitude (ºW)
RMMA20210812	*Parascalops breweri*	1	3 Ridges Ecological Farm	42.697	-81.026
RMMA20210824	*Peromyscus leucopus*	1	3 Ridges Ecological Farm	42.697	-81.026
RMMA20210816	*Peromyscus leucopus*	2	New New Age Farm	42.730	-80.835
RMMA20210825	*Napaeozapus insignis*	2	New New Age Farm	42.730	-80.835
RMMA20210813	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210814	*Peromyscus leucopus*	2	New New Age Farm	42.731	-80.836
RMMA20210817	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210818	*Peromyscus leucopus*	2	New New Age Farm	42.731	-80.836
RMMA20210819	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210820	*Peromyscus leucopus*	2	New New Age Farm	42.731	-80.836

RMMA20210821	*Peromyscus leucopus*	2	New New Age Farm	42.731	-80.836
RMMA20210822	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.836
RMMA20210823	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210826	*Parascalops breweri*	2	New New Age Farm	42.731	-80.835
RMMA20210827	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210828	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210829	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210830	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210831	*Napaeozapus insignis*	2	New New Age Farm	42.731	-80.837
RMMA20210815	*Peromyscus leucopus*	2	New New Age Farm	42.771	-80.835
RMMA20210838	*Napaeozapus insignis*	3	North Tract	44.081	-79.311
RMMA20210840	*Microtus pennsylvanicus*	3	North Tract	44.081	-79.310
RMMA20210841	*Napaeozapus insignis*	3	North Tract	44.081	-79.311

RMMA20210839	*Napaeozapus insignis*	3	North Tract	44.090	-79.311
RMMA20210833	*Napaeozapus insignis*	3	North Tract	44.081	-79.310
RMMA20210832	*Peromyscus leucopus*	3	North Tract	44.082	-79.313
RMMA20210834	*Peromyscus leucopus*	4	Brown Hill Tract	44.209	-79.366
RMMA20210835	*Peromyscus leucopus*	4	Brown Hill Tract	44.209	-79.366
RMMA20210842	*Peromyscus leucopus*	4	Brown Hill Tract	44.209	-79.366
RMMA20210836	*Peromyscus leucopus*	4	Brown Hill Tract	44.210	-79.366
RMMA20210837	*Peromyscus leucopus*	4	Brown Hill Tract	44.210	-79.366
RMMA20210809	*Peromyscus leucopus*	5	Upjohn Nature Reserve	45.076	-79.360
RMMA20210810	*Peromyscus leucopus*	5	Upjohn Nature Reserve	45.076	-79.360
RMMA20210808	*Peromyscus maniculatus*	6	Dyer Memorial Nature Reserve	45.404	-79.149
RMMA20210811	*Napaeozapus insignis*	6	Dyer Memorial Nature Reserve	45.404	-79.149
RMMA20210803	*Myodes gapperi*	7	Rose Hill Nature Reserve	45.159	-77.226

RMMA20210806	*Myodes gapperi*	7	Rose Hill Nature Reserve	45.159	-77.227
RMMA20210807	*Peromyscus maniculatus*	7	Rose Hill Nature Reserve	45.159	-77.227
RMMA20210801	*Peromyscus maniculatus*	7	Rose Hill Nature Reserve	45.160	-77.227
RMMA20210802	*Napaeozapus insignis*	7	Rose Hill Nature Reserve	45.160	-77.226
RMMA20210804	*Peromyscus maniculatus*	7	Rose Hill Nature Reserve	45.160	-77.227
RMMA20210805	*Napaeozapus insignis*	7	Rose Hill Nature Reserve	45.160	-77.226
RMMA20210843	*Peromyscus leucopus*	8	Kirkview Farm	45.422	-74.670
RMMA20210844	*Peromyscus leucopus*	8	Kirkview Farm	45.422	-74.670
RMMA20210846	*Peromyscus leucopus*	8	Kirkview Farm	45.422	-74.670
RMMA20210847	*Peromyscus leucopus*	8	Kirkview Farm	45.422	-74.670
RMMA20210848	*Peromyscus leucopus*	8	Kirkview Farm	45.422	-74.670
RMMA20210852	*Blarina brevicauda*	9	Saint-Polycarpe	45.329	-74.394
RMMA20210855	*Peromyscus leucopus*	9	Saint-Polycarpe	45.329	-74.394

RMMA20210856	*Peromyscus leucopus*	9	Saint-Polycarpe	45.329	-74.394
RMMA20210845	*Peromyscus leucopus*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210849	*Peromyscus leucopus*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210850	*Peromyscus leucopus*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210851	*Peromyscus leucopus*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210853	*Peromyscus leucopus*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210854	*Blarina brevicauda*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210857	*Peromyscus leucopus*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210858	*Blarina brevicauda*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210859	*Blarina brevicauda*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210860	*Blarina brevicauda*	9	Saint-Polycarpe	45.330	-74.394
RMMA20210866	*Peromyscus leucopus*	10	Saint-Valentin	45.185	-73.348
RMMA20210867	*Peromyscus leucopus*	10	Saint-Valentin	45.185	-73.348

RMMA20210868	*Peromyscus leucopus*	10	Saint-Valentin	45.185	-73.347
RMMA20210869	*Myodes gapperi*	10	Saint-Valentin	45.185	-73.347
RMMA20210870	*Napaeozapus insignis*	10	Saint-Valentin	45.185	-73.347
RMMA20210871	*Myodes gapperi*	10	Saint-Valentin	45.185	-73.347
RMMA20210872	*Myodes gapperi*	10	Saint-Valentin	45.185	-73.347
RMMA20210874	*Myodes gapperi*	10	Saint-Valentin	45.185	-73.348
RMMA20210875	*Myodes gapperi*	10	Saint-Valentin	45.185	-73.347
RMMA20210861	*Peromyscus maniculatus*	11	Henryville	45.117	-73.212
RMMA20210862	*Peromyscus maniculatus*	11	Henryville	45.117	-73.212
RMMA20210864	*Peromyscus maniculatus*	11	Henryville	45.117	-73.210
RMMA20210873	*Myodes gapperi*	11	Henryville	45.117	-73.211
RMMA20210863	*Peromyscus leucopus*	11	Henryville	45.118	-73.211
RMMA20210865	*Peromyscus leucopus*	11	Henryville	45.118	-73.211

RMMA20210883	*Myodes gapperi*	12	Lefebvre	45.738	-72.406
RMMA20210884	*Myodes gapperi*	12	Lefebvre	45.738	-72.406
RMMA20210885	*Blarina brevicauda*	12	Lefebvre	45.738	-72.406
RMMA20210886	*Blarina brevicauda*	12	Lefebvre	45.738	-72.406
RMMA20210892	*Blarina brevicauda*	12	Lefebvre	45.738	-72.406
RMMA20210888	*Myodes gapperi*	13	Parc du Sanctuaire Saint-Majorique	45.943	72.530
RMMA20210891	*Napaeozapus insignis*	13	Parc du Sanctuaire Saint-Majorique	45.943	-72.529
RMMA20210893	*Blarina brevicauda*	13	Parc du Sanctuaire Saint-Majorique	45.943	-72.530
RMMA20210894	*Myodes gapperi*	13	Parc du Sanctuaire Saint-Majorique	45.943	-72.531
RMMA20210881	*Peromyscus maniculatus*	13	Parc du Sanctuaire Saint-Majorique	45.944	-72.530
RMMA20210882	*Myodes gapperi*	13	Parc du Sanctuaire Saint-Majorique	45.944	-72.531
RMMA20210887	*Napaeozapus insignis*	13	Parc du Sanctuaire Saint-Majorique	45.944	-72.530

RMMA20210889	*Napaeozapus insignis*	13	Parc du Sanctuaire Saint-Majorique	45.944	-72.530
RMMA20210890	*Myodes gapperi*	13	Parc du Sanctuaire Saint-Majorique	45.944	-72.529
RMMA20210895	*Napaeozapus insignis*	13	Parc du Sanctuaire Saint-Majorique	45.944	-72.530
RMMA20210877	*Peromyscus maniculatus*	14	Serpentine-de-Coleraine Ecological Reserve	45.978	-71.370
RMMA20210878	*Peromyscus maniculatus*	14	Serpentine-de-Coleraine Ecological Reserve	45.978	-71.371
RMMA20210879	*Myodes gapperi*	14	Serpentine-de-Coleraine Ecological Reserve	45.978	-71.370
RMMA20210880	*Peromyscus maniculatus*	14	Serpentine-de-Coleraine Ecological Reserve	45.978	-71.370
RMMA20210876	*Sorex cinereus*	15	Frontenac National Park	45.815	-71.203
RMMA20210896	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.119
RMMA20210897	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.118
RMMA20210898	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.118
RMMA20210899	*Napaeozapus insignis*	16	Saint-Sylvestre	46.368	-71.118

RMMA202108100	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.118
RMMA202108101	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.119
RMMA202108102	*Myodes gapperi*	16	Saint-Sylvestre	46.368	-71.119
RMMA202108103	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.118
RMMA202108104	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.118
RMMA202108105	*Blarina brevicauda*	16	Saint-Sylvestre	46.368	-71.118

Table D3. Primers and conditions used in genetic testing of tick and mammal samples with nested PCRs for *Anaplasma phagocytophilum*, *Babesia odocoilei*, *Babesia microti*, *Borrelia burgdorferi*, and *Borrelia miyamotoi*. For pathogen testing workflow, see Figure C1 and C2.

Species name	Primer name	Reference	Target gene
Anaplasma phagocytophilum	AnaP44OutL1-F AnaP44OutL1-R	In house	p44
	AnaP44InF AnaP44InR	Holden et al. 2003	
Babesia spp.	BabGenPCRF BabGenPCRR	Scott et al. 2021	18S rRNA
Babesia microti	Mic494 BabGenInR1	In house	
Babesia odocoilei	Odo563 BabGenInR1	In house	
Babesia microti	BabMicOutF BabMicOutR BabMicInF BabMicInR	Persing et al. 1992	18S rRNA
Babesia odocoilei	Bab1F Out Bab4R Out Bab2F In Bab3R In	National Microbiology Lab (Canada)	18S rRNA
Borrelia burgdorferi s.l.	MLP-0035 MLP-0036 MLP-0037 MLP-0038	Wodecka 2011	Flagellin B (*flaB*)
Borrelia spp.	VETTBOROUTF VETTBOROUTR	Dibernardo et al. 2014	5S-23S Intergenic Space Region

Borrelia burgdorferi s.s.	VETTBURGINF VETTBURGINR	Dibernardo et al. 2014	5S-23S Intergenic Space Region
Borrelia miyamotoi	VETTMIYINF VETTMIYINR	Zinck et al. 2021	18S rRNA

Sequence (5’ > 3’)	Amplicon size	Initial Denaturation		Denaturation		Annealing		Elongation	
		(s)	(°C)	(s)	(°C)	(s)	(°C)	(s)	(°C)
gtagaagaaaccgccctaat tctatgttggtttggattacag	850	300	95	30	95	30	53	60	72
gcccagtaacaacatcataagc ccagcgtttagcaagataagag	334	300	95	30	95	30	53	60	72
gtcttgtaattggaatgatgg tagtttatggttaggactacg	488	300	95	30	95	30	55	45	72
ccgtctcggctctttgcc tctgatcgtcttcgatcccc	308	300	95	30	95	15	63	20	72
ccgtattttgacttttgtcgactgt tctgatcgtcttcgatcccc	311	300	95	30	95	15	63	20	72
cttagtataagcttttatacagc ataggtcagaaacttgaatgataca	238	300	94	30	94	30	55	30	72
gttatagtttatttgatgttc aagccatgcgattcgctaat	155	300	94	30	94	30	55	30	72
ccgtcgtagtcctaacyataaac ccttgttacgacttctccttcc	767	300	95	30	95	60	52	60	72
ttcttgattctytgggtrgtgg ctaggcattcctcgttcawgat	343	300	95	30	95	60	55	60	72
tggtatgggagtttctgg tctgtcattgtagcatcttt	774	600	94	30	94	45	50	60	72
cagacaacagagggaaat tcaagtctattttggaaagcacc	605	600	94	30	94	45	54	60	72
gtatgtttagtgaggggggtg ggatcatagctcaggtggttag	1029 (*B. burgdorferi*); 588 (*B. miyamotoi*)	240	94	60	94	60	50	60	72

atgtattccattgttttaattacg gacaagtattgtagcgagc	340	300	95	30	95	30	51	30	72
ataaacctgaggtcggagg aaagtgtggctggatcacc	507	300	95	30	95	30	60	30	72

Repetitions	Final Elongation (s)	(°C)
35	300	72
40	600	72
40	600	72
40	600	72
40	600	72
30	300	72
30	300	72
30	600	72
30	600	72
40	420	72
40	420	72
35	600	72

40	600	72
40	600	72

References

Dibernardo, A., T. Cote, N. Ogden, and L. Lindsay. 2014. The prevalence of *Borrelia miyamotoi* infection, and co-infections with other *Borrelia* spp. in *Ixodes scapularis* ticks collected in Canada. Parasites & Vectors 7.

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Table D4. Summary of collected small mammals at our sites in Ontario and Quebec, Canada, where the abundances of infected and total small mammal individuals are indicated. Only two small mammals were infected with pathogens across our sites including one *Peromyscus leucopus* (*Babesia microti*) and one *Blarina brevicauda* (*Babesia odocoilei*).

Site ID	Site	Collected specimens					
		P. leucopus	*P. maniculatus*	*B. brevicauda*	*S. cinereus*	*M. pennsylvanicus*	*M. gapperi*
1	3 Ridges Farm	0/1	0	0	0	0	0
2	New New Age Farm	0/6	0	0	0	0	0
3	North Tract	0/1	0	0	0	0/1	0
4	Brown Hill Tract	0/5	0	0	0	0	0
5	Upjohn Nature Reserve	0/2	0	0	0	0	0
6	Dyer Memorial Nature Reserve	0	0/1	0	0	0	0
7	Rose Hill Nature Reserve	0	0/3	0	0	0	0/2
8	Kirkview Farm	0/5	0	0	0	0	0
9	Saint-Polycarpe	0/8	0	0/5	0	0	0
10	Saint-Valentin	0/3	0	0	0	0	0/5
11	Henryville	1/2	0/3	0	0	0	0/1
12	Lefebvre	0	0	0/3	0	0	0/2
13	Parc du Sanctuaire Saint-Majorique	0	0/1	0/1	0	0	0/4

14	Serpentine-de-Coleraine Ecological Reserve	0	0/3	0	0	0	0/1
15	Frontenac National Park	0	0	0	0/1	0	0
16	Saint-Sylvestre	0	0	1/8	0	0	0/1

P. breweri	*N. insignis*	Pathogen(s) detected
0/1	0	None
0/1	0/11	None
0	0/4	None
0	0	None
0	0	None
0	0/1	None
0	0/2	None
0	0	None
0	0	None
0	0/1	None
0	0	*B. microti* (*P. leucopus*)
0	0	None
0	0/4	None
0	0	None
0	0	None
0	0/1	*B. odocoilei* (*B. brevicauda*)

Table D5. Summary of the released small mammal species at our sites in Ontario and Quebec, Canada. The presence of released small mammals including juveniles and non-targeted species is indicated with an "X".

Site ID	Site	*Peromyscus spp.*	*B. brevicauda*	*N. insignis*	*G. volans*	*S. carolinensis*	*T. striatus*	*T. hudsonicus*
1	3 Ridges Farm	-	-	-	X	-	X	-
2	New New Age Farm	X	-	-	-	-	X	-
3	North Tract	X	-	X	-	-	X	-
4	Brown Hill Tract	X	-	-	-	-	-	-
5	Upjohn Nature Reserve	X	-	-	X	-	-	-
6	Dyer Memorial Nature Reserve	-	-	-	-	-	-	-
7	Rose Hill Nature Reserve	-	-	-	X	-	-	-
8	Kirkview Farm	-	-	-	-	-	X	-
9	Saint-Polycarpe	X	X	-	-	-	X	-
10	Saint-Valentin	X	X	-	-	-	-	-
11	Henryville	X	X	-	-	-	X	-
12	Lefebvre	X	-	-	-	-	-	-
13	Parc du Sanctuaire Saint-Majorique	X	-	-	X	X	-	X
14	Serpentine-de-Coleraine Ecological Reserve	X	-	-	-	-	-	-
15	Frontenac National Park	X	-	-	-	-	X	-
16	Saint-Sylvestre	X	X	X	-	-	-	X

Table D6. The abundance of infected and total questing and feeding *Ixodes scapularis* at our sites in Ontario and Quebec, Canada, with infection prevalence in parentheses. Tick abundance included pools of larvae separated by grids (questing) and hosts (feeding), individual nymphs, and individual adults. No questing or feeding ticks tested positive for *Anaplasma phagocytophilum*, *Babesia microti*, or *Borrelia miyamotoi*.

Site ID	Site	*Babesia odocoilei*		*Borrelia burgdorferi*	
		Questing	Feeding	Questing	Feeding
1	3 Ridges Farm	0	0	0	0
2	New New Age Farm	1/27 (3.7%)	0/9 (0%)	1/27 (3.7%)	0/9 (0%)
3	North Tract	0	0/2 (0%)	0	0/2 (0%)
4	Brown Hill Tract	0	0	0	0
5	Upjohn Nature Reserve	0	0	0	0
6	Dyer Memorial Nature Reserve	0	0	0	0
7	Rose Hill Nature Reserve	0	0/1 (0%)	0	0/1 (0%)
8	Kirkview Farm	0/4 (0%)	0/1 (0%)	0/4 (0%)	1/1 (100%)
9	Saint-Polycarpe	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
10	Saint-Valentin	1/71 (1.4%)	0/5 (0%)	18/71 (25.4%)	0/5 (0%)
11	Henryville	1/37 (2.7%)	1/5 (20.0%)	3/37 (8.1%)	0/5 (0%)
12	Lefebvre	0/1 (0%)	0	0/1 (0%)	0
13	Parc du Sanctuaire Saint-Majorique	2/14 (14.3%)	0/3 (0%)	0/14 (0%)	1/3 (33.3%)
14	Serpentine-de-Coleraine Ecological Reserve	0/22 (0%)	0	0/22 (0%)	0
15	Frontenac National Park	0/1 (0%)	0	0/1 (0%)	0
16	Saint-Sylvestre	0/1 (0%)	0	0/1 (0%)	0

Table D7. The abundance of infected and total small mammal hosts at our sites in Ontario and Quebec, Canada, with infection prevalence in parentheses. No small mammal hosts tested positive for *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, or *Borrelia miyamotoi*.

Site ID	Site	*Babesia odocoilei*	*Babesia microti*
1	3 Ridges Farm	0/2 (0%)	0/2 (0%)
2	New New Age Farm	0/18 (0%)	0/18 (0%)
3	North Tract	0/6 (0%)	0/6 (0%)
4	Brown Hill Tract	0/5 (0%)	0/5 (0%)
5	Upjohn Nature Reserve	0/2 (0%)	0/2 (0%)
6	Dyer Memorial Nature Reserve	0/2 (0%)	0/2 (0%)
7	Rose Hill Nature Reserve	0/7 (0%)	0/7 (0%)
8	Kirkview Farm	0/5 (0%)	0/5 (0%)
9	Saint-Polycarpe	0/13 (0%)	0/13 (0%)
10	Saint-Valentin	0/9 (0%)	0/9 (0%)
11	Henryville	0/6 (0%)	1/6 (16.7%)
12	Lefebvre	0/5 (0%)	0/5 (0%)
13	Parc du Sanctuaire Saint-Majorique	0/10 (0%)	0/10 (0%)
14	Serpentine-de-Coleraine Ecological Reserve	0/4 (0%)	0/4 (0%)
15	Frontenac National Park	0/1 (0%)	0/1 (0%)
16	Saint-Sylvestre	1/10 (10%)	0/10 (0%)

Table D8. Parameter estimates for the binomial generalized linear mixed models assessing the impact of the abundance of questing and feeding *Ixodes scapularis* on pathogen presence across our sites in Ontario and Quebec, Canada. Pathogen presence indicated whether pathogens were present (1) or absent (0) in *I. scapularis* or in small mammal specimens at a locality. All biotic factors were centred and standardized. A random factor of Site was also included in the models. The model formula for *I. scapularis* is Pathogen presence (0/1) ~ abundance of *I. scapularis* + (1|Site). Significance of terms is denoted by *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Binomial – cloglog link function (AIC: 14.71)

	Estimate	Standard error	*z* value	*P* value
Intercept	14.050	10.500	1.339	0.181
I. scapularis abundance	32.560	22.260	1.462	0.144

Site – Variance = 0, Std. Dev = 0

Binomial – logit link function (AIC: 14.98)

	Estimate	Standard error	*z* value	*P* value
Intercept	14.360	12.260	1.171	0.242
I. scapularis abundance	32.910	25.580	1.287	0.198

Site – Variance < 0.001, Std. Dev < 0.001

Table D9. Parameter estimates for the binomial generalized linear mixed models assessing the impact of the relative abundance of *Peromyscus leucopus* and mammal species richness on pathogen presence across our sites in Ontario and Quebec, Canada. Pathogen presence indicated whether pathogens were present (1) or absent (0) in *I. scapularis* or in small mammal specimens at a locality. All biotic factors were centred and standardized. A random factor of Site was also included in the models. The model formula for mammal hosts is Pathogen presence (0/1) ~ Relative abundance *P. leucopus* + No. mammal species + (1|Site). Significance of terms is denoted by *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Binomial – cloglog link function (AIC: 20.49)

	Estimate	Standard error	*z* value	*P* value
Intercept	-1.353	0.839	-1.614	0.107
Relative abundance *P. leucopus*	0.114	0.718	0.159	0.874
No. mammal species	1.939	1.485	1.306	0.192

Site – Variance = 0.405, Std. Dev = 0.636

Binomial – logit link function (AIC: 15.19)

	Estimate	Standard error	*z* value	*P* value
Intercept	-11.436	5.098	-2.243	0.024*
Relative abundance *P. leucopus*	0.342	4.162	0.082	0.934
No. mammal species	44.440	14.658	3.032	0.002**

Site – Variance = 10795, Std. Dev = 103.9

Table D10. Parameter estimates for the binomial generalized linear mixed model assessing the effect of the relative abundance of *Peromyscus leucopus* and mammal species richness on pathogen prevalence in questing *Ixodes scapularis* across our sites in Ontario and Quebec, Canada. All biotic factors were centred and standardized. In addition, a random factor of Site was included in the model. The model formula is Pathogen prevalence ~ Relative abundance *P. leucopus* + No. mammal species + (1|Site). Significance of terms is denoted by *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Binomial – cloglog link function (AIC: 28.15)

	Estimate	Standard error	*z* value	*P* value
Intercept	-1.813	0.421	-4.305	< 0.001
Relative abundance *P. leucopus*	-0.111	0.498	-0.224	0.824
No. mammal species	0.294	0.990	0.297	0.767

Site – Variance = 0.1289, Std. Dev = 0.359

Binomial – logit link function (AIC: 28.15)

	Estimate	Standard error	*z* value	*P* value
Intercept	-1.718	0.461	-3.724	< 0.001
Relative abundance *P. leucopus*	-0.112	0.550	-0.204	0.838
No. mammal species	0.337	1.109	0.304	0.761

Site – Variance = 0.1507, Std. Dev = 0.3882

Table D11. Parameter estimates for the ordinal logistic regression assessing the effect of the relative abundance of *Peromyscus leucopus* and mammal species richness on the number of pathogen species detected in *Ixodes scapularis* and small mammal specimens across our sites in Ontario and Quebec, Canada. The number of pathogen species (or pathogen diversity) ranged from 0 to 3 distinct species, which represents the four levels of our ordinal variable. All biotic factors were centred and standardized. The model formula is Pathogen diversity ~ Relative abundance *P. leucopus* + No. mammal species. One additional model was assessed after employing the *stepAIC* function from the *cAIC4* package on our full model (Säfken et al. 2021). The additional model was Pathogen diversity ~ No. mammal species. Significance of terms is denoted by *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

	Estimate	Standard error	*t* value	*P* value
Relative abundance *P. leucopus*	0.143	0.633	0.226	0.822
No. mammal species	2.485	1,055	2.355	0.019*
0|1	1.088	0.818	1.330	0.184
1|2	2.160	0.980	2.205	0.028
2|3	4.540	1.593	2.850	0.004**

AIC: 33.152, residual deviance = 23.152

	Estimate	Standard error	*t* value	*P* value
No. mammal species	2.470	1.056	2.339	0.019*
0|1	1.079	0.810	1.333	0.183
1|2	2.147	0.973	2.206	0.027
2|3	4.559	1.606	2.839	0.004**

AIC: 31.203, residual deviance = 23.202

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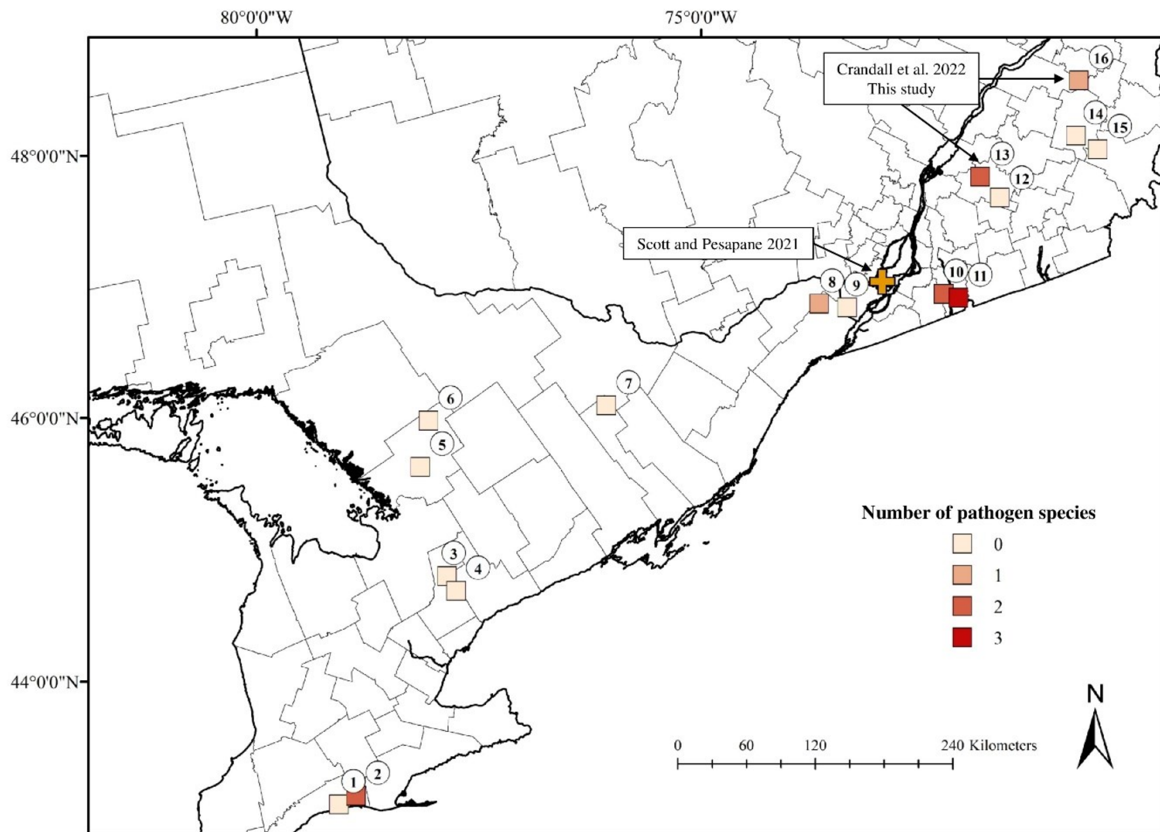

Figure D1. The range limits of *Babesia odocoilei* in Quebec, Canada. Scott and Pesapane (2021) found that *B. odocoilei* was present in *Ixodes scapularis* at Sainte-Anne-de-Bellevue, which previously represented the most poleward detection of this pathogen in Quebec. This study demonstrates that *B. odocoilei* is located further northeast than previously detected, with two questing *I. scapularis* nymphs and one shrew (*Blarina brevicauda*) testing positive for the pathogen near Saint-Majorique-de-Grantham (Site 13) and Saint-Sylvestre (Site 16), respectively.

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Appendix E

Environmental factors limiting the overwintering survival of *Ixodes scapularis* nymphs in Quebec, Canada

Environmental factors limiting the overwintering survival of *Ixodes scapularis* nymphs in Quebec, Canada

Kirsten E. Crandall, Jeremy T. Kerr, and Virginie Millien

Abstract

In temperate regions of North America, the activity, survival, and range expansion of *Ixodes scapularis* are limited by environmental conditions such as temperature, relative humidity, and snow cover. However, most studies examining the relationship between abiotic conditions and the survival of *I. scapularis* have been conducted in a laboratory or in the field using temperature acclimated or reared colony ticks. Using a translocation experiment, we assessed the impact of abiotic conditions on the overwintering survival of *I. scapularis* nymphs in Quebec, Canada. Two treatments were included in our experiment: location (South versus North) and snow cover (snow versus no snow). We monitored the temperature and relative humidity inside tick housing units using iButton data loggers, while the total accumulated snow cover was calculated using weather tower data. As no *I. scapularis* nymphs successfully overwintered, we also assessed the effect of abiotic conditions on the state of decay of our specimens using an ordinal logistic regression. Maximum temperature was significantly related to decay class, with higher temperatures associated with greater decay. The survival of *I. scapularis* nymphs may have been impacted by several factors, including the use of non-acclimated tick specimens, limited energy reserves, and unfavourable abiotic conditions. Therefore, the rate of range expansion and survival of *I. scapularis* nymphs may be impacted by extreme weather events such as heat waves or cold snaps, the frequency and intensity of which are increasing with global change. Future work should further assess the vulnerability of the overwintering life stages of *I. scapularis* (larvae, nymphs, and adults) to abiotic factors across consecutive years at different latitudes.

Keywords: *Ixodes scapularis*, overwintering, survival, temperature, snow cover, climate change, Quebec

Introduction

With climate warming, many species are shifting or expanding their geographic ranges poleward where conditions are more favourable (Roy-Dufresne et al. 2013, Coristine and Kerr 2015). In the Northern Hemisphere, some species may require a distributional shift of up to 20 kilometers poleward per year to remain within their suitable thermal limits (Chen et al. 2011, Roy-Dufresne et al. 2013, Hällfors et al. 2021). Populations at the northern range edge of a species are facing novel environmental conditions (Rehm et al. 2015, Oldfather et al. 2020). These leading edge populations are thus important for the establishment and maintenance of a species in a newly suitable area (Rehm et al. 2015).

Zoonotic disease vectors, such as mosquitoes and ticks, are expected to become more widely distributed with changes in climate, land use, and habitat (Ogden and Lindsay 2016, Rocklöv and Dubrow 2020). The increase in abundance, activity, and geographical distribution of mosquitoes and tick populations in temperate zones has been attributed to climate warming (Ogden and Lindsay 2016). The greater survival and activity of these disease vectors in new locations may result in increased pathogen prevalence and transmission (Ogden and Lindsay 2016, Rocklöv and Dubrow 2020). Therefore, newly established mosquito and tick populations may increase the risk for emerging vector-borne diseases such as West Nile Virus and Lyme disease, respectively (Kulkarni et al. 2015, Ogden and Lindsay 2016, Bouchard et al. 2019).

In temperate regions of North America, the blacklegged tick (*Ixodes scapularis*) is a disease vector of significant public health concern. The life cycle of *I. scapularis* takes two to three years to complete, where the development period between the four life stages (eggs, larvae, nymphs, and adults) may take months (Ogden and Lindsay 2016). This tick vector can transmit several tick-borne pathogens known to cause anaplasmosis, babesiosis, Lyme disease, and Powassan virus (Kulkarni et al. 2015, Bouchard et al. 2019). Ticks rely on the movement of their vertebrate hosts to expand their distributions past their current limits (Caminade et al. 2019, Bouchard et al. 2019). In Canada, the northward range expansion of *I. scapularis* is expected to be facilitated through changes in the behaviour and movements of their vertebrate hosts with climate warming (Ogden and Lindsay 2016, Bouchard et al. 2019, Alkishe et al. 2021). Currently, infected *I. scapularis* occur all across Canada, with long-established populations in Manitoba, Ontario, Quebec, New Brunswick, and Nova Scotia (Kulkarni et al. 2015, Chilton et al. 2019, Foley-Eby et al. 2020, Sperling et al. 2020).

The activity and survival of the different life stages of *I. scapularis* in Canada are limited by environmental variables such as relative humidity (RH) and temperature (Eisen et al. 2016, Ogden and Lindsay 2016, Linske et al. 2019, Volk et al. 2022). Decreased tick activity times and survival occur when these abiotic conditions are outside the optimal thermal or humidity thresholds (Eisen et al. 2016, Ogden and Lindsay 2016). Microclimate refuges can help ticks maintain optimal thermal and humidity conditions due to their insulative properties, especially with variable winter and summer conditions (Bertrand and Wilson 1996, Lindsay et al. 1999, Linske et al. 2019, Volk et al. 2022).

These arthropods are particularly prone to desiccation in the summer months, or freezing during the winter. With the high temperatures and low RH in the summer, ticks are more likely to die due to water loss (Stafford 1994, Ogden et al. 2004, Eisen et al. 2016). To avoid desiccation, ticks will limit their activity by questing at lower vegetation heights, closer to the leaf litter (Vail and Smith 2002, Burtis et al. 2019). Similarly, milder winters may result in limited or inexistant snow cover, which decreases overwintering tick survival because of inoculative freezing (Eisen et al. 2016, Linske et al. 2019, Volk et al. 2022). However, greater amounts of leaf litter and snow may increase tick survival (Eisen et al. 2016, Linske et al. 2019, Volk et al. 2022).

The relationships between temperature, humidity, and *I. scapularis* survival have previously been assessed in a laboratory setting. Nymphs were found to successfully survive at 85% RH for long time periods, with survival dropping below 75% RH (Stafford 1994, Ginsberg et al. 2017). Nymphal *I. scapularis* were found to not survive an incubation temperature of 32°C (Ogden et al. 2004); in contrast, unfed *I. scapularis* nymphs survived short-term cold exposure below their optimal thermal thresholds (Vandyk et al. 1996).

Previous field experiments have also assessed the overwintering survival of *I. scapularis* nymphs. Between 38.6% and 74.3% of unfed *I. scapularis* nymphs were able to successfully overwinter in Ontario, Canada (Lindsay et al. 1995). Studies in the northeastern United States have found variable percentages in overwintering survival of *I. scapularis* nymphs ranging from 27.0% to 93.0% (Brunner et al. 2012, Linske et al. 2019, Tufts et al. 2020, Volk et al. 2022). Of importance, leaf litter and snow cover were found to contribute significantly to greater overwintering survival of *I. scapularis* nymphs (Linske et al. 2019, Volk et al. 2022).

However, most studies examining the relationship between environmental conditions and tick survival were conducted in laboratories or in the field using either temperature acclimated or reared colony ticks (e.g., Lindsay et al. 1995, Vandyk et al. 1996, Bertrand and Wilson 1996, Ogden et al. 2004, Ginsberg et al. 2017, Burtis et al. 2019, Linske et al. 2019, Tufts et al. 2020, Volk et al. 2022). Here, we conducted a field-based translocation experiment to test for the impact of abiotic conditions on the overwintering survival of naturally occurring (i.e., field collected and not temperature acclimated) *I. scapularis* nymphs in Quebec, Canada. We found that winter conditions including temperature extremes and variability as well as desiccation stress impact the overwintering *I. scapularis* survival and decay. This study helps identify potential environmental factors such as temperatures extremes and low relative humidity that may impact overwintering survival and potential range expansion of *I. scapularis* in Quebec and, more generally, in Canada.

Materials and Methods

Tick collection

In August 2021, 118 nymphs were collected by tick dragging, where a 1m^2 cotton flannel was dragged over low-lying vegetation at two nearby sites in Saint-Valentin, Quebec (45.185°N, 73.347°W) and Henryville, Quebec (45.118°N, 73.211°W). Collected ticks were not acclimated in a laboratory, and were placed in aerated vials that were embedded in natal soil in housing units for less than 24 hours until they were installed at the two field sites. Ticks not active after a breath test were removed from the experiment, resulting in 100 remaining ticks.

Treatments

We tested two treatments: location and snow cover. A total of 50 ticks were set at each of our field site located approximately 520 kilometers apart: a southern natal site in Saint-Valentin, Quebec (45.185°N, 73.347°W) and a northern transplant site in Chibougamau, Quebec (49.809°N, 74.469°W). Saint-Valentin is located in Montérégie, an endemic region for *I. scapularis*, while Chibougamau is located in the Nord-du-Québec, where *I. scapularis* are not detected (Institut national de santé publique du Québec 2022). At each location, we tested the impact of snow cover with 25 ticks per treatment (snow versus no snow).

Placement of tick housing units

The design of housing units was tested in 2019, confirming the containment of ticks and the possibility of overwintering survival of *I. scapularis* (Crandall 2020, unpublished data). Eight 5-litre plastic buckets with snap-on lids were modified to simulate the natural environment and test the overwintering survival of *I. scapularis* nymphs (Figure S1 and S2; Lindsay et al. 1995). These units were filled with 80 mm of natal soil, where openings were cut in the sides and bottom and covered with mesh to maintain adequate aeration of the soil throughout the duration of the experiment. Ticks were placed into 90 mL polypropylene vials with 50 mL of soil, with mesh covered openings in the top and bottom. The mesh allowed for water and air flow, while preventing ticks from escaping. Two to three vials containing three to seven nymphs were embedded into the soil in each housing unit. We then embedded four tick housing units into 80 mm deep holes in Chibougamau on August 26th and in Saint-Valentin on August 27th. For the treatment with no snow cover, units were placed under a raised 104-litre container leaving a 5 cm gap with the leaf litter (Figure S3).

Abiotic variables

In each housing unit, temperature and RH were monitored using DS 1923-F5# Hygrochron Temperature and Relative Humidity iButtons (Maxim Integrated Products, California, United States) placed on the soil inside the unit. Data loggers were programmed to take high resolution detections at six-hour intervals (6 AM/PM and 12 AM/PM) for temperature and RH (0.0625°C and 0.04%, respectively). A layer of plastic wrap was placed around the seal of the iButtons to prevent malfunctioning due to water. For each unit, the mean, maximum, and minimum temperature and RH (compensated by temperature) were calculated.

Between August 2021 and May 2022, we extracted historical measurements for snow cover from weather towers using snow on the ground (SOG) in cm, discarding estimated and flagged values (Environment and Climate Change Canada 2022). We calculated the daily snow accumulation as the difference in snow on the ground between two days (i.e., (n+1) – n). If the difference was greater than 0, then this value was used. Otherwise, the value became zero. These calculated values were then summed for the total snow accumulation over the duration of the experiment.

Assessing overwintering survival

In May 2022, housing units were removed from the field and warmed to room temperature to assess the overwintering survival of *I. scapularis* nymphs. The contents of each vial were thoroughly examined by hand using a white pan (Burtis 2017). A tick was designated as alive if it moved out of line within 10 minutes or responded to direct breathing during this time (Vandyk et al. 1996). Ticks not found in vials after searching were considered dead.

Assessing the state of decay

As no nymphs successfully overwintered, we also assessed the impact of the treatments on the state of decay of our specimens, where greater decay should occur with a longer estimated time since death or warmer abiotic conditions (Duncan et al. 2003). Using a stereomicroscope, each tick specimen's state of decay was examined and categorized into 4 classes based on the presence of body parts ranging from class 1 (full body and partial or full legs) to class 4 (intact mouthparts or head or body). An ordinal logistic regression was conducted using the *polr* function in the MASS package (Venables and Ripley 2002) to assess the impact of abiotic factors on the specimen's decay class. Fixed factors included mean, maximum, and minimum temperature and mean RH from iButtons as well as total accumulation of snow on the ground. The *stepAIC* function was used to identify the best model with the lowest AIC (R Core Team 2021).

Results

Temperature and relative humidity values differed in the housing units across treatments (Figure E1 and E2; Table E1). Warmer maximum temperatures were detected in Saint-Valentin with snow cover (27.0°C versus 18.2°C) and without snow cover (23.0°C versus 17.2°C) compared to Chibougamau. Cooler minimum temperatures were found in Chibougamau without snow cover (-9.6°C versus -7.4°C) and with snow cover (-6.2°C versus -3.1°C) compared to Saint-Valentin. The consecutive number of days with snow cover differed between locations, with 89 days in Saint-Valentin and 166 days in Chibougamau. Similarly, a greater accumulation of snow occurred in Chibougamau with 164 cm versus 110 cm in Saint-Valentin. Humidity remained above 80% for the majority of the duration of the experiment, with RH dropping below this value in some housing units in spring (Figure E2).

No nymphal ticks successfully overwintered in our translocation experiment. We were unable to recover 33 ticks, which were presumed dead as we found no evidence that these nymphs could have escaped from the housing units. In Saint-Valentin, 32 *I. scapularis* nymphs and 1 *Haemaphysalis leporispalustris* nymph were identified. In Chibougamau, 33 *I. scapularis* nymphs were detected and one specimen that could not be identified to species due to degradation. We removed these two non-*Ixodes* specimens from further analyses.

There was a greater variability in the decay class of *I. scapularis* nymphs in Saint-Valentin, but there was no significant difference between the frequencies across the location and snow cover treatments ($X^2 = 13.11$, $p = 0.158$; Figure S4). In Saint-Valentin, the counts of each decay class (class 1 to 4) included 22 nymphs, 8 nymphs, one nymph, and one nymph, respectively. In Chibougamau, the counts per decay class (class 1, 2, and 4) included 29 nymphs, three nymphs, and one nymph, respectively. The best model identified through stepwise selection demonstrated a significant effect of maximum temperature on decay class ($t = 2.395$, $p = 0.017$; Figure E3).

Discussion

We found that environmental factors related to winter conditions such as temperature, relative humidity, and snow cover may limit overwinter survival rate in *I. scapularis* nymphs. Between locations, we found a five to eight degree difference in maximum temperature and a three degree difference in mean temperature, with warmer temperatures in Saint-Valentin (Table E1). Within locations, there was a three to five degree difference in minimum temperature between snow cover treatments. While none of the ticks in our experiment successfully overwintered, we found that the maximum temperature was significantly related to the state of decay of our specimens. As decay proceeds after death, a sequence of morphological stages occurs with the disarticulation of body parts through time (Duncan et al. 2003). At higher temperatures, specimens were less likely to be categorized as class 1 (i.e., specimen fully intact) and more likely to be identified as class 2 or above (i.e., specimen partially intact; Figure E3). At Saint-Valentin, *I. scapularis* nymphs may be more susceptible to decay, as higher temperatures were recorded at this site throughout the experiment compared to Chibougamau. Furthermore, an earlier spring thaw and sustained high temperatures in April and May in Saint-Valentin likely led to greater condensation levels in the housing units, resulting in an accelerated decay process.

Several factors might have contributed to the *I. scapularis* nymphs being unable to successfully overwinter. First, the *I. scapularis* nymphs used in our experiment were not reared from a laboratory colony, but they were collected in the field. These specimens were not temperature acclimated before our experiment, which rarely occurs in overwintering studies of *I. scapularis* (e.g., Scott and Scott 2018). In studies with laboratory acclimated ticks, the overwintering survival of unfed *I. scapularis* nymphs ranged from 27.0% to 93.0% in the northeastern United States and from 38.6% and 74.3% in Ontario (Lindsay et al. 1995, Brunner et al. 2012, Linske et al. 2019, Tufts et al. 2020, Volk et al. 2022). Therefore, acclimation to cooler temperatures before a field experiment may allow *I. scapularis* nymphs to decrease their low temperature limit for overwintering survival (Yu et al. 2014). However, the majority of ticks used in our experiment were field collected from Saint-Valentin, Quebec, so we expected them to be relatively capable of tolerating local environmental conditions.

A more likely hypothesis for the lack of overwintering survival of *I. scapularis* nymphs in our experiment was due to a shortage of sufficient energy reserves. Stafford (1994) found that the maximum number of days survived by *I. scapularis* nymphs in a laboratory study ranged from 162 days at 85% RH and 210 days at 100% RH. The rapid decline in the number of surviving *I. scapularis* nymphs after long-term survival may be due to a depleted energy reserves. In a more natural setting, field collected nymphs survived over a year under ambient field conditions in Massachusetts (Yuval and Spielman 1990). Finally, the median time to death of engorged *I. scapularis* larvae in Connecticut and Rhode Island was 211 days, with 100% mortality after 11 months (Tufts et al. 2020). Therefore, the high mortality level we observed may be due to the length of our experiment (265 and 269 days), a duration at which the energy stores of nymphal ticks might have been entirely depleted.

Next, changes in humidity levels and snow cover observed throughout the experiment at both our field sites may have increased the mortality rate of the nymphs. Overall, the average RH in the housing units was above 80% during the experiment. Brunner et al. (2012) found that mortality risk was greatest at high RH combined with low temperature, especially during winter as ticks can be directly exposed to ice crystals. Similarly, nymphal *I. scapularis* survival decreases when leaf litter and snow are removed, as they provide an insulating barrier to harsher environmental conditions (Linske et al. 2019, Volk et al. 2022). Therefore, the lower

temperatures of housing units without snow cover, especially in Chibougamau, may have led to inoculative freezing and subsequent mortality.

Finally, we observed considerable variation in RH at our study locations, with sustained periods of RH below 80% during late spring, similar to Brunner et al. (2012). Although some *I. scapularis* nymphs may be able to survive up to a week at 65% RH (Stafford 1994, Berger et al. 2014), a drop in RH below 80% for several consecutive days can be detrimental to tick activity and survival because of a greater chance of desiccation. In our experiment, continuous periods of low humidity lasting longer than a week occurred in April and May, in conjunction with very high temperatures (up to 27°C) in Saint-Valentin. These warmer temperatures with lower humidity levels require higher energy use by ticks, which may have contributed to higher mortality (Burtis et al. 2019, Volk et al. 2022).

While the distribution range of *I. scapularis* is expected to shift poleward under global change (Ripoche et al. 2022), the increase in frequency and severity of extreme weather events may limit the survival of overwintering nymphs, altering the rate of establishment of tick populations at the leading range edge of this species. With climate warming, milder temperatures or short warming periods during the winter may lead to intermittent snow cover and decreased insulation, further decreasing tick survival (Volk et al. 2022). Conversely, cold snaps that occur during periods of limited snow cover may lead to increased tick mortality due to inoculative freezing. In addition, heat waves during the spring and summer at lower latitudes are associated with low RH, which may lead to increased tick mortality due to desiccation stress (Eisen et al. 2016, Ogden and Lindsay 2016, Ginsberg et al. 2017, Burtis et al. 2019). The nature of the litter may also influence tick survival, as deciduous litter found at lower latitudes is more favourable for ticks than the coniferous litter found at higher latitudes (Volk et al. 2022). It is unknown if *I. scapularis* ticks that have dispersed to areas outside of their current range (adventitious populations) can overwinter successfully. While a greater snow accumulation in these northern regions can provide positive insulating properties, less favourable environmental and habitat conditions such as colder winter temperatures and coniferous litter may negatively affect tick survival (Brunner et al. 2012). As a result, the rate of range expansion of *I. scapularis* may be slowed by weather variability and extremes including heat waves and cold snaps.

Future work should assess the vulnerability of the overwintering *I. scapularis* life stages (larvae, nymphs, and adults) to temperature, RH, and snow cover across consecutive years at

different latitudes. Here, we found that no *I. scapularis* nymphs successfully overwintered in our translocation experiment, indicating that the survival and range expansion of this life stage may be vulnerable. Lindsay et al. (1995) found that unfed nymphs and fed females generally had higher overwintering survival than unfed adults across four localities in Ontario, Canada. Unfed nymphs are also thought to have the greatest cold hardiness based on previous laboratory experiments (Vandyk et al. 1996). However, nymphal *I. scapularis* survivorship is found to be lower in spring and early summer when they have depleted energy reserves (Burtis et al. 2019). Therefore, future studies should strive to untangle the impact of abiotic conditions on the overwintering survival of *I. scapularis* ticks in Canada. If climatic variability and extremes negatively impact the survival and geographic extent of tick populations, it may decrease the likelihood of pathogen spread and transmission in areas outside their current range. As a result, weather variability and extreme climatic events should be considered when modelling the current and future distributions of zoonotic disease vectors under global change to better estimate the rate of their range expansion.

Acknowledgements

We express our thanks to the landowners and stakeholders that allowed access to their property to run the translocation experiment. We want to gratefully thank our assistants for their invaluable help collecting ticks. K.E.C. is supported by Hydro-Québec and NSERC fellowships. V.M. is supported by a NSERC Discovery Grant. J.T.K. is supported by funds from an NSERC Discovery Grant, NSERC Discovery Accelerator Supplement, and the University Research Chair in Macroecology and Conservation at the University of Ottawa.

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Table

Table E1. Temperature and relative humidity values by location and snow treatment extracted from iButtons (DS 1923-F5#) placed inside tick housing units during the translocation experiment in Quebec, Canada. At each study site, accumulated snow on the ground was calculated using snow cover data extracted from nearby weather towers (Environment and Climate Change Canada 2022).

Location	House	Snow	Maximum temperature (°C)	Minimum temperature (°C)	Mean temperature (°C)	Mean relative humidity (%)	Accumulated snow on ground (cm)
Chibougamau	H1	Snow	18.63	-6.67	2.23	99.20	164
Chibougamau	H2	Snow	17.82	-5.73	2.16	95.83	164
Chibougamau	H1	No snow	17.13	-9.60	1.09	98.26	164
Chibougamau	H2	No snow	17.33	-9.53	1.07	96.96	164
Saint-Valentin	H1	Snow	27.49	-3.46	5.44	99.45	110
Saint-Valentin	H2	Snow	26.35	-2.66	5.59	97.55	110
Saint-Valentin	H1	No snow	23.45	-6.90	4.77	86.68	110
Saint-Valentin	H2	No snow	22.48	-7.89	4.70	83.65	110

Figures

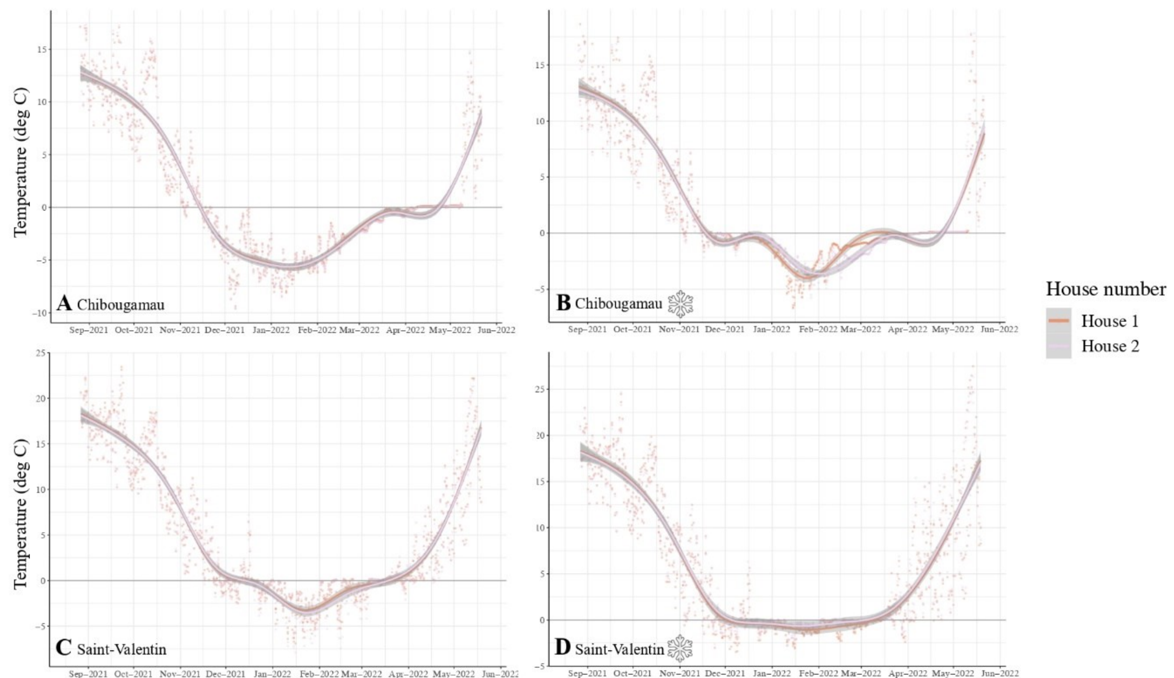

Figure E1. Temperature values obtained by iButtons (DS 1923-F5#) placed inside tick housing units containing *Ixodes scapularis* nymphs during the translocation experiment in Quebec, Canada. Housing units were placed at two different study sites in Quebec: Chibougamau (A-B) located at 49.809°N, 74.469°W and Saint-Valentin (C-D) located at 45.185°N, 73.347°W. Snow cover was the second treatment that was manipulated, with no snow cover (A and C) versus natural snow cover (B and D) at each study site. Generally, temperatures in housing units were lower in Chibougamau and when no snow cover was present.

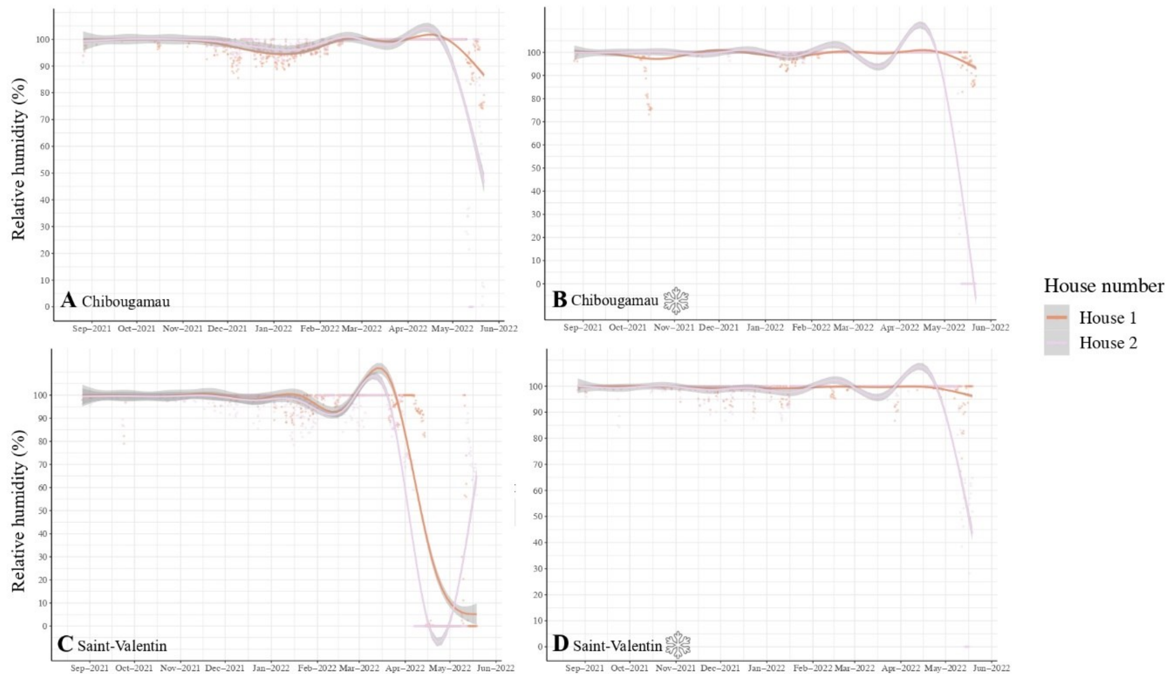

Figure E2. Relative humidity (RH) values obtained by iButtons (DS 1923-F5#) placed inside tick housing units containing *Ixodes scapularis* nymphs during the translocation experiment in Quebec, Canada. Housing units were placed at two different study sites in Quebec: Chibougamau (A-B) located at 49.809°N, 74.469°W and Saint-Valentin (C-D) located at 45.185°N, 73.347°W. Snow cover was the second treatment that was manipulated, with no snow cover (A and C) versus natural snow cover (B and D) at each study site. Generally, RH values in housing units were stable and above 80% RH throughout the experiment until early spring.

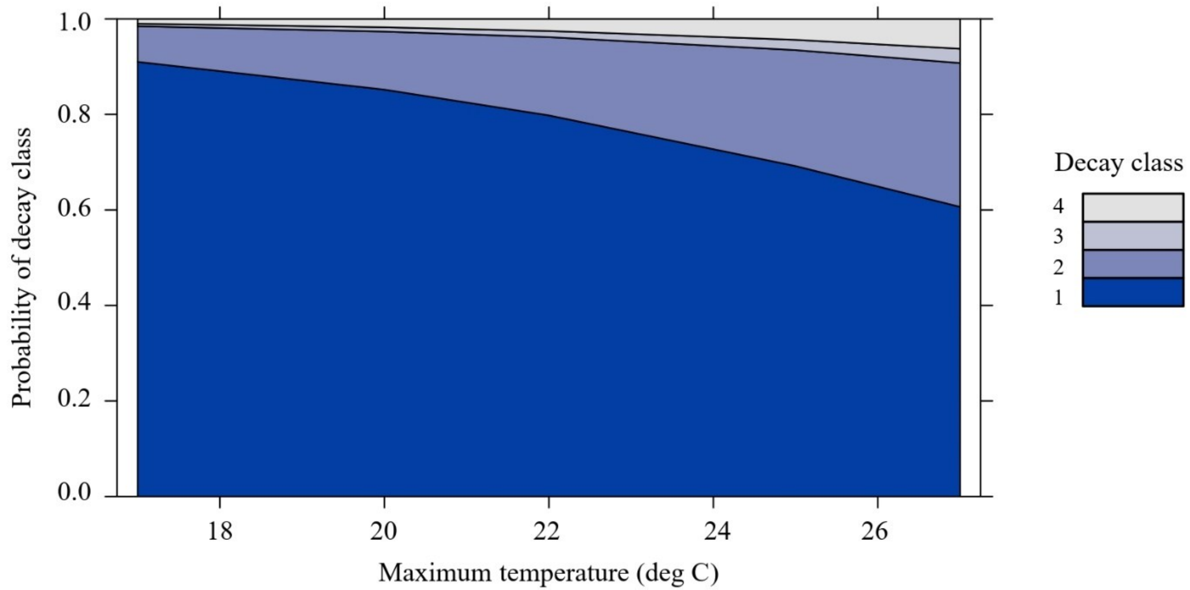

Figure E3. State of decay of *Ixodes scapularis* specimens is significantly related to maximum temperature, with varying probabilities based on distinct classifications of decay class. At lower temperatures, tick specimens have the highest probability of being less degraded and classified as class 1. In contrast, tick specimens were more likely to have increased degradation at higher temperatures, with greater probabilities of being defined as class 2 to 4.

Supplementary information for Appendix E

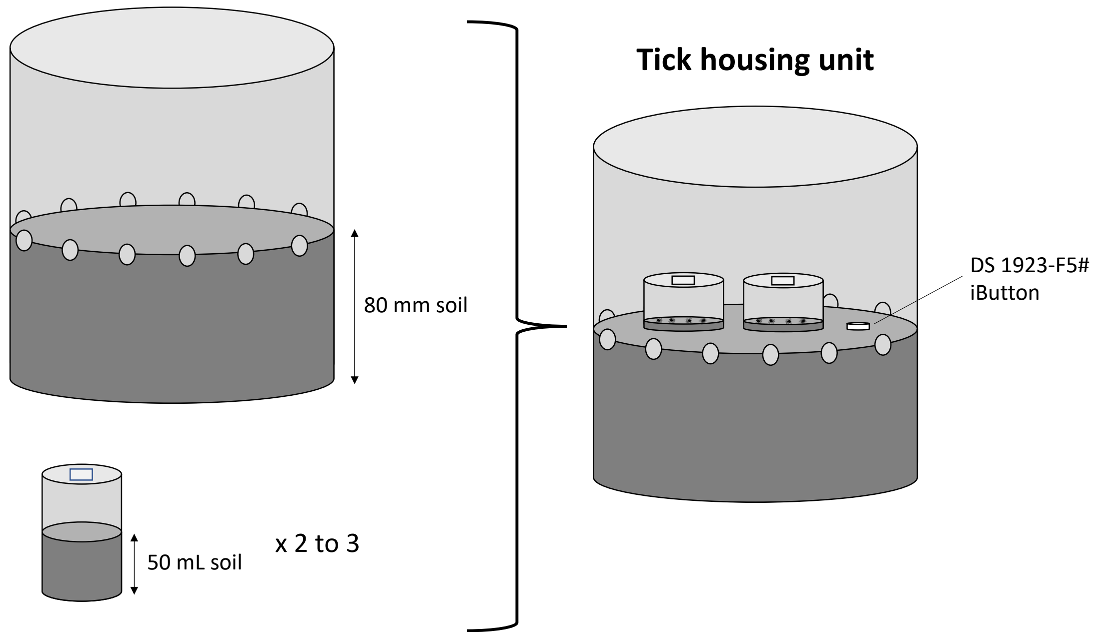

Figure S1. Schematic of the tick housing units created for our translocation experiment in Quebec, Canada. Each housing unit was created with 5-litre plastic buckets with snap-on lids, where two to three polypropylene vials were placed inside and embedded in soil. A DS 1923-F5# Hygrochron Temperature and Relative Humidity ibutton was placed on the soil inside the unit.

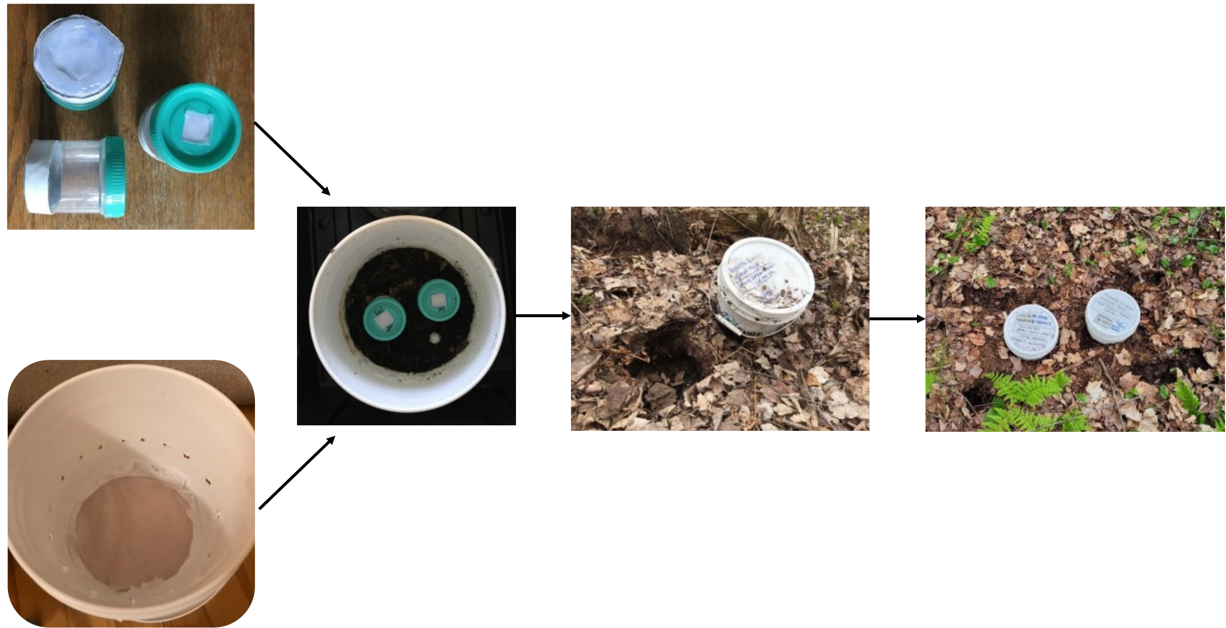

Figure S2.– Tick housing units were created using a 5-litre plastic bucket with a snap-on lid, where openings were cut and covered with mesh in the sides and bottom. Ticks were placed into 90 mL polypropylene vials that had mesh covering openings in the top and bottom. Two to three vials containing nymphs were embedded into the soil in each housing unit. At each study site, housing units were embedded into 80 mm deep holes.

Chibougamau, Quebec

Saint-Valentin, Quebec

Figure S3. Two treatments were tested in our translocation experiment: location and snow cover. The two locations were a southern natal site in Saint-Valentin, Quebec (45.185°N, 73.347°W) and a northern transplant site in Chibougamau, Quebec (49.809°N, 74.469°W). At each location, the treatment with no snow cover placed housing units under a raised 104-litre container leaving a 5 cm gap with the leaf litter (see bottom row).

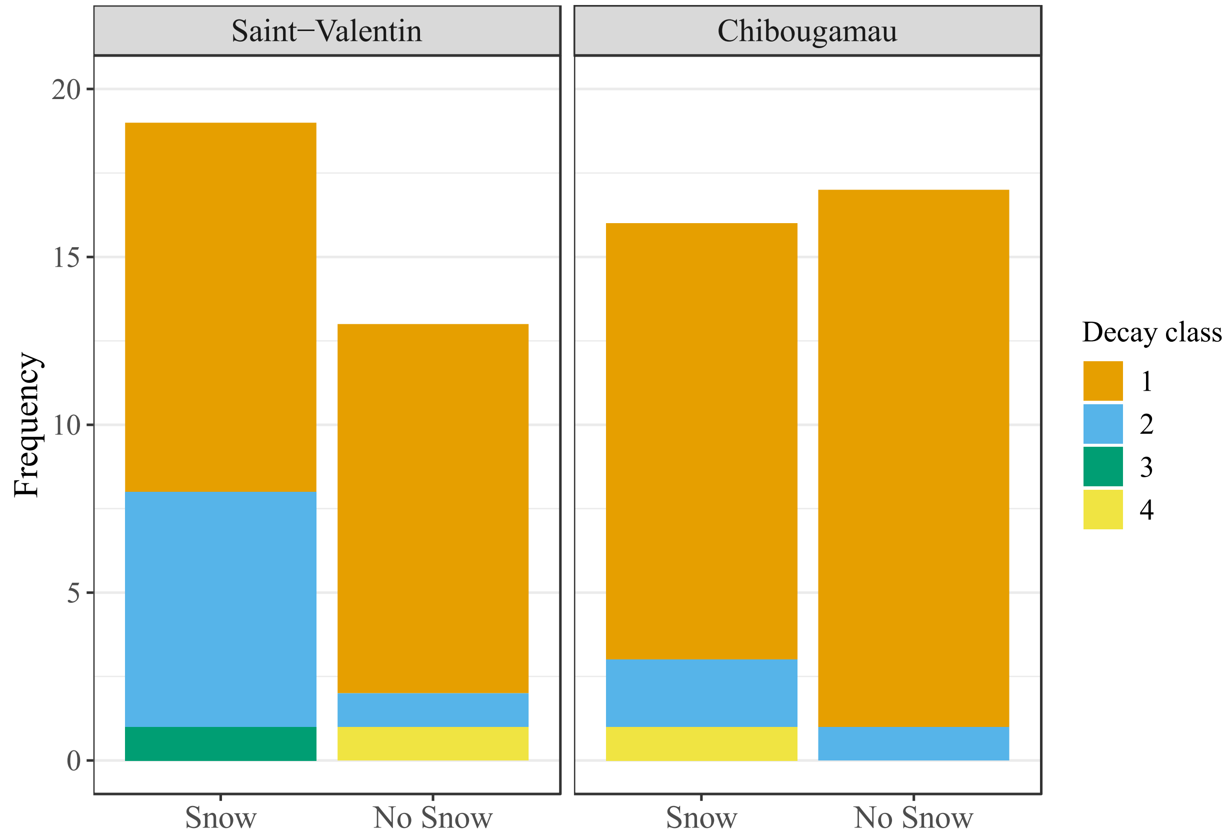

Figure S4. Frequency of decay classes identified for each *Ixodes scapularis* specimen retrieved from the tick housing units. State of decay was classified into 4 classes based on the presence of body parts at examination: complete head, body, and partial or full legs (class 1, orange), partial or full mouthparts, head, and body (class 2, blue), mouthparts and head or head and body (class 3, green), and intact mouthparts or head or body (class 4, yellow). The majority of *I. scapularis* nymphs were categorized into class 1 and 2.