Presence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum*., *Babesia spp*., *Bartonella spp.* and Powassan virus within *I. scapularis* in Halifax Regional Municipality, Nova Scotia, Canada

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Introduction

Host:

Ixodes scapularis, known by the common names “deer tick” and “blacklegged tick” is a hardbacked tick in the family Ixodidae (Lindquist et al. 2016).
These arachnids feed on a variety of animals including birds and mammals, including dogs, cats, deer, cows, and sometimes humans (Anderson 1988)

Figure 2. Adult female *Ixodes scapularis* tick.

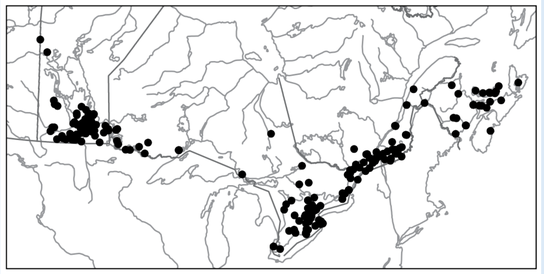

Figure 1. Collection localities for *I. scapularis* in Canada (map 13, Lindquist et al.).

Range:

- Expected to expand Northward in all future climate scenarios (McPherson et al. 2017)
- Their populations in Nova Scotia have also been increasing in recent years, increasing the risk of contracting tick-borne diseases (Ogden et al. 2014)

Pathogens of concern:

I. scapularis is known to be a vector for 16 human pathogens (Nelder et al. 2016). The following are being studied in this report:

***Borrelia burgdorferi*:**

- Causative agent of Lyme disease
- Spirochete bacteria
- 26.2% disease prevalence within *I. scapularis* in Nova Scotia as of 2019 (Wilson et al. 2022)
- Cases of Lyme disease in Nova Scotia have been increasing in recent years, with 595 being reported in 2021 alone (PHAC 2021)

***Bartonella spp.*:**

- Causative agent of bartonellosis (including trench fever and cat scratch disease)
- Gram-negative hemoparasitic bacteria
- There is no evidence of tick-human transmission (Telford and Wormser 2010)
-75.16% disease prevalence within I. scapularis in Nova Scotia as of 2021 (Kho et al. 2021)

***Anaplasma phagocytophilum*:**

- Causative agent of anaplasmosis
- Rickettsial bacteria
- 3.9% disease prevalence within *I. scapularis* in Nova Scotia as of 2019 (Wilson et al. 2022)
- There have been 2 confirmed cases of anaplasmosis in humans within Nova Scotia (NS ZWG 2021)

***Babesia spp.*:**

- Some species are causative agents of babesiosis
- Intracellular apicomplexan (protozoan) parasites
- *Babesia microti* has not been found within *I. scapularis* in Nova Scotia (Wilson et al 2022)
- A single locally acquired case of babesiosis was reported in 2021 (Allehebi et al. 2022)

Powassan Virus:

- Causative agent of Powassan encephalitis
- Flavivirus in the family *Flaviviridae*
- Only one tick found with POWV in Nova Scotia (translates to 0.6% prevalence) (Wilson et al. 2022)
- No case of Powassan virus have been reported in Nova Scotia (NS ZWG 2021)

Aim of study and Hypothesis:

Determine the prevalence of these pathogens in Halifax Regional Municipality (HRM), and if there are any significant correlations with sex and location. Coinfection rates between the pathogens will also be determined.

I hypothesize that the prevalence of pathogens will increase compared to previous years.

Methods

Field Collection:

Approximately 250 unfed adult *I. scapularis* ticks were collected from 5 locations around Halifax Regional Municipality in Nova Scotia, Canada between the months of June and October 2023.

Ticks were caught using the “flag dragging” method and kept in plastic bags with a wet tissue to maintain moisture.

Figure 4. Author demonstrating “flag dragging” method.

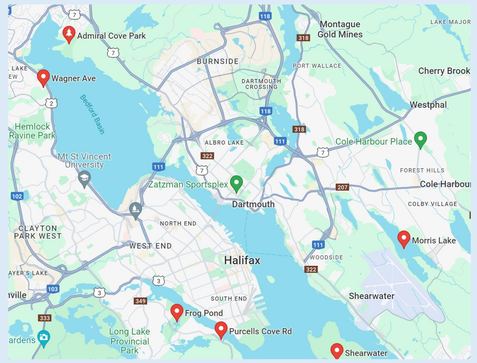

Figure 3. Collection sites for I. scapularis within Halifax Regional Municipality (Google Maps).

DNA and RNA Extraction:

The ticks were cut in half along the sagittal plane using a scalpel, with one half being used for DNA extraction, and the other half being used for RNA extraction.

DNA:
-Extracted using AquaGenomic solution as per developer's instructions

RNA:
- Preserved using RNAlater stabilization solution
- Extracted using RNeasy® Mini Kit as per developer’s instructions

PCR and gel electrophoresis:

- Master mix was created by combining primers, denucleated water, and GoTaq Green
- Nested PCR was used for *B. burgdorferi* to increase sensitivity and ensure positive prevalence
- RT-PCR was used to test for Powassan virus
- Amplicons were visualized on a 2% agarose gel along with a 100 bp ladder, run for approximately 30 minutes at 100V, and photographed

Table 1. Primers used in PCR.

Pathogen	Primer	Sequence (5’-3’)	Length of Amplicon	Target Gene	Source
Borrelia burgdorferi	OspA_Out_R1	GTTAGCAGCCTTGACGAGA	272 bp	OspA	Ogden et al. 2006
	OspA_Out_F1	GATACTAGTGTTTTGCCATC			
	OspA_In_R1	GCGTTTCAGTAGATTTGCCTG	214 bp		Patterson et al. 2017
	OspA_In_F1	TCAAGTGTGGTTTGACCTAG			
	FlaB_Out_R1	AATTGCATACTCAGTACTATTCTTTATAGAT	612 bp	FlaB	
	FlaB_Out_F1	AAGTAGAAAAAGTCT TAGTAAGAATGAAGGA			
	FlaB_In_R1	AAGGTGCTGTAGCAGGTGCTGGCTGT	390 bp		
	FlaB_In_F1	CACATATTCAGATGCA GACAGAGGTTCTA			
Powassan Virus	POW-6	TTGTGTTTCCAGGGCAGCGCCA	689 bp	TBE virus envelope gene (env)	Anderson and Armstrong 2012
	ENV-A	GTCGACGACGAGGTGCACGCATCTTGA			
Anaplasma phagocytophilum	16SANA-F	CAGAGTTTGATCCTGGCTCAGAAC	421 bp	16S rRNA	Stuen et al. 2003
	16SANA-R	GAGTTTGCCGGG ACTTCTTCTGTA			
Babesia spp.	BJ1	GTCTTGTAATTGGAATGATGG	488-952 bp	18S rRNA	Stensvold et al. 2015
	BN2	TAGTTTATGGTTAGGACTACG			
Bartonella spp.	P24-E	GGAATTCCCTCCTTCAGTTAGGCTGG	279 bp	16S-rRNA	Eskow et al. (2001)
	P-12B	CGGGATCCCGAGATGGCTTTTGGAGATTA			Carey et al. (2019)

Controls:

Negative: Denucleated water and master mix
Positive: Concentrated form or DNA from ticks previously found to be positive for each pathogen

Results

Table 2. Locations and sexes of *I. scapularis* collected between June and October 2023.

	McNabs Island	Morris Lake	Wagner Ave.	Frog Pond	Admiral's Cove	Spryfield
Males	33	1	48	93	1	6
Female	23	2	44	76	6	8
Total	56	3	92	169	7	14

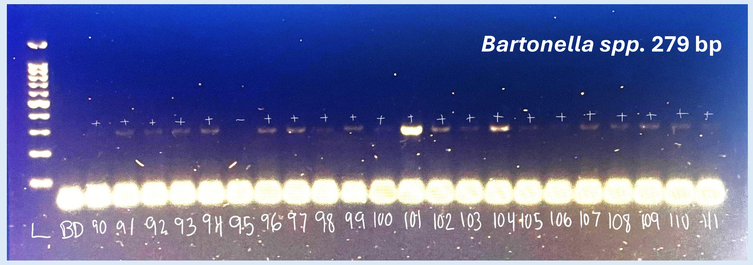

Figure 5. Gel electrophoresis results for *Bartonella spp.*

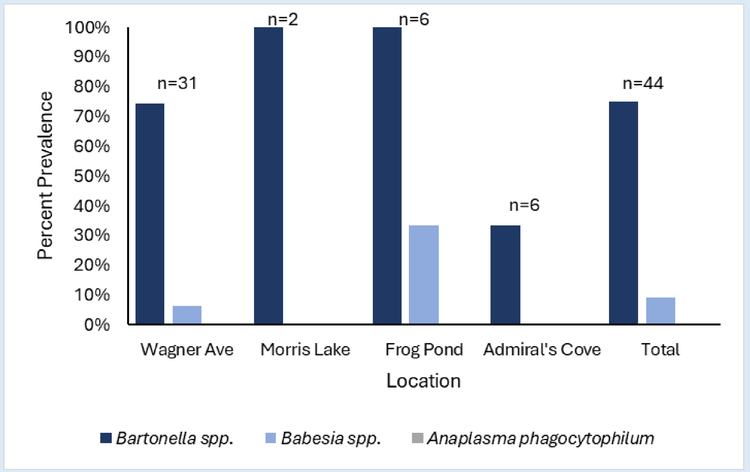

Figure 6. Percent prevalence for three of the pathogens for five locations in the HRM.

Discussion and Next Steps

- Prevalence of *Bartonalla spp.* (75%) is already appearing very similar to what was observed in 2021 (75.16%) (Kho et al. 2021)
- Currently, no ticks are positive for *Anaplasma phagocytophilium*, which is only slightly lower than the 3.9% prevalence observed in 2019 (Wilson et al. 2022)
- *Babesia spp*. have a prevalence of approximately 9% so far. This is a large increase compared to the 0% expected (Wilson et al 2022)
    - *Babesia spp.* has been observed in racoons in Nova Scotia at a prevalence of 6% between 2015 and 2018, with all of the infections being from *B. microti* or a *B. microti* like species (Garrett et al 2019)

Next Steps

- Extract and test DNA from more ticks
- Test ticks for Powassan virus and *Borrelia burgdorferi*

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