

ORIGINAL ARTICLE

Active surveillance of pathogens from ticks collected in New York State suburban parks and schoolyards

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Abstract

Schoolyards and suburban parks are two environments where active tick surveillance may inform local management approaches. Even in a state such as New York with a robust active tick surveillance programme operated by the state Department of Health, these settings are not routinely covered. The goal of this study was to highlight the importance of active surveillance for tick-borne pathogens by describing their prevalence in ticks collected from schoolyards and suburban parks and to guide the use of integrated pest management in these settings. Tick dragging was performed in three regions of New York State: Long Island, the Lower Hudson Valley and the Capital Region. A total of 19 schoolyards and 32 parks were sampled. The location, habitat and weather at the time of tick collection were recorded. Ticks were speciated and tested for the presence of 17 pathogens with a novel application of nanoscale real-time PCR. The causative agents of Lyme disease, anaplasmosis, babesiosis and Powassan virus disease were all detected from *Ixodes scapularis* in various sites throughout the capital region and south-eastern counties of New York state. The most common agent detected was *Borrelia burgdorferi*, and coinfection rates were as high as 36%. This surveillance study also captured the first of the invasive Asian longhorned tick species, *Haemaphysalis longicornis*, in New York state (collected 2 June 2017). Results from this study highlight the importance of collaborative efforts and data sharing for improvement of surveillance for tick-borne disease agents.

Yuan and Llanos-Soto contributed equally.

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KEYWORDS

Asian longhorned tick, Blacklegged tick, school health and safety, surveillance, Tick-borne infections

1 | INTRODUCTION

Tick-borne disease remains a crucial focus for public health institutions in the United States. Over 300,000 human cases of Lyme disease are estimated to occur per year (Centers for Disease Control and Prevention, 2019b), and the disease incidence is rising as a result of both improved diagnostics and geographical expansion (Bacon, Kugeler, Mead, & Centers for Disease Control and Prevention (CDC), 2008). Reported cases of human granulocytic anaplasmosis have also increased from 348 in 2000 to 5,762 in 2017 (Aliota et al., 2014; Centers for Disease Control and Prevention, 2019a; Dantas-Torres, 2007). The increasing number of human cases of tick-borne diseases in the United States has prompted tick surveillance studies as well as medical case evaluations across states where Lyme disease and other tick-borne diseases are endemic (Aliota et al., 2014; Dupuis et al., 2013; Fatmi, Zehra, & Carpenter, 2017; Sung et al., 2013).

Ixodes scapularis is considered one of the most medically important tick species in the United States because it is responsible for 90% of all reported tick-borne diseases in humans (Rosenberg et al., 2018; Spielman, Clifford, Piesman, & Corwin, 1979; Tokarz et al., 2019). In addition to transmitting *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, causative agents of Lyme disease and anaplasmosis, it is also a vector for *Babesia microti* (Burgdorfer et al., 1982; Pancholi et al., 1995) and *Borrelia miyamotoi* (Scoles, Papero, Beati, & Fish, 2001). *Ixodes scapularis* is also responsible for disseminating deer tick virus (DTV) or Powassan virus lineage II, a causative agent of tick-borne encephalitis that can induce severe health consequences if transmitted to humans (Dupuis et al., 2013; Telford et al., 1997). While *I. scapularis* is the predominant tick species responsible for disease agent transmission in the United States, other species including *Dermacentor variabilis* are also involved in the transmission of human and animal pathogens (Ammerman et al., 2004; Staples, Kubota, Chalcraft, Mead, & Petersen, 2006). *Haemaphysalis longicornis* is an invasive species in eastern North America that appears to be parthenogenetic. This aspect of the tick's biology, along with its propensity to feed on a wide variety of hosts in large numbers (Greay et al., 2016; Oakes et al., 2019; Rainey, Occi, Robbins, & Egizi, 2018), has raised concern among public health officials, wildlife conservationists and vector biologists.

Tick-borne diseases have historically been a major public health issue in New York State (NYS), an area considered as hyperendemic for *I. scapularis* (Aliota et al., 2014; Centers for Disease Control and Prevention, 2019b, 2019a; Piedmonte, Shaw, Prusinski, & Fierke, 2018; Tokarz, Jain, Bennett, Briese, & Lipkin, 2010; Tokarz et al., 2019; Varde, Beckley, & Schwartz, 1998). Thanks to rigorous active surveillance and open data sharing by the NYS Department of Health (NYSDOH), the distribution and density of *I. scapularis* in NYS are well appreciated. They have been associated with that of the

Impacts

- Identifies potential vector-borne disease health risks in areas frequented by children and other vulnerable populations.
- Documents deer tick virus presence in Nassau County, New York.
- Compiles tick-borne pathogen data for the New York capital region and understudied counties in the southeastern part of the state.

white-tailed deer (*Odocoileus virginianus*), which are crucial hosts for the adult tick stage (Wilson, Ducey, Litwin, Gavin, & Spielman, 1990). Efforts to survey ticks and their pathogens have mostly focused in rural areas of the state where high prevalence rates and diversity of pathogens have been recorded (Aliota et al., 2014; Dupuis et al., 2013). However, findings from a study carried out in public parks of New York City have provided strong evidence that human populations are at risk of being bitten by pathogen-infected blacklegged ticks in highly urbanized landscapes (VanAcker, Little, Molaei, Bajwa, & Diuk-Wasser, 2019). Thus, tick-borne disease risk is not limited to natural areas in suburban or rural communities. Nonetheless, surveillance and scientific research involving ticks and their pathogens have focused in certain counties and natural areas in NYS (Aliota et al., 2014; Duffy et al., 1994; Ginsberg & Zhioua, 1996; Maupin, Fish, Zultowsky, Campos, & Piesman, 1991; New York Department of Health, 2019; Piedmonte et al., 2018; Tokarz et al., 2010; Tokarz et al., 2019), leaving some surveillance gaps.

This study aims to improve the understanding of tick-borne pathogen prevalence on school properties and in parks in NYS. This risk was assessed by determining the prevalence of pathogens in *I. scapularis* residing in and around schoolyards, school athletic fields and nearby wooded areas on Long Island (Nassau County), in the Hudson Valley (Westchester County) and in the Capital (Albany, Rensselaer, Saratoga and Schoharie counties) regions. A novel application of nanoscale PCR was used for simultaneous and high-throughput detection of bacterial, viral and parasitic tick-borne pathogens. The pathogens included in the panel were *A. phagocytophilum*, *Anaplasma marginale*, *B. microti*, *Bartonella* spp., *B. burgdorferi* sensu stricto, *Borrelia mayonii*, *B. miyamotoi*, *Ehrlichia canis*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, Heartland virus, *Mycoplasma haemocanis*, Powassan virus/Deer tick virus, *Rickettsia* spp., *Rickettsia rickettsii*, Severe Fever with Thrombocytopenia Syndrome virus (SFTSV) and *Theileria orientalis*. Factors such as the life stage and sex of the tick and site-specific conditions were recorded in order

to explore possible variables affecting prevalence of *B. burgdorferi*, *B. microti*, *A. phagocytophilum*, *B. miyamotoi* and Powassan virus.

This study presents critical surveillance data for health officials, healthcare employees, extension specialists and the public. Up to this point, there have been no large-scale tick-borne pathogen surveillance projects in areas surrounding schools in NYS or in any part of Nassau County. Nassau County is home to more than 1.3 million residents and is situated in the Western central region of Long Island, bordering New York City to the west. To the east is Suffolk County, which has large tick and deer populations and is a hot spot for Lyme and other tick-borne diseases in humans (New York Department of Health, 2017). Practically, no surveillance data exist for ticks from Nassau County, despite its proximity to other tick-infested communities, including parts of New York City. The dense human population, intense urbanization, lack of tick surveillance data and unknown numbers of large wild animals (namely white-tailed deer) were motivations to examine parks and preserves in this county for the presence, but not relative abundance, of black-legged ticks. Given that almost 30,000 human cases of Lyme disease have been officially reported in NYS from 2009 to 2018 (Centers for Disease Control and Prevention, 2019b), along with the concern of Powassan virus (Dupuis et al., 2013), additional preventive and surveillance initiatives need to be developed in and around schools and parks in order to protect students and school personnel, as well as suburban park and preserve users, from ticks that may be coinfecting with a range of pathogens.

2 | MATERIALS AND METHODS

2.1 | Selection of study area sites

2.1.1 | Long Island

Sites sampled included state and county parks, public and private wildlife preserves, and school grounds, all of which featured wooded areas that could support wildlife and ticks. Tick surveillance in athletic fields was also attempted at several Long Island school properties; however, the nearly universal presence of tall fences around school grounds restricted access to the wooded edge where ticks are normally found.

2.1.2 | Lower Hudson Valley

Public parks and schools in Westchester, Putnam, Dutchess, Orange and Rockland counties were identified using two approaches in Google maps. With the map scale set to two miles, the "Search this area" function was used to look for "park" or "recreation." Sites were evaluated with the satellite view to determine whether open fields were adjacent to wooded areas on at least two sides. The second approach used the satellite view to search for baseball diamonds across each county. Baseball fields on public property, adjacent

to wooded areas on at least two sides, were included. Ticks were also collected as part of a separate study of the effects of mulching leaves on greenhouse gas emissions in fourteen sites (<https://hdl.handle.net/1813/44244>).

2.1.3 | Capital Region

To perform surveillance on school grounds, the cooperation of local public schools was solicited through the use of contacts such as the Board of Cooperative Educational Services (BOCES) and Cornell Cooperative Extension (CCE) offices. School properties were chosen for surveillance if athletic fields or mowed turf areas were directly adjacent to woodlots or wooded areas without fencing. Ticks were collected from a total of 15 school properties from 23 October 2018 to 29 November 2018. Fourteen properties were visited once, and one property was visited four times.

2.2 | Tick collection and identification

Collection sites are mapped in Figure S1. A transect of each habitat was created perpendicular to the direction of sampling, with one sample pathway 3 m into the woods, one at the edge of the woods and two additional paths three to 6 m into the turf (Falco & Fish, 1992). For schools, one to three areas along the edge of woods or woodlots on each school ground were chosen. A drag cloth composed of one square yard (0.91 x 0.91 m) of white flannel attached to a 3/4" (1.9 cm) diameter wooden dowel and fitted with a rope handle about 7 feet (2.13 m) long, end to end, was dragged for 20 m in all four pathways. Drags were checked every 10 m. All adults and nymphs from both the drag cloth and on the collectors were collected. Sampling occurred on days when the weather was above 37°F (2.8°C) with no rainfall. Several locations were sampled more than once if either no ticks were collected the first time, or first round drags were saturated.

Ticks were examined by personnel experienced in tick taxonomy and identified to life stage and species (Clifford, Anastos, & Elbl, 1961; Durden & Keirans, 1996; Egizi et al., 2019; Keirans & Clifford, 1978; Keirans & Litwak, 1989) with the aid of a stereomicroscope.

2.3 | Pathogen testing

Ticks were homogenized individually in 96-well racked minitubes (Axygen) containing 400 µl of phosphate-buffered saline, PBS, pH 7.4 (Gibco) and a single 4 mm hollow brass bead (Hareline Dubbin). Minitube plates were sealed with TPE Capcluster mats (USA Scientific) and subjected to 5 min of homogenization at 2,100 oscillations per min in a Mini-Beadbeater-96 (BioSpec Products, Inc.). Homogenates were centrifuged for 5 min at 1,048 g, and 175 µl of the processed samples was extracted in high throughput

using MagMAX Total Nucleic Acid Isolation (AM1840, Applied Biosystems) on the Kingfisher Flex (Thermo Fisher Scientific) automated extraction instrument. The manufacturer's instructions were altered with an added mechanical lysis step of 2 x 2.5 min with zirconia beads in the Mini-Beadbeater at 2,100 oscillations per min, with a 5-min rest between. Bacteriophage MS2 was added to the lysis buffer as an internal control to monitor for inhibition and extraction efficiency (Dreier, Störmer, & Kleesiek, 2005). Two negative extraction controls, consisting of PBS, were included on each extraction plate.

Tick nucleic acid was tested for pathogens on either the 7500-FAST Real-Time PCR or QuantStudio 12K Flex OpenArray platform (Thermo Fisher Scientific) using the assays listed in Table S1. The OpenArray Tick Nanochip workflow followed methods described previously (Goodman et al., 2016) on a custom printed plate containing assays for *A. phagocytophilum*, *A. marginale*, *B. microti*, *Bartonella spp.*, *B. burgdorferi*, *B. mayonii*, *B. miyamotoi*, *E. canis*, *E. chaffeensis*, *E. ewingii*, *M. haemocanis*, Powassan virus, *Rickettsia spp.*, *R. rickettsii*, Severe Fever with Thrombocytopenia Syndrome virus (SFTSV), *T. orientalis* and Heartland virus with each assay printed in triplicate.

2.4 | Powassan virus subtyping and *Rickettsia* species identification

To confirm and lineage type specimens identified as positive by PCR for Powassan virus, a region of the non-structural protein NS-5, encoded by the polyprotein gene, was amplified and sequenced. An amplicon of 415 bases was generated with forward primer (5' AGAATGGCCATGACAGACACAA 3'), reverse primer (5' ACATGTACCArATrGCCCTGCT 3') and Superscript III One-Step RT-PCR System with Platinum Taq (Invitrogen). Amplification cycling conditions were 45°C for 30 min, 94°C for 5 min, 35 cycles of (94°C for 30 s, 56°C for 1 min, 72°C for 1 min), followed by a final extension at 72°C for 10 min.

To identify the *Rickettsia* species carried by ticks collected in this study, a subset of ten ticks with strong positive results from the *Rickettsia* spp. assay were selected for amplification and sequencing of the 23S-5S intergenic spacer region. Nested PCR was performed as described by Kakumanu et al. (2016).

Amplicons were visualized by electrophoresis on 1.5% agarose gels stained with GelRed nucleic acid gel stain (Biotium) and the ChemiDoc Imaging system (Bio-Rad) using Quantity One software version 4.6.9. Gel bands were excised and purified with the QIAquick Gel Extraction Kit (Qiagen). Purified PCR products were quantified with a Qubit 3.0 fluorometer (Thermo Fisher Scientific) and submitted for Sanger sequencing at the Institute of Biotechnology at Cornell University, Ithaca, NY. Chromatograms were visualized and trimmed with Geneious Prime® 2019.1.3 (Biomatters Ltd.). These sequences are available in GenBank under accession numbers MN447639–MN447651 and MN704870–MN704879.

2.5 | Statistical analysis

For the 2017 Westchester County samples, Fisher's exact test was used to evaluate whether life stage (adult or nymph) affected the presence of any pathogen that was found to be carried. For each of the five sample sets (Albany and Westchester counties in 2017 and the Capital Region, Long Island and Lower Hudson Valley in 2018), the C-score was calculated to assess the co-occurrence of *A. phagocytophilum*, *B. burgdorferi*, *B. miyamotoi*, *B. microti* and POWV. These values were calculated using EcoSimR with significance assessed by comparing the observed value to the central 95% of null simulation values. All statistical analyses were performed using R v. 3.5.1 (R Core Team, 2017).

2.6 | Phylogenetic analysis

Phylogenetic trees were generated using the neighbour-joining method (Saitou & Nei, 1987) with a maximum composite likelihood model and bootstrap test of phylogeny with 1,000 replications (Felsenstein, 1985) in MEGA version X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). Phylogenetic analysis was performed with the sequences obtained from Powassan-positive ticks, excluding the primer sequences and reference NS-5 sequences representing Powassan lineages I and II, obtained from NCBI, as follows: Lineage 1 (Pow-24 MG652438, POWNad91 EU670438, POWNYA64 HM440563, POWNYB64 AF310945, POWOn58 NC_003687, POWOn60s AF310940, POWOn62 AF310942, POWOn64B AF310939, POWOn65 AF310937, POWOn81 AF310943, POWPa06 EU543649, POWSp75 EU770575); Lineage 2 (DTVCo52 AF310950, DTVct94 AF311056, DTVMa94 AF310947, DTVMa96 HM440559, DT-NY-07 EU338403, DTVNY13 KJ746872, DTVWi97 AF310938, DTVWi99 HM440558, DTVWiA08 HM440560, DTVWiB08 HM440561, DTVWiC08 HM440562, DTVWV77 AF310949, P0375 KU886216, R59266 AF310948) (Corrin et al., 2018; Pesko, Torres-Perez, Hjelle, & Ebel, 2010; Robich et al., 2019). In the case of *Rickettsia* intergenic spacer region sequences, phylogenetic analysis was performed with the sequences obtained from ticks in this study and sequences from previously characterized *Rickettsia* species: *Rickettsia africana* (CP001612), *Rickettsia amblyommatis* (CP015012), *Rickettsia bellii* (U11015), *Rickettsia buchneri* (NZ_JFKF01000080), *Rickettsia canadensis* (CP003304), *Rickettsia conorii* (AE006914), *Rickettsia felis* (CP000053), *Rickettsia honei* (AY125015), *Rickettsia japonica* (AP011533), *Rickettsia massiliae* (CP003319), *Rickettsia monacensis* (JQ796867), *Rickettsia mongolotimonae* (HQ710799), *Rickettsia montanensis* (KJ796429), *Rickettsia parkeri* (CP003341), *Rickettsia peacockii* (CP001227), *Rickettsia philipii* (CP003308), *Rickettsia prowazekii* (CP014865), *Rickettsia raoultii* (CP010969), *Rickettsia rhipicephali* (CP003342), *R. rickettsii* (CP000766), *Rickettsia slovacica* (CP002428), *Rickettsia* sp. Clone 205 12 (JQ688104), *Rickettsia tamurae* (NZ_CCMG01000015) and *Rickettsia typhi* (LS992663). Two *Rickettsia* endosymbiont sequences were also included:

Rickettsia endosymbiont of *Ixodes scapularis* (NZ_CM000770) and *Rickettsia* endosymbiont of *Ixodes pacificus* strain Humboldt (NZ_LAOP01000001).

3 | RESULTS

3.1 | Tick collection

In 2017, 92 *I. scapularis* (1 larva, 18 nymphs, 33 females and 40 males) were collected from six sites in two counties. A total of 677 *I. scapularis* ticks were collected in 2018 (351 females and 325 males, & 1 nymph), from 29 sites in nine counties. Additional tick species collected in Westchester County in 2017 included nymphs from *H. longicornis* and *Ixodes dentatus* and two male *Dermacentor variabilis*. Details about tick collection in each county can be found in Table 1, and locations are mapped in Figure 1 (ticks positive for pathogens) and Figure S1 (all collection sites).

3.2 | Pathogen prevalence

Overall, 40% to 73% of ticks were positive for any pathogen across sampling sites in 2017 and 2018 (Figure 1, Table 2). Tick-borne pathogen prevalence rates appeared to be highest in the Lower Hudson Valley, although this may be due to the small number of ticks collected from this region. The prevalence of detection of any pathogen was significantly lower [$p < .001$; two-tailed Fisher's exact test with odds ratio 0.034 (0.0007–0.28)] in nymphs compared to that of adults collected from Westchester County in 2017.

Of the pathogens known to be transmitted by *I. scapularis* and endemic to NYS, *B. burgdorferi* was the most frequently detected pathogen among the regions surveilled in both 2017 and 2018. *Borrelia burgdorferi* was present in 38%–64% of ticks tested in each region (Table 2, Table S2). *Anaplasma phagocytophilum* was detected in each region sampled in 2017 and 2018, with a prevalence range of 2% to 27% (Table 2, Table S2). *Babesia microti* was detected in ticks at a rate of 4% to 21% from each region sampled in 2017 and 2018 (Table 2, Table S2). The prevalence of *B. miyamotoi* ranged from 2% to 9% among the included regions for 2017 and 2018 (Table 2, Table S2). Powassan virus was detected in all three regions; however, it was only detected in 2018. Notably, 15 ticks from the Long Island collection sites were positive (2.6%), and 1 tick from each of the other regions was positive (Table 2). Additionally, *Bartonella* spp. and *M. haemocanis* were detected in Nassau County in 2 and 1 ticks, respectively. The following pathogens were not detected from any ticks in this study: *A. marginale*, *B. mayonii*, *E. canis*, *E. chaffeensis*, *E. ewingii*, Heartland virus, *R. rickettsii*, SFTSV and *T. orientalis*. All non-*I. scapularis* ticks were negative for all pathogens tested.

Coinfections of ticks with two or three pathogens were detected in each region during 2017 and 2018. Coinfections were detected in 61 out of all 769 (7.9%) ticks tested; the detected coinfections by region are provided in Table 2. *Borrelia burgdorferi* was present in all instances of coinfection, and *B. microti* was also detected in 52% of these (32 of 61). Other pathogens detected in coinfections were *A. phagocytophilum* (16/61), *B. miyamotoi* (9/61), Powassan virus (7/61) and *Bartonella* spp. (2/61). C-scores for all sites were within the central 95% of null simulations, indicating that coinfection did not differ from what would be expected by chance given the prevalence of each individual pathogen.

TABLE 1 Location, collection period, life stage, collection time and sky cover by county

Year	Location	Collection period	Life stage			Collected in the morning (%)	Clear sky cover (%)
			Female (%)	Male (%)	Nymph (%)		
2017	Albany	Oct 25	38.3	61.7	0	0	0
	Westchester ^a	Apr 26–Jun 23	32.6	23.9	41.3	42.2	0
2018	Long Island	Oct 18–Dec 4	51.7	48.1	0	40.9	67.3
	Nassau	Oct 18–Dec 4	51.3	48.5	0.2	38.9	68.8
	Queens	Nov 12	81.8	18.2	0	100	0
	Suffolk	Nov 1–Nov 7	40	60	0	90	60
	Capital Region	Oct 23–Nov 11	50	50	0	8.3	30.6
	Albany	Oct 23	50	50	0	0	75
	Rensselaer	Nov 8–Nov 11	50	50	0	14.3	0
	Saratoga	Oct 25–Oct 30	47.4	52.6	0	0	63.2
	Schoharie	Oct 31	57.1	42.9	0	0	100
	Lower Hudson Valley	Nov 29–Dec 4	72.7	27.3	0	72.7	72.7
	Westchester	Nov 29–Dec 4	71.4	28.6	0	85.7	75
	Rockland	Nov 29–Dec 3	75	25	0	50	71.4

^a1 nymph was lost and not tested.

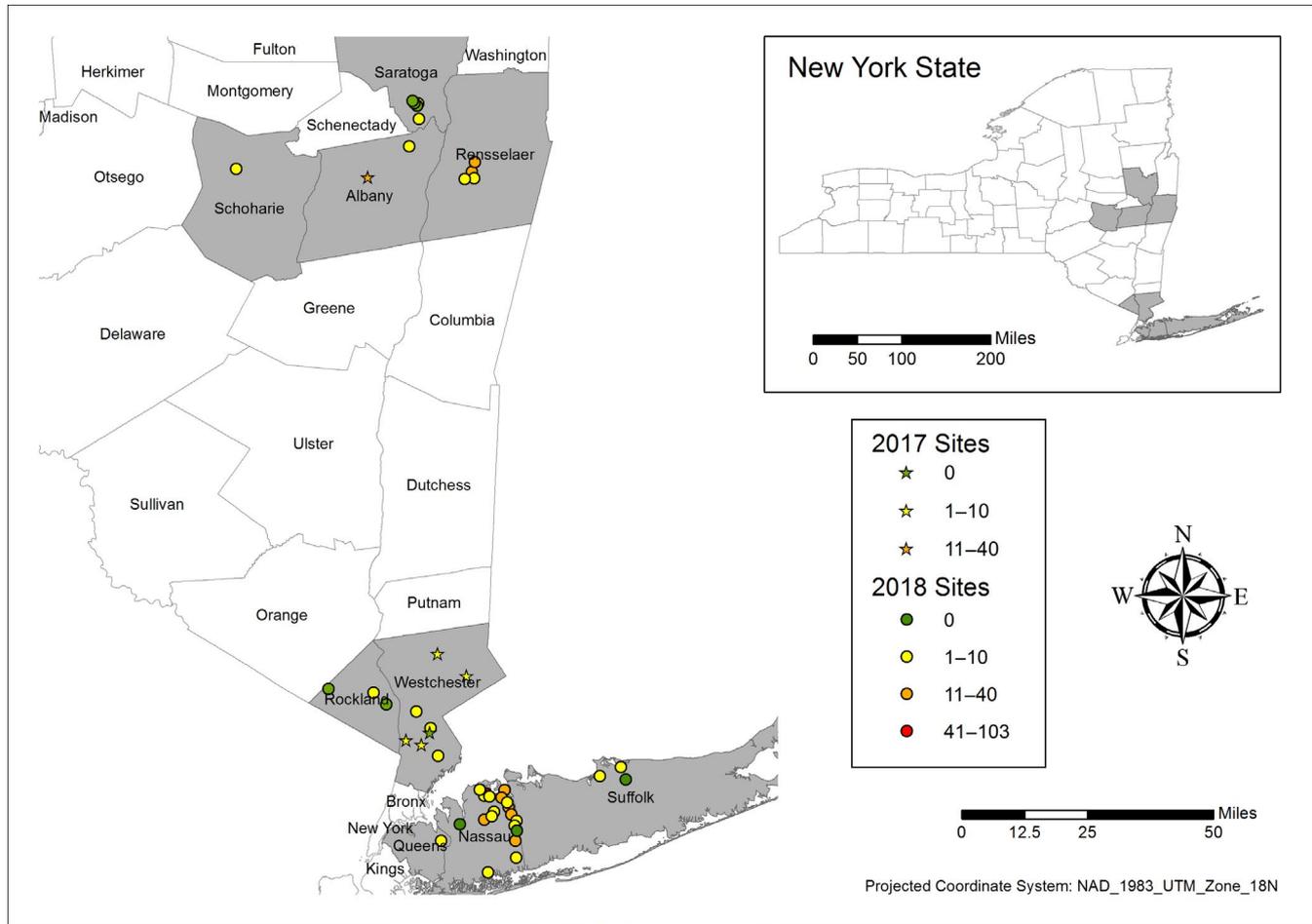


FIGURE 1 Locations of ticks collected with number of ticks positive for any pathogen indicated by colour. Sampling year is indicated by point shape. GPS coordinates of sample locations were projected to NAD_1983_UTM_Zone_18N using ArcGIS v. 10.6.1 [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

3.3 | Powassan virus subtyping and *Rickettsia* identification

A region of the NS-5 gene was amplified from 13 Powassan-positive ticks and sequenced. Sequencing was not performed on the other two Powassan-positive ticks because very little viral RNA was present in those samples. Eight NS-5 gene sequences were identical to each other as well as deer tick virus strain DT-NY-07 (EU338403) and Powassan virus strain P0375 (KU886216.1). Two sequences, DTVNY-HV175-2016 and DTVNY-LI212-2018, varied by one nucleotide in different sites. The three remaining sequences, DTVNY-LI108-2018, DTVNY-LI171-2018 and DTVNY-LI192-2018, were identical to each other but differed from the other 10 NS-5 sequences by 1 nucleotide. To determine the lineage of these Powassan virus sequences, phylogenetic analysis was performed along with 14 sequences previously identified as deer tick virus or Powassan virus lineage 2 and 12 Powassan virus lineage 1 sequences (Corrin et al., 2018; Pesko et al., 2010; Robich et al., 2019). The phylogenetic tree revealed that all of the sequences determined herein cluster with Powassan virus lineage 2 and deer tick virus sequences (Figure 2).

Rickettsia spp. were detected in 80% (545/678) of the ticks collected in 2018. Ticks collected in 2017 were not tested for the

presence of *Rickettsia* spp. This high prevalence led us to sequence the 23S-5S intergenic spacer region from a subset of 10 ticks with the highest load in order to identify which *Rickettsia* spp. were present. All ten sequences were identical to each other. Searches of NCBI RefSeq Genome and nr databases using BLAST revealed they were also identical to *R. buchneri* (NZ_JFKF01000080), *Rickettsia* endosymbiont of *Ixodes scapularis* (REIS, NZ_CM000770) and *Rickettsia* species C sequences (AaFT07-8 and Is2FT68-2 [NCBI KJ796407, KJ796403]). Further, the sequences determined here shared 99.38% nucleotide identity with *Rickettsia* endosymbiont of *Ixodes pacificus* (REIP, NZ_LAOP01000001) and 98.46% identity with *R. tamurae* (NZ_CCMG01000015). Phylogenetic analysis showed that the *Rickettsia* sequences determined here were on the same branch as *R. buchneri* and REIS. This cluster was positioned closest to REIP and *R. tamurae* and near the *R. monacensis* branch (Figure 3).

4 | DISCUSSION

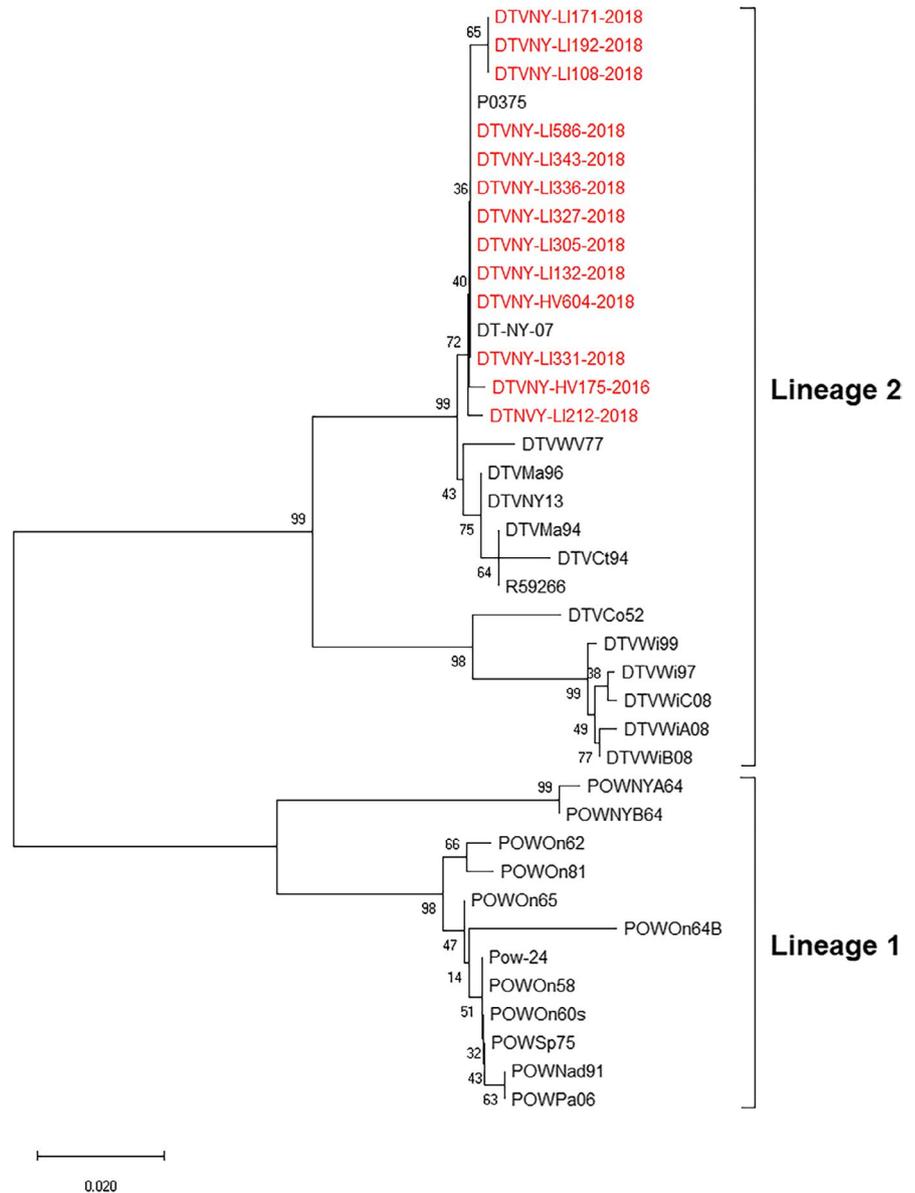
New York State is considered a hyperendemic area for *I. scapularis*, which transmits a wide range of bacterial, protozoal and viral pathogens of public health concern (Aliota et al., 2014;

TABLE 2 Detected pathogens by county.

Collection (n)	Pathogens detected (%; 95% C.I.)						
	<i>A. phagocytophilum</i>	<i>B. microti</i>	<i>B. burgdorferi</i>	<i>B. miyamotoi</i>	POWV	Any Pathogen	≥ 2 Pathogens
2017							
Albany (47)	2.1 (0.1–11.3)	21.3 (10.7–35.7)	42.6 (28.3–57.8)	2.1 (0.1–11.3)	0 (0–7.5)	51.1 (36.1–65.9)	17 (7.6–30.8)
Westchester (45)	2.2 (0.1–11.8)	8.9 (2.5–21.2)	37.8 (23.8–53.5)	2.2 (0.1–11.8)	0 (0–7.9)	40 (25.7–55.7)	8.9 (2.5–21.2)
2018							
Long Island (594)	1.9 (0.9–3.3)	3.7 (2.3–5.6)	50.7 (46.6–54.8)	2 (1–3.5)	2.5 (1.4–4.1)	54.9 (50.8–58.9)	6.2 (4.4–8.5)
Nassau (573)	1.9 (1–3.4)	3.5 (2.1–5.3)	50.4 (46.3–54.6)	2.1 (1.1–3.6)	2.6 (1.5–4.3)	54.6 (50.4–58.8)	6.3 (4.4–8.6)
Queens (11)	0 (0–28.5)	0 (0–28.5)	63.6 (30.8–89.1)	0 (0–28.5)	0 (0–28.5)	63.6 (30.8–89.1)	0 (0–28.5)
Suffolk (10)	0 (0–30.8)	20 (2.5–55.6)	50 (18.7–81.3)	0 (0–30.8)	0 (0–30.8)	60 (26.2–87.8)	10 (0.3–44.5)
Capital Region (72)	9.7 (4–19)	9.7 (4–19)	51.4 (39.3–63.3)	4.2 (0.9–11.7)	1.4 (0–7.5)	56.9 (44.7–68.6)	16.7 (8.9–27.3)
Albany (4)	0 (0–60.2)	0 (0–60.2)	25 (0.6–80.6)	50 (6.8–93.2)	0 (0–60.2)	50 (6.8–93.2)	25 (0.6–80.6)
Rensselaer (42)	14.3 (5.4–28.5)	16.7 (7–31.4)	57.1 (41–72.3)	2.4 (0.1–12.6)	2.4 (0.1–12.6)	64.3 (48–78.4)	23.8 (12.1–39.5)
Saratoga (19)	5.3 (0.1–26)	0 (0–17.6)	26.3 (9.1–51.2)	0 (0–17.6)	0 (0–17.6)	26.3 (9.1–51.2)	5.3 (0.1–26)
Schoharie (7)	0 (0–41)	0 (0–41)	100 (59–100)	0 (0–41)	0 (0–41)	100 (59–100)	0 (0–41)
Lower Hudson Valley (11)	27.3 (6–61)	9.1 (0.2–41.3)	63.6 (30.8–89.1)	9.1 (0.2–41.3)	9.1 (0.2–41.3)	72.7 (39–94)	36.4 (10.9–69.2)
Westchester (7)	28.6 (3.7–71)	14.3 (0.4–57.9)	85.7 (42.1–99.6)	14.3 (0.4–57.9)	14.3 (0.4–57.9)	100 (59–100)	42.9 (9.9–81.6)
Rockland (4)	25 (0.6–80.6)	0 (0–60.2)	25 (0.6–80.6)	0 (0–60.2)	0 (0–60.2)	25 (0.6–80.6)	25 (0.6–80.6)

Abbreviation: POWV: Powassan virus.

FIGURE 2 Phylogenetic tree of Powassan virus NS-5 gene sequences. Numbers at the nodes of the tree indicate bootstrap values. Sequences determined in this study are shown in red font. NCBI accession numbers for reference sequences, indicated by black font, are listed in the Materials and methods section



Dantas-Torres, 2007; Dupuis et al., 2013). In this study, active surveillance was performed in Albany and Westchester counties in 2017 and Long Island, the Lower Hudson Valley and the New York Capital Region in 2018. This data revealed that 38%–64% of *I. scapularis* ticks carried *B. burgdorferi* in each region. These values were generally similar to those for the corresponding counties published by the NYSDOH with the exception of Albany County in 2017, for which our estimate (95% CI of 28.3%–57.8%) was lower than the 64% reported by NYSDOH. Table S2 provides a comparison of the data described here with public records from the NYSDOH. Our overall Long Island *B. burgdorferi* estimates fall between the values reported by NYSDOH for 2017 and 2018 in Suffolk County, the only Long Island county for which NYSDOH data were available. *Anaplasma phagocytophilum* prevalence was notably lower in our 2017 Albany County (0.1%–11.3%) and 2018 Long Island (0.9%–3.3%) collections than in NYSDOH data for Albany County in 2017 (15.1%) and Suffolk County in 2018 (13.0%). Our values for *B. microti* and *B. miyamotoi* in

Long Island (2.3%–5.6% and 1.0%–3.5%) were also lower than the 2018 NYSDOH Suffolk County data (11% and 4.5%). Our data were similar to those for other collection sites (NYSDOH, 2019).

Two similar studies found somewhat different values for pathogen prevalence, though neither are directly comparable to our study. One of these tested over 4,800 adult *I. scapularis* collected in the Hudson Valley in 2003 and 2004 and found *B. burgdorferi*, *A. phagocytophilum* and *B. microti* prevalences of 46.6%, 11.3% and 1.9% in eastern counties and 39.0%, 4.8% and 0.4% in Western counties (Prusinski et al., 2014). While our Lower Hudson Valley point estimates were consistently higher, most of the values were within our confidence bounds. However, differences would be expected given the differences in sampling time and counties sampled. A more recent study tested 411 adult and nymphal *I. scapularis* collected from hunter-harvested deer in the Capital Region in 2013 and 2014 and found *B. burgdorferi* in 19.7%, *A. phagocytophilum* in 27.0% and *B. miyamotoi* in 1.5% (Wroblewski et al., 2017). The lower prevalence of

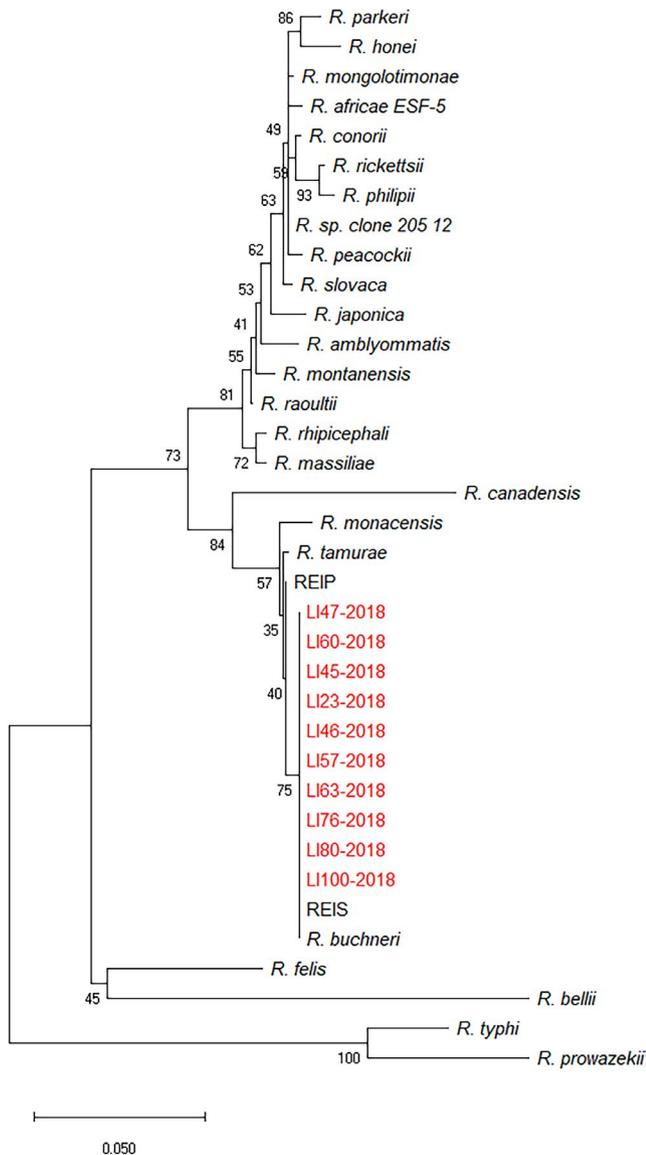


FIGURE 3 Phylogenetic tree of *Rickettsia* intergenic spacer region sequences. Numbers at the nodes of the tree indicate bootstrap values. Sequences determined in this study are shown in red font, and reference sequences are indicated by black font. NCBI accession numbers are listed in the Materials and methods section. 'REIS' stands for *Rickettsia* endosymbiont of *Ixodes scapularis*, and 'REIP' stands for *Rickettsia* endosymbiont of *Ixodes pacificus*

B. burgdorferi and higher prevalence of *A. phagocytophilum* compared to our study could result from the inclusion of adults and nymphs or differences in collection and testing methods.

Both tick population density and pathogen infection prevalence can vary greatly over a small geographical area, and even more so within a particular county (Holman et al., 2004; Prusinski et al., 2014). It is possible that surveys targeted areas (i.e. habitats) that differed in the density of ticks, host reservoirs and other factors that contribute to pathogen prevalence (Frank, Fish, & Moy, 1998; Mannelli, Kitron, Jones, & Slajchert, 1994; Maupin et al., 1991; Piedmonte et al., 2018). Variation in prevalence between years could be driven by differences in climatic conditions, which could

have potentially affected reservoir demographics (Ostfeld, Canham, Oggenfuss, Winchcombe, & Keesing, 2006), pathogen fitness (Ogden et al., 2008) and tick populations (Diuk-Wasser et al., 2010; Ogden et al., 2004).

Neither the NYSDOH database nor peer-reviewed literature have information available about tick-borne pathogen prevalence in Nassau County, which was a motivating factor in choosing certain counties for surveillance in this study. During 2018, this study included the collection of adult ticks in urban and peri-urban locations of Nassau County. This is an area in which tick-borne pathogen surveillance has been less intensive and the context in which pathogens are being transmitted to humans needs to be investigated in detail. Prevalence of *B. burgdorferi* in *I. scapularis* was high in Nassau (50.44%), similar to adult tick surveys performed in other counties in southern NYS (Aliota et al., 2014; New York Department of Health, 2019; Prusinski et al., 2014; Tokarz et al., 2010). In contrast, *A. phagocytophilum* and *B. microti* showed a low prevalence in comparison with neighbouring counties (Aliota et al., 2014; Prusinski et al., 2014; Tokarz et al., 2010); meanwhile, results for *B. miyamotoi* were similar to previous surveys done in the state (Tokarz et al., 2010; Wroblewski et al., 2017).

Additionally, 15 *I. scapularis* collected in Nassau were positive for Powassan virus, 13 of which were further identified to be Powassan virus lineage II (deer tick virus; DTV), a highly pathogenic emergent human virus whose incidence has been increasing in south-eastern NYS throughout the previous decade (Hinten et al., 2008; Tavakoli et al., 2009). Detection of DTV in questing adult and nymph *I. scapularis* has already been reported in Suffolk, Dutchess, Putnam, Westchester and Rockland counties (Aliota et al., 2014; Dupuis et al., 2013; Tokarz et al., 2010). It is important to highlight that the results from Nassau are extremely relevant because ticks were collected in areas frequently visited by adults and children for recreational and educational activities (i.e. parks and soccer fields). This constitutes an important public health issue that requires appropriate preventive measures to avoid human cases of tick-borne diseases, particularly in children. Furthermore, medical diagnosis of tick-borne diseases in children can be challenging due to the occurrence of common paediatric conditions showing similar signs and symptoms of disease (Esposito, Bosis, Sabatini, Tagliaferri, & Principi, 2013). The risk of tick-borne illnesses in children can be severe; in the case of bites from a DTV-infected tick, children may develop an infection that culminates in severe lifelong health consequences, or even death (McLean & Donohue, 1959; Smith et al., 1974).

The *Rickettsia* species sequences amplified from *I. scapularis* ticks in this study were identical to (a) the *R. buchneri* strain ISO7^T genome assembly, (b) the *Rickettsia* endosymbiont of *Ixodes scapularis* (REIS) genome assembly and (c) *Rickettsia* species C intergenic spacer region sequences (Gillespie et al., 2012; Kurtti et al., 2015; Lee et al., 2014). It appears that *R. buchneri* and REIS represent the same genome (Kurtti et al., 2015). Considering that these identical *Rickettsia* sequences were identified in disparate locations, our findings support the premise that this *Rickettsia* endosymbiont is widespread in *I. scapularis* ticks (Kurtti et al., 2015). The phylogenetic analysis of

Rickettsia sequences in this study places them on the same branch as *R. buchneri* and REIS, clustering near REIP and *R. tamurae*. Consistent with other phylogenetic analyses, this branch is near but distinct from *R. monacensis* (Kurtti et al., 2015; Lee et al., 2014). No apparent pathogenicity has been associated with this *Rickettsia* endosymbiont (Gillespie et al., 2012).

Only a low number of nymphs were collected during field surveys reported here. Nymphs can pose a greater transmission risk than adults because they are not seen easily, which allows more time for pathogen transmission to occur (Barbour & Fish, 1993). DTV can be transmitted within 15 min (Ebel & Kramer, 2004) and *A. phagocytophilum* within 12 hr of attachment (Katavolos, Armstrong, Dawson, & Telford, 1998), which underscores the need for awareness and surveillance of different life stages. All DTV-positive ticks in this study were *I. scapularis* adults.

Part of this study was conducted to highlight the importance of active surveillance for tick-borne pathogens by describing their prevalence and distribution in settings frequented by children and to guide the use of integrated pest management for schoolyards and parks. Data for relative tick abundance between adjacent woodlot, field edge and athletic fields were inconclusive due to time of year and low collection numbers on school grounds. The presence of tick-borne pathogens at rates similar to other studies helps define health risks and the need for more investigation and careful management of ticks on school properties. Although not reported, tick abundance was too low to sample on school properties with high fences, suggesting that fences that exclude white-tailed deer may be protective.

The 2 June 2017 collection of *H. longicornis* is to date the earliest documented detection in NYS. Two recently published *H. longicornis* distribution models indicate broad regions of eastern United States to be suitable habitats with pockets extending into Canada and along the Western coastline (Raghavan et al., 2019; Rochlin, 2019). Experimental evidence suggests that small mammals such as the white-footed mouse are not a preferred host, that larvae tend to move off of mice (Ronai, Tufts, & Diuk-Wasser, 2019), and *H. longicornis* is not a competent vector of *B. burgdorferi* sensu stricto (Breuner et al., 2020). For the purposes of this surveillance study, and in particular to inform future research on this vector, ticks were tested for a larger panel of pathogens than what would be indicated based on evidence of transmission potential. The high-throughput PCR Nanochip method applied here used a fixed panel of 18 assays.

The NYSDOH has established a robust surveillance programme in the state to actively screen for pathogens of public health concern in ticks, and data are openly shared in spreadsheet format (<https://health.data.ny.gov>). Nonetheless, gaps still exist. This study aimed to fill some of those missing areas using a combination of direct molecular surveillance of competent vectors in addition to indirect surveillance for additional pathogens. The novel and open-source application of nanoscale PCR on the OpenArray platform used for this study can inform future efforts to collect this type of information in a cost-effective and reliable manner. Coordinated strategic surveillance collaborations among academic and governmental institutions is essential to improve surveillance

of tick-borne diseases in endemic and poorly characterized counties, particularly areas that have been either recently colonized by vectors or are projected to be in the future (Kugeler, Farley, Forrester, & Mead, 2015; Sonenshine, 2018). Harmonization of tick data collection, processing and reporting from active and passive surveillance would provide a more robust screening of pathogens and disease risk assessment.

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CONFLICT OF INTERESTS

The authors declare no conflicts.

ETHICAL APPROVAL

No human or animal subjects were involved in this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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