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Infection Prevalence of *Borrelia burgdorferi* in Adult Blacklegged Ticks (*Ixodes scapularis*) from Pittsburgh Regional City Parks

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Abstract

Although Lyme disease is the most common vector-borne illness in the United States, its risk has mostly been studied in rural and suburban landscapes and not adequately in urban green spaces. Therefore, we investigated the relative abundance and distribution of adult blacklegged ticks (*Ixodes scapularis*) infected with Lyme disease-causing bacteria (*Borrelia burgdorferi*) in four Pittsburgh regional city parks. Ticks were collected using drags in fall 2015 and spring 2016. For each park twenty randomly chosen sites, which contained 10x10 m wooded plots and 100 m adjacent edges, were surveyed. The DNA of each tick was extracted and tested for the *B. burgdorferi*-specific OspA gene using real-time PCR. The real-time PCR results were validated using conventional PCR and gel electrophoresis for a *B. burgdorferi*-specific region of the fla gene. There was a significant difference between parks in the density of ticks as well as in the number of sites with ticks (P<0.001). Highland Park had the highest density (2.70±0.64 ticks/100 m) and number of sites with ticks (90%), whereas Frick Park had the lowest density (0.10±0.06 ticks/100m) and number of sites with ticks (10%). The overall density of ticks along edges (1.7±3.5 ticks/100m) was significantly greater than in plots (0.40±1.0 ticks/100m) (P<0.001). The overall infection prevalence of *B. burgdorferi* was 53.6% and not significantly different between parks (P=0.198). However, the density of infected ticks was significantly different with Highland Park having the highest (1.48±0.39 ticks/100m) and Frick Park the lowest (0.05±0.03 ticks/100m) densities (P<0.001). In conclusion, the density of ticks and infection prevalence of *B. burgdorferi* in these urban green spaces are comparable to rural and suburban areas highly endemic for Lyme disease. A recommendation is that preventative measures, such as posting trails with warning signs, be taken to reduce Lyme disease risk in the Pittsburgh regional city parks.

Keywords: Lyme Disease, PCR, Urban Green Spaces

1. Introduction

Lyme disease is caused by the spirochete bacterium, *Borrelia burgdorferi*. Early symptoms are typically flu-like and may be accompanied by a bulls-eye rash. If left untreated, the infection can cause serious joint, heart, and nervous system disorders[1]. In the northeastern, mid-Atlantic, and north-central United States, *B. burgdorferi* is primarily transmitted to humans by the blacklegged tick, *Ixodes scapularis*[2]. Therefore, the density of *I. scapularis*, infection prevalence of *B. burgdorferi*, and the rate of tick encounters are important determinants of Lyme disease risk.

Lyme disease is one of the fastest growing and spreading infectious diseases in the United States. In 2015, 38,069 confirmed or probable cases were reported in the United States, a 63% increase since 2005[3]. The number of counties with a high incidence of Lyme disease has increased from 69 in 1993-1997 to 260 in 2008-2012[4]. The blacklegged tick has exhibited a similar geographic expansion. In 2015, *I. scapularis* was established in 842 counties in 35 states, compared to 1998 when it was established in 396 counties in 32 states[5]. Currently, ninety-five percent of confirmed Lyme disease cases are reported by fourteen northeastern and northcentral states[1].

Within these Lyme disease and blacklegged tick-endemic regions, Lyme disease risk has been perceived as more of a suburban, exurban, and rural, and less of an urban public health problem[6,2]. This is partly because more Lyme disease risk studies have been done in non-urban landscapes. For example, Aliota et al. and Prusinski et al. collected ticks from recreational lands in the Hudson Valley Region of New York State to determine the prevalence and distribution of tick-borne pathogens[7,8]. Most Lyme disease risk studies in urban landscapes have been conducted in Europe. The prevalence of *B. burgdorferi* sensu lato (s.l.) in the sheep tick (*Ixodes ricinus*), which is the primary European vector of the pathogen, has been investigated in and around cities of the Czech Republic (Prague and Brno), Finland (Helsinki), France (Paris), Germany (Berlin and Bonn), Hungary (Budapest), Italy (Imolo), Lithuania (Vilnius), The Netherlands (Bijlmerweide), Poland (Gdansk, Sopo, Gdynia, Szczecin and Warsaw), Serbia (Belgrade), Slovakia (Bratislava and Košice), Switzerland (Basel), and the United Kingdom (London and Salsbury)[9,10]. In the United States, blacklegged ticks collected by either drag sampling or removing from small mammals, deer, or bird hosts in urban areas of Baltimore (MD)[11], Bridgeport (CT)[12], Chicago area (IL)[13,14], New York (NY)[15], and Philadelphia (PA)[16], have been tested for *B. burgdorferi*. In some studies, antibody tests for *B. burgdorferi* were used to determine the prevalence of seropositive hosts[11,12,17].

There is a growing interest in managing vector-borne disease risks in urban settings, and a realization that there are substantial gaps in our knowledge of the ecological and social factors determining these risks[18]. Many studies have investigated Lyme disease risk in semi-urban fragmented and intact forests with mixed findings[19]. This uncertainty is due to our limited understanding of the relationships between density of blacklegged ticks, prevalence of *B. burgdorferi*, and exposure of humans to infected blacklegged ticks across urbanization and forest fragmentation gradients[19]. Although it is assumed that Lyme disease risk is minimal in highly urbanized areas due to lack of suitable habitat (e.g., leaf litter) and hosts (e.g., deer) for ticks[19], there is a paucity of studies on insular greenspaces including gardens, parks and zoos within cities in the United States. This oversight could increase Lyme disease risk for the large number of urban greenspace users including hikers, nature watchers and dog walkers, due to lack of awareness and failure to take preventative measures.

The relative abundance and distribution of adult blacklegged ticks infected with *B. burgdorferi* in four Pittsburgh regional city parks were investigated. This study is important because approximately five million people use these parks each year[20], since 2011 Pennsylvania has reported the most Lyme disease cases in the United States[3], and the incidence of Lyme disease in southwestern Pennsylvania where Pittsburgh is located has more than tripled[21].

2. Methodology

The Pittsburgh regional city parks are located in the northern and northeastern sections of the city (Fig. 1). The size of the smallest park is 287 acres (Riverview Park) and largest is 455 acres (Frick Park), and the shortest distance between boundaries of adjacent parks is 1.5 km (Frick and Schenley Parks) and furthest is 7.2 km (Highland and Riverview Parks). The parks represent the highest and steepest portions of the city and have ravines and valleys with runs. They also have a mixture of woodlands with trails, sports fields, and turfs and lawns with scattered ornamental and shade trees. Within each of the four parks, twenty forested sites were randomly chosen using a geographic information system (GIS). Each site consisted of a 10 x 10 m wooded plot and 100 m of nearest edge, which included trails, roads, and fields. The ticks were collected in fall 2015 and spring 2016 by dragging a BioQuip® 1m^2 canvas sailcloth along the forest floor through and over vegetation at a rate of ~18m/min ensuring full coverage of the plots and edges. Every 10 m, the ticks were transferred and stored in microfuge tubes containing 70% ethanol. All ticks were identified using the key of Keirans and Litwak[22].

The DNA extraction method is based on quadrisectioning of ticks to physically disrupt the exoskeleton as described by Ammazzalorso et al.[23], followed by DNA purification from tissues using the Qiagen QIAamp Mini Kit. A NanoDrop spectrophotometer was used to assess the purity and quantitate the DNA extracts.

The *Ixodes* spp. internal transcribed spacer (ITS2) region and *B. burgdorferi* outer surface protein A (OspA) plasmid gene were detected using real-time PCR with TaqMan® Gene Expression Assay-Fast reaction conditions, primers, and probe sequences as described by Edwards et al.[24]. The oligonucleotide primers and TaqMan® probes used are presented in Table 1.

The real-time PCR results were validated using conventional PCR and gel electrophoresis to detect a 276 base pair (bp) product within the *B. burgdorferi* chromosomal flagellin (fla) gene as described by Hutchinson et al.[25] (Table 1). Each set of reactions included no template controls (NTC) that did not contain the template DNA and positive controls, which were samples supplied by the Pennsylvania Department of Environmental Protection (PADEP). The oligonucleotide primers used are presented in Table 1.

All statistical analyses were done using VassarStats (http://vassarstats.net/). A one-way ANOVA was used to compare differences between parks regarding observed abundance of ticks. A paired t-test was used to compare differences in tick abundance between samples from edges and wooded plots. Differences in the number of sites with ticks, and in prevalence of *B. burgdorferi* among parks were compared using a chi-square test. Maps were created using ArcMap software with base maps obtained from the Pennsylvania Spatial Data Access (PASDA) spatial information clearinghouse (http://www.pasda.psu.edu/).

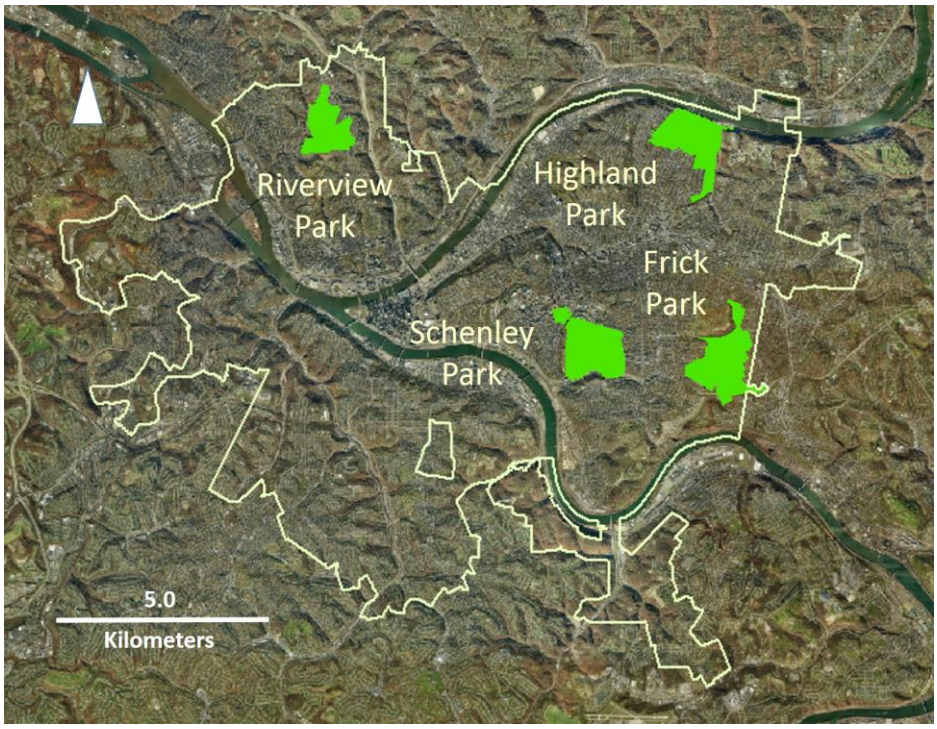

Figure 1. Locations of regional city parks in Pittsburgh.

Table 1. List of oligonucleotide primers and TaqMan® probes used in this study.

Species	Target	Sequence 5' to 3'	Amplicon
B. burgdorferi	Osp A F	CTGGGGAAGTTTCAGTTGAAC	181 bp
B. burgdorferi	Osp A R	TTGGTGCCATTTGAGTCGTA	
B. burgdorferi	Osp A Probe	6-FAM-CAGCTTGGAATTCAGGCA-MGBNFQ	
B. burgdorferi	fla F	CTGGGGAAGTTTCAGTTGAAC	276 bp
B. burgdorferi	fla R	TTGGTGCCATTTGAGTCGTA	
Ixodes spp.	ITS2 F	TGCGTCGTAGCCTTC	77 bp
Ixodes spp.	ITS2 R	AACGGCATTCCCCTAC	
Ixodes spp.	ITS2 Probe	6-FAM-TCTAAGACCTTCGCG-MGBNFQ	

3. Results

3.1 Real-time PCR

The real-time PCR method was optimized for the detection and quantification of the OspA gene. The real-time PCR results from analysis of a sub-sample of four *B. burgdorferi*-infected and four non-infected ticks collected from

Highland Park, and a positive control from the Pennsylvania Department of Environmental Protection (PADEP) are shown in Fig. 2. The Ct values for positive OspA ranged from 27.2 to 34.8. No amplification was seen for the NTCs and negative OspA samples. All samples tested positive for ITS2 verifying presence of tick DNA (data not shown).

3.2 Conventional PCR and gel electrophoresis

Conventional PCR results for the same nine tick samples analyzed above using real-time PCR are shown in Fig. 3. The PCR fla gene products for *B. burgdorferi*-infected ticks from Highland Park (Lanes 3 to 6) were approximately 234.5 base pairs, which matched the positive control from the PADEP (Lane 2). All samples that tested positive for the OspA gene using real-time PCR (Fig. 2) were also positive for the fla gene using conventional PCR and gel electrophoresis (Fig. 3). Furthermore, there was an inverse relationship between Ct values and fluorescence on the gel. From lowest to highest Ct values, the samples were HP35Trb, HP14Trb, HP32Trb, and HP18Pla (Fig. 2), whereas qualitatively the band intensities were in the reverse order (Fig. 3).

3.3 Abundance, distribution and infection prevalence

One hundred eighty adult ticks were collected and all but one, which was excluded from data analyses, were blacklegged ticks. There was a significant difference in the abundance and distribution of total ticks (both uninfected and *B. burgdorferi*-infected considered together) and infected ticks (considered alone) between and within the parks. Highland Park had the highest density of total ticks (Table 2) [F (3,76) = 10.0, P < 0.001] and greatest number of sites with total ticks (Table 3, Fig. 4) [χ^2 (3, N = 80) = 28.3, P < 0.001], whereas Frick Park had the lowest. The density of total ticks along edges was significantly greater than in wooded plots (Table 2) [t (79) = 3.78, P < 0.001]. The overall prevalence of *B. burgdorferi* was 53.6%, with no significant difference between parks (Table 4) [χ^2 (3, N = 179) = 4.67, P = 0.198]. Highland Park had the highest density of infected ticks (Table 4) [F (3,76) = 7.71, P < 0.001], and greatest number of sites with infected ticks (Table 3, Fig. 4) [χ^2 (3, N = 80) = 28.2, P < 0.001], whereas Frick Park had the lowest. The density of infected ticks along edges was significantly greater than in wooded plots (Table 3) [t (79) = 3.23, P < 0.001].

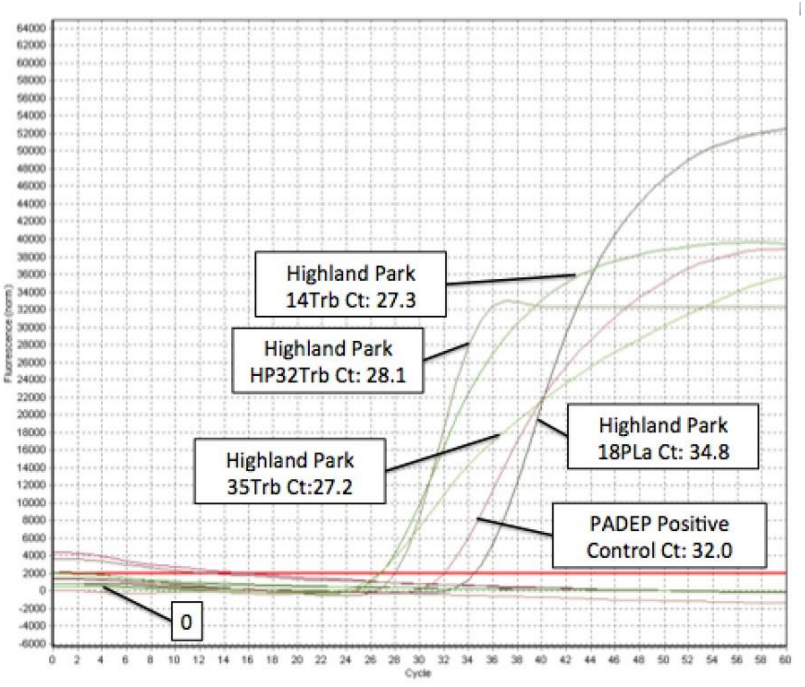

Figure 2. Real-time PCR assays showing amplification of an oligonucleotide segment from the *Borrelia burgdoferi* Osp A gene along with the Ct (threshold cycle) values from analysis of four infected and four non-infected blacklegged ticks from Highland Park and a positive control from the PADEP.

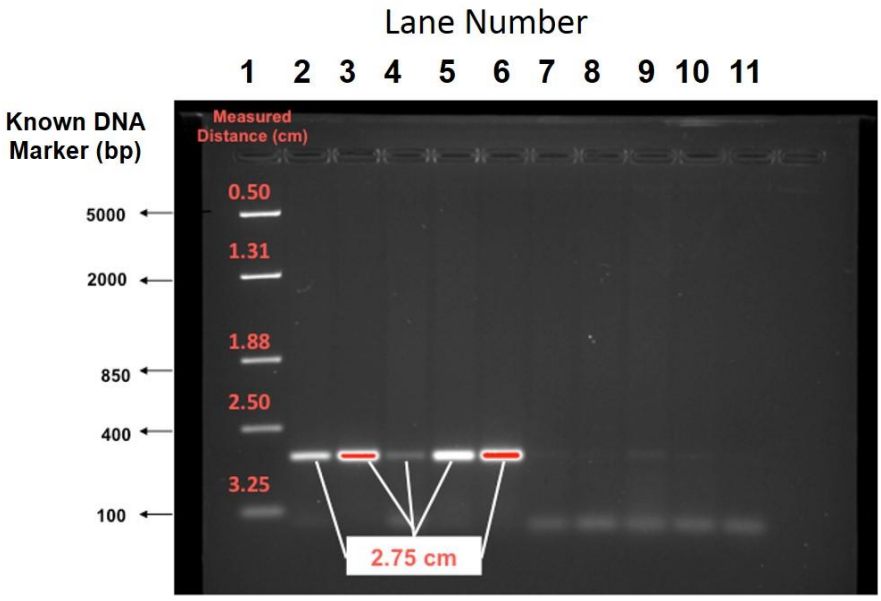

Figure 3. Ethidium bromide stained 2% agarose gel of conventional PCR fla gene products. Lane 1 – standard DNA ladder, lane 2 – PADEP positive control, lanes 3 to 6 –Highland Park *B. burgdorferi*-positive ticks (samples 14Trb, 18PLa, 32Trb, 35Trb, respectively, see Figure 2), lanes 7 to 10 – Highland park *B. burgdorferi*-negative ticks.

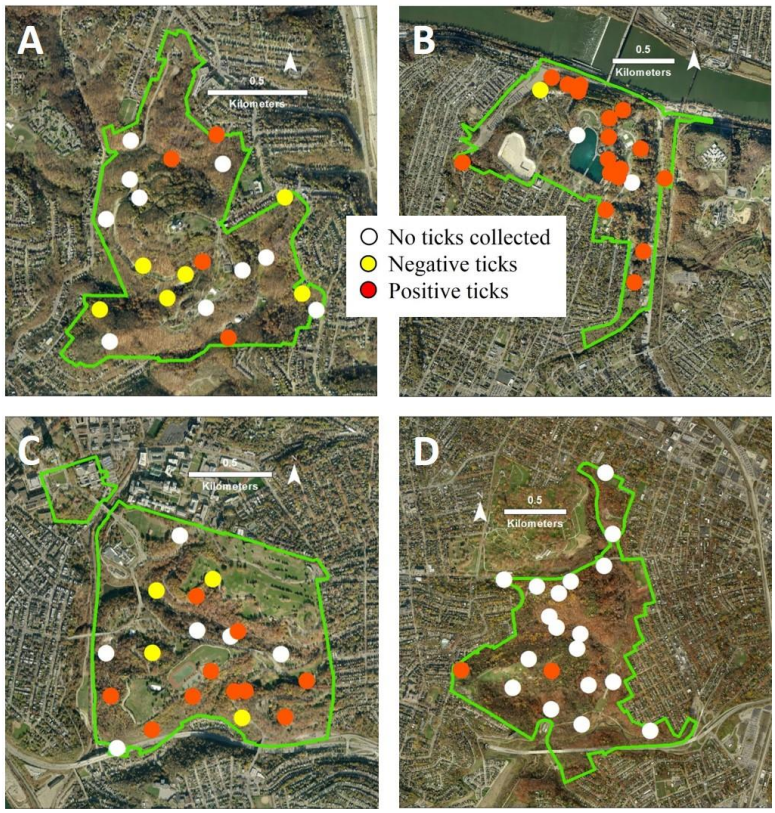

Figure 4. Location of collection sites and presence and absence of uninfected and *B. burgdorferi*-infected adult blacklegged ticks (*Ixodes scapularis*) in the Pittsburgh regional city parks. Each collection site consisted of 10x10 m wooded plot and 100 m of nearest edge. A. Riverview Park, B. Highland Park, C. Schenley Park, and D. Frick Park.

Table 2. Density of total (uninfected and *B. burgdorferi*-infected considered together) adult blacklegged ticks (X ± SE). Each site consisted of a 10 x 10 m plot and 100 m of nearest edge, n = 20 sites per park.

Park	Number of Ticks/100m^2/Site (Plot + Edge)	Number of Ticks/100m^2/Plot	Number of Ticks/100m^2/Edge
Frick	0.10 ± 0.06	0	0.15 ± 0.12
Highland	2.70 ± 0.64	1.15 ± 0.34	4.55 ± 1.20
Riverview	0.48 ± 0.16	0.25 ± 0.10	0.70 ±0.28
Schenley	1.20 ± 0.30	0.35 ± 0.15	2.05 ± 0.62
Total	**1.12 ± 0.21**	**0.44 ± 0.12**	**1.86 ± 0.39**

Table 3. Number of collection sites in Pittsburgh Regional City Parks with total (uninfected and *B. burgdorferi*-infected considered together) or infected (considered alone) adult blacklegged ticks. Each site consisted of a plot and adjacent edge, n = 20 sites per park.

Pittsburgh Regional City Park	Number of Sites With Ticks	Number of Sites With Infected Ticks
Frick	2	2
Highland	18	17
Riverview	10	4
Schenley	14	10
Total	**44**	**33**

Table 4. Density of infected adult blacklegged ticks and infection prevalence of *B. burgdorferi* (X ± SE). Each site consisted of a 10 x 10 m plot and 100 m adjacent edge, n = 20 sites per park.

Park	Number of Infected Ticks/100m^2/Site (Plot + Edge)	Number of Infected Ticks/100m^2/Plot	Number of Infected Ticks/100m^2/Edge	% Infection Prevalence (Positive/Total Ticks)
Frick	0.05 ± 0.03	0	0.10 ± 0.07	50.0 (2/4)
Highland	1.48 ± 0.39	0.75 ± 0.24	2.32 ± 0.07	54.6 (59/108)
Riverview	0.15 ± 0.07	0.10 ± 0.07	0.20 ± 0.12	31.6 (6/19)
Schenley	0.73 ± 0.25	0.10 ± 0.50	1.35 ± 0.50	60.4 (29/48)
Total	**0.60 ± 0.13**	**0.24 ± 0.07**	**0.96 ± 0.23**	**53.6 (96/179)**

4. Discussion

Questing adult blacklegged ticks were collected in the four Pittsburgh regional city parks and tested for *B. burgdorferi* using real-time PCR to evaluate acarological Lyme disease risk. The density of total (uninfected and *B. burgdorferi*-infected considered together) and infected (considered alone) ticks varied within and between parks with the greatest densities and infection prevalence comparable to highly Lyme disease endemic rural and suburban areas in the northeastern United States.

The densities of total ticks in two of the Pittsburgh regional city parks were close to densities in the public conservation lands of Indiana County in mid-western Pennsylvania. Indiana County has a high incidence of Lyme

disease attributable to an abundance of blacklegged ticks and high prevalence of *B. burgdorferi*[25,26]. The density of ticks in Schenley and Highland Parks were 1.2 and 2.7 per m^2, respectively. In comparison, the average density for twelve Indiana County public conservation lands was 3.2 per m^2 (0.3 to 10.9 m^2)[26].

Real-time PCR was a rapid and efficient method for detecting *B. burgdorferi* in ticks by targeting the OspA plasmid gene. This method was validated by conventional PCR targeting the chromosomal fla gene. Not only was there consistency between results when comparing samples that tested positive or negative with each method, but also when comparing real-time PCR Ct values and conventional PCR gel band intensities. There was an inverse relationship between the two, which was to be expected based on differences in data generated by these techniques. Basically, the higher the concentration of amplified target DNA the lower the Ct value and greater the gel band intensity.

The overall *B. burgdorferi* prevalence of 53.6% in ticks from the Pittsburgh regional city parks is comparable to rural and suburban areas in the northeastern United States highly endemic for Lyme disease. For example, Aliota et al. and Prusinski et al. reported prevalence rates of 46% and 55%, respectively, for adult ticks from recreational areas in the Hudson Valley region of New York[7,8]. Although there was no significant difference in the prevalence of *B. burgdorferi* between the Pittsburgh regional city parks, there were marked differences in the densities and distributions of infected ticks. The density of infected ticks in Highland Park was 30 times greater than Frick Park, and the number of sites with infected ticks eight and one-half times as great. Prusinski et al. also reported geographic variation between recreational areas of the Hudson Valley region of southeastern New York State, and noted its significance in terms of differences in Lyme disease risk[8]. The density of infected ticks in the Pittsburgh regional city parks was four times greater along edges than in wooded plots. Because edges included trails, walkways and sidewalks, the number of infected ticks was greatest in locations most traversed by people. Other studies in rural and suburban settings have also documented more adult blacklegged ticks along edges of woodlands[26, 27].

It is not known why the density of ticks varied between the parks, especially Frick Park compared to Highland Park. The effect of urbanization and forest fragmentation on factors that determine tick abundance and distribution such as vegetation structure, leaf litter, microclimate, presence of predators, and availability of hosts is complex[18,19]. White-tailed deer (*Odocoileus virginianus*), which are an important host for adult ticks, were observed in all of the parks as well as damage to plants due to over browsing. More research needs to be done within the parks on small mammal communities (e.g., mice, chipmunks and shrews) which are important hosts for tick larvae and nymphs, and reservoirs for *B. burgdorferi*.

Rizzoli et al. reviewed all of the literature on the occurrence of *B. burdorferi* s.l. in questing *I. ricinus* ticks in urban and in some cases suburban settings across Europe[9]. Based on their review of 24 studies, they concluded that prevalence of *B. burgdorferi* s.l. for ticks is similar across urban, suburban and natural environments, and therefore so is the risk of contracting Lyme borreliosis. A similar conclusion cannot be made for urban environments in the United States because information on the prevalence of *B. burgdorferi* for *I. scapularis* collected from greenspaces in cities is more limited. Previous blacklegged tick surveys have found that 32 of 114 females (28.1%) removed from deer in Bridgeport (CT)[12], one of 22 pools of larvae (4.5% minimum infection rate) and three of six nymphs (50%) removed from birds near Chicago (IL)[13], 61 of 172 questing adults (35.5%) collected near Chicago (IL)[14], two of three host-seeking adults (66.7%) collected in New York (NY)[15], and 37 of 42 (88.1%) questing adults collected in the greater Philadelphia (PA) area were infected with *B. burgdorferi*[16]. These studies along with the present study in the Pittsburgh regional city parks suggest that acarological Lyme disease risk in urban settings can be as great as in suburban and rural environments for the United States, as indicated for Europe.

Adult *I. scapularis* ticks were studied because they have a higher *B. burgdorferi* infection rate than do nymphs, so it is easier to determine the pathogen pool in the population[28]. However, nymphs are generally surveyed to determine entomological (or acarological) Lyme disease risk because they are responsible for most cases of Lyme disease[29]. Subsequently, nymphs collected from the parks are also being tested to determine prevalence of *B. burgdorferi*. Nonetheless, given the high density of adult ticks and infection prevalence of *B. burgdorferi* in some of Pittsburgh regional city parks, a recommendation is that preventative measures such as posting warning signs along trails be taken to reduce Lyme disease risk.

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