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Spatial Heterogeneity of Sympatric Tick Species and Tick-Borne Pathogens Emphasizes the Need for Surveillance for Effective Tick Control

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Abstract

Three tick species that can transmit pathogen causing disease are commonly found parasitizing people and animals in the mid-Atlantic United States: the blacklegged tick (*Ixodes scapularis* Say), the American dog tick (*Dermacentor variabilis* [Say]), and the lone star tick (*Amblyomma americanum* [L.]) (Acari: Ixodidae). The potential risk of pathogen transmission from tick bites acquired at schools in tick-endemic areas is a concern, as school-aged children are a high-risk group for tick-borne disease. Integrated pest management (IPM) is often required in school districts, and continued tick range expansion and population growth will likely necessitate IPM strategies to manage ticks on school grounds. However, an often-overlooked step of tick management is monitoring and assessment of local tick species assemblages to inform the selection of control methodologies. The purpose of this study was to evaluate tick species presence, abundance, and distribution and the prevalence of tick-borne pathogens in both questing ticks and those removed from rodent hosts on six school properties in Maryland. Overall, there was extensive heterogeneity in tick species dominance, abundance, and evenness across the field

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Authors' Contributions

Authors E.T.M. and A.Y.I. designed the experiment. Authors E.T.M., L.E., A.H., R.M.N., S.H., C.C., and S.H. conducted the data acquisition. Author B.T.V. conducted the data analysis. All authors contributed to the development of the article, final review, and approval for submission.

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sites. *A. americanum* and *I. scapularis* were found on all sites in all years. Overall, *A. americanum* was the dominant tick species. *D. variabilis* was collected in limited numbers. Several pathogens were found in both questing ticks and those removed from rodent hosts, although prevalence of infection was not consistent between years. Borrelia burgdorferi, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, and *Ehrlichia* "Panola Mountain" were identified in questing ticks, and *B. burgdorferi* and *Borrelia miyamotoi* were detected in trapped *Peromyscus* spp. mice. *B. burgdorferi* was the dominant pathogen detected. The impact of tick diversity on IPM of ticks is discussed.

Keywords

tick-borne pathogens; spatial distribution; rodents; ticks; school IPM; *Ixodes*

Introduction

Three human-biting tick species, the blacklegged tick (*Ixodes scapularis* Say), the American dog tick (Dermacentor variabilis [Say]), and the lone star tick (Amblyomma americanum [L.]) (Acari: Ixodidae), are commonly found in the Mid-Atlantic states (Eisen and Paddock 2021). These ticks transmit pathogens that can cause diseases in human beings and animals such as Lyme disease, Rocky Mountain spotted fever, and ehrlichiosis, respectively.

The risk of pathogen transmission from tick bites at schools in tick-endemic areas is a particular concern. School children, specifically ages 5 to 9, have been identified as a high risk group for cases of Lyme disease (Schwartz et al. 2017), likely in part because of their time spent outside at home or at school engaging in recreational activities that may result in exposure to ticks. School properties in rural and suburban areas often encompass many hectares of recreation space, including athletic fields and playgrounds with wooded perimeters and brushy vegetation that can harbor ticks and their animal hosts.

Integrated pest management (IPM) is encouraged for schools in most states, and for others, like Maryland, is mandated by State law. However, a critical component of an IPM plan is the identification of the target pest(s). It is well known that ticks of the same species are not distributed evenly throughout the environment, and factors such as landscape cover (Schmidt and Ostfeld 2003) and host community composition (Ostfeld and Keesing 2000) can influence tick density and infection with pathogens. However, prior studies have focused generally on a single tick species even though multiple sympatric species can be present. In addition, some tick species are expanding from native ranges (Sonenshine 2018). It is unknown how these species will compete with established species, and whether they will be found in tandem or separated by ecological niche. This has important control implications as some tick control methods target specific vertebrate hosts, which are parasitized by different combinations of tick species. As noted previously (Eisen and Stafford 2021), rodent-targeted approaches can be expected to impact *I. scapularis* and *D. variabilis* but not *A. americanum*, whereas deer-targeted approaches will impact *I. scapularis* and *A. americanum* but not *D. variabilis*. In contrast, broadcast application of acaricides will impact all three tick species.

There are limited data on the spatial distribution of sympatric tick species and their associated pathogens on school grounds. The purpose of this study was to evaluate spatial

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distribution and species composition of ticks and prevalence of tick-borne pathogens on school properties in the Mid-Atlantic. This will enable the recognition of pathogen risks in this area, and the information on tick assemblages can provide guidance for which tick control methods to include in local IPM programs.

Materials and Methods

Study sites

Six public school sites in Prince George's County, Maryland, USA were selected (Fig. 1). Sites were chosen based on facility design and landscape, including cleared space (athletic fields, playgrounds, or open space) adjacent to a minimum of 12 ha of undeveloped forest. Edge habitats (the boundary between the open space and forested habitat) ranged from 550 to 750 linear m per school. Sites were designated by their city and included Laurel A (latitude/longitude 39.109430/–76.895222), Laurel B (39.070900/–76.844810), College Park (38.988363/–76.904353), Bowie (38.919284/–76.758991), Greenbelt (39.012312/–76.877186), and Upper Marlboro (38.827810/–76.710910). Each study site was a minimum of 10 km from any other site in the study. Laurel A, Bowie, and College Park had deployed tick control during 2016 and 2017, including Select TCS™ bait boxes (Tick Box Technology Corporation, Norwalk, CT), for topical application of the acaricide fipronil to control ticks on rodents, four-poster feeders (C.R. Daniels, Ellicott City, MD) for topical application of the acaricide permethrin to control ticks on deer, and broadcast spraying of the natural product Essentria IC^3 (Zoëcon, Schaumburg, IL) to kill questing ticks. The other school sites (Laurel B, Greenbelt, Upper Marlboro) did not have any method of tick control. Sites were in suburban areas surrounded by a mix of residential and commercial land uses in addition to the forested area. Sites were composed of primarily oak–hickory and oak–pine dominant areas and included species such as American beech (*Fagus grandifolia*), white oak and red oak group (*Quercus spp.*), American holly (*Ilex opaca*), greenbrier (*Smilax rotundifolia*), and coarse woody debris (*e.g.*, fallen twigs and logs).

Questing tick surveillance

Tick collections were made monthly from April through September in 2016 and 2017 by sweeping vegetation and leaf litter as described by Carroll and Schmidtmann (1992). Briefly, a 0.5 × 0.5 meter flannel cloth was held by a metal pole in front of the collector walking at a moderate pace and moving the flag back and forth, parallel to the ground. The cloth was checked on both sides after each 10-meter interval by the collector by laying the cloth on the ground to check the first side and slowly folding over to check the second side to avoid ticks dropping off. Ticks were removed with fine-tipped forceps and placed in plastic vials. Sweeping occurred along two parallel transects per site, one transect along the perimeter edge of each site just outside the improved land and another transect ~ 10 meters within the wooded habitat. The area surveyed by sweeping was estimated at 100 m^2 per transect (2 meters side to side motion with a 0.5^2 flannel cloth along a 100 meter transect).

Collected questing ticks were placed in microcentrifuge tubes with 80% ethanol for storage in the laboratory. Individual ticks were identified to species and life stage using

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morphological characteristics (Clifford et al. 1961, Keirans and Litwak 1989, Keirans and Durden 1998) before DNA extraction and pathogen testing were conducted.

Tick surveillance on rodent hosts

Rodent trapping occurred on each site monthly from April through September in 2016 and 2017 using Sherman live traps (LFAHD folding trap, H.B. Sherman Traps, Inc., Tallahassee, FL). Sherman traps were placed along the perimeter of each site within 10 meters of the interface between the wooded and open areas and at ~ 10 meter intervals. Traps included cotton for nesting and were baited with an oat-peanut butter-bird seed mix and apple slice for hydration. Traps were set in the late afternoon and opened at dawn the next morning. Each monthly trapping event consisted of two trap nights. Both white-footed mice (*Peromyscus leucopus*) and deer mice (*Peromyscus maniculatus*) occur in Maryland, and both species are known to have morphologic variations based on location (Kamler et al. 1998, Grieco and Rizk 2010). Morphological measurements have performed poorly in the field in other circumstances (Bruseo et al. 1999), and it has been suggested that only molecular or biochemical methods are truly reliable at distinguishing by species (Rich et al. 1996, Lindquist et al. 2003). Thus, to avoid confusion or conclusions regarding tick ecology with questionable host species identification, the captured rodents for the current study will be referred together as *Peromyscus* spp.

Rodents were temporarily sedated with isoflurane after which ticks were removed and placed in 80% ethanol. Blood was collected using submandibular puncture on Whatman #4 filter paper (100 μL) (GE Healthcare, Chicago, IL), and an ear tissue sample was taken with a mouse ear punch (Integra Miltex, York, PA). Each captured animal was given a unique identifying ear tag (Stoelting, Inc., Wood Dale, IL). Blood and tissue samples were stored and transported in an insulated cooler with an ice pack. Ear samples were placed in RNAlater (Qiagen, Venlo, the Netherlands), and both ear and blood samples were stored at 4°C until processing. Captured rodents recovered from the isoflurane in their respective traps or in a recovery cage with HotHands® hand warmers (Kobayashi Healthcare International, Inc., Dalton, GA) depending on the outside temperatures. Once recovered, rodents were released at the location of their capture. All capture and handling procedures were approved by the U.S. Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center Institutional Animal Care and Use Committee (#15–030).

Peromyscus infection with tick-borne pathogens

Nucleic acids were isolated from rodent blood samples on filter paper by first adding 400 μL of lysis buffer (376 μL ATL; 20 μL proteinase K; 2 μL Reagent DX; and 2 μL Carrier RNA, 1 μg/μL) (Qiagen, Valencia, CA) to each tube containing the blood sample and then incubating for 20 min at 56°C. Nucleic acids were isolated from rodent ear tissue by first placing the ear tissue sample in a tube containing 100 mL phosphate-buffered saline/collagenase A (100 μg collagenase A/mL; Roche Applied Science, Indianapolis, IN) and then incubating the ear tissue sample for 4 h at 37°C. Thereafter, 300 μL of lysis buffer (276 μL ATL; 20 μL proteinase K; 2 μL Reagent DX; and 2 μL Carrier RNA, 1 μg/μL) was added to each tube containing the ear tissue sample, and the tubes were incubated overnight

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at 56°C. Following the final incubation step for each of the sample types, 300 μL lysate of either the blood sample or the ear tissue sample was processed using the KingFisher DNA extraction system and the MagMAX™ Pathogen RNA/DNA Kit (Thermo Fisher Scientific, Houston, TX).

The subsequent multiplex TaqMan PCRs included Centers for Disease Control and Prevention in-house primer and probe master mixes M73 (targeting *Anaplasma* M4 and M78; targeting *Borrelia burgdorferi sensu* lato, *B. burgdorferi sensu* stricto, *Borrelia mayonii, Borrelia miyamotoi*, and rodent glyceraldehyde 3-phosphate dehydrogenase [GAPDH] for ear tissue samples) (as described in Graham et al. 2018, Fedele et al. 2020, Milholland et al. 2021). The rodent GAPDH target (Applied Biosystems® TaqMan® Rodent GAPDH Control Reagents Kit; Thermo Fisher Scientific) was included as a PCR and DNA purification control. PCRs for M73 and M78 were performed in 15 μL solutions with 7.5 μL iQ Multiplex Powermix (Bio-Rad, Hercules, CA), 5 μL DNA extract, primers/probes, and water. PCRs for M4 were performed in 25 μL with 12.5 μL iQ Multiplex Powermix, 5 μL DNA extract, primers/probes, and water. All PCR cycling conditions are as described by Milholland et al. (2021).

Tick infection with tick-borne pathogens

Nucleic acids were isolated from ticks using the Qiagen DNeasy Blood and Tissue Kit and the Tissues and Rodent Tail protocol on the QIAcube instrument (Qiagen) and were processed by the Centers for Disease Control and Prevention laboratory. For questing ticks, DNA from *I. scapularis* was isolated from individual ticks because of expected high pathogen prevalence. Questing *A. americanum* and *D. variabilis* ticks were pooled in groups of five, so that aliquots of five individual tick homogenates were combined, isolated, and tested together. Individual lysates were archived at −20°C to be isolated singly later if the pool tested positive for a pathogen. For ticks removed from a single mouse, ticks were separated by species and combined in one tube for isolation with all life stages.

Each tick sample received lysis buffer (20 μL proteinase K and 200 μL ATL) and a 5 mm stainless steel bead. Following bead beating for 30 s, samples were centrifuged at 10,000 rpm for 5 min followed by an incubation for a minimum of 15 min at room temperature. Ticks with low pathogen prevalence (*D. variabilis* and *A. americanum*) were isolated in pools, where 24 μL of each lysate was combined in a pool of five samples for a total volume of 120 μL, and then DNA isolation was completed using a QIAcube instrument. *I. scapularis* ticks were isolated individually, so 120 μL of lysate was transferred to a QIAcube. Individual or pooled samples were eluted in Buffer AE at a final volume of 200 μL.

Ticks were only tested for human pathogens for which their species are known to serve as vectors and were tested at the U.S. Army Public Health Center. All PCR cycling conditions were performed as previously described (Milholland et al. 2021). *I. scapularis* was tested for *B. burgdorferi* s.l., *Ba. microti*, and *Anaplasma phagocytophilum*; *A. americanum* was tested for *Ehrlichia ewingii, Ehrlichia chaffeensis*, and *Ehrlichia* "Panola Mountain"; and *D. variabilis* was screened for any bacteria in spotted fever group or typhus group in the genus *Rickettsia*. All initial positive results were confirmed by testing the DNA extract with

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a second PCR for a different genetic target within the tested pathogen. Positive specimens were defined as samples that produced at least two separate PCR positive results.

Statistical analysis

The effect of tick treatment deployed at three of the study sites was not evaluated during this study because of the extreme differences in observed tick populations and short-term surveillance. In addition, the evaluation of the study sites was done in year 1 and 2 after treatment deployment. Due to the multiyear life cycle of ticks, impacts on tick populations would not be fully realized until year 3 after treatment based on the combination of treatment methods used.

Tick assemblage characteristics were evaluated using total number of species (S) and tick density per meter^2 (D). Tick assemblages at each site were also evaluated by:

Hurlbert's Probability of Interspecific Encounter (PIE) is a measure of evenness of a community and assesses the probability that two individuals selected at random from a sample will belong to a different species (Hurlbert 1971). This formula is like Simpson's index, but with a correction factor based on total abundance of organisms and is calculated as:

$$\text{PIE}=\left(\frac{N}{N-1}\right)\left(\sum_{i=1}^{s}\left(\frac{N_i}{N}\right)^2\right)$$

where N is the total number of individuals in a community, N_i is the number of individuals of the ith species in the community, and S is the number of species in the community. Generally, communities with a PIE <0.70 have an uneven abundance distribution meaning that they have a dominant species.

Shannon index (H') considers the numbers of organisms of each species present in a given sample (Shannon 1948). Lower values indicate little or no diversity, and higher numbers indicate that the number of individuals recovered is evenly distributed among all species. This value is calculated from the equation:

$$H' = -\sum_{i=1}^{R}(p_i \text{In} p_i)$$

where p_i is the proportion of individuals belonging to species i, and R is the number of species in the sample.

The Berger–Parker dominance index (d) is the proportional abundance of the most abundant species (Berger and Parker 1970). Values of d closer to 1 indicate species dominance of a single species and are calculated as:

$$d = N_{\text{max}}/N$$

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where N_{max} is the number of individuals in the most abundant species, and N is the total number of individuals in the sample.

Statistical evaluation of all questing tick and mouse-infesting tick infection measurements was accomplished with a Site and Year main-effects generalized linear ANOVA utilizing Site × Year interaction as each model's error term; a reasonable procedure because visual examination of the Site × Year plots indicated no predominant Site × Year interaction. All statistical analyses were conducted using *R version 4.0.2* (*package::function*). A negative binomial (*MASS::glm.nb*) model with log link was fit to ground tick abundance and mouse total infection prevalence, specifying offset log(sample area) and log(total mice), respectively (Ripley et al. 2013). A Beta (*betareg*) model with logit link was fit to all other variables. F-tests for significance of Site and Year main effects were obtained using *car::Anova* and subsequent mean comparisons through the *emmeans* and *multcomp* packages. Approximately 95% confidence intervals for % prevalence of pathogenic ticks for each school site and year were calculated using Agresti and Caffo (2000).

Results

Overall, 759 ticks were collected by flagging over the 2-year study, including 657 *A. americanum* ticks, 12 *D. variabilis*, and 89 *I. scapularis* (Table 1). Total ticks per site ranged from 8 at Bowie to 506 at College Park. Over nine times more total ticks were recovered at College Park than the next highest recovery at Laurel B. Generally, *A. americanum* was the dominant species recovered across all years and sites, except for 2016 (Laurel A, Upper Marlboro, and Bowie) and 2017 (Bowie) where *I. scapularis* was dominant (Table 1).

Assemblage covariates were calculated and are presented in Table 2. Tick density was similar between years for most sites. The exception was Laurel B where tick density doubled from 2016 to 2017, and Upper Marlboro where the opposite was true tick density decreased by half. There were both site ($F = 18.43$; df = 5,3; $p = 0.0182$) and year ($F = 21.92$; df = 1,3; $p = 0.0184$) differences of Hurlbert's PIE (Table 3). Overall, College Park was the most specialized in tick species numbers, having primarily *A. americanum* recovered. Most sites had a PIE ranging from 0.25 to 0.48 over both years. The only exceptions were Laurel B and Bowie in 2016 with PIE of 0.52 and 0.71, respectively. There were differences in the Shannon diversity index among sites ($F = 22.36$; df = 5,3; $p = 0.0138$) but not years. College Park had significantly lower diversity than the remaining sites. Diversity and evenness values were low for College Park both years (2016 $H' = 0.114$, 2017 $H' = 0.040$). H' varied on all sites between years. The diversity (H') of tick species increased in some cases (Laurel A, Upper Marlboro) and decreased in others (College Park, Greenbelt, Laurel B, Bowie). Noticeably, H' decreased by nearly half at Laurel B from 2016 ($H' = 0.342$) to 2017 ($H' = 0.184$). d Values were different among sites ($F = 34.31$; df = 5,3; $p = 0.0074$) and between years ($F = 42.00$; df = 1,3; $p = 0.0075$). In general, most sites had d values over 0.75 for both years, which discriminated that the most abundant tick species was proportionally very dominant. College Park differed significantly from the other sites because nearly all recovered ticks were *A. americanum* (Table 1), and even though there were three species of ticks recovered, d was 0.942 and 0.984 for 2016 and 2017, respectively. In 2016, four sites

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had *d* values <0.75; Laurel A (*d* = 0.667), Laurel B (*d* = 0.600), Upper Marlboro (*d* = 0.694), and Bowie (d = 0.429).

There were significant differences in tick abundance by area among sites (F = 125.60; df = 5,5; $p < 0.0001$) but not between years (F = 0.00; df = 5,5; $p = 0.9981$) (Table 4). Mean tick recovery was highest at College Park with 9.3 ticks per 100 m^2. This was over four times greater than the Laurel B site, which had the next highest recoveries per area. Laurel B and Greenbelt had similar mean tick collections at 2.02 and 1.50 per 100 m^2, respectively. These sites were not significantly different from Upper Marlboro, which had a mean recovery of 1.18 ticks per 100 m^2. Laurel A was not significantly different from Upper Marlboro but had nearly half as many ticks recovered per 100 m^2 at 0.62. Bowie had the fewest mean ticks recovered per 100 m^2 at 0.18.

Over both years, 25 *A. americanum* and 22 *I. scapularis* ticks were infected with at least 1 pathogen (Table 1). *B. burgdorferi* s.l. infection of *I. scapularis* ticks ranged from 9.1% to 50.0% and was most prevalent in 2016 at the Laurel A. Only one *I. scapularis* tick was infected with *A. phagocytophilum* which was a coinfection with *B. burgdorferi* s.l. One *A. americanum* tick was recovered coinfected with *E. ewingii* and *E. chaffeensis*, and one *A. americanum* was infected with Panola Mountain *Ehrlichia*. Overall infection with *E. ewingii* ranged from 2.9% to 33.3%, although in the latter case only three ticks were tested and one was positive. *E. chaffeensis* infection ranged from 0.8% to 5.6% of collected *A. americanum*.

Over both years, there were 366 *Peromyscus* spp. captured, including recaptured individuals that were sampled for ticks and pathogens. Overall, *Peromyscus* spp. infection prevalence was 32.5% for *B. burgdorferi* s.s. and 1.3% for *B. miyamotoi* for all sites and years (Table 5). There were significant differences of pathogen prevalence in *Peromyscus* spp. among sites (F = 168.32; df = 5,4; $p < 0.0001$) but not years (Table 6). *Peromyscus* spp. infection with *B. burgdorferi* s.s. was highest at Greenbelt and Upper Marlboro with close to or over 50% infection of mice captured both years, but this did not differ from College Park, Upper Marlboro, or Bowie (Table 5). *Peromyscus* spp. infection at Greenbelt was greater than Laurel A (22.0%) and Laurel B (0%). Pathogen prevalence in mice captured all sites that decreased from 2016 to 2017.

Discussion

Understanding tick species population dynamics, including diversity, dominance, and abundance, is an important first step for IPM on school grounds. Our results demonstrate that tick species dominance differed at some schools, and there were often large differences in tick population sizes. The heterogeneous distribution of ticks, even in ecologically similar and geographically close areas, has important implications for tick management and control. In addition, several medically important pathogens were detected from the tick populations collected on school grounds in Maryland.

Tick species diversity and abundance were distinctly different by study site. In some cases, questing tick recoveries were dominated by *A. americanum*, and in other cases

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A. americanum and *I. scapularis* presence was comparable or dominance was reversed. These two species of ticks overlap geographically in some areas, and both species are expanding their ranges (Sonenshine 2018). Considerable efforts have been made in recent years to identify tick species climate and habitat preferences to help explain location-specific differences in abundance of sympatric tick species (Estrada-Peña 2008, Walter et al. 2016). However, understanding the species-specific ecological niches of ticks is complex due to the many variables influencing each life stage of the tick. Evidence suggests that tick assemblages may be influenced by interspecific competition in some cases (Norval and Short 1984, Berkvens et al. 1998, Tønnesen et al. 2004). These interactions are likely influential to IPM decisions, especially with regards to host-targeted tick control methods and could become increasingly important as tick species interact in new habitat ranges.

Predictions of tick species assemblages could be broadly useful for planning tick surveillance but are likely not suitable for management of ticks on individual school grounds or other habitats with ticks. On large scales, climate data can often be used to predict tick species-specific ranges; but while using habitat assessments to predict tick species assemblages has been successful in some modeling approaches (Raghavan et al. 2016), field studies have failed to confirm modeled habitat predictions (Trout-Fryxell et al. 2015). On smaller spatial scales, bioclimatic temperature and precipitation predictors are likely not sensitive enough to explain assemblage differences (Tkadlec et al. 2018). This emphasizes the importance of conducting preliminary surveillance and tick identification on target sites as the first step of IPM before establishing a control program in areas where multiple tick vectors may be present.

Multiple species of ticks on a target property may complicate control efforts. Previous evaluations of IPM methods have resulted in reductions of *I. scapularis* or *A. americanum* (Bloemer et al. 1990, Mount et al. 1997a,b, 1999, Schulze et al. 2007, 2017, Williams et al. 2018), but these evaluations either were done in geographical areas where only one of these species was abundant or only reported results for one of the species. The purpose of IPM is to break the pest life cycles in multiple points to increase the efficacy of control. Select TCS bait boxes will impact only immature *I. scapularis* and have no effect on *A. americanum*, whereas four-poster feeders and broadcast of natural or synthetic acaricides can target both immature and adult *I. scapularis* and *A. americanum* depending on the seasonal application scheme. Similarly, four-poster feeders would be ineffective against *D. variabilis* ticks. As demonstrated in the current study, population and species distribution of sympatric tick species may differ considerably from school to school, so it is important to identify the target species before control methods are selected. Implementing IPM programs, including technologies targeting multiple tick species, could be more successful at reducing questing tick numbers in areas with *multiple species*.

The duration of this study was not sufficient to determine if the previously implemented control measures had an impact on the tick assemblage or if differences were from yearly variations because of the multiyear life cycle of the targeted tick species. Three of the six sites had tick control implemented at the beginning of the study, which may have impacted the assemblage characteristics of year 2 sampling at College Park, Laurel A, and Bowie. The abundance and diversity of the tick population did not change notably at College Park. At

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the Laurel A site, the dominant species changed from *I. scapularis* to *A. americanum*, but all other metrics remained similar or increased. There were notable decreases in tick species density at Bowie where *I. scapularis* was dominant, but this is likely due to low sample sizes. Changes in host community (Vuong et al. 2017), precipitation, temperature (Hayes et al. 2015), and other environmental factors like acorn production (Ostfeld et al. 2006) and habitat structure (Horobik et al. 2006) can influence tick populations. Because of this, short-term surveillance studies are also unlikely to demonstrate the stability needed to assess patterns in tick populations (Christie et al. 2021) for evaluating efficacy of control methods.

Tick-borne pathogens were present in questing ticks, but in some cases, these risks were localized. Only one *I. scapularis* tick was found infected with *A. phagocytophilum*. Previous studies of tick infection rates in Maryland have found a low (1.5%) infection rate of *A. phagocytophilum* in *I. scapularis* nymphs (Feldman et al. 2015, Johnson et al. 2016). All sites sampled produced *I. scapularis* ticks or *Peromyscus* spp. mice infected with *B. burgdorferi* s.s., and four of the six sites also produced mice infected with the relapsing fever spirochete, *B. miyamotoi* (Table 1). The percentage of *B. burgdorferi* s.l.-positive *I. scapularis* ticks ranged from 9.1% to 100% across sites, although high infection prevalence was often related to low sample numbers. These infections are like previous studies in eastern Maryland (Feldman et al. 2015, Johnson et al. 2016, Kuchinsky et al. 2019). It is worth noting that most of the ticks collected and tested in this study were nymphs due to sampling months, and higher infection prevalence would be expected in adult ticks. Coinfections in questing *I. scapularis* were not detected, which is like other studies on Maryland ticks (Feldman et al. 2015). However, given that a small number of *I. scapularis* ticks were detected harboring other pathogens, coinfections may be a risk in Maryland and may have been detected with a larger sample size or increased numbers of adult ticks. *B. burgdorferi* remains the most common tick-borne pathogen present in Maryland questing ticks and continues to be a significant public health risk in the state.

Lone star ticks were infected with *E. chaffensis* and *E. ewingii*, including one coinfection at similar levels to previous studies in Maryland (Stromdahl et al. 2000) and neighboring states (Wright et al. 2014, Miller et al. 2016). The site with a 20% *E. ewingii* prevalence in the current study was likely an artifact of the small sample size but serves as a useful reminder that ehrlichial human pathogens are present throughout Maryland. As the lone star tick is a common human-biting tick in this state and is spreading toward the western regions of Maryland, ehrlichiosis should be considered as an alternative to Lyme disease when diagnosing locally acquired tick-borne infections.

B. burgdorferi was found in collected I. scapularis, and *B. burgdorferi* and *B. miyamotoi* were found in blood and tissues collected from captured *Peromyscus* spp. mice. This aligns with other studies suggesting that *Peromyscus* mice are an important reservoir host in the enzootic cycle of these *Borrelia* species in Maryland (Hofmeister et al. 1999, Anderson and Norris 2006). Cats have previously been found to play a role in the enzootic circulation of *B. miyamotoi* in Maryland (Shannon et al. 2017), and this study adds to the body of evidence on the ecology of this relatively newly described human pathogen.

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Conclusion

As tick-borne disease cases continue to rise in the United States (Rosenberg et al. 2018), the need for tick control on school grounds will likely become increasingly urgent. Schools are often mandated to include IPM principles into any control program. Surveillance to identify the pest species and monitoring to establish baseline levels of pests are two of the first steps of IPM. Major differences in tick species assemblages and densities were identified at the study locations. These results confirm the need for school-specific surveillance and monitoring, regardless of habitat similarity or adjacency to nearby properties with surveillance and monitoring programs. In addition, the range of medically important pathogens detected from questing ticks confirms the risk of tick-borne pathogen transmission from ticks on school grounds in Maryland. Control methods encompassing multiple tick species should be considered in at-risk schools in areas and in regions with range overlap of medically important tick species.

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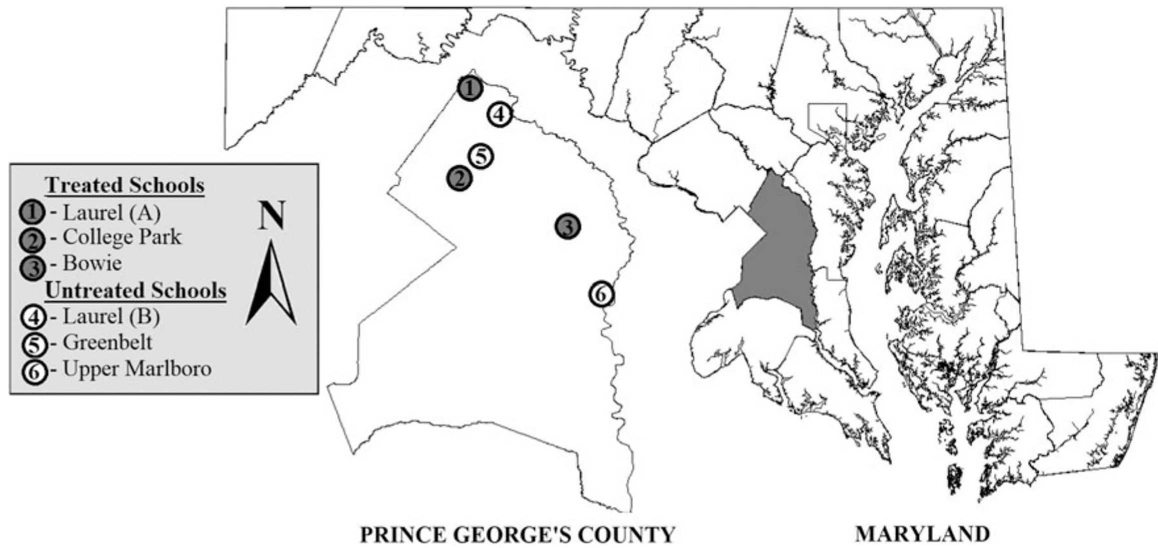

FIG. 1.

School sites included in this study in Prince George's County, Maryland, USA. Tick sampling and mouse trapping occurred during the spring, summer, and fall of 2016 and 2017.

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Table 1.

Total Sweep-Collected Adult and Nymphal Tick (*Amblyomma americanum; Dermacentor variabilis*; *Ixodes scapularis*) Abundance and Pathogen Prevalence at School Sites in Prince George's County, Maryland, USA During the Spring, Summer, and Fall of 2016 and 2017

							Infected by tick species (n) [a]		*Pathogen infection prevalence* [b]						
Site	***Year***	***Tick species***	**♀ *(n)***	**♂*(n)***	***Nymphs* (n)**	***Total ticks (n)***	**Aa**	**Is**	**Bb *(%)***	**Ap *(%)***	**Ee *(%)***	**Ec *(%)***	***PME (%)***	**Ee+Ec *(%)***	***Total prevalence [95% CI]*** [c] ***(%)***
College Park	2016	*Aa*	20	37	185	242	11	0	—	—	8 (3.3)	2 (0.8)	1 (0.4)	1 (0.4)	6.2 [3.8–10.0]
		Dv	4	2	0	6	0	0	—	—	—	—	—	—	
		Is	1	2	6	9	0	5	4 (44.4)	1 (11.1)	—	—	—	—	
	2017	*Aa*	26	39	180	245	9	0	—	—	7 (2.9)	2 (0.8)	—	—	3.6 [1.8–6.9]
		Dv	1	0	0	1	0	0	—	—	—	—	—	—	
		Is	0	0	3	3	0	0	—	—	—	—	—	—	
Totals			52	80	374	506	20	5	4 (33.3)	1 (8.3)	15 (3.1)	4 (0.8)	1 (0.2)	1 (0.2)	
Greenbelt	2016	*Aa*	5	2	18	25	0	0	—	—	—	—	—	—	0.0 [0–12.4]
		Dv	0	1	0	1	0	0	—	—	—	—	—	—	
		Is	2	5	1	8	0	0	—	—	—	—	—	—	
	2017	*Aa*	7	1	23	31	1	0	—	—	1 (3.2)	—	—	—	5.6 [0.7–19.3]
		Dv	0	0	0	0	0	0	—	—	—	—	—	—	
		Is	0	1	4	5	0	1	1 (20.0)	—	—	—	—	—	
Totals			14	10	46	70	1	1	1 (7.7)	—	1 (1.7)	—	—	—	
Laurel A	2016	*Aa*	0	1	4	5	0	0	—	—	—	—	—	—	33.3 [15.2–58.5]
		Dv	0	0	0	0	0	0	—	—	—	—	—	—	
		Is	0	3	7	10	0	5	5 (50.0)	—	—	—	—	—	
	2017	*Aa*	1	2	10	13	0	0	—	—	—	—	—	—	0.0 [0–22.1]
		Dv	1	0	0	1	0	0	—	—	—	—	—	—	
		Is	0	0	3	3	0	0	—	—	—	—	—	—	
Totals			2	6	24	32	0	5	5 (38.5)	—	—	—	—	—	
Laurel B	2016	*Aa*	7	2	9	18	1	0	—	—	—	1 (5.6)	—	—	6.7 [0.9–22.6]
		Dv	0	1	0	1	0	0	—	—	—	—	—	—	
		Is	0	1	10	11	0	1	1 (9.1)	—	—	—	—	—	

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Site	Year	Tick species	♀ (n)	♂(n)	Nymphs (n)	Total ticks (n)	Infected by tick species (n) [a]		Pathogen infection prevalence [b]						Total prevalence [95% CI][c] (%)
							Aa	Is	Bb (%)	Ap (%)	Ee (%)	Ec (%)	PME (%)	Ee+Ec (%)	
	2017	*Aa*	1	6	44	51	2	0	—	—	2 (3.9)	—	—	—	6.7 [2.2–16.5]
		Dv	0	0	0	0	0	0	—	—	—	—	—	—	
		Is	0	0	9	9	0	2	2 (22.2)	—	—	—	—	—	
Totals			8	10	72	90	3	3	3 (15.0)	—	2 (1.6)	1 (1.6)	—	—	
Upper Marlboro	2016	*Aa*	0	0	11	11	0	0	—	—	—	—	—	—	19.4 [9.6–35.4]
		Dv	0	0	0	0	0	0	—	—	—	—	—	—	
		Is	2	1	22	25	0	7	7 (28.0)	—	—	—	—	—	
	2017	*Aa*	1	1	11	13	0	0	—	—	—	—	—	—	0 [0–22.1]
		Dv	1	1	0	2	0	0	—	—	—	—	—	—	
		Is	0	0	2	2	0	0	—	—	—	—	—	—	
Totals			4	3	46	53	0	7	7 (25.9)	—	—	—	—	—	
Bowie	2016	*Aa*	1	0	2	3	1	0	—	—	1 (33.3)	—	—	—	14.3 [1.0–53.6]
		Dv	0	1	0	1	0	0	—	—	—	—	—	—	
		Is	0	1	2	3	0	0	—	—	—	—	—	—	
	2017	*Aa*	0	0	0	0	0	0	—	—	—	—	—	—	100.0 [17.1–100]
		Dv	0	0	0	0	0	0	—	—	—	—	—	—	
		Is	0	0	1	1	0	1	1 (100.0)	—	—	—	—	—	
Totals			1	2	5	8	1	1	1 (25.0)	—	1 (33.3)	—	—	—	

[a]There were no pathogens detected from *D. variabilis.*

[b]Pathogen surveillance included: *Ap, Anaplasma phagocytophilum; Bb, Borrelia burgdorferi sensu lato; Ec, Ehrlichia chaffeensis; Ee, Ehrlichia ewingii*; PME, Panola mountain *Ehrlichia. Ee+Ec* indicates a tick coinfected with both pathogens. Within species pathogen prevalence was calculated independently for each tick assemblage in each year.

[c][Lower–upper] 95% confidence limits for % prevalence.

Aa, A. americanum; Dv, D. variabilis; Is, I. scapularis.

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Table 2.

Site Level Tick Assemblage Characteristics from Sweep-Collected Ticks in 2016 and 2017 at Schools in Prince George's County, Maryland, USA

Site	*Sampling area* (m^2)	*Year*	*Dominant tick species*	*D/100m* ^{2}	S	*PIE*	H′	d
College Park	2720	2016	*Aa*	9.4	3	0.112	0.114	0.942
		2017	*Aa*	9.2	3	0.032	0.040	0.984
Greenbelt	2328	2016	*Aa*	1.5	3	0.415	0.291	0.735
		2017	*Aa*	1.5	2	0.246	0.175	0.861
Laurel A	2588	2016	*Is*	0.6	2	0.476	0.276	0.667
		2017	*Aa*	0.7	3	0.404	0.294	0.765
Laurel B	2224	2016	*Aa*	1.3	3	0.522	0.342	0.600
		2017	*Aa*	2.7	2	0.259	0.184	0.850
Upper Marlboro	2244	2016	*Is*	1.6	2	0.437	0.267	0.694
		2017	*Aa*	0.8	3	0.412	0.308	0.765
Bowie	2244	2016	*Is*	0.3	3	0.714	0.436	0.429
		2017	*Is*	0.0	1	0.000	0.000	1.000

Assemblage covariates include: total tick density per meter^2 (D/m^2); Hurlbert's PIE; number of species present at a site (*S*); Shannon's index (*H′*); and the Berger–Parker dominance index (*d*).

PIE, Probability of Interspecific Encounter.

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Table 3.

Model Predicted Mean Site Level Tick Assemblage Characteristics from Sweep-Collected Ticks in 2016 and 2017 at Schools in Prince George's County, Maryland, USA

	PIE			**H′**			**d**		
Main effect	***Mean***	***Lower 95% CI***	***Upper 95% CI***	***Mean***	***Lower 95% CI***	***Upper 95% CI***	***Mean***	***Lower 95% CI***	***Upper 95% CI***
Site									
College Park	0.07[c]	0.03	0.12	0.08[c]	0.03	0.12	0.96[a]	0.94	0.99
Greenbelt	0.33[b]	0.25	0.41	0.23[b]	0.16	0.31	0.80[b]	0.74	0.85
Laurel A	0.44[b]	0.36	0.52	0.29[ab]	0.21	0.37	0.72[b]	0.66	0.78
Laurel B	0.39[b]	0.31	0.47	0.26[ab]	0.19	0.34	0.73[b]	0.67	0.79
Upper Marlboro	0.42[b]	0.34	0.51	0.29[ab]	0.21	0.37	0.73[b]	0.67	0.79
Bowie *	0.64[a]	0.52	0.77	0.40[a]	0.27	0.52	0.52[c]	0.42	0.62
Year									
2016	0.45[a]	0.41	0.49	0.29[a]	0.25	0.33	0.68[a]	0.65	0.71
2017	0.32[b]	0.28	0.37	0.23[a]	0.18	0.27	0.81[b]	0.78	0.84

Assemblage covariates include: Hurlbert's PIE; Shannon's index (H'); and the Berger–Parker dominance index (d).

Generalized linear site and year main effect ANOVA using a beta distribution with logit link. Means with different letters for each main effect are statistically different ($\alpha = 0.05$) within each main effect.

* The one tick observed in 2017 was excluded.

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Table 4.

Model Predicted Sweep-Collected Tick (*Amblyomma americanum; Dermacentor variabilis*; *Ixodes scapularis*) Mean Abundance per 100 m^2 on School Sites in Prince George's County, Maryland, USA During the Spring, Summer, and Fall of 2016 and 2017

Main effect	*Mean total ticks/100m ^{2} **	*Lower 95% CI*	*Upper 95% CI*
Site			
College Park	9.30[a]	7.20	12.01
Greenbelt	1.50[b]	1.02	2.22
Laurel A	0.62[c]	0.37	1.04
Laurel B	2.02[b]	1.41	2.90
Upper Marlboro	1.10[bc]	0.77	0.18
Bowie	0.10[d]	0.07	0.46
Year			
2016	1.24[a]	0.99	0.16
2017	1.24[a]	0.99	0.16

Generalized linear site and year main effect ANOVA using negative binomial distribution, log link, and log sample area offset. Means with different letters for each main effect are statistically different ($\alpha = 0.05$) within each main effect.

*Concurrent sweep transects occurred along the perimeter of the school's wooded and cleared areas and paralleled ~20 meters within forest interior.

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Table 5.

Pathogen Prevalence of Tissue and Blood Samples Taken from Trapped *Peromyscus* spp. and Pathogens Identified from Ticks Removed from Rodents at Schools in Prince George's County, Maryland, USA

			Peromyscus *spp. pathogen prevalence % (n)*					*Tick pool pathogen positive % (n)* [b]		
Site	*Year*	*Peromyscus samples tested* (n)[a]	Bm	Bb	*Total [95% CI]* [c]	*Tick species* [a]	*Total ticks (n pools)*	Ap	Bam	Bb
College Park	2016	23	4.3 (1)	30.4 (7)	34.8 (8) [18.8–55.3]	*Dv*	0	—	—	—
						Is	5 (5)	0	0	0
	2017	45	2.2 (1)	24.4 (11)	26.7 (12) [15.9–41.2]	*Dv*	0	—	—	—
						Is	2 (1)	0	0	0
Greenbelt	2016	11	—	54.5 (6)	54.5 (6) [28.1–78.6]	*Dv*	1 (1)	—	—	—
						Is	28 (4)	0	0	16.7 (1)
	2017	54	1.9 (1)	51.9 (28)	53.7 (29) [40.6–66.3]	*Dv*	2 (1)	—	—	—
						Is	14 (9)	0	0	22.2 (2)
Laurel A	2016	49	—	26.5 (13)	26.5 (13) [16.2–40.4]	*Dv*	0	—	—	—
						Is	11 (6)	0	0	50.0 (3)
	2017	66	—	18.9 (12)	18.2 (12) [10.6–29.4]	*Dv*	0	—	—	—
						Is	1 (1)	0	0	0
Laurel B	2016	1	—	—	0 (0) [0–82.9]	*Dv*	0	—	—	—
						Is	16 (4)	0	0	16.7 (1)
	2017	0	—	—	0 (0)—	*Dv*	6 (5)	—	—	—
						Is	0	0	0	0
Upper Marlboro	2016	37	2.7 (1)	51.4 (19)	54.1 (20) [38.4–68.9]	*Dv*	1 (1)	—	—	—
						Is	24 (6)	0	0	37.5 (3)
	2017	16	—	43.8 (7)	43.8 (7) [23.2–66.8]	*Dv*	2 (2)	—	—	—
						Is	21 (13)	0	0	23.1 (3)
Bowie	2016	18	—	33.3 (6)	33.3 (6) [16.3–56.5]	*Dv*	1 (1)	—	—	—
						Is	11 (4)	0	0	33.3 (2)
	2017	46	2.2 (1)	21.7 (10)	23.9 (11) [13.8–38.2]	*Dv*	0	—	—	—
						Is	2 (1)	0	0	0
Total		366	1.3 (5)	32.5 (119)	33.9 (124) [29.2–38.9]		146 (60)	0	0	25.0 (15)

[a]One *Amblyomma americanum* tick was removed from a *Peromyscus* spp. at the college park site in 2017, but it was not positive for any pathogens.

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[b]Pathogen surveillance included for rodents included *Bm, Borrelia miyamotoi*; and *Bb, Borrelia burgdorferi sensu* stricto; pathogen screening in ticks removed from rodents included *Ap, Anaplasma phagocytophilum*; *Bam, Babesia microti*; and *Bb, B. burgdorferi sensu* lato.

[c][Lower–upper] 95% confidence limits for % prevalence.

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Table 6.

Model Predicted Mean Site Level Infection Prevalence (All Pathogens) in *Peromyscus* spp. Trapped at Schools in Prince George's County, Maryland, USA

Main effect	*Mouse total infection*	*Lower 95% CI*	*Upper 95% CI*
Site			
College Park	0.30[ab]	0.17	0.54
Greenbelt	0.58[a]	0.37	0.92
Laurel A	0.22[b]	0.13	0.37
Laurel B	0.00[c]	0.00	0.00
Upper Marlboro	0.48[a]	0.29	0.80
Bowie	0.28[ab]	0.15	0.52
Year			
2016	0.31[a]	0.23	0.41
2017	0.39[a]	0.28	0.55

Generalized linear site and year main effect ANOVA using negative binomial distribution, log link, and log sample area offset. Means with different letters for each main effect are statistically different ($\alpha = 0.05$) within each main effect.