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Predicting spatiotemporal patterns of Lyme disease incidence from passively collected surveillance data for *Borrelia burgdorferi* sensu lato-infected *Ixodes scapularis* ticks

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ABSTRACT

Lyme disease is the most prevalent vector-borne disease in the United States. *Ixodes scapularis*, commonly referred to as the blacklegged tick, is the primary vector of Lyme disease spirochetes, *Borrelia burgdorferi* sensu lato (s.l.), in the eastern United States. Connecticut has pervasive populations of *I. scapularis* and remains a hotspot for Lyme disease. A primary aim of this study was to determine if passively collected data on human-biting *I. scapularis* ticks in Connecticut could serve as a useful proxy for Lyme disease incidence based on the cases reported by the Connecticut Department of Public Health (CDPH). Data for human-biting *I. scapularis* ticks submitted to the Tick Testing Laboratory at the Connecticut Agricultural Experiment Station (CAES-TTL), and tested for infection with *B. burgdorferi* s.l., were used to estimate the rate of submitted nymphs, nymphal infection prevalence, and the rate of submitted infected nymphs. We assessed spatiotemporal patterns in tick-based measures and Lyme disease incidence with generalized linear and spatial models. In conjunction with land cover and household income data, we used generalized linear mixed effects models to examine the association between tick-based risk estimates and Lyme disease incidence. Between 2007 and 2017, the CAES-TTL received 26,116 *I. scapularis* tick submissions and the CDPH reported 23,423 Lyme disease cases. The rate of submitted nymphs, nymphal infection prevalence, the rate of submitted infected nymphs, and Lyme disease incidence all decreased over time during this eleven-year period. The rate of submitted nymphs, the rate of submitted infected nymphs, and Lyme disease incidence were spatially correlated, but nymphal infection prevalence was not. Using a mixed modeling approach to predict Lyme disease incidence and account for spatiotemporal structuring of the data, we found the best fitting tested model included a strong, positive association with the rate of submitted infected nymphs and a negative association with the percent of developed land for each county. We show that within counties, submissions of *B. burgdorferi* s.l. infected nymphs were strongly and positively associated with inter-annual variation in reported Lyme disease cases. Tick-based passive surveillance programs may be useful in providing independent measures of entomological risk, particularly in settings where Lyme disease case reporting practices change substantially over time.

1. Introduction

First described in 1977 following the investigation of a cluster of children with arthritis-like symptoms in Lyme, Connecticut (Steere et al., 1977), Lyme disease is now the most prevalent vector-borne disease in the United States, with an estimated 330,000 human cases occurring annually (Hinckley et al., 2014; Nelson et al., 2015; Schwartz et al., 2017). *Ixodes scapularis*, commonly referred to as the blacklegged

Abbreviations: AIC, Akaike Information Criterion; CAES, Connecticut Agricultural Experiment Station; CDPH, Connecticut Department of Public Health; DIN, density of infected nymphs; DON, density of nymphs; GLMER, Generalized Linear Mixed Effects Model; LOO, leave-one-out; NIP, nymph infection prevalence; NLCD, National Land Cover Database; PCR, polymerase chain reaction; RMSE, root mean square error; TTL, Tick Testing Laboratory; US, United States

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tick or deer tick, is the primary vector of Lyme disease spirochetes, *Borrelia burgdorferi* sensu lato (s.l.), and several other human disease-causing pathogens in the Eastern United States (Burgdorfer et al., 1982; Eisen and Eisen, 2018). Connecticut has pervasive populations of *I. scapularis* (Dennis et al., 1998; Eisen et al., 2016), and remains a high-incidence state for Lyme disease (Schwartz et al., 2017). In 2015, Connecticut was among the 14 states from which 95% of Lyme disease cases in the United States were reported, had the 5th highest number of reported cases ($n = 1873$), and concurrently has the 5th highest incidence (52.2 per 100,000 population) (Centers for Disease Control and Prevention, 2017).

Surveillance for Lyme disease cases can be complemented by conducting active or passive tick surveys to better understand spatial and temporal risk of human exposure to tick bites. Active tick surveillance is the collection of ticks in the environment, for example through drag or flag sampling or examination of captured rodents. Entomological risk measures generated through active tick surveillance include the density of host-seeking infected nymphal ticks (DIN), calculated as the product of the density of nymphs (DON) and nymphal infection prevalence (NIP) which is the proportion of nymphs that test positive for *B. burgdorferi* s.l. (or another pathogen of interest). DIN is generally considered the best predictor of human Lyme disease risk (Mather et al., 1996; Diuk-Wasser et al., 2012; Pepin et al., 2012).

Active tick surveillance is labor intensive, which limits the geographic coverage of sampling locations. Moreover, tick abundance and density estimated through active tick surveillance (i.e., tick dragging) is highly variable and unreliable if not based on repeated measures (Clow et al., 2018). Additionally, human behavior (such as how humans use the landscape, to what extent they take protective measures, and for how long ticks remain attached before detection and removal) mediates the relationship between DIN and Lyme disease acquisition (Rossi et al., 2015; Eisen and Eisen, 2016). Several studies have found a positive relationship between DIN and Lyme disease cases (Mather et al., 1996; Nicholson and Mather, 1996; Stafford et al., 1998; Pepin et al., 2012). However, in some cases the relationship was weak or equivocal (Nicholson and Mather, 1996; Pepin et al., 2012; Ripoche et al., 2018), and in other studies no association was reported (Connally et al., 2006; Prusinski et al., 2014). These discrepant findings likely reflect differences across studies in human behavior or the scale of the analysis, with the strength of the relationship between DIN and Lyme disease weakening with increased spatial resolution (Connally et al., 2006; Pepin et al., 2012).

Compared with active surveillance, there has been less focus on understanding how well tick measures obtained through passive surveillance estimate reported Lyme disease cases. Passive surveillance can include assessing tick abundance or infection rates in ticks submitted from the public, physicians or veterinarians. Testing for pathogens in ticks engorged or partially engorged with human blood is offered at no cost to residents of Connecticut by the Tick Testing Laboratory at the Connecticut Agricultural Experiment Station (CAES-TTL). This testing service promotes voluntary tick submissions from Connecticut residents. Secondarily, it provides passive surveillance data to estimate the frequency of human exposure to ticks, as well as tick infection prevalence, on a broader scale than more focal active tick surveillance (Xu et al., 2016). Compared to active surveillance of ticks in the environment, passive surveillance is economical, more epidemiologically relevant, covers a larger geographical area and may better detect tick populations at low densities. Drawbacks of passive surveillance include (1) limitations of a presence-only dataset, (2) potential for waning interest over time (participation fatigue) or variable knowledge across communities of the surveillance program, (3) spatial bias to more versus less populated areas, and (4) difficulty in detecting immature tick life stages on humans and pets (Koffi et al., 2012; Nelder et al., 2014; Soucy et al., 2018). Nevertheless, passive tick surveillance has been used to better understand the epidemiology of tick-borne diseases and assess the risk of human infection (Stromdahl et al., 2001; Ogden et al., 2006, 2010; Koffi et al., 2012; Nelder et al., 2014; Rossi et al., 2015; Gasmi et al., 2016; Xu et al., 2016; Ripoche et al., 2018). Previous studies have found associations between passive tick surveillance metrics and Lyme disease cases, and provided insights into spatiotemporal trends of actual human exposure to bites by infected ticks (Johnson et al., 2004; Rand et al., 2007; Waller et al., 2007; Rossi et al., 2015; Shelton, 2015; Ripoche et al., 2018; Gasmi et al., 2019; Jordan and Egizi, 2019).

Here we use passive surveillance data, based on *I. scapularis* tick submissions to the CAES-TTL and tick testing results for *B. burgdorferi* s.l., and reported Lyme disease cases to describe spatiotemporal patterns of disease risk at two spatial scales (town and county) in Connecticut between 2007 and 2017. Over this eleven-year period, we aim to describe tick-based risk measures and Lyme disease incidence and examine the relationship between passive tick surveillance-derived tick-based risk metrics and Lyme disease incidence.

2. Materials and methods

2.1. Study area

Connecticut is the southernmost state in New England, a small state of about 14,356 km^2 and a population of 3.6 million people (United States Census Bureau, 2017). The state has eight counties and 169 towns. Overall, approximately 58% of the state is forested and even in the most urban counties forest cover is roughly 50% (Wharton et al., 2004; The Community Health Foundation, 2007; Butler, 2017).

2.2. Lyme disease data

Lyme disease case data for each town and year were provided by the Connecticut Department of Public Health (CDPH) Epidemiology and Emerging Infections Program. Notably, Lyme disease surveillance methods in Connecticut have changed over time. Mandatory laboratory reporting was instated in 1998 to monitor the efficacy of the Lyme disease vaccine, but this requirement ended when the vaccine was withdrawn in 2002 and was not reinstated until 2007 (Ertel et al., 2012).

Between 1996 and 2007, 16% more Lyme disease cases were reported by physicians in years when laboratory reporting was mandated (Ertel et al., 2012). Therefore it is pragmatic to restrict the epidemiological data to 2007–2017 when both laboratory and physician surveillance were conducted. Physician reported cases tend to include early onset manifestations (e.g., erythema migrans), whereas laboratory reported cases tend to comprise later manifestations such as those involving the musculoskeletal, neurological, or cardiovascular systems (Ertel et al., 2012). We therefore use the combined surveillance metric, which we call total cases (confirmed and probable physician and laboratory-based surveillance cases) for analysis as it provides a more comprehensive estimate of Lyme disease cases (Ertel et al., 2012). We used the US Census estimates from 2000 to calculate incidence per 100,000 population for each year from 2007 to 2009 and the 2010 US Census estimates to calculate incidence per 100,00 population for each year from 2000 to 2017 (United States Census Bureau, 2017).

2.3. Tick-based data

The CAES-TTL started testing ticks for evidence of infection with *B. burgdorferi* s.l. in 1996. Ticks are submitted by residents, health departments, and physicians' offices. All submitted ticks are examined under a dissecting microscope and identified with standard morphological keys and taxonomic references (Keirans and Litwak, 1989; Durden and Keirans, 1996). Engorged or partially engorged female and nymphal *I. scapularis* ticks (showing evidence of at least some ingested blood) are screened for infection with *B. burgdorferi* s.l. as described below.

Two methodologies have been used for screening of *I. scapularis* ticks for evidence of infection with *B. burgdorferi* s.l. from 1996 to 2017. From 1996 to 2014, polymerase chain reaction (PCR) amplification combined with Southern blot hybridization was used. Briefly, ticks were homogenized, genomic DNA extracted, and a portion of the OspA gene was amplified (Persing et al., 1990). PCR-amplified products were then analyzed by gel electrophoresis, followed by Southern blot hybridization (Persing et al., 1990). In 2014, Southern blot hybridization was removed from the methodology due to the potential health and safety hazards associated with using ^{32}P-labled probes. Since 2014, screening of engorged or partially engorged ticks was conducted by extracting genomic DNA using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA), or DNA-zol BD (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturers' recommendations with some modifications (Molaei et al., 2006), followed by PCR amplification of the flagellin (Barbour et al., 1996), 16S rRNA (Gazumyan et al., 1994), and OspA (Persing et al., 1990) genes. A more detailed description of these methods is provided elsewhere (Williams et al., 2018). Comparison between the two methods, PCR-Southern blot hybridization and PCR using three diagnostic genes on a subset of DNA extracts from ticks with known and unknown infection status with *B. burgdorferi* s.l. produced comparable results (data not shown). Although this assay is not specific to *B. burgdorferi* sensu stricto (s.s.), a human-pathogenic member of the bacterial genospecies complex *B. burgdorferi* s.l., it is agreed upon that *B. burgdorferi* s.s. accounts for the vast majority of Lyme disease infections in Connecticut and throughout North America (Waddell et al., 2016). Moreover, a recent study capable of distinguishing *B. burgdorferi* s.s. from other *B. burgdorferi* s.l. spirochetes found all infected *I. scapularis* nymphs from Connecticut, and nearly all from neighboring New York, to represent *B. burgdorferi* s.s. (Feldman et al., 2015).

On the submission form to the CAES-TTL, the person submitting the tick must enter their, or their patient's town of residence and provide information on the likely town the tick was acquired if it is known to be different from the town of residence. Ticks acquired outside of Connecticut or from a Connecticut county other than the county of the submitter's residence were excluded from the analysis. These actions served to minimize error introduced by travel-related tick exposures, which can be problematic in a passive surveillance program based on human tick bites (Xu et al., 2018). We further narrowed the dataset to submissions of female and nymphal ticks, excluding males and larvae. Because nymphs are considered the primary vectors of Lyme disease spirochetes to humans in the Northeast (Falco et al., 1999), we estimated the rate of submitted nymphs per 100,000 population, NIP, and the rate of submitted infected nymphs per 100,000 population at two spatial scales (town and county) for each year from 2007 to 2017. To calculate the rate of submitted nymphs per 100,000 population, we used the 2000 and 2010 United States Census estimates (United States Census Bureau, 2017). NIP was calculated as the number of positive nymphs divided by the total number of tested nymphs. The rate of submitted infected nymphs recovered from humans was calculated as the rate of submitted nymphs multiplied by the NIP.

2.4. Covariates

To assess the influence of selected underlying conditions on the variability in the (infected) rate of submitted nymphs and Lyme disease incidence in Connecticut, we measured median household income and extent of developed land cover. We speculated that these variables influence tick submission to the CAES-TTL and/or Lyme disease incidence. Median household income may underlie access to or knowledge of services for tick testing or Lyme disease diagnosis and the degree of developed land cover may explain some of the variability in human-tick encounters (Cortinas and Spomer, 2014). To estimate town and county level median household income, we used United States Census (2012–2016) American Community Survey 5-year estimates of median household income (United States Census Bureau, 2017). To determine the extent of developed land cover for each town and county, we used the 2011 National Land Cover Database (NLCD) (Homer et al., 2015). We used the land cover classes considered developed (developed open space, developed low intensity, developed medium intensity, and developed high intensity) to create a binary raster grid at 30 meter spatial resolution of developed and undeveloped land. Using this binary raster grid we then determined the percentage of developed land for each town and county using the "zonal statistics as table" tool from the spatial analysis toolbox in ArcGIS 10.1 (ESRI, 2011). We investigated the relationship of these two covariates to tick-based risk measures and Lyme disease incidence through correlation analyses.

2.5. Data analysis

Passive surveillance data from the CAES-TTL is available since 1996 and we used the full record (1996–2017) to describe submission patterns including seasonality of submissions. To compare tick-based risk measures to Lyme disease incidence, we restricted the analyses to the years 2007–2017. To ensure that this restricted dataset was reflective of the entire dataset, we performed a Spearman's rank correlation test.

To assess temporal patterns in tick-based risk metrics and Lyme disease incidence, we summarized the data across the state for annual estimates. To test for temporal differences in the rate of submitted nymphs, NIP, the rate of submitted infected nymphs, and Lyme disease incidence, we used generalized linear models (family = Poisson; link = log) with year structured as an ordinal integer. To test for spatial patterns, we summarized the data across all years for each town (n = 169) and calculated the Global Moran's I in ArcGIS 10.1. For robust estimation of Global Moran's I at least thirty observations are needed; therefore, we were unable to calculate spatial clustering at the county (n = 8) level.

To assess the relationship between Lyme disease incidence and tick-based metrics, we used generalized linear mixed effects models (GLMER; family = Poisson; link = log) with year and county as grouping variables to explicitly account for spatiotemporal structure in the data. We compared GLMER model fits by Akaike Information Criterion (AIC). Lower scores indicate better model fits; a two-point difference is significant. To determine how accurately the GLMER models predicted Lyme disease incidence, we calculated Spearman's rank correlation coefficient between predicted and observed Lyme disease cases. Further, we used leave-one-out (LOO) cross validations across years and counties. Each year (or county) of data was iteratively omitted from the analysis and the compiled sets of predictions from the LOO models were then compared with predictions based on the full record using root mean square error (RMSE). RMSE gives the standard deviation of the model prediction error; smaller values indicate better model performance. For data processing and analyses we used R (R Core Team, 2017) and for mixed effects modeling we employed the lme4 package (Bates et al., 2014).

3. Results and discussion

3.1. Lyme disease data, 2007–2017

A total of 31,471 Lyme disease cases (including confirmed and probable) has been reported from Connecticut during 2007 to 2017. Of these, 8048 were excluded due to unknown town of residence. Of the remaining 23,423 cases, 13,331 (57%) were initiated through laboratory-based surveillance and 10,092 (43%) through physician-based reporting.

3.2. Tick-based data, 1996–2017

A total of 91,671 *I. scapularis* ticks was submitted to the CAES-TTL between 1996 and 2017, most of which (91,409; 99.7%) by Connecticut

Table 1
Annual *Ixodes scapularis* tick submissions to the CAES-TTL, 1996–2017.

	No. submitted		No. tested (% positive)	
Year	Nymph	Adult	Nymph	Adult
1996	2563	1789	2403 (15%)	1565 (29%)
1997	1195	1133	1113 (12%)	1041 (27%)
1998	1877	1938	1764 (19%)	1824 (33%)
1999	3235	2870	3138 (16%)	2737 (32%)
2000	3178	2545	3085 (17%)	2402 (32%)
2001	2464	2550	2388 (17%)	2448 (36%)
2002	3401	2481	3386 (21%)	2447 (39%)
2003	1684	3768	1673 (23%)	3694 (35%)
2004	1599	2478	1596 (35%)	2438 (42%)
2005	3193	1983	3174 (23%)	1936 (36%)
2006	1557	2525	857 (16%)	1149 (27%)
2007	806	1358	540 (36%)	684 (33%)
2008	996	1606	566 (20%)	731 (26%)
2009	1094	1979	659 (41%)	905 (34%)
2010	663	1221	461 (34%)	597 (29%)
2011	622	1716	424 (16%)	824 (27%)
2012	366	1210	270 (15%)	556 (20%)
2013	1142	959	824 (29%)	520 (33%)
2014	520	1492	339 (28%)	789 (27%)
2015	847	1646	718 (27%)	1297 (33%)
2016	740	1543	561 (19%)	1239 (33%)
2017	758	2832	693 (16%)	2610 (36%)
Total	34500	43622	30632 (21%)	34433 (33%)

Total numbers of *I. scapularis* submitted and/or tested for *B. burgdorferi* s.l. by life stage (nymph and adult female) for each year 1996–2017.

residents. The majority of these ticks were females (48,747) or nymphs (39,236) but there were also submissions of males (1027) and larvae (2399).

Although we did not assess the precise location the tick was acquired, human tick encounters were traced to the town of residence or the likely town the tick was acquired, if known (see Methods). We found a high degree of agreement between the locations of a submitter's residence and where the tick was thought to be acquired – 73,312 (80%) ticks were acquired and submitted from the same town and 81,171 (89%) were acquired and submitted from the same county. The finding that the vast majority of ticks were acquired and submitted in the same town supports the importance of peridomestic risk for tick-borne disease transmission (Connally et al., 2006; Eisen et al., 2016; Jordan and Egizi, 2019). Nymphal submissions were markedly higher between 1996 and 2006 compared with between 2007 and 2017 (Table 1); however we have no explanation for this change.

Of those ticks that were submitted and acquired from the same county between 1996 and 2017, 43,622 were adult females and 34,500 were nymphs (Table 1). A total of 65,056 partially or fully engorged ticks (34,433 females and 30,632 nymphs) recovered while biting humans were tested for the presence of *B. burgdorferi* s.l. The overall prevalence of *B. burgdorferi* s.l. infection in *I. scapularis* ticks was 21% for nymphs and 33% for adult females (see Table 1 for annual values). These results are similar to passive surveillance-derived *I. scapularis* infection prevalence (all stages combined) in Massachusetts (30% between 2006 and 2012) (Xu et al., 2016) and in New Jersey (38% of adult females and 22% of nymphs between 2006 and 2016) (Jordan and Egizi, 2019)

Submissions of nymphal and adult female *I. scapularis* ticks followed a distinct seasonal pattern (Fig. 1). Nymphal tick submissions peaked in June, while submissions of adult female ticks showed a bimodal pattern with a major peak in April-May and a minor peak in November. The June peak of nymphal submissions coincides with the June–July peak in reported Lyme disease cases in Connecticut (Ertel et al., 2012). This finding further supports the understanding that nymphal bites are responsible for the majority of Lyme disease cases in the Northeast (Mather et al., 1996; Falco et al., 1999). Nymphal tick submissions in June alone represented 25% of the total *I. scapularis* submissions,

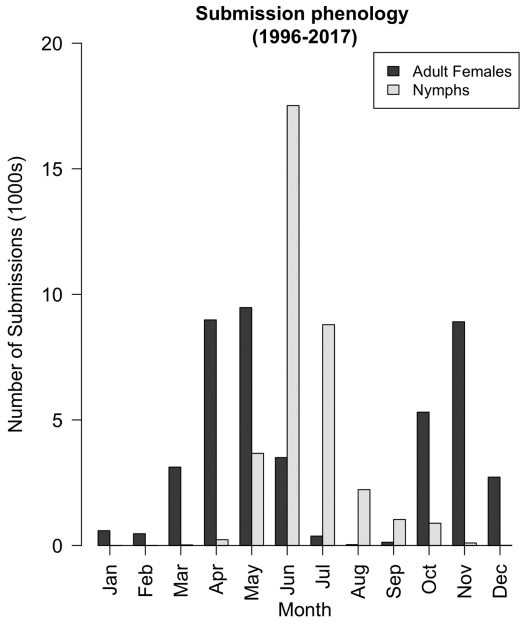

Fig. 1. Submission phenology. Submission phenology of adult female and nymph *Ixodes scapularis* ticks to the CAES-TTL by month (1996–2017).

underscoring the temporally focused nature of Lyme disease risk in Connecticut and throughout the Northeastern United States.

3.3. Tick-based data, 2007–2017

When comparing the tick-based risk measures to Lyme disease incidence, we restricted the analyses to the years 2007–2017. Over this eleven-year period there were 26,116 submissions of female and nymphal *I. scapularis* ticks that were submitted and acquired from the same county in Connecticut. Partially or fully engorged ticks tested for presence of *B. burgdorferi* s.l. (n = 16,807; 64% of all submitted ticks) included 10,752 females and 6055 nymphs. Tick-based risk measures calculated for this temporally restricted dataset were well correlated, assessed with Spearman's rank correlation coefficient, with those calculated for the 1996–2017 period at both the town and county levels (town rate of submitted nymphs: $\rho = 0.79$, $p < 0.001$; town NIP: $\rho = 0.59$, $p < 0.001$; county rate of submitted nymphs: $\rho = 0.98$, $p < 0.001$; and county NIP: $\rho = 0.90$, $p = 0.002$).

The rate of submitted nymphs, calculated as nymphal tick submissions per 100,000 population, ranged from 10.24 in 2012 to 32.12 in 2009 across the eleven-year period (mean = 22.12, SD = 6.99). Generally we note a slight decline in the annual rate of submitted nymphs, albeit with fluctuations (Fig. 2). We note that the rate of submitted nymphs per 100,000 population was much higher in Fairfield County compared to all other counties (Fig. 2). The rate of submitted infected nymphs, follows a similar trajectory – decreasing over time and showing substantial spatial variability across counties (Fig. 2). NIP also generally decreased over time but remained markedly steady across counties (Fig. 2).

We assessed the association between NIP and the rate of submitted nymphs to determine if the downward trend in NIP over time is a result of decreasing submission rates. However, by testing for associations using Pearson's product–moment correlations, we did not find an association at either the town ($r = 0.003$; $p = 0.930$) or the county ($r = 0.028$, $p = 0.799$) spatial scale.

3.4. Association of Lyme disease incidence and tick-based measures with household income and land cover

We found positive correlations between median household income and the rate of submitted nymphs ($r = 0.50$, $p < 0.001$) and the rate of

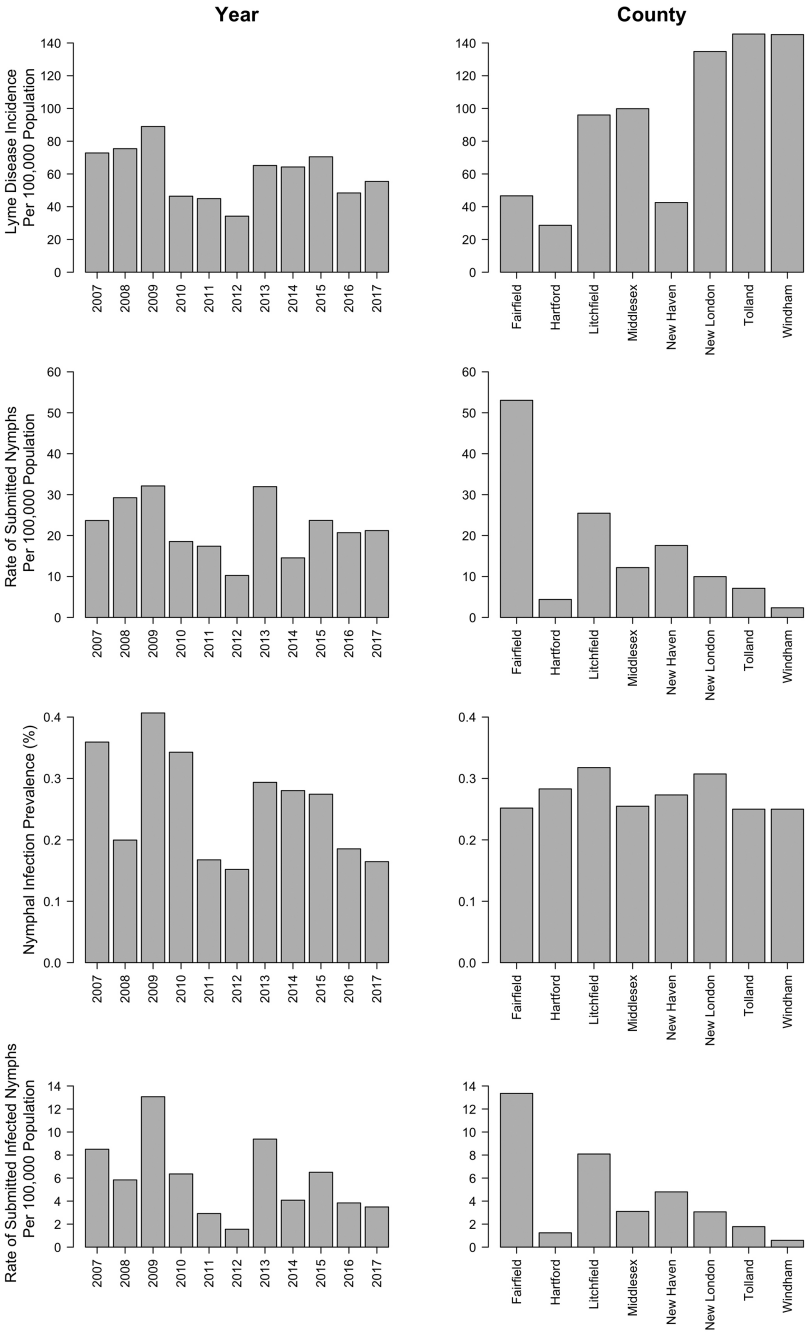

Fig. 2. Descriptive spatial and temporal Lyme disease and tick-based risk measures. Cumulative Lyme disease incidence per 100,000 population, cumulative rate of submitted nymphs per 100,000 population, cumulative nymphal infection prevalence (%), and the cumulative rate of submitted infected nymphs by year and county for the years 2007–2017.

submitted infected nymphs ($r = 0.48$, $p < 0.001$) at the town spatial scale but not at the county level. We did not find a relationship between NIP and median household income at either spatial scale, nor did we find a relationship between any tick-based risk measure and the degree of developed land at either spatial scale. We did not find a significant association between median household income and reported number of Lyme disease cases at either spatial scale. However, we did find a strong negative correlation between Lyme disease incidence and the degree of developed land at both the scale of town ($r = -0.61$, $p < 0.001$) and county ($r = -0.91$, $p = 0.002$).

The positive associations between the rate of submitted nymphs and the rate of submitted infected nymphs with median household income imply that participation in the tick submission program increases with income. Perhaps wealthier communities have more knowledge of or access to the CAES-TTL. In contrast, the lack of an association between reported Lyme disease incidence and median household income suggests that Lyme disease case reporting is independent of the community's wealth. Lot size has been shown to be associated with tick infestation and Lyme disease risk, with larger lots more likely to have a wooded area, higher numbers of ticks, and Lyme disease cases (Maupin et al., 1991; Cromley et al., 1998). The association between the rate of submitted infected nymphs and median household income may indicate that households with higher income tend to have larger lots with greater likelihood of including wooded areas. The degree of developed land use was associated with Lyme disease incidence but none of the tick-based metrics. The increase in reported Lyme disease incidence in less developed areas may therefore be due to human behavioral differences in urban versus rural areas. While we can only speculate on the

Table 2
Temporal trends.

	Year β (95% CI)
Rate of submitted nymphs	0.974 (0.968, 0.981)
Nymphal infection prevalence	0.950 (0.936, 0.964)
rate of submitted infected nymphs	0.924 (0.855, 0.999)
Lyme disease incidence	0.972 (0.968, 0.976)

Temporal trends of tick-based risk metrics (rate of submitted nymphs, nymphal infection prevalence, and rate of submitted infected nymphs) and Lyme disease incidence across Connecticut. Here we report the coefficient estimate (β) for year. β Values under 1 support a decrease in each tick-based risk metric and Lyme disease incidence over time.

differential mechanisms underlying these relationships, we are assured that, at least as they were measured, neither covariate confounds the relationship between these tick-based risk metrics and Lyme disease incidence.

3.5. Spatiotemporal patterns, 2007–2017

Overall, annual nymphal submissions were correlated (Spearman's rank correlation) with annual reported Lyme disease incidence both at the town ($\rho = 0.26$, $p < 0.001$, $n = 1859$ observations) and the county ($\rho = 0.66$, $p < 0.001$, $n = 88$ observations) scales.

To explicitly assess temporal changes in the rate of submitted nymphs, NIP, the rate of submitted infected nymphs, and Lyme disease incidence, we used generalized linear models with year as an ordinal integer (Table 2). The models suggest that the rate of submitted nymphs, NIP, the rate of submitted infected nymphs, and Lyme disease incidence decreased over time between 2007 and 2017 (Table 2; βs < 1).

While Lyme disease cases have increased overall in the United States (Centers for Disease Control and Prevention, 2015), other researchers have noted a downward trend in Lyme disease incidence in states previously classified as high incidence (Schwartz et al., 2017). Such downward trends may be due to reporting fatigue, human behavioral changes (e.g., improved prevention and control), decreasing tick densities, among other factors.

The observation that NIP decreased over time between 2007 and 2017 differs from reports where infection prevalence in field-collected nymphs (Diuk-Wasser et al., 2012; Feldman et al., 2015) and passively collected *I. scapularis* ticks (Xu et al., 2016; Jordan and Egizi, 2019) remain relatively stable over time. In contrast to endemic areas, in areas of emergence infection prevalence has been shown to increase over time (Nelder et al., 2014; Gasmi et al., 2016). The fluctuations in rates of submitted (infected) nymphs are in agreement with changes in tick densities and the density of infected ticks over time, which in turn may be due to changes in host populations and climatic conditions (Stafford et al., 1998; Wilson, 1998; Killilea et al., 2008). However, in a hyperendemic Lyme disease state such as Connecticut we cannot rule out the possibility that tick submissions to the CAES-TTL have declined due to waning public interest.

We note differences in Lyme disease incidence across counties in Connecticut. Lyme disease incidence was highest in Windham, Tolland, and New London counties and lowest in New Haven, Fairfield, and Hartford counties (Fig. 2). At the town scale, we found evidence of spatial clustering for Lyme disease incidence (Moran's I: 0.547, $z = 10.307$, $p < 0.001$); specifically, we note high incidence towns at the intersection of Tolland, Windham and New London Counties and low incidence towns in southwestern Hartford and northeastern New Haven Counties (Fig. 3).

At the town scale, we found evidence of spatial clustering for the rate of submitted nymphs (Fig. 4; Moran's *I*: 0.447, $z = 8.776$, $p < 0.001$), and the rate of submitted infected nymphs (Fig. 5; Moran's I: 0.412, $z = 7.997$, $p < 0.001$). Indeed, the majority (81%) of submitted nymphs were from Fairfield and New Haven Counties (Fig. 2). There was little difference in NIP across towns (21.1%, 95%CI: 20.0%, 22.1%) or counties (21.0%, 95%CI: 19.4%, 22.5%) in Connecticut between 2007 and 2017 (Fig. 2) and NIP did not display spatial clustering (Fig. 6; Moran's I: 0.07, $z = 1.52$, $p = 0.13$). NIP may be near uniform, at least at the spatial scale of counties or towns, in states or regions where *I. scapularis* is long established and ubiquitous (New York City Department of Health and Mental Hygiene, 2018). Of course, there is aggregation of estimates at the county and town levels. At smaller spatial scales, such as for individual households, there is likely a great deal of variability in tick-based risk measures (Ostfeld et al., 1996; Pardanani and Mather, 2004; Killilea et al., 2008). Interestingly the finding that NIP is relatively steady across Connecticut is different from previous study in Connecticut showing that before 1991 ticks infected with *B. burgdorferi* were concentrated to the coastline (Magnarelli et al., 1993), indicating a shift from emergent to endemic populations of *I. scapularis*. If it is true that NIP is fairly stable across the state within any year but changes over time, then repeated annual sampling in a few locations in an active tick surveillance program might provide sufficient information to quantify risk especially when resources are limited.

After accounting for population, we note higher Lyme disease incidence in more rural counties of Connecticut as has been noted previously (Cromley et al., 1998), such as Windham and Tolland, yet lower rates of submitted (infected) nymphs – estimates that similarly account for population – and similar NIP across counties (Fig. 2). Collectively, these findings suggest that human behavior is playing a large part in encounters with infected ticks and Lyme disease transmission risk (Nicholson and Mather, 1996). There may also be a need to better promote the CAES-TTL program in more rural parts of the state.

Future research should assess whether the rates of submitted nymphs are associated with the density of host-seeking nymphs. Furthermore, a comparison of infection prevalence in nymphal ticks collected from humans versus from the environment would be needed to determine if the trend for infection prevalence in nymphs removed from humans (in this case a decreasing trend) directly reflect that of nymphs in the environment, or if changes in human use of the landscape over time could have led to increased exposure to nymphs residing in microhabitats with lower tick density and less intense enzootic transmission of *B. burgdorferi* s.l., or if decreasing submission and case reports are simply explained by fatigue or reduced participation. Future studies should also explore whether passive (ticks on people) or active (drag sampling) surveillance provides better estimates of human disease risk. This comparison should also include a cost analysis to determine if any predictive improvement in active surveillance outweighs the added costs of these programs (Nelder et al., 2014). Finally, the findings that NIP decreases temporally between 2007 and 2017 but is geographically uniform, warrants further investigation.

3.6. Spatiotemporal modeling, 2007–2017

We found general declines in tick-based risk measures as well as Lyme disease incidence during the period 2007–2017. We also found divergent spatial patterns in the rates of submitted (infected) nymphs with those for Lyme disease incidence. We used a generalized linear mixed effects model to explicitly account for these spatiotemporal differences in tick-based risk measures and Lyme disease incidence to determine (1) if within each county (or town), there is a relationship between these tick-based risk measures and Lyme disease incidence and (2) if we can use these tick-based risk measures to predict Lyme disease for each county (or town).

At both the county and town spatial scales, we found that over the eleven years investigated an increase in the rate of submitted (infected) nymphs was predictive of increased Lyme disease incidence for each county (or town). Table 3 shows the coefficient estimates for each tick-based risk metric, the associated AIC score, and Spearman's rank correlation coefficient for the model-predicted and observed Lyme disease

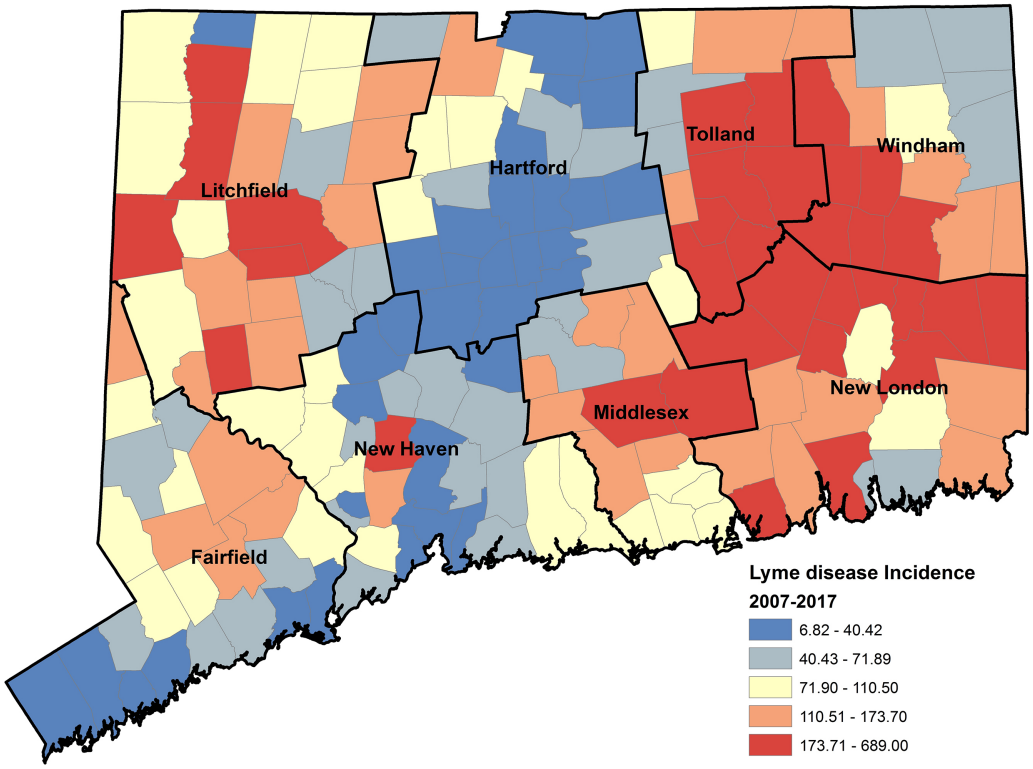

Fig. 3. Lyme disease incidence. Cumulative (2007–2017) total Lyme disease incidence (per 100,000) broken into quartiles and mapped by town.

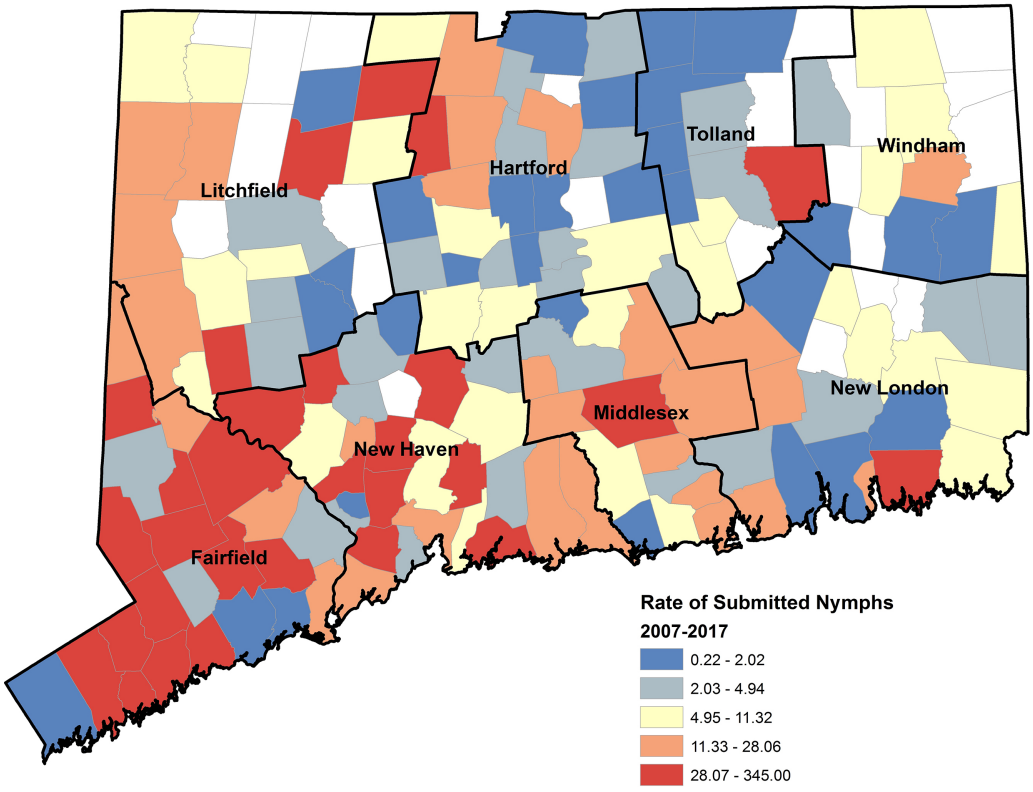

Fig. 4. Rate of submitted nymphs. Cumulative (2007–2017) rate of submitted nymphs per 100,000 populations broken into quartiles and mapped by town.

incidence. Overall, we find better model performance at the county compared to the town spatial scale. We note that the models with NIP are not significant, but that inclusion of NIP with the rate of submitted nymphs in the tick-based risk metric rate of submitted infected nymphs is an improvement over the predictive value of just the rate of submitted nymphs. Moreover the inclusion of the percent of developed land further explains variability in Lyme disease incidence and improves model fit. We conducted chi-squared tests to assess whether the inclusion of predictors

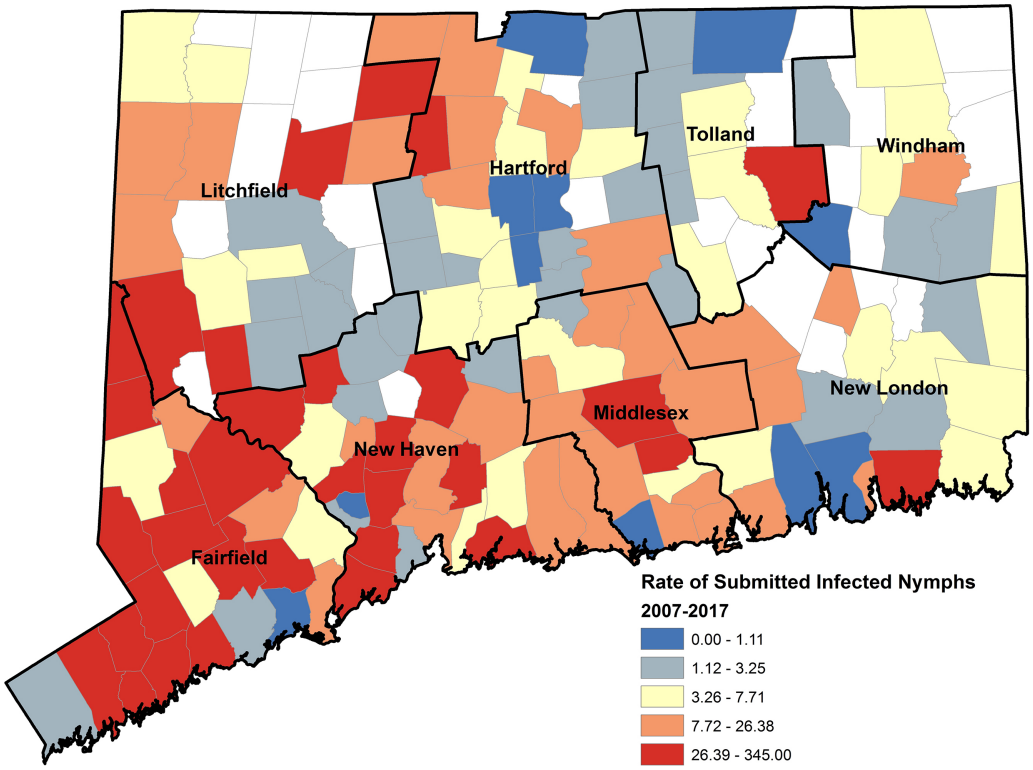

Fig. 5. Rate of submitted infected nymphs. Cumulative (2007–2017) rate of submitted infected nymphs per 100,000 population broken into quartiles and mapped by town.

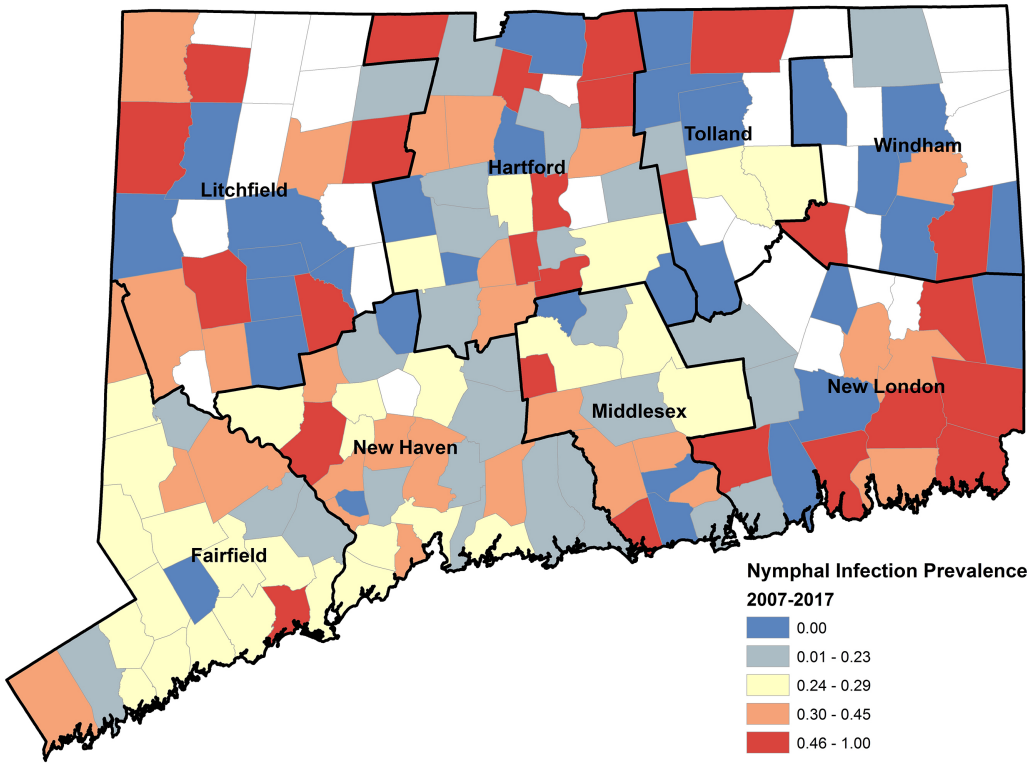

Fig. 6. Nymphal infection prevalence. Cumulative (2007–2017) nymphal infection prevalence broken into quartiles and mapped by town.

led to statistically significant improvements in model fit as measured by a reduction in the residual sum of squares. Compared to a null model, the rate of submitted infected nymphs improved model performance ($\chi^2 = 12.874$, $p < 0.001$). Inclusion of the percent of developed land in the county model further improved model fit without influencing the effect estimate for the rate of submitted infected nymphs ($\chi^2 = 15.599$, $p < 0.001$). Of the models tested, the rate of submitted infected nymphs along with the percent of developed land as a covariate at the county scale provided the best model fit for predicting Lyme disease incidence as measured by AIC (AIC = 1267, Table 3).

Table 3
Model results comparing tick-based risk metric predictive value.

Model parameters	β (95% CI)	AIC	ρ
Town spatial scale (n = 1859)			
Rate of submitted nymphs	1.200 (1.180, 1.221)	10,711	0.598
Nymphal infection prevalence	0.988 (0.969, 1.007)	10,263	0.598
Rate of submitted infected nymphs	1.187 (1.166, 1.208)	9970	0.595
Rate of submitted nymphs + degree developed	1.017 (0.999, 1.036)	7271	0.724
Nymphal infection prevalence + degree developed	0.985 (0.966, 1.004)	6762	0.720
Rate of submitted infected nymphs + degree developed	1.021 (1.002, 1.041)	6760	0.720
County spatial scale (n = 88)			
Rate of submitted nymphs	1.050 (1.015, 1.087)	1304	0.946
Nymphal infection prevalence	0.998 (0.976, 1.020)	1294	0.944
Rate of submitted infected nymphs	1.050 (1.022, 1.078)	1281	0.945
Rate of submitted nymphs + degree developed	1.051 (1.016, 1.088)	1290	0.946
Nymphal infection prevalence + degree developed	0.998 (0.976, 1.020)	1281	0.944
Rate of submitted infected nymphs + degree developed	1.051 (1.023, 1.079)	1267	0.945

Generalized linear mixed effect models (family = Poisson, link = log) with year and county as crossed random effects. For each set of model parameters tested we compare: the coefficient (β) estimate for the tick-based risk metric is given along with the 95% confidence interval; AIC is the Akaike Information Criterion for the model, lower is better; and Spearman's rank correlation coefficient (ρ) for the model-predicted and observed Lyme disease incidence are given. The models were conducted at two spatial scales, town and county. There were 1859 observations at the town spatial scale (169 towns and 11 years); and 88 observations at the county spatial scale (8 counties and 11 years).

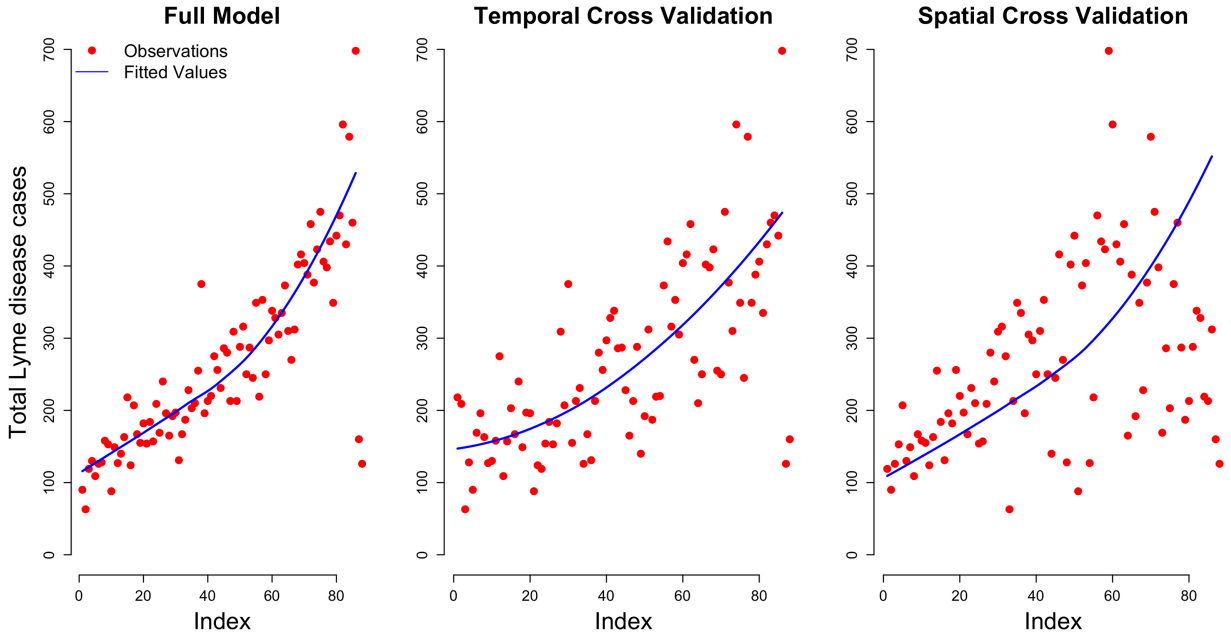

Fig. 7. Model fits. Relationship of observed Lyme disease cases (red dots) and model predictions of Lyme disease cases (blue line). Predictions based on best fitting model by AIC – the model including the rate of submitted infected nymphs and the degree of developed land use at the county spatial scale. (For interpretation of the references to color in the text, the reader is referred to the web version of this article.)

Fitted model values (predicted values) were strongly and positively correlated with observed values of Lyme disease incidence at the county scale (Table 3, ρs range from 0.945 to 0.946, $p < 0.001$; Fig. 7, Full Model). This indicates a signal between the rate of submitted (infected) nymphs with Lyme disease incidence regardless of potential spatio-temporal biases in passive tick or Lyme disease surveillance.

3.7. Spatiotemporal model validation, 2007–2017

By conducting leave-one-out temporal and spatial cross validations (LOOTCV and LOOSCV, respectively), we found the full model (RMSE = 40.91) performed better than either the LOOTCV model (RMSE = 73.27) or the LOOSCV model (RMSE = 136.70) (Fig. 7). The lower RMSE for the LOOTCV suggests that out of sample predictions (i.e. model predictions of a set of observations different than those that the model was fitted on) is better year-to-year than county-to-county. Models trained on data from certain counties (such as counties with more observations) may provide better predictions than models trained on data from others.

3.8. Conclusion

While Lyme disease has been endemic in Connecticut for over three decades, disease occurrence is still spreading geographically in other parts of the Eastern United States (Eisen and Eisen, 2018). We can learn from this Connecticut based research and employ the results in emergent areas facing a growing threat of Lyme disease (Stone et al., 2017). Results from this longitudinal analysis in an endemic setting suggest

that the rate of submitted infected nymphs are highly predictive of Lyme disease incidence for each town or county. These metrics could be calculated from other passive surveillance datasets in emergent areas, but their accuracy in predicting Lyme disease occurrence would need to be evaluated. There are some very important caveats to passive tick surveillance programs, which were well accounted for in this study but can be difficult to achieve: tick identification being done by trained individuals and exclusion of ticks acquired while traveling out of county or state.

The use of passive surveillance to build predictive models for public health decision-making is limited, as it has been asserted that passive surveillance data are biased (Beck et al., 2014). However, tick submissions through passive surveillance were shown to predict Lyme disease cases at a town level in an emergent region in Canada (Ripoche et al., 2018). Moreover, a predictive model for Lyme disease based on passive surveillance data was successfully validated using active surveillance data in Canada (Soucy et al., 2018).

In this study we analyzed an eleven-year record of passive surveillance data with 23,432 reported Lyme disease cases and 26,116 tick submissions and found a strong relationship between the rate of submitted infected nymphs with Lyme disease incidence for each county over time. Our findings underscore the relevance of using passive surveillance based on ticks recovered from humans to guide informed decisions concerning prevention and treatment of tick-borne diseases.

Authors' contributions

GM and JFA oversaw the Tick Testing Laboratory. GM and KCS conceived of the paper. EAHL ran the analysis. EAHL, LE, RJE, and GM wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are mostly available online at: http://www.ct.gov/caes/cwp/view.asp?a=2837&q=378212&caesNav=|. More detailed information is also available from the CAES TTL on reasonable request by contacting the Tick.Testing.Laboratory@ct.gov.

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