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Comparison of acarological risk metrics derived from active and passive surveillance and their concordance with tick-borne disease incidence

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Abstract

Tick-borne diseases continue to threaten human health across the United States. Both active and passive tick surveillance can complement human case surveillance, providing spatio-temporal information on when and where humans are at risk for encounters with ticks and tick-borne pathogens. However, little work has been done to assess the concordance of the acarological risk metrics from each surveillance method. We used data on *Ixodes scapularis* and its associated human pathogens from Connecticut (2019-2021) collected through active collections (drag sampling) or passive submissions from the public to compare county estimates of tick and pathogen presence, infection prevalence, and tick abundance by life stage. Between the surveillance strategies, we found complete agreement in estimates of tick and pathogen presence, high concordance in infection prevalence estimates for *Anaplasma phagocytophilum, Borrelia burgdorferi* sensu stricto, and *Babesia microti*, but no consistent relationships between actively and passively derived estimates of tick abundance or abundance of infected ticks by life stage. We also compared nymphal metrics (i.e., pathogen prevalence in nymphs, nymphal abundance, and abundance of infected nymphs) with reported incidence of Lyme disease, anaplasmosis, and babesiosis, but did not find any consistent relationships with any of these metrics. The small spatial and temporal scale for which we had consistently collected active and passive data limited our ability to find significant relationships. Findings are likely to differ if examined across a broader spatial or temporal coverage with greater variation in acarological and epidemiological outcomes. Our results indicate similar outcomes between some actively and passively derived tick

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Author's contributions:

Karen M Holcomb: Formal analysis, Writing – Original Draft, Writing – Review & Editing, Visualization; **Noelle Khalil**: Investigation, Writing – Review & Editing; **Duncan W Cozens**: Investigation, Writing – Review & Editing; **Jamie Cantoni**: Investigation, Writing – Review & Editing; **Doug E Brackney**: Investigation, Writing – Review & Editing; **Megan A Linske**: Investigation, Writing – Review & Editing; **Scott C. Williams**: Investigation, Writing – Review & Editing; **Goudarz Molaei**: Funding acquisition, Project administration, Resources, Data curation, Investigation, Writing – Review & Editing; **Rebecca J Eisen**: Conceptualization, Writing – Review & Editing, Supervision

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surveillance metrics (tick and pathogen presence, pathogen prevalence), but comparisons were variable for abundance estimates.

Keywords

active surveillance; passive surveillance; *Ixodes scapularis* ; Lyme disease; anaplasmosis; babesiosis

Introduction:

Tick-borne diseases are the leading cause of locally acquired vector-borne disease in the United States. In 2019, 91% of all vector-borne disease cases reported to the Centers for Disease Control and Prevention (CDC; 55,858 cases) were tick-borne, with Lyme disease cases representing 63% of all reported vector-bone disease cases (69% of reported tick-borne cases) (Centers for Disease Control and Prevention, 2021). The blacklegged tick (*Ixodes scapularis*) serves as a vector of the causative agent of Lyme disease, *Borrelia burgdorferi* sensu stricto (s.s.) (Burgdorfer et al., 1982). Blacklegged ticks are also capable of vectoring a growing number of human pathogens, including *Anaplasma phagocytophilum* (anaplasmosis), *Babesia microti* (babesiosis), *Borrelia miyamotoi* (hard tick relapsing fever), *Borrelia mayonii* (Lyme disease), and *Ehrlichia muris eauclairensis* (ehrlichiosis) (Eisen and Eisen, 2018). The majority of tick-borne disease cases are reported in the northeastern and north central states (Schwartz et al., 2017) with the number of counties classified as high incidence continuing to increase; in 2008-2012, 260 counties were classified as high incidence (Kugeler et al., 2015) and this number rose to 593 by 2020 (Centers for Disease Control and Prevention, 2022a).

To monitor changes in human risk of exposure to ticks and tick-borne human pathogens across the United States, CDC initiated a national tick surveillance program in 2018 with a special emphasis on *Ixodes* spp. ticks and their associated pathogens (Centers for Disease Control and Prevention, 2022b; Eisen and Paddock, 2021). The program outlined surveillance objectives and developed a common electronic database to generate and report standardized tick-based data (i.e., the ArboNET Tick Module). Surveillance objectives increase in complexity, knowledge gained, and also in the time and cost to quantify each metric. The most basic objective aims to identify the presence of tick species in order to update vector distribution maps. Counties are categorized as having no records, reported (fewer than six ticks of the same species and life stage submitted) or established populations (more than one life stage or ≥ 6 ticks of a single life stage and species submitted within a 12-month period). The second objective documents pathogen presence per county (at least a single record of the pathogen in ticks) and estimating the respective infection prevalence by pathogen and tick species and life stage. Demonstrating the value of more complex metrics (pathogen data compared with vector data alone), presence of *Bo. burgdorferi* s.s. in host-seeking ticks was found to be a better predictor of high Lyme disease incidence counties compared with tick presence alone (Burtis et al., 2022). Additional objectives seek to estimate the density of host-seeking ticks by species and life stage and to derive a combined estimate of the density of infected host-seeking nymphs or adults. The density

of host-seeking *Bo. burgdorferi*-infected nymphs has been shown to correlate with Lyme disease incidence, with density of infected nymphs being a better predictor than density estimates alone (Pepin et al., 2012). Finally, a single objective addresses the timing of risk of human encounters with ticks by documenting regional differences in the host-seeking phenology of ticks by life stage.

Active and passive methods have been used to address these objectives (Eisen and Eisen, 2021; Mader et al., 2021; Nieto et al., 2018). Active surveillance uses dragging or flagging to collect ticks from the environment while in passive surveillance, people submit ticks found on their body. Due to the availability of resources, one strategy may be preferred over another. Active surveillance can produce standardized metrics of presence, infection prevalence, density, and density of infected ticks that are comparable across space and time. However, dragging and flagging surveys are labor intensive, thereby reducing the number and size of sampling locations that can be feasibly sampled. Additionally, repeated samples are needed to obtain robust estimates of local population density (Clow et al., 2018; Dobson, 2013) with peak density (i.e., max density calculated) often used to capture the highest level of risk. Human behaviors modulating tick-encounters are not captured by active surveillance so these metrics may not accurately reflect zoonotic risk (Eisen and Eisen, 2016). In contrast, metrics derived from passive surveillance (ticks on people) directly provide information on human-tick encounters. Testing submitted ticks can identify infection prevalence from a generally broader geographic area than can be sampled through active surveillance (Xu et al., 2016). Similar to active surveillance, these passively-derived estimates can provide spatio-temporal data on pathogen spread over time (Walter et al., 2016). Limitations of passive surveillance include less spatial precision compared with drag sampling in estimating where ticks are present, waning interest over time (participation fatigue) or variable knowledge of and willingness to participate in the program between communities in the surveillance area, spatial bias in submissions to more densely populated communities, difficulty among submitters in detecting immature life stages resulting in bias towards submission of adult ticks, presence-only information, and concerns about data quality (Eisen and Eisen, 2021; Koffi et al., 2012; Nelder et al., 2014; Soucy et al., 2018). While all life stages of ticks can be collected via active and passive surveillance, nymphs are the most epidemiologically relevant life stage as most human cases of *I. scapularis*-borne diseases (Lyme disease, anaplasmosis, and babesiosis) result from the bite of a nymphal tick (Falco et al., 1996; Mead, 2015; Spielman et al., 1985).

While metrics derived from active or passive surveillance have been found to be independently associated with the incidence of tick-borne diseases, little work has been done to concurrently compare the metrics with each other and with human disease incidence. Connecticut is one of few states that have conducted both active and passive tick and tick-borne pathogen surveillance across all counties in the state and during the same time periods, making it an ideal location for comparing concordance in surveillance objectives derived by active or passive methods. Using data from 2019-2021, we assessed the concordance of *I. scapularis* adult and nymphal metrics (i.e., tick presence; pathogen presence and infection prevalence; density vs. submission rate; and density of infected ticks vs. submission rate of infected ticks) derived from active and passive surveillance as well as

the relationship of nymphal metrics to human incidence of Lyme disease, anaplasmosis, and babesiosis.

Methods:

Study area

Connecticut is a northeastern state in the United States. Its 13,023 km^2 land area is divided into eight counties, which range in size from 956 km^2 (Middlesex County) to 2,383 km^2 (Litchfield County) (United States Census Bureau, 2021). Connecticut reports high annual incidence of Lyme disease (average of 75.4 cases/100,000, 1995-2020), anaplasmosis (average of 1.9 cases/100,000, 2008-2020), and babesiosis (average of 6.0 cases/100,000, 2011-2020) (Connecticut State Department of Health, 2021). The state also has a longstanding passive surveillance program with the Connecticut Agricultural Experiment Station (CAES, located in New Haven County) offering free tick testing to residents since 1996 (Connecticut State Government, 2023). In 2019, CAES and the Connecticut Department of Public Health jointly received support through the CDC Epidemiology and Laboratory Capacity (ELC) cooperative agreement to initiate ongoing active surveillance.

Tick-based data and metrics

We obtained active and passive surveillance data from Connecticut for 2019-2021, the period of time in which passive and active surveillance were conducted, and calculated acarological risk metrics from each method. We then compared metrics to each other as well as with reported human incidence of Lyme disease, anaplasmosis, and babesiosis for the same period. From passive surveillance, we calculated the proportion of ticks (nymph and adult female) infected with *Bo. burgdorferi* sensu lato (s.l.), *A. phagocytophilum*, and *Ba. microti*, the submission rate (total and peak for nymph and adult female), and the rate of infected nymphal submissions (INS). From active surveillance, we calculated infection prevalence for the three pathogens (in nymph and adult females), the peak density (nymph and adult female), and density of infected nymphs (DIN).

Passive tick surveillance

We obtained records of ticks submitted to CAES by Connecticut residents. Each record included a submitted tick's species, life stage, date of submission, location where the tick was likely acquired (town, neighborhood, or area) if different than the submitter's town of residence, and pathogen testing results. We used date of tick submission instead of date of removal as the former has been found to be more reliable. We excluded records if the location of tick acquisition could not be resolved at the county level or travel was reported within the previous ten days. All submitted ticks were examined under a dissecting microscope and identified to species using standard morphological keys and taxonomic references (Durden and Keirans, 1996; Keirans and Litwak, 1989). We filtered data to exclude any species that was not identified as *I. scapularis*. Each *I. scapularis* tick was individually tested for *Bo. burgdorferi* s.l., *A. phagocytophilum*, and *Ba. microti* using a previously described methodology (Williams et al., 2018). Briefly, genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) or

DNAzol (Molecular Research Center, Cincinnati, OH) according to the manufacturer's recommendations, with some modifications (Molaei et al., 2006), followed by a PCR amplification of flagellin and 16S rRNA genes for *Bo. burgdorferi* s.l. (Barbour et al., 1996), the 16S rRNA gene for *A. phagocytophilum* (Massung et al., 1998), and the 18S rRNA gene for *Ba. microti* (Persing et al., 1992). Assays for *A. phagocytophilum* did not discriminate between human-active (ha) variant or variant 1 (v1). CAES did not test ticks that were unengorged or found on a pet. Unengorged ticks were not tested due to limited resources and the high number of submissions CAES received each year (~4% submitted ticks annually are unengorged).

Assessing tick presence—Since the travel histories of tick submitters were assessed, we classified each county as 'established' or 'reported' for *I. scapularis*, following the widely accepted and standardized definitions used by Dennis et al. (1998) and Eisen et al. (2016). Specifically, we classified a county as 'established' if at least six individual *I. scapularis* ticks or two host-seeking life stages (i.e., adult and nymph) were submitted within a one-year time period.

Documenting pathogen presence—Using the pathogen testing records, we identified pathogen (*Bo. burgdorferi* s.l., *A. phagocytophilum*, and *Ba. microti*) presence by county. We classified a pathogen as being present in a county if it was detected in at least one submitted *I. scapularis* tick during the three-year study period.

Estimating pathogen prevalence—We calculated infection prevalence of *Bo. burgdorferi* s.l., *A. phagocytophilum*, and *Ba. microti* from passive data as the proportion of ticks testing positive for the respective pathogens. We calculated infection prevalence by life stage, year, and county of submission. For comparing infection prevalence estimates from active and passive surveillance, we assumed that all positive *Bo. burgdorferi* s.l. results were *Bo. burgdorferi* s.s. based on previous findings that all *Bo. burgdorferi* s.l. isolated from ticks collected in Connecticut was *Bo. burgdorferi* s.s. (Feldman et al., 2015). We calculated 95% confidence intervals around infection prevalence estimates using Wilson score intervals (Wilson, 1927) and the *pooledBin* function in the R package PooledInfRate (Biggerstaff, 2002; R Core Team, 2022).

Estimating I. scapularis abundance by life stage (submission rate)—To calculate peak submission rates, we first aggregated submissions by life stage (i.e., adult female and nymph), and county of submission and totaled the number of ticks submitted per week of the year to calculate weekly submission volume. We then calculated a weekly per capita submission rate (ticks submitted per 10,000 residents) using the county-level estimates for total population from the 2020 Census (i.e., dividing weekly submission volume by county population and multiplying by 10,000) (United States Census Bureau, 2021). Constraining the submissions to peak activity months (January-May and October-December for adults and March-September for nymphs), we identified the week with the highest rate of submissions ("peak week") per county, year, and life stage. For adult females, we selected two peaks (spring (January-May) and fall (October-December)) corresponding to the observed bimodal phenology of host-seeking adults; the spring peak corresponded to the cohort of adults that

took a bloodmeal as nymphs during the previous summer, while the fall peak corresponded to the cohort of adults that took a bloodmeal as nymphs in the summer of the observation year.

We also calculate the total annual per capita submission rate (submissions per 10,000 residents) for nymphs and both cohorts of adults. For this, we used the total number of ticks submitted per county, year, and cohort (i.e., adult females submitted from January-June (spring cohort), adult females submitted from July-December (fall cohort), and nymphs submitted from January-December).

Estimating abundance of infected ticks (infected nymphal submission rate)— We then calculated the rate of submission of infected nymphs (INS) per county, year, and pathogen in two ways. For the first, we multiplied the per capita rate of submission for the peak week with nymphal infection prevalence (peak number of infected nymphs submitted per week and 10,000 residents, “peak INS”). For the second, we used the total annual per capita rate of submission instead in the multiplication with nymphal infection prevalence (number of infected nymphs submitted per year and 10,000 residents, “total INS”). We focused on nymphs because we expected this metric to be the best predictor of human risk as the majority of human cases of Lyme disease, anaplasmosis, and babesiosis result from nymphal bites (Falco et al., 1996; Mead, 2015; Spielman et al., 1985).

Active tick surveillance

We obtained active tick surveillance data reported by the Connecticut Department of Public Health and CAES to the ArboNET Tick Module. Data consisted of records of site-level tick drags detailing the location, date, area dragged, and the number of nymphal and adult female *I. scapularis* ticks collected. Tick drags were conducted at 5-6 collection sites per county with 2-8 visits per collection site per year (Fig. 1, Table A.1). For each site, collections occurred approximately every 3-4 weeks from April to November each year. On each sampling occasion, 750 m^2 were dragged per collection site. Sites were selected based on public accessibility and presence of suitable tick habitat.

Due to resource limitations, a subset of collected ticks (an average of 64% of nymphs and 74% of adult females collected state-wide per year) were tested using a previously described multiplex real-time reverse transcription-PCR assay (Tokarz et al., 2017) for *Bo. burgdorferi* s.s., *A. phagocytophilum*, and *Ba. microti*. Briefly, ticks were identified to species, individually placed into microcentrifuge tubes containing 500 μl PBS, and homogenized on a mixer mill (30 Hz for 4 min.). Subsequently, 200 μl total nucleic acid was extracted using the MagMax Viral/Pathogen Nucleic Acid Isolation Kit (Applied Biosystems) on a Kingfisher Flex high-throughput extraction device and eluted in 50 μl according to manufacturer’s recommendations. Testing results for each life stage were reported at the county-year aggregation level to the ArboNET Tick Module; date of collection for tested ticks was thus not available. The assay for *Bo. burgdorferi* was specific for *Bo. burgdorferi* s.s. while the assay for *A. phagocytophilum* did not discriminate between human-active (ha) variant or variant 1 (v1).

Assessing tick presence—We classified each county as 'established' or 'reported' for *I. scapularis*, following the definitions used by Dennis et al. (1998) and Eisen et al. (2016). As with passive surveillance, we classified a county as 'established' if at least six individual *I. scapularis* ticks or two host-seeking life stages (i.e., adult and nymph) were collected within one year.

Documenting pathogen presence—Similar to passive surveillance, we identified pathogen (*Bo. burgdorferi* s.s., *A. phagocytophilum*, and *Ba. microti*) presence by county based on testing results of ticks collected via drag samples. We classified a pathogen as being present in a county if it was detected in at least one host-seeking *I. scapularis* tick during the three-year study period.

Estimating pathogen prevalence—From the county-year testing results, we calculated infection prevalence estimates (with 95% CI using Wilson score tests (Wilson, 1927)) by pathogen, life stage, county, and year. Due to aggregation of the reported testing result data, we could not calculate prevalence estimates separately for the spring and fall cohorts of adult females, but rather calculated a single yearly estimate for adult females.

Estimating abundance (peak density)—We estimated peak density of host-seeking ticks per life stage from site-level drag sampling. For each site, we calculated the density of nymphs (DON) and adult females (DOF) as the number of each life stage collected per 1,000 m^2 of dragging. Constraining collections to within months of peak activity (as defined above), we identified the peak density of each tick cohort per year and county (i.e., peak DON, peak spring DOF, and peak fall DOF).

Estimating abundance of infected ticks (density of infected nymphs)—Multiplying infection prevalence with peak density, we calculated density of infected nymphs (DIN) by pathogen, cohort, county, and year. We again focused on nymphs because DIN has been shown to be a robust estimator of human Lyme disease cases (Diuk-Wasser et al., 2012; Eisen and Eisen, 2016; Mather et al., 1996; Pepin et al., 2012) and to complement our choice of risk metrics from passive surveillance.

Human disease data

We obtained from the Connecticut Department of Public Health (Connecticut State Department of Health, 2021) counts of human cases of Lyme disease, anaplasmosis, and babesiosis that met the criteria for reported and confirmed (Council of State and Territorial Epidemiologists, 2022) by county and year. All three tick-borne diseases are reportable in Connecticut with medical professionals and clinical laboratories required to submit reports (Connecticut State Department of Health, 2023). For each county, we calculated the annual incidence (cases per 100,000 residents) using the total population size per county from the 2020 Census (United States Census Bureau, 2021).

Data analysis

We compared each set of metrics (i.e., tick presence, pathogen presence and infection prevalence, peak density or submission rate (peak or total), and DIN or INS (peak or total))

derived from active and passive surveillance to assess their concordance. To compare tick presence, we compared the number of counties classified as 'established' and 'reported' from active vs. passive surveillance using a Fisher's exact test (*fisher.test* function in R (R Core Team, 2022)). Similarly, to compare pathogen presence derived from active and passive surveillance, we compared the number of counties for which each of the three pathogens were reported as present using a Fisher's exact test. For this and the following comparison of infection prevalence estimates, we assumed that all ticks testing positive for *Bo. burgdorferi* s.l. from passive surveillance were infected with *Bo. burgdorferi* s.s. For infection prevalence, we assessed if the estimates for each county, year, pathogen, and life stage differed using Fisher's exact tests. To compare peak density with peak or total per capita submission rate, we fit linear models to the scatter of the separate density-submission rate metrics by individual county by tick cohort (i.e., spring adult females, summer nymphs, and fall adult females) to identify the direction (slope) and significance (Wald test *P*-value that the slope ≠ 0) of the relationship (*lm* function in R (R Core Team, 2022)). We also estimated the linear relationship across all county-year estimates to capture the relationship at a larger spatial scale. Similarly, to compare DIN and INS, we fit linear models per individual county and all counties together and determined the direction and significance of the slope of the relationship. We assessed the relationship of peak INS and total INS with DIN separately.

We then compared nymph-specific metrics to human disease incidence to identify their concordance. Similar to above, we fit linear models to incidence of tick-borne disease (Lyme disease, anaplasmosis, and babesiosis) and each nymphal metric (i.e., nymphal infection prevalence, peak nymphal density or nymphal submission rate (peak and total), and DIN or INS (peak or total)) derived from active and passive surveillance for each county individually and all counties together. For each model, we identified the slope (direction of association) and strength of association (significance of Wald test *P*-value for the slope).

We conducted all analyses using R statistical software (version 4.2.0 (R Core Team, 2022)).

Results:

Tick submissions and collections

From 2019-2021, Connecticut residents submitted 7,651 adult female and 3,356 nymphal *I. scapularis* to CAES (Table 1). The magnitude of submissions varied across counties, with the largest number of submissions from Fairfield (45.2-52% of submitted females; 46.1-50% of submitted nymphs) and New Haven (20.4-21.4% of submitted females; 17.2-21.7% of submitted nymphs) each year. Of the submitted ticks, 7,383 females and 3,330 nymphs were individually tested for *A. phagocytophilum*, *Bo. burgdorferi* s.l., and *Ba. microti*. Thirty-six ticks (7 adult females (0.09%) and 29 nymphs (0.86%)) were inconclusive for *Ba. microti* so we excluded these results from subsequent analyses.

Submission rates of ticks resolved into distinct seasonal curves that were similar in shape across counties and years (Fig. 2). Submissions of adult female *I. scapularis* exhibited a bimodal distribution with peaks around week 16 (April) and 45 (November) while submissions of nymphs peaked around week 26 (June).

During the same period, 2,136 adult female and 2,907 nymphal *I. scapularis* were collected through drag sampling (204-278 collection events per year, Table A.1). Of those ticks collected, 1,559 adults and 1,787 nymphs were individually tested for *A. phagocytophilum*, *Ba. microti*, and *Bo. burgdorferi* s.s.

Tick status comparison

We independently categorized eight (100%) counties as established for *I. scapularis* ticks based on records from active and passive surveillance. We did not find a difference in presence classifications derived from surveillance methods (Fisher's exact $P = 1$).

Pathogen presence comparison

For both active and passive surveillance, we identified *Bo. burgdorferi* s.s., *A. phagocytophilum*, and *Ba. microti* as present in all eight counties of Connecticut. Thus, we found exact concordance in pathogen presence (Fisher's exact $P = 1$ for difference by individual pathogen).

Infection prevalence comparison

We found high correspondence of infection prevalence estimates derived from active and passive surveillance (Fig. 3). Across both surveillance methods, we estimated an average infection prevalence of *A. phagocytophilum* in adult females of 7.9% (range: 0-20.8%) and 4.0% (range: 0-13.3%) in nymphs during our study period. We estimated an average infection prevalence for *Ba. microti* in adult females of 11.7% (range: 3.8-28.1%) and 6.2% (range: 0-19.2%) in nymphs. For *Bo. burgdorferi* s.s., we estimated an average infection prevalence in adult females of 43.7% (range: 29.6-69.8%) and 19.4% (range: 7.7-35.9%) in nymphs. Of the 144 comparisons across years, pathogens, counties, and life stages, 17 pairs (11.8%) of infection prevalence estimates were significantly different for active vs. passively derived ticks by Fisher's exact test. Of these discordant pairs, estimates from active surveillance tended to be higher than those derived from passive surveillance.

Peak density vs. submission rate comparisons

While the exact week identified as the peak in terms of density or submission rate varied, we found some broad consistency in the timing of when ticks are active and in their observed peaks of activity for each cohort from active (Fig. 2) and passive (Fig. A.1) surveillance. The spring cohort of adult females peaked between week 12 and 20 (March-May) while the fall cohort of adult females peaked between week 40 and 50 (October-December). Nymphs peaked between week 20 and 29 (May-July).

When comparing peak density (ticks/1,000m^2) and peak submission rate (submission/10,000 residents), we did not find a consistent significant relationship for either nymphs or adults on the scale of the individual county or all counties together. Overall, we found variation in the direction and significance of the linear relationship at the individual county-year (Fig. 4B, Fig. A.2) and across all county-year estimates (Fig. 4). Similarly, we did not find a consistent significant relationship when comparing peak density and total submission rates at the county or all-county levels (Fig. A.3).

Comparison of infected nymphal density and submission rate

We found variation in the direction and significance of the relationship of DIN and peak INS across pathogens and spatial aggregations (Fig. 5, Fig. A.4). We did not find a consistent relationship between DIN and peak INS for *A. phagocytophilum* or *Bo. burgdorferi* s.s. In contrast, we did find a consistent positive relationship for *Ba. microti*, but only the relationship for Tolland County was statistically significant (Wald *P*-value < 0.05). We were unable to determine the slope of the relationship in Windham County for *A. phagocytophilum* infected nymphs because none of the passively submitted nymphs from this county tested positive for *A. phagocytophilum* leading to no variation in the estimated submission rate of infected nymphs (Fig. A.4A).

We found very similar results when comparing DIN and total INS (Fig. A.5) as for DIN and peak INS with no consistent relationships identified per county or all counties together. The direction of the relationships was often the same, but significance of the relationship varied. While we again found a significant positive association for *Ba. microti* in Tolland County, the only other significant relationship was with *Bo. burgdorferi* s.s. in Fairfield County (positive).

Comparison of nymphal metrics and human disease incidence

We did not find consistent relationships between any of the nymphal metrics derived from either active or passive surveillance and human incidence of Lyme disease, anaplasmosis, or babesiosis in Connecticut.

For nymphal infection prevalence of dragged or submitted ticks, we found variation in the direction and significance of the respective relationships and human incidence of the corresponding tick-borne disease (Fig. 6, Fig. A.6). For infection prevalence estimated from active surveillance, we found a consistent, but non-significant positive linear relationship with all county-year estimates and incidence of human cases of the corresponding diseases (Fig. 6A–B). However, there was not a consistent direction of relationship across the individual counties for each disease. For infection prevalence estimates from passive surveillance (Fig. 6C–D), the only significant linear relationship we identified was a positive relationship between the proportion of submitted nymphs positive for *A. phagocytophilum* and the incidence of anaplasmosis in Litchfield County. We could not estimate a linear relationship with *A. phagocytophilum*-infected nymphs in Windham County because no nymphs submitted between 2019-2021 were positive for *A. phagocytophilum*. Across counties and pathogens, the direction of the slope of the relationship of nymphal infection prevalence and human incidence of disease were not consistent (Fig. 6D, see Fig. A.6C–D for county-level plots).

We did not estimate consistent relationships for either peak nymphal density nor peak nymphal submission rate and human incidence of tick-borne disease (Fig. 7, Fig. A.7). For peak density (active surveillance), we estimated a significant positive linear relationship with the incidence of babesiosis at the all county-year scale as well as in Fairfield County (Fig. 7A–B), but the direction of the relationship was not consistent across the other seven counties. For peak nymphal submission rate (passive surveillance) vs. tick-borne disease

incidence, we did not find a consistent direction of association across counties (Fig. 7C–D and Fig. A.7C–D). We only estimated a significant relationship in Hartford County (positive relationship with anaplasmosis). In both Hartford and Litchfield counties, we did find a consistent positive linear relationship between peak nymphal submission rate and human incidence of all three diseases, but the relationships were not consistently significant. In contrast, we found consistent, yet non-significant, negative relationships with submission rate and human incidence for all three tick-borne diseases in Middlesex and New London counties.

The estimated relationships of annual submission rate and human incidence of tick-borne disease mirrored that of the relationships with peak nymphal submission rate and incidence (Fig. A.8). The direction of the linear relationship was often the same, but significance varied. The only significant relationships we identified were for anaplasmosis at the all county-year scale (positive) and for babesiosis in Windham County (positive).

Similarly, we found variation in the relationship between peak density of infected nymphs (DIN) and peak infected nymphal submission rate (peak INS) and human incidence. We did not find any significant relationships with DIN and incidence as well as inconsistent directions of relationships across counties for each tick-borne disease (Fig. 8A–B). While non-significant, the only consistent relationship we observed was in Middlesex County (negative relationship between DIN and incidence of each disease; Fig. 8B and Fig. A.9A–B).

For peak INS, we found several significant linear relationships with incidence, but the relationships were not consistent across counties (Fig. 8C–D, Fig. A.9C–D). We were unable to determine the direction of the relationship of peak INS and anaplasmosis incidence in Windham County because no nymphs submitted during our study were positive for *A. phagocytophilum*; therefore, there was no variation in the submission rate of infected nymphs.

The relationships for total INS and human incidence (Fig. A.10) mirrored those of with peak INS with no consistent linear relationships. As seen previously, the direction of the linear relationships was similar for total INS and peak INS, but the significance of the relationships varied.

Discussion:

Given the practical considerations of implementing active or passive tick surveillance, one method may be preferred over the other and choices are likely to differ across public health jurisdictions. With the aim of disseminating accurate and current estimates of human encounters with ticks and tick-borne pathogens, we sought to assess the congruency of common tick surveillance metrics derived from passive or active data collection strategies. We compared commonly derived acarological risk metrics from active and passive surveillance from Connecticut counties (2019-2021) to determine their correspondence to each other as well as their relationship to human disease incidence. We found high concordance between active and passive estimates of tick establishment, pathogen

presence status, and infection prevalence for *Bo. burgdorferi* s.s., *A. phagocytophilum*, and *Ba. microti* in *I. scapularis* nymphs and adults. However, we did not find consistent relationships between active and passive surveillance in estimated host-seeking density and tick submission rate nor density of infected host-seeking nymphs and submission rate of infected nymphs. Similarly, we did not find consistent relationships between any nymphal metrics and incidence of Lyme disease, anaplasmosis, or babesiosis.

Comparison of active and passive surveillance resulted in concordant estimates on the presence of *I. scapularis* and pathogen infection in female and nymphal ticks. Given that *I. scapularis* ticks have been reported to be established in Connecticut prior to 1996 (Dennis et al., 1998; Eisen et al., 2016), it comes as no surprise that both surveillance methods agree here, indicating the robust nature of each method for detection in endemic locations. When ticks or pathogens are rare, like in emerging locations, passive surveillance may identify the tick or pathogen before active surveillance collections do, potentially due to insufficient collections, range expansion, or travel importation (Nieto et al., 2018). Conversely, a pathogen might not be identified in passive submissions when it has been identified through active surveillance simply due its low prevalence in the tick population. Therefore, the criteria we used to classify pathogen presence (i.e., pathogen detected in at least one tick during the study) may not provide consistent and accurate classification in emerging areas. Recognizing that humans often travel outside their county of residence, any new detections from passive submissions should be followed up with active sampling to provide standardized information on the tick or pathogen status, increasing the spatial precision of established populations and pathogen presence in emerging locations.

We found a high degree of concordance in estimated infection prevalence between active and passive surveillance methods. When estimates differed statistically, the resulting public health action would not differ; practically the estimates were not different. In general, estimates from active surveillance were higher than those from passive surveillance. This discrepancy could reflect a propensity to select “higher risk” sites for drag sampling, the fact that unengorged ticks were not tested from passive surveillance, or perhaps differences in testing methodologies between surveillance programs (not compared). Estimates derived from passively submitted ticks may be a closer indication of the overall infection prevalence in *I. scapularis* populations found in areas where people spend the most time in Connecticut since ticks were derived from a wider geographic area than those visited for drag sampling and represent actual encounters between humans and ticks. Nonetheless, the resulting infection prevalence estimate from a relatively larger number of ticks submitted across a wide region (7,383 adult and 3,330 nymphs tested) compared with those collected via dragging from 40-41 sites per year (1,559 adult and 1,787 nymphs tested) did not meaningfully differ from each other for three important tick-borne pathogens. Additionally, the estimated infection prevalence of each pathogen and life stage were consistent with estimates from across the northeastern US. For example, Lehane et al. (2021) estimated an average infection prevalence of *A. phagocytophilum* in adults of 8.1% (95% CI: 7.0-9.3%) and 5.8% (95% CI: 4.6-7.2%) in nymphs which were very similar to our estimates (average 7.9% in adults and 4.0% in nymphs). Similar consistency in results held across life stages for the other two pathogens, but with a somewhat higher estimate of *Ba. microti* in adults (11.7% vs. 3.5%) and a somewhat lower estimate of *Bo. burgdorferi* s.s. in adults

(43.7% vs. 58.0%) in Connecticut than the northeastern US. One limitation of our infection prevalence calculations was that we were unable to resolve infection prevalence by cohort of adult females. Thus, we were unable to investigate differences in infection prevalence and any variation in risk between the spring and fall cohorts. However, since nymphs are more epidemiologically relevant for human infections, differences in female infection prevalence between cohorts likely would not meaningfully modulate the risk from human-tick encounters.

Other passive surveillance programs have also reported similar infection prevalence rates in *I. scapularis* and *I. pacificus* as those estimated by active collections, illustrating the repeatability of this concordance between active and passive surveillance beyond our study and across spatial scales. Of nymphal *I. scapularis* submitted to TickReport from Massachusetts, 23.1% (95% CI: 21.6-24.7%) were positive for *Bo. burgdorferi* s.s., 6.4% (95% CI: 5.6-7.4%) for *Ba. microti*, and 4.9% (95% CI: 4.2-5.8%) for *A. phagocytophilum* (Sack et al., 2023). In comparison, Lehane et al. (2021) estimated an average nymphal prevalence across the northeastern US (Maine, New York, Pennsylvania, and Vermont) of 21.3% (95% CI: 19.1-23.6%) for *Bo. burgdorferi* s.s., 5.7% (95% CI: 4.5-7.1%) for *Ba. microti*, and 5.8% (95% CI: 4.6-7.2%) for *A. phagocytophilum*. Like our findings in Connecticut, estimates for adult prevalence in Massachusetts vs. the Northeast region were somewhat higher for *Ba. microti* [8.1% (95% CI: 7.6-8.6%) vs. 3.35% (95% CI: 2.5-4.4%)], somewhat lower *Bo. burgdorferi* s.s. [39.0% (95% CI: 38.1-39.9%) vs. 58.0% (95% CI: 55.9-60.1%)], and very similar for *A. phagocytophilum* [7.6% (95% CI: 7.1-8.1%) vs. 8.1% (95% CI: 7.0-9.3%)]. Similar consistency in estimates were also found in actively and passively collected *I. pacificus* ticks. In ticks submitted from the western US (Arizona, California, Nevada, Oregon, Utah, and Washington) to Northern Arizona University for free identification and pathogen testing (passive surveillance), Nieto et al. (2018) reported 1.2% (95% CI: 0.7-1.8%) of adults and 0.9% (95% CI: 0.2-3.5%) of nymphs were infected with *A. phagocytophilum* while 3.5% (95% CI: 2.7-4.5%) of adults and 1.8% (95% CI: 0.6-4.8%) of nymphs were infected with *Bo. burgdorferi* s.s. As a comparison, Lehane et al. (2021) estimated an average of 1.1% (95% CI: 0.6-2.1%) of adults and 0% (95% CI: 0-16.1%) of nymphs were infected with *A. phagocytophilum* while 2.3% (95% CI: 1.4-3.6%) of adults and 5.0% (95% CI: 0.9-23.6%) of nymphs were infected with *Bo. burgdorferi* s.s. in the Northwest (Oregon and Washington; data were not available for other western states). Previous studies suggest that prevalence of infection increases over time in emerging areas, but remains fairly consistent in endemic areas (Foster et al., 2022; Tran et al., 2022), suggesting we are likely to see a high degree of concordance between active and passive measures of infection prevalence in endemic areas, but might see differences in emerging areas.

We did not find that adult or nymphal submission rates related to corresponding drag sampling density estimates. We found similar results using peak and total submission rates. In contrast, using two years of active and passive tick surveillance data at the county level ($N = 57$) in New York State, Tran et al. (2021) found a high correlation between total annual submissions of *I. scapularis* ticks and density estimated from drag samples. The authors also demonstrated that the strength of the association improved when accounting for collector-associated factors like demographics, human activity level, and experience with

Lyme disease. Thus, the number of counties in our study ($N = 8$) was likely too small, or the degree of variation among counties too limited, to identify a significant relationship; a larger number of sampling units (e.g., census tracts or counties across multiple states) would likely be needed. Additionally, the variation in the direction of the linear relationship we estimated across counties provides support that other factors, like demographics or outdoor activity rates, may be modulating the relationship across Connecticut. However, while density and submission rate estimates varied quantitatively, both indicated that there was qualitatively "high risk" for encountering a tick across the state. Although it was beyond the scope of this study, thorough examination of a larger and more spatially expansive data set we may be able to identify meaningful categories of risk that may be interchangeable between passive and active metrics (e.g., binning data as elevated, moderate, and high risk). A greater degree of concordance between passive and active may be observed based on categorical, rather than continuous variables.

Similarly, we did not find that density of infected nymphs consistently related to either the peak or total annual submission rate of infected nymphs. In contrast to nymphs infected with *A. phagocytophilum* and *Bo. burgdorferi* s.s., estimates for *Ba. microti*-infected nymphs were all consistently positive across spatial scales (individual counties and across county-year estimates) for both peak and total submission rates. The lack of significance of the relationships could be attributed to reduced power due to small sample sizes.

In contrast to previous studies, we did not find that either density of infected nymphs or submission rate of infected nymphs (peak or annual rates) consistently corresponded to reported incidence of tick-borne diseases in humans, potentially due to only using three years of data in a state endemic for tick-borne diseases. Under-reporting of human cases in endemic locations, as has been seen previously (Schiffman et al., 2018; White et al., 2018), could have introduced bias if the under-reporting rate varies year-to-year, limiting our ability to find a significant relationship. Also, using data that captures a larger amount of variation (e.g., a longer timeseries, larger spatial area, or finer spatial scale) may be required to identify significant associations. For example, using ten years (2007-2017) of submissions to CAES at the town- and county-level, Little et al. (2019) illustrated that submission rates of *Bo. burgdorferi* s.l. infected *I. scapularis* nymphs had a strong, positive association with Lyme disease incidence in Connecticut. Mather et al. (1996) found that DIN was strongly predictive of Lyme disease incidence at the town-level in Rhode Island and Stafford et al. (1998) found that both nymphal density and DIN were strongly correlated with Lyme disease cases at the sub-state and state levels in Connecticut. In contrast, using three-years (2004-2006) of drag samples across 36 states in the eastern United States, Pepin et al. (2012) did not find a significant relationship between density of *Bo. Burgdorferi* s.s. infected nymphs and Lyme disease in Connecticut. The authors hypothesized that this was due to small sample sizes as they also failed to find significant relationships in other states with small numbers of counties (i.e., Delaware and Rhode Island). Other weak or non-significant relationships have been reported elsewhere (Connally et al., 2006; Nicholson and Mather, 1996; Prusinski et al., 2014), potentially due to variation in human behavior or too fine of a spatial scale assessed. However, strong associations between DIN and Lyme disease cases have also been found with finer spatial scales (Johnson et al., 2004).

A larger spatial area, longer temporal series, or finer spatial scale may be needed to find associations with acarological metrics and human incidence in endemic locations because of a decoupling of acarological metrics and incidence (Elias et al., 2020). Densities of established *I. scapularis* have been found to fluctuate year to year, but without an upward trend, while emerging populations have increasing trends over time (Elias et al., 2020; Foster et al., 2022; Rand et al., 2007). Thus, while metrics from active (Mather et al., 1996; Stafford et al., 1998) and passive (Gasmi et al., 2019; Koffi et al., 2012; Nelder et al., 2014; Rand et al., 2007) surveillance may track the increase in incidence very well during emergence, variation in acarological metrics no longer clearly relate to variation in incidence after establishment (Elias et al., 2020). However, for our analysis, we were limited by the amount of data available as active surveillance in Connecticut only began in 2019. Further work done across a wider geographic region encompassing both emerging and endemic locations may find more conclusive trends.

Estimates of relationships with peak or total submission rates yielded similar results, indicating that both passively-derived metrics encapsulate similar information. Peak submission rate is comparable in construction to peak density estimates from drag sampling, in theory producing similar estimates of abundance. Total submission rates by cohort could closer approximate the total exposure risk than a single peak estimate. However, we did not find consistent relationships with any of these metrics or with human incidence of tick-borne diseases. Further work with larger sample sizes or across emerging and endemic locations may identify strengths and correspondence of total vs. peak submission rate metrics.

The significant positive associations of anaplasmosis incidence and nymphal infection prevalence and submission rate of *A. phagocytophilum* infected nymphs was unexpected since the testing assays did not distinguish between human (ha) and non-human (v1) variants. Spatial variation, but geographic co-occurrence of each of these variants has been previously documented in the Northeast (Courtney et al., 2003; Massung et al., 2002) with evidence that the ha-variant was up to twice as prevalent than the non-pathogenic variant in *I. scapularis* (Keesing et al., 2014; Massung et al., 2002). Notably, Prusinski et al. (2023) showed a higher degree of concordance in prevalence of *A. phagocytophilum*-ha variants compared with non-genotyped *A. phagocytophilum* with incidence of anaplasmosis in New York. Thus, while estimates of infection prevalence of *A. phagocytophilum* without differentiating variants could overestimate risk to some degree, overestimation may not be profound in areas where the tick and pathogen have been long-established, but differences might be observed in areas where the tick and pathogen are emerging; further work is needed to identify the degree of overestimation across a broad geographic coverage.

Contrary to our expectation, we did not find acarological metrics from passive surveillance as better predictors of human incidence of tick-borne disease than metrics derived from active surveillance. While previously associated with tick-borne disease cases (Mather et al., 1996; Stafford et al., 1998), estimates of the density of host-seeking nymphs do not take into account the likelihood of human contact with sampled host-seeking nymphs (Eisen and Eisen, 2016). In contrast, ticks submitted in passive surveillance have encountered a human since they are found crawling or attached to someone (Eisen and Eisen, 2021; Hook et al., 2021). Thus, submission rate of infected nymphs should more closely reflect human

risk of encounters with infected nymphs. Hook et al. (2021) demonstrated that reported tick encounters were strongly associated with tick-borne disease at the individual and household level in Connecticut and New York. We may not have been able to find the same association because we did not have paired data on tick-borne disease outcomes and tick encounters in those who submitted ticks nor in those who did not submit ticks; we just had cross-sectional incidence data. Those submitting ticks to CAES may not have represented the average population so their experiences and risks during this relatively short study may not reflect the population-level risk.

The week of the year identified as peak in terms of density or submission rate spanned up to ten weeks for both nymphs and adults, resulting in a wide risk season. However, the methodologies employed by the active surveillance program were not designed to describe host-seeking phenology so should not be used to identify the exact timing of peak densities. An active surveillance system to adequately describe phenology would require an extensive amount of time and resources with sampling performed consistently (often bi-weekly) throughout the year (Falco and Fish, 1992; Lord, 1995; Piesman et al., 1987; Schulze et al., 1986; Wilson and Spielman, 1985).

Passive surveillance provided good data on phenology of human encounters with host-seeking *I. scapularis* nymphal and adult ticks. Temporal variation in submissions throughout the year depended on both tick questing behavior and human activity patterns so these data could be used to temporally define the likelihood a person will encounter ticks by life stage. Since we used date of submission instead of data of tick removal and the difference between the two could vary over time, some amount of uncertainty in the timing exists. Also, adults were more commonly submitted than nymphs, which is consistent with other studies (Nieto et al., 2018; Sack et al., 2023; Salkeld et al., 2019). Owing to their larger size and perhaps relatively longer activity season, adults are more likely to be identified and submitted than nymphs. Thus, the magnitude of human-nymphal tick encounter rate may be underestimated from passive surveillance. Nevertheless, the broad temporal trends likely still hold. Deriving prevalence estimates from the passive tick submissions across a wide geographic area can be resource intensive. Although similar county level estimates were derived for life-stage and pathogen specific estimates of infection prevalence, 4.7-times and 1.9-times as many adults and nymphs, respectively, were tested through the passive submission program compared with active surveillance submissions. While active surveillance also includes personnel costs for tick collections, these costs may not exceed those resulting from the much larger volume of submission from passive surveillance. Additionally, the documentation of the individual ticks received, promptly reporting the test results back to submitters, and responding to each submitter’s inquiries added additional resource-intensity to the passive surveillance program.

Conclusion:

Using active and passive surveillance data for *I. scapularis* in Connecticut (2019-2021), we investigated the relationship between corresponding acarological risk metrics derived from each method. While both methods agreed on the establishment status of the tick, presence of the pathogens in ticks, and infection prevalence of *Bo. burgdorferi* s.s., *A. phagocytophilum*, and *Ba, microti*, we did not find a consistent relationship between host-seeking nymphal

density and nymphal submission rate nor the host-seeking density and submission rate of infected nymphs. Considering nymphal metrics from both active and passive surveillance, we did not find consistent relationships with incidence of Lyme disease, anaplasmosis, or babesiosis. Limiting the numbers of ticks tested per county may be economical without compromising estimates of pathogen prevalence. Binning tick abundance estimates (derived from passive and active measures) into categories may provide meaningful risk estimates that might provide consistent associations with epidemiological outcomes. Surveillance methods could be used synergistically with targeted active surveillance informed by passive surveillance. Further work is needed to effectively integrate both active and passive surveillance into public health response for tick-borne diseases, including evaluating the correspondence of metrics across methods in emerging locations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Disclaimer:

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention or the Department of Health and Human Services.

Availability of data and materials:

Records from active and passive surveillance used in the analysis are available from the Connecticut Agricultural Experiment Station (CAES) on reasonable request by contacting Tick.Testing.Laboratory@ct.gov. Tick-borne incidence data used in the analysis are mostly available online at: https://portal.ct.gov/DPH/Epidemiology-and-Emerging-Infections/Infectious-Diseases-Statistics.

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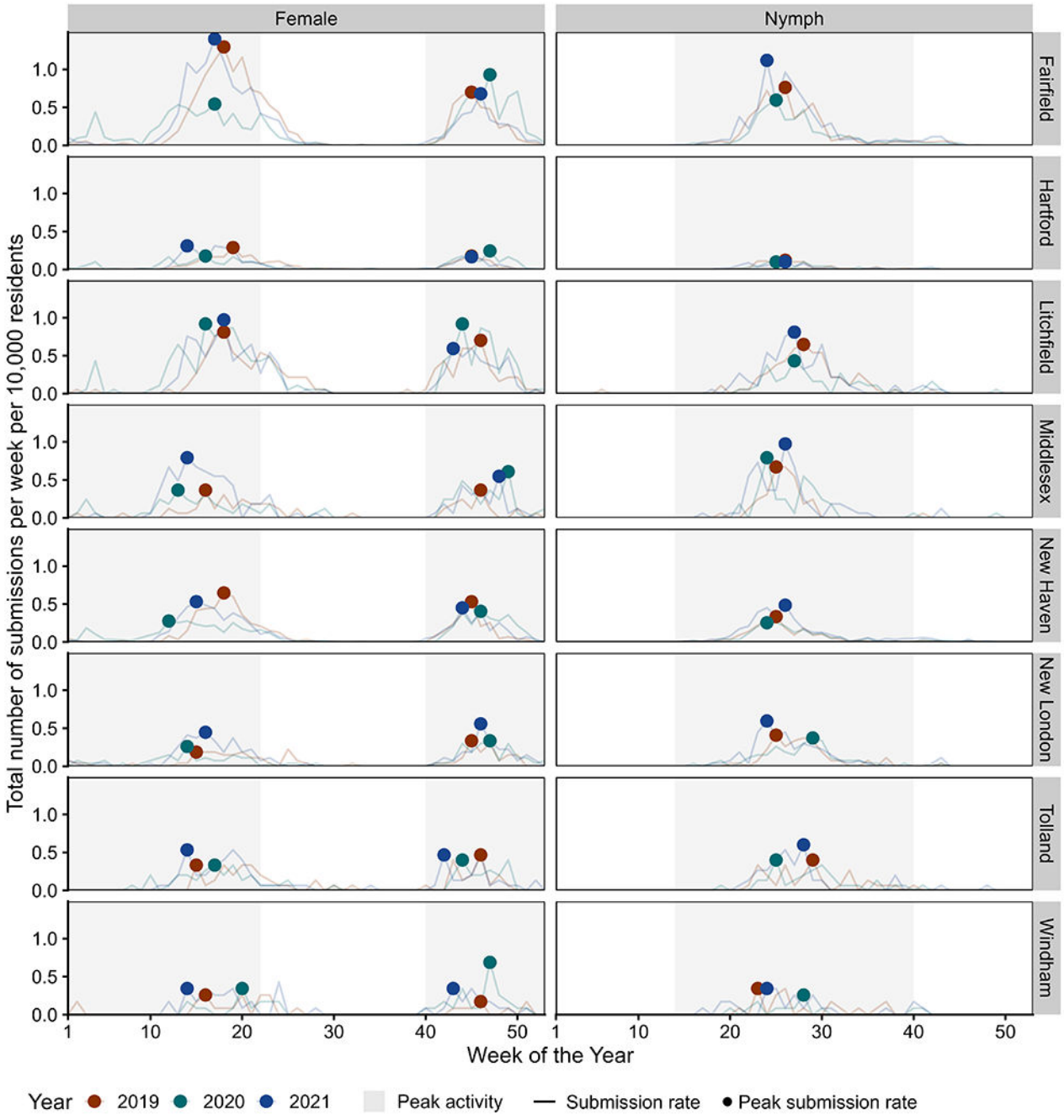

Fig. 1. Location of drag sample collection sites in Connecticut, USA (2019-2021).
County boundaries indicated in grey. See Table A.1 for number of visits per site per year.

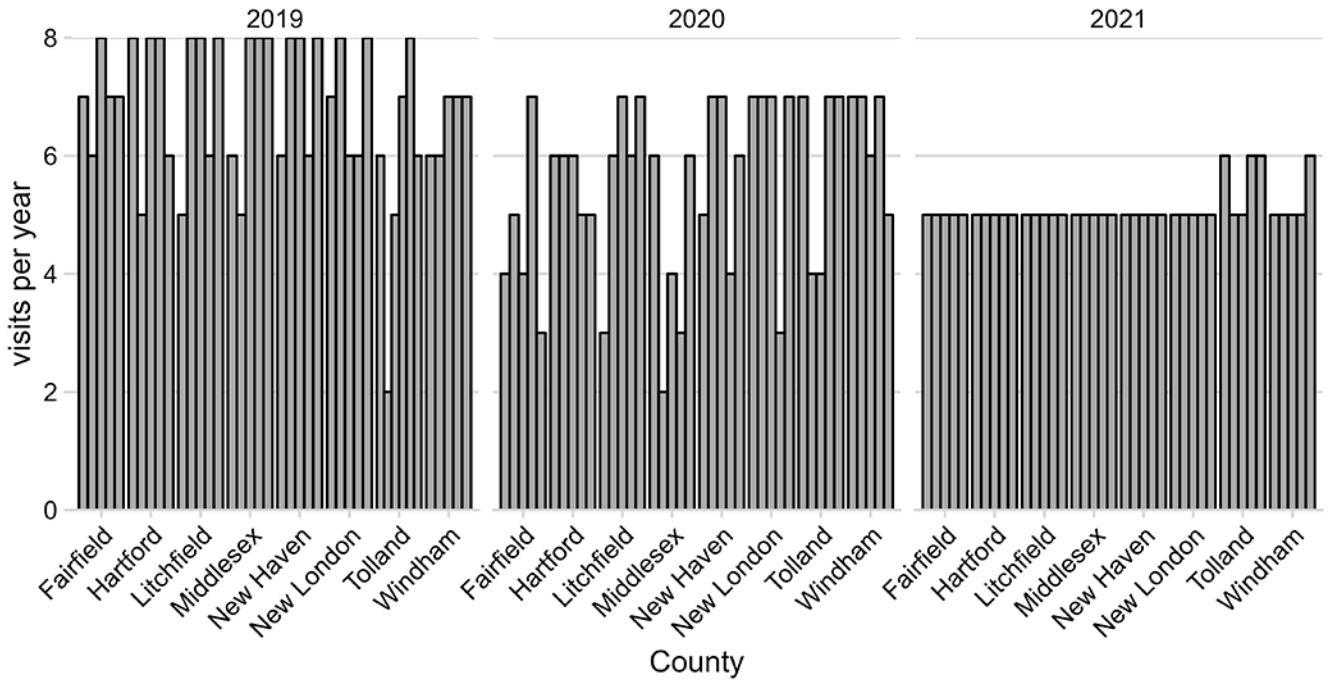

Fig. 2. Weekly submission rate of *Ixodes scapularis* ticks to the Connecticut Agricultural Experiment Station by county (2019-2021).

Peak months of activity for each life stage indicated by the grey shading. Weekly (line) and peak (dot) submission rate indicated per county, year, and cohort (i.e., spring female, fall female, and nymph).

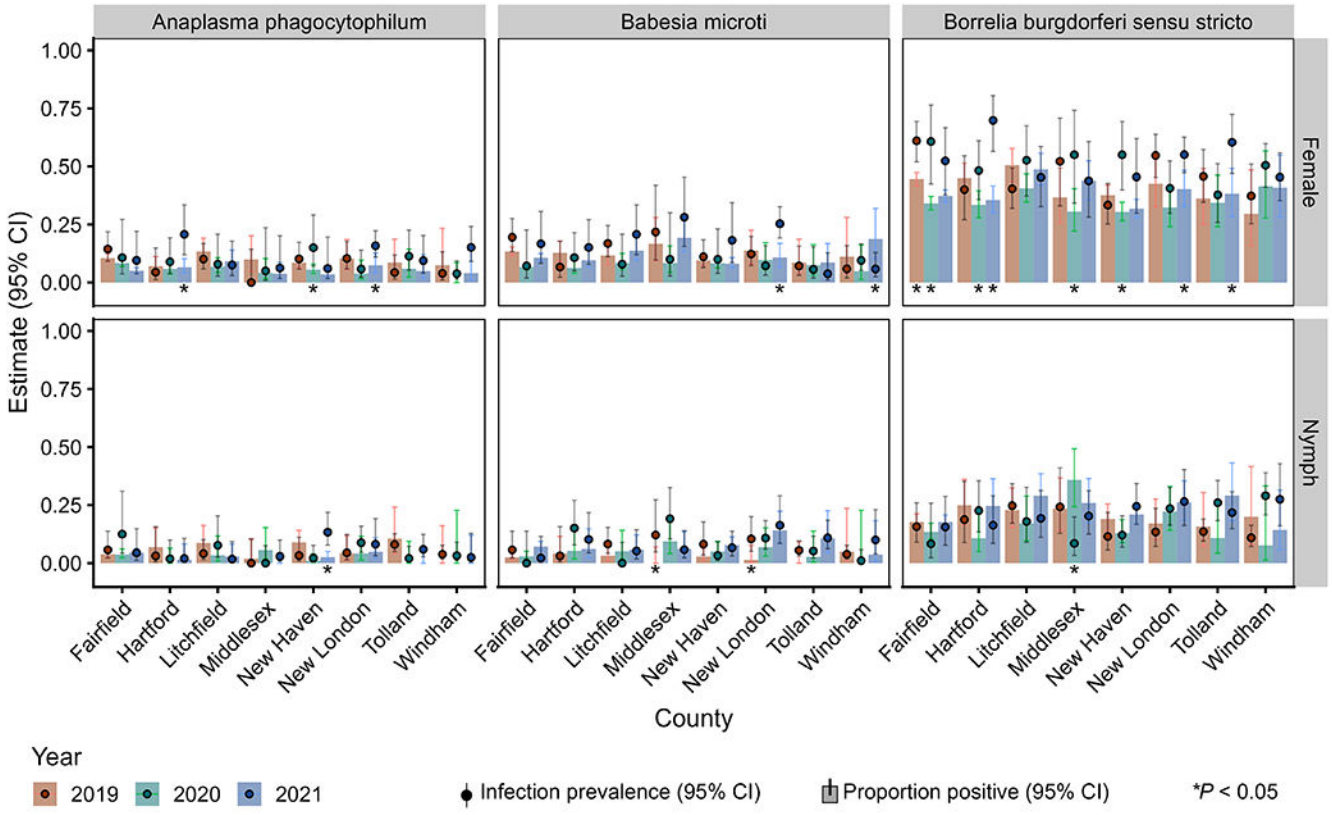

Fig. 3. Estimated infection prevalence (95% CI) of *I. scapularis* from active and passive surveillance in Connecticut, USA (2019-2021).

Infection prevalence estimated per pathogen and life stage for all ticks dragged (active surveillance; point) or submitted (passive surveillance; bar) per year and county. Statistically significant differences in estimates indicated by * (Fisher's exact test *P*-value < 0.05).

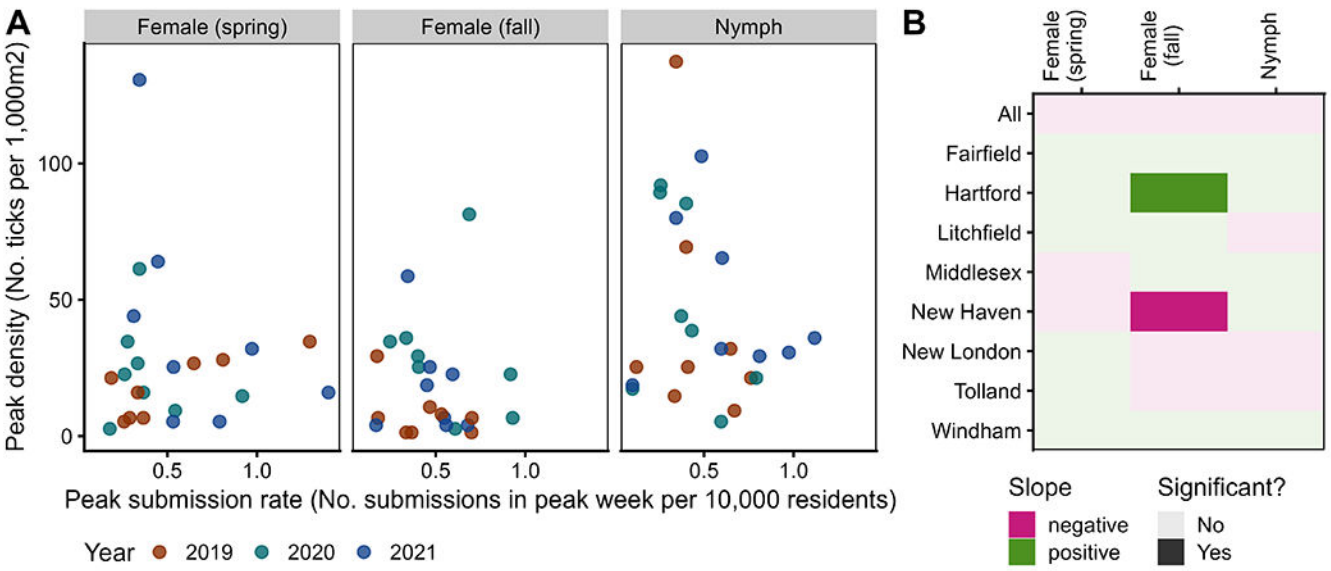

Fig. 4. Peak density vs. peak submission rate of ticks in Connecticut, USA (2019-2021).

A) All county-year scatter plot of the relationship of peak density (active surveillance) and peak submission rate (passive surveillance) of *I. scapularis* ticks by cohort. Each point represents a single county and year. See Fig. A.2 for county-level plots per cohort. B) Direction (slope) and significance (Wald *P-value* < 0.05) of the linear relationship between peak density and peak submission rate per cohort at the individual county or aggregated county level. Top row ("All") corresponds to slope and significance of relationships in A).

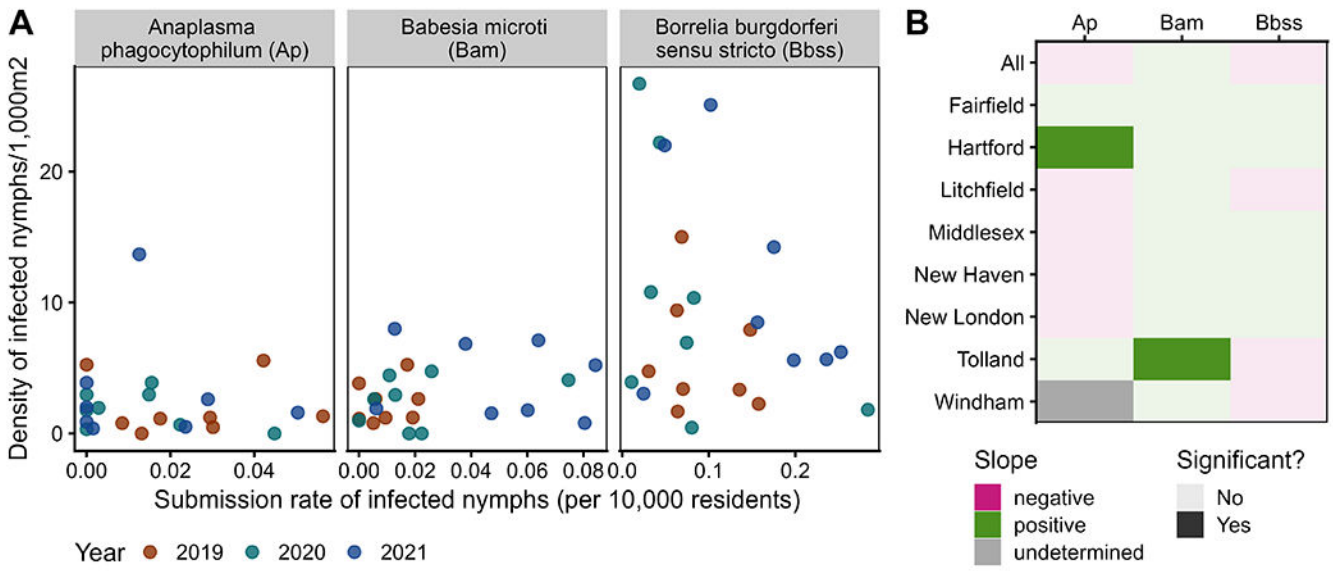

Fig. 5. Peak density of infected nymphs vs. peak submission rate of infected nymphs in Connecticut, USA (2019-2021).

A) All county-year scatter plot of the relationship of peak density of infected *I. scapularis* nymphs (active surveillance) and peak weekly submission rate of infected *I. scapularis* nymphs (passive surveillance) by pathogen. Each point represents a single county and year estimate. See Fig. A.4 for county-level plots per pathogen. B) Direction (slope) and significance (Wald $P\text{-}value < 0.05$) of the linear relationship between peak density and peak submission rate of infected nymphs per pathogen at the individual county or aggregated county level. Top row ("All") corresponds to the slope and significance of relationships in A).

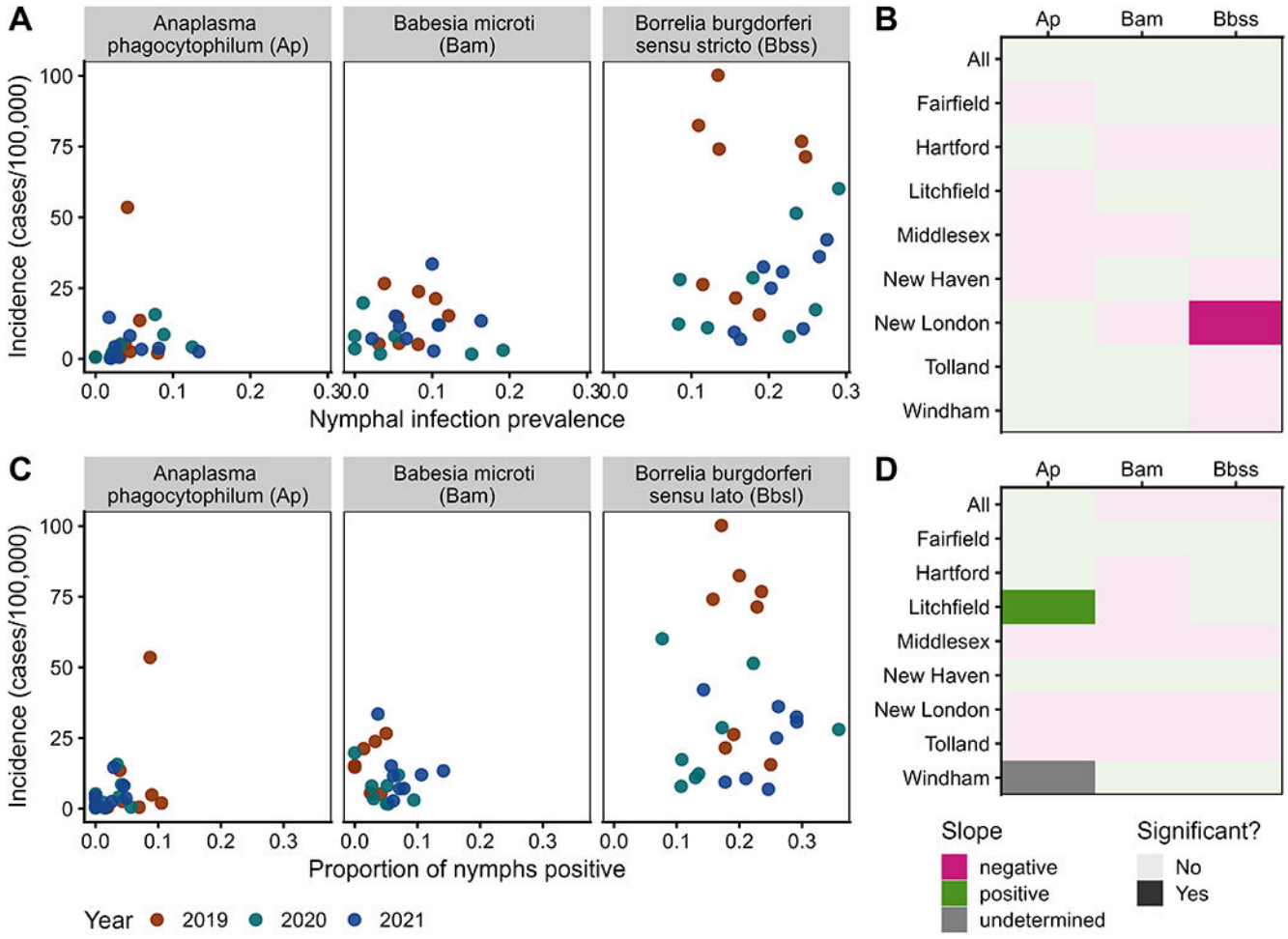

Fig. 6. Nymphal infection prevalence vs. human incidence of the corresponding tick-borne disease.

All county-year scatter plot of the relationship of infection prevalence of *I. scapularis* nymphs collected via dragging (A) or passive submission (C). Each point represents a single county and year infection prevalence and corresponding human incidence. See Fig. A.6 for county-level plots. Direction (slope) and significance (Wald *P-value* < 0.05) of the linear relationship between drag sample (B) and passive submission (D) of infected nymphs per pathogen at the individual county or aggregated county level. Top row ("All") in B) and D) corresponds to the slope and significance of relationships in A) and C), respectively.

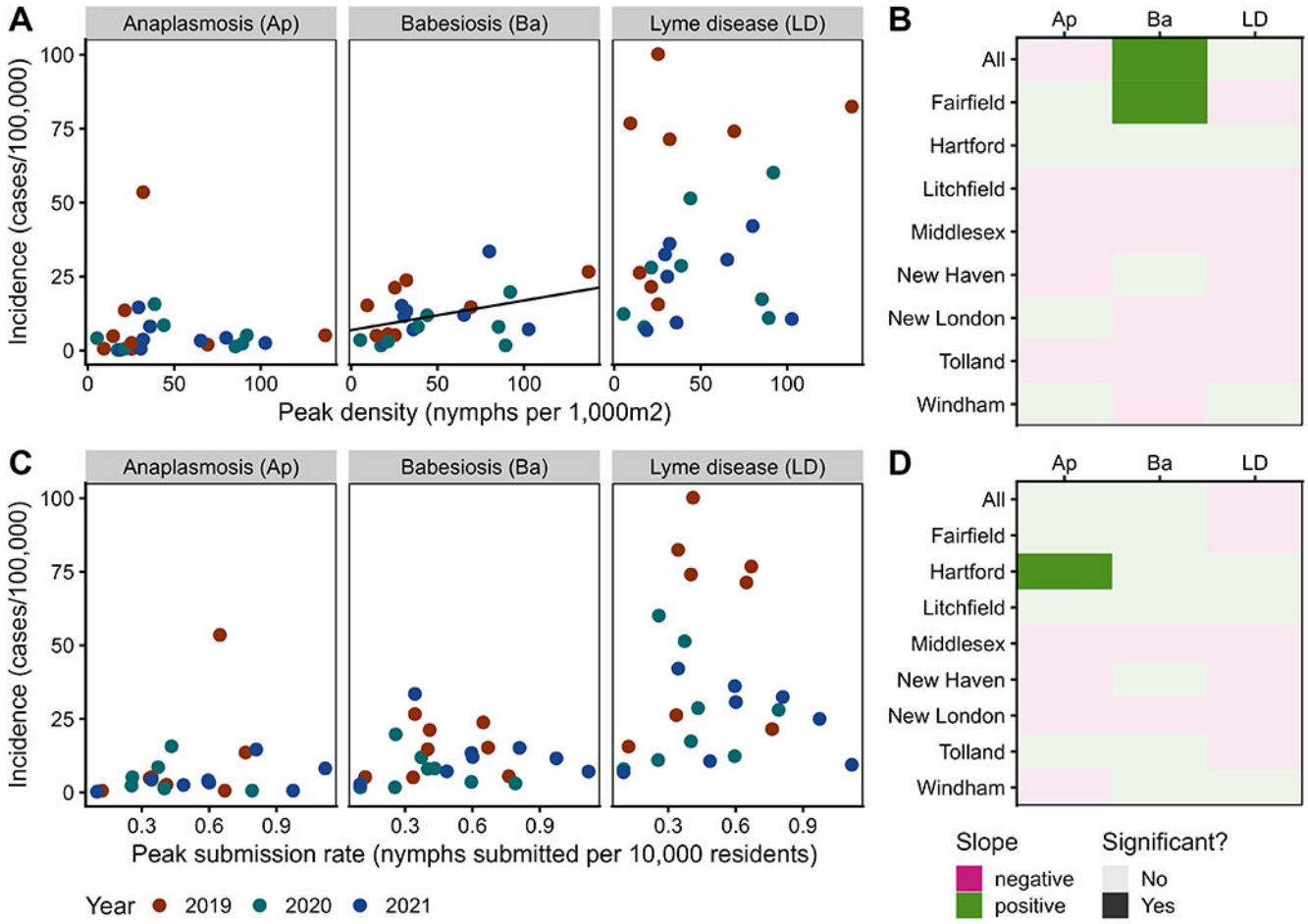

Fig. 7. Peak nymphal density or submission rate vs. human incidence of tick-borne disease.

All county-year scatter plot of the relationship of peak density of *I. scapularis* nymphs collected via dragging (A) or peak nymphal submission rate (C). Each point represents a single county and year estimate and corresponding human incidence. See Fig. A.7 for county-level plots. Direction (slope) and significance (Wald *P-value* < 0.05) of the linear relationship between density (B) or submission rate (D) at the individual county or aggregated county level. Top row ("All") in B) and D) corresponds to the slope and significance of relationships in A) or C), respectively.

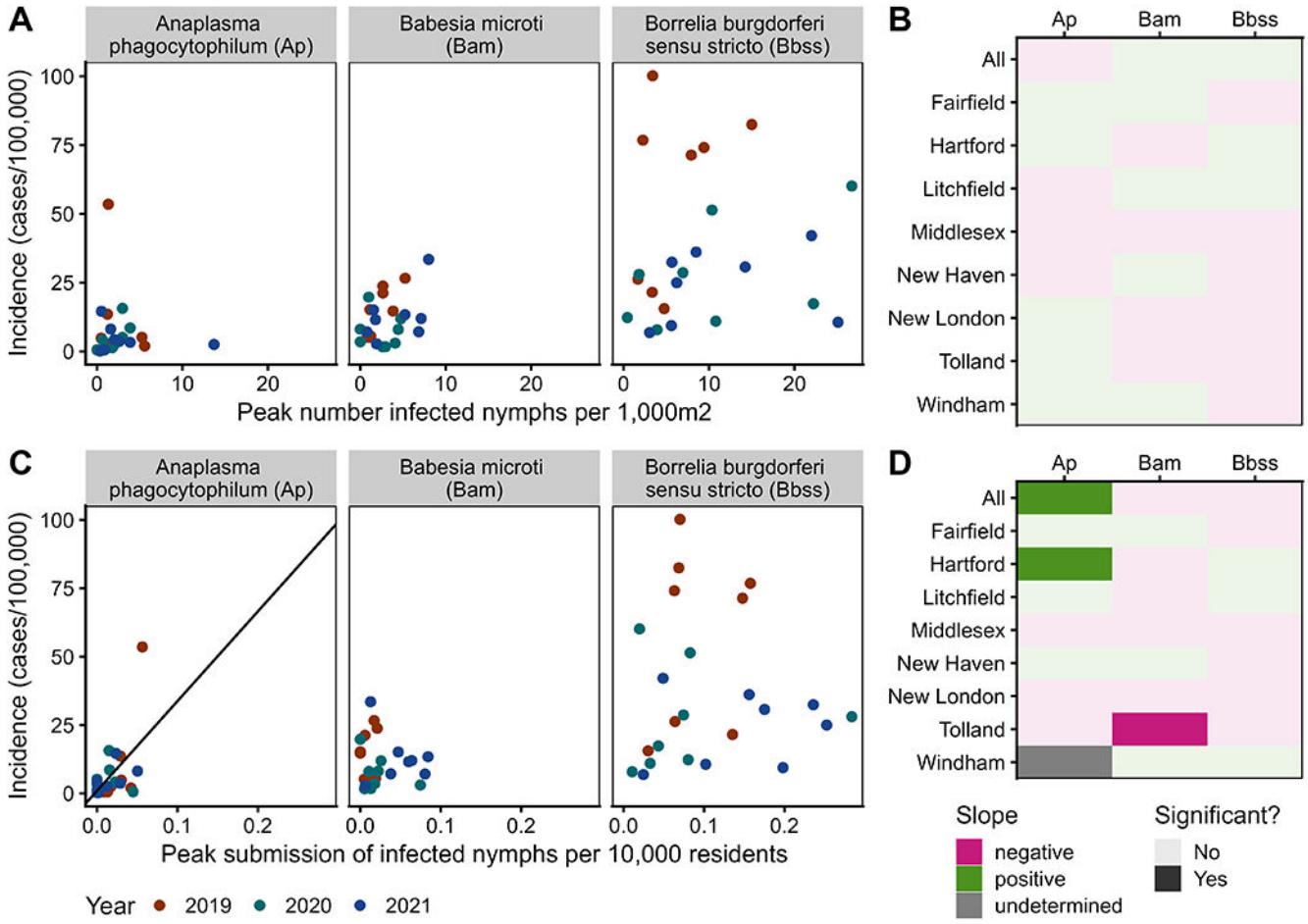

Fig. 8. Density of infected nymphs or submission rate of infected nymphs vs. human incidence of the corresponding tick-borne disease.

All county-year scatter plot of the relationship of the peak density of infected nymphal *I. scapularis* (A) or peak submission rate of infected nymphs (C). Each point represents a single county and year estimate and corresponding human incidence. See Fig. A.9 for county-level plots. Direction (slope) and significance (Wald *P-value* < 0.05) of the linear relationship between density (B) and submission rate (D) of infected nymphs per pathogen at the individual county or aggregated county level. Top row ("All") in B) and D) corresponds to the slope and significance of relationships in A) or C), respectively.

Table 1.

Number of *Ixodes scapularis* nymphs and adults submitted though passive surveillance in Connecticut, 2019-2021.

Submissions by year, life stage, and county to the Connecticut Agricultural Experiment Station (CAES). Percentage contribution per county to state totals are shown per year.

	Number of adult females submitted (%‡)				Number of nymphs submitted (%‡)			
County†	2019	2020	2021	Total	2019	2020	2021	Total
Fairfield	1,292 (52.0)	1,106 (45.2)	1,279 (47.1)	3,677	527 (50.0)	404 (46.1)	689 (48.3)	1,620
Hartford	236 (9.5)	259 (10.6)	264 (9.7)	759	73 (6.9)	56 (6.4)	65 (4.6)	194
Litchfield	185 (7.4)	255 (10.4)	198 (7.3)	638	93 (8.8)	59 (6.7)	103 (7.2)	255
Middlesex	63 (2.5)	100 (4.1)	138 (5.1)	301	51 (4.8)	56 (6.4)	81 (5.7)	188
New Haven	531 (21.4)	515 (21.0)	554 (20.4)	1,600	181 (17.2)	179 (20.4)	309 (21.7)	669
New London	90 (3.6)	102 (4.2)	151 (5.6)	343	71 (6.7)	72 (8.2)	103 (7.2)	246
Tolland	61 (2.5)	69 (2.8)	83 (3.1)	213	38 (3.6)	37 (4.2)	48 (3.4)	123
Windham	28 (1.1)	42 (1.7)	50 (1.8)	120	20 (1.9)	13 (1.5)	28 (2.0)	61
Total	2,486	2,448	2,717	7,651	1,054	876	1,426	3,356

†County where the submitted tick was most likely acquired if different than the submitter's county of residence.

‡Percent of ticks per life stage submitted per year. Due to rounding, totals may not add to 100%.