Journal of Medical Entomology, XX(XX), 2025, 1–6
https://doi.org/10.1093/jme/tjaf055
Short Communication

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Using geospatial analysis to describe the association between active tick surveillance data and clinical cases of anaplasmosis in Connecticut

Julia Desiato[1,2], Grace Chan[1], Marco Palmeri[1], Jamie L. Cantoni[3], Duncan W. Cozens[3], Megan A. Linske[3], Doug E. Brackney[3], Kirby C. Stafford III[3], and David B. Banach[1,*]

[1]University of Connecticut School of Medicine, Farmington, CT, USA
[2]Emerging Infections Program, Yale School of Public Health, New Haven, CT, USA
[3]Department of Entomology, Connecticut Agricultural Experiment Station, New Haven, CT, USA

[*]Corresponding author. University of Connecticut School of Medicine, 263 Farmington Ave., Farmington, CT, USA (Email: DBanach@uchc.edu).

Subject Editor: Erika Machtinger

Received on 4 December 2023; revised on 17 March 2025; accepted on 29 March 2025

Anaplasmosis is a vector-borne disease caused by the bacterium *Anaplasma phagocytophilum* and is vectored by *Ixodes scapularis* ticks primarily in the northeastern United States. The Connecticut Department of Public Health designated anaplasmosis a state-wide reportable disease in 2008 and a large increase in cases was witnessed in Connecticut between 2014 and 2019. This study used clinical cases of anaplasmosis reported to the Connecticut Department of Public Health and *A. phagocytophilum* prevalence data in questing *I. scapularis* to understand emerging geographic disease hotspots and evaluate potential association between human and *I. scapularis* infections. Human incidence rates were calculated per 100,000 people by county. *I. scapularis* infection prevalence was calculated as an acarological risk index using active tick surveillance data from the Connecticut Agricultural Experiment Station. The potential association between incidence rates and acarological risk index was analyzed using Spearman Rank correlation. From 2019 to 2020, 420 human cases of anaplasmosis were reported and 148 *A. phagocytophilum*-infected *I. scapularis* were identified in Connecticut and a significant positive correlation was identified between acarological risk index and incidence rates. Active tick surveillance is a helpful tool for identifying geographic areas with increased risk of anaplasmosis and can be useful in guiding public health interventions to prevent cases before they occur while also identifying potential locations where underreporting may occur.

Keywords: active tick surveillance, anaplasmosis, Connecticut, geospatial analysis

Introduction

Anaplasmosis is a disease caused by the bacterium *Anaplasma phagocytophilum* transmitted to humans through the bite of infected *Ixodes scapularis* ticks. In 1999, human anaplasmosis became a nationally notifiable disease by the United States. Centers for Disease Control and Prevention (CDC). Anaplasmosis cases have increased in the United States from 348 cases in 2000 to the peak of 5,762 cases in 2017 since becoming a reportable disease (CDC 2022). Similar trends have been observed in Connecticut (CT) as anaplasmosis cases have continued to increase annually. Following the recommendation by the Council of State and Territorial Epidemiologists, the CDC established a case definition for anaplasmosis and the Connecticut Department of Public Health (CTDPH) designated anaplasmosis a state-wide reportable disease based on positive laboratory tests in 2008 (CTDPH 2022). In CT, there has been an increase from 76 cases in 2014 to 297 reported cases in 2019 (CTDPH 2022).

In CT, surveillance for tick-borne pathogens, including *A. phagocytophilum*, consists of both active and passive surveillance activities. Passive surveillance is conducted by the CT Agricultural Experiment Station (CAES) and the CT Veterinary Medical Diagnostic Laboratory (CVMDL). Individuals from the public, healthcare providers, Town Departments of Public Health (or Health

Districts) submit ticks to the CVMDL and CAES where they are identified, screened for pathogens as needed, and timely results reported back to submitters. The CT Active Tick Surveillance Program (ATSP) was established in 2019 by the CAES and relies on standard tick dragging techniques at five locations within each of CT's 8 counties (CAES 2021).

There is increasing recognition of the importance of evaluating the spatial relationship between data from active tick surveillance (ATS) and incidence of tick-borne infections in human populations.

The New York State Department of Health conducted a spatial emergence study on the incidence of anaplasmosis between 2010 and 2018 using human case surveillance and ATS data to understand geographical patterns of increased cases (Russell et al. 2021). Surveillance data were analyzed to determine tick population density at each site, collected ticks were screened for multiple pathogens, and then an entomologic risk index (ERI) was calculated by measuring density of pathogen-infected ticks (Russell et al. 2021). Human cases were then compared to the ERI to understand how increased prevalence of infected ticks overlapped with human cases. The study determined that case hotspots bordered the neighboring states of NY, including CT, which highlighted the need for further study (Russell et al. 2021).

The CAES has previously studied the utility of passive surveillance methods in understanding the incidence of Lyme disease in CT by analyzing data for *Borrelia burgdorferi* infected *I. scapularis* ticks in CT. The authors noted a future aim to determine if passive or ATS can better estimate the risk of clinical cases of tick-borne infections (Little et al. 2019).

In this study, we evaluated the spatial relationship between cases of human anaplasmosis and data derived from ATS for *A. phagocytophilum* in ticks to determine if the ATSP in CT can identify geographic regions with increased anaplasmosis risk that could benefit most from targeted infection prevention activities and education.

Materials and Methods

Human Case Surveillance

Human anaplasmosis cases included all individuals who tested positive in a CT laboratory for anaplasmosis in 2019 and 2020. Laboratories are mandated to report all polymerase chain reaction (PCR) positive cases to the CTDPH (CTDPH 2023). For this study, both probable and confirmed cases were considered positive cases. Summary tables of cases by county were obtained from the CTDPH Infectious Disease Statistics website (CTDPH 2019, 2020). Population estimates for CT counties in 2019 and 2020 were obtained from the CTDPH (CTDPH 2020, Backus 2021).

Active Tick Surveillance

The CAES began their ATS program in spring 2019. While the CAES ATS program documented numerous species of ticks collected, only *I. scapularis* was of interest for the purpose of this study. Data recorded for collected *I. scapularis* included date of collection, town, county, life stage, total area sampled, and standardized density of adult and nymphal ticks per 100 m^2 (Cantoni 2021). A piece of 1 m^2 cloth attached to a wooden dowel was used to drag sample 750 m^2 along publicly accessible trail systems at 5 locations throughout each of CT's 8 counties.

I. scapularis obtained from the field were returned to the lab and tested for pathogens by organism-specific PCR. Adult females and nymphs were individually sorted into 2.0 ml microcentrifuge tubes containing 450 µL PBS-G and a copper bead. Ticks were homogenized in a mixer mill at 25 1iter/s. Bacterial DNA and viral RNA were then extracted from 200 µL of tick homogenate using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (ThermoFisher, Waltham, MA) and eluted into 50 µL of elution solution. Nucleic acid was subjected to a multiplex real-time reverse-transcription polymerase chain reaction (RT-PCR) on a Bio-Rad C1000 with a CFX96 optical module (Bio-Rad Laboratories, Hercules, CA). *A. phagocytophilum* was targeted using primers and probes specific for the 16S rRNA gene using established primer and probe sets (Tokarz et al. 2017). The RT-PCR took place in a 20 µL reaction using 5.0 µL 4× TaqPath One-Step Multiplex Mix (ThermoFisher, Waltham, MA), 1.0 µL of our *Ixodes* primer mix (10 µM), 0.4 µL of our *Ixodes* probe mix (10 µM), 11.6 µL of nuclease-free water and 2.0 µL of tick extract. The reactions then underwent UNG inhibition at 25 °C for 2 min, followed by reverse transcription at 53 °C for 10 min and polymerase activation at 95 °C for 2 min. The samples were then cycled 40 times at 95 °C for 15 s and 60 °C for 1 min. Positive samples were determined using the Bio-Rad CFX Maestro software (Bio-Rad Laboratories, Hercules, CA).

Data Analysis

County-level incidence rates (IR) of human anaplasmosis per 100,000 residents were calculated by the CTDPH using reported case numbers and population estimates for 2019 and 2020 (Backus 2021). Active surveillance data from the CAES were formatted to combine all 2019 and 2020 data by county, town, site, date, number of *I. scapularis* in each life stage tested and the positivity percentage. ArcGIS Online (ESRI 2024) was used to create a map to display the sites, number of *I. scapularis* collected, and the positivity percentage. The average acarological risk index (ARI) by county was calculated by averaging all the ARI from each sampling event within a county. Summary data of *I. scapularis* infection positivity rates were calculated and analyzed using SAS Version 9.4 (SAS Institute, Cary, NC). To understand the anaplasmosis risk for humans, an ARI was calculated using *I. scapularis* population density (number of *I. scapularis* collected/area dragged) multiplied by the bacterium prevalence (number of *I. scapularis* positive for anaplasmosis/number of *I. scapularis* tested) at each site based on CDC recommendations for effective active surveillance (U.S. Centers for Disease Control and Prevention 2021). Average ARI was compared with anaplasmosis IR using a Spearman rank correlation in SAS with statistical significance determined at 5% (Zar 2005). The magnitude of the correlation between the 2 sets of variables was analyzed based on their ranks. ArcMap (ESRI 2021) and ArcGIS Pro (ESRI 2022) were used to map the IR of anaplasmosis and the ARI by county for each year to determine where pathogen prevalence in *I. scapularis* and human incidence of disease overlap.

Results

Human Disease Incidence

There were 297 anaplasmosis cases reported to the CTDPH in 2019, of which 4 were excluded from analysis due to unknown location, and 123 reported cases state-wide in 2020. Fairfield County had the most reported cases with 130 and 40 in 2019 and 2020, respectively, and Middlesex County reported the fewest. Anaplasmosis IR ranged from 0.56 and 0.22 in Hartford County to 54.90 and 15.68 in Lichfield County for 2019 and 2020, respectively (Table 1).

Pathogen Prevalence in Ticks

There were 642 adult *I. scapularis* tested throughout CT in 2019 of which 58 tested positive for infection with *A. phagocytophilum* with the highest percent positivity in Fairfield County (14.4%) and the lowest in Middlesex County (0%). There were 745 nymphal *I. scapularis* tested throughout CT in 2019 and 35 tested positive for infection with *A. phagocytophilum* with the highest percent positivity in Tolland County (8.0%) and the lowest in Middlesex County (0%) (Table 2).

There were 409 adult *I. scapularis* tested throughout CT in 2020 and 32 tested positive for infection with *A. phagocytophilum* with the highest percent positivity in New Haven County (15%) and the lowest in Windham County (3.8%). There were 545 nymphal *I. scapularis* tested throughout CT in 2020 and 23 tested positive for infection with *A. phagocytophilum* with the highest percent positivity in Fairfield County (12.5%) and the lowest percent positivity in Middlesex County (0%) (Table 2).

ARI was calculated by county and year. In 2019, Fairfield County had the highest ARI (0.122) and Middlesex County the lowest (0). In 2020, New London County had the highest ARI (0.108) and Middlesex County again had the lowest (0.011) (Table 2).

Figure 1 illustrates the total number of *I. scapularis* collected and percent positive with *A. phagocytophilum* by site and county across both years of data collection.

Association between Human IR and Tick ARI

Spearman rank correlations were calculated for 2019, 2020, and 2019–2020 combined (Table 3). During 2019 to 2020, there was a positive correlation between the IR and ARI ($n = 16$ counties, $R = 0.5430$, $P = 0.0297$). In 2019, there was a positive correlation between IR and ARI ($n = 8$ counties, $R = 0.5952$, $P = 0.1195$) and in 2020, there was also a positive correlation ($n = 8$ counties, $R = 0.5476$, $P = 0.1600$), though neither reached statistical significance.

Spatial analyses revealed that anaplasmosis IR was related to *A. phagocytophilum* ARI at the county level in CT during our study period (Fig. 2). Areas at highest risk were Litchfield, Fairfield, and New London Counties. Certain areas such as New Haven, Tolland, and Windham Counties had moderate risk. Areas at lowest risk were Hartford and Middlesex Counties. Other counties maintained relatively stable levels of anaplasmosis between 2019 and 2020 as illustrated in Fig. 3. Middlesex, Hartford, and Windham Counties saw fairly constant IRs of anaplasmosis, and consistent increases in ARI from 2019 to 2020. Tolland County had a slight decrease in both IR and ARI. New Haven County saw a decrease in IR from 2019 to 2020, but an increase in ARI.

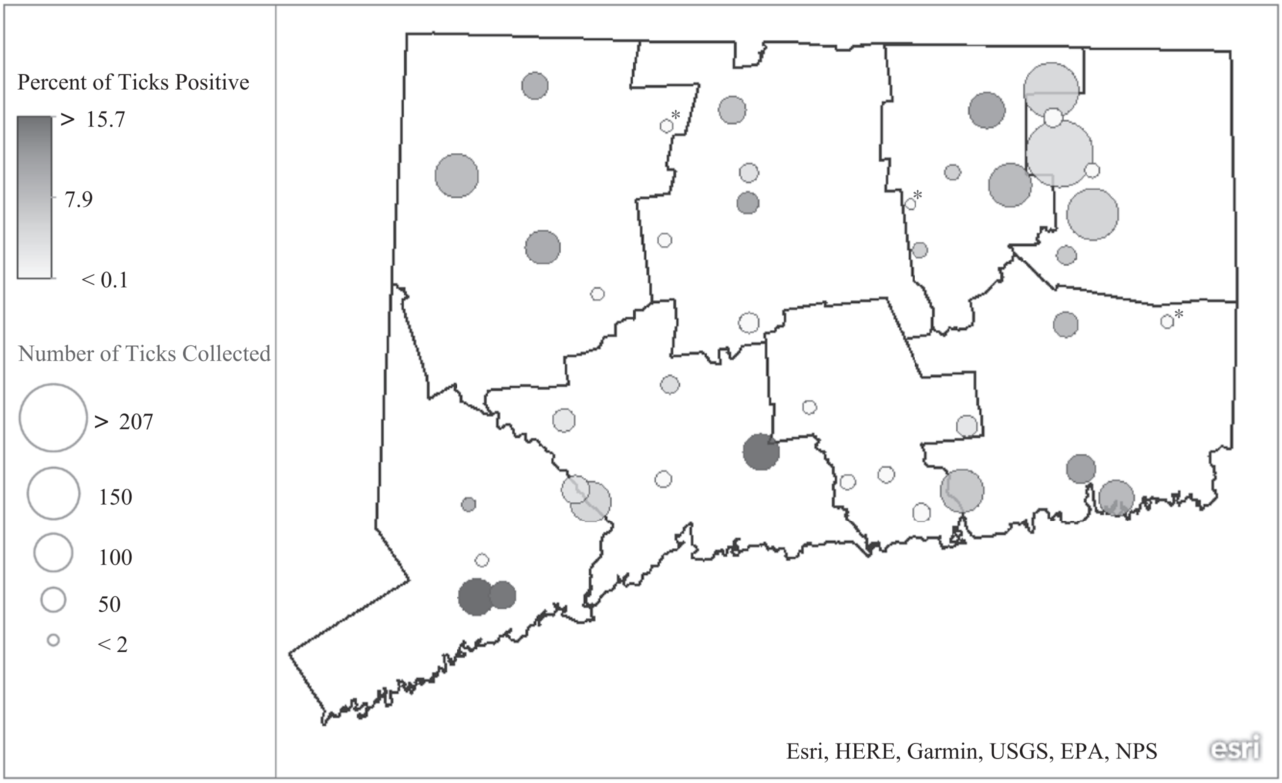
Table 1. Human anaplasmosis incidence rate (IR) per 100,000 by CT county, 2019 and 2020

	2019			2020		
County	Number of cases	Total population	IR	Number of cases	Total population	IR
Fairfield	130	943,332	13.78	40	957,050	4.18
Hartford	5	891,720	0.56	2	898,682	0.22
Litchfield	99	180,333	54.90	29	184,938	15.68
Middlesex	1	162,436	0.62	1	164,063	0.61
New Haven	42	854,757	4.91	20	864,094	2.31
New London	7	265,206	2.64	23	268,450	8.57
Tolland	3	150,721	1.99	2	149,767	1.34
Windham	6	116,782	5.14	6	116,404	5.15

Table 2. Summary of ATS data and *A. phagocytophilum* acarological risk index (ARI) by CT county, 2019 and 2020

	2019				2020			
County	# of sites	Number of adult ticks tested (% positive)	Number of nymph ticks tested (% positive)	Average ARI (SD [a])	# of sites	Number of adult ticks tested (% positive)	Number of nymph ticks tested (% positive)	Average ARI (SD [a])
Fairfield	5	118 (14.4)	73 (5.5)	0.122 (0.17)	5	28 (10.7)	24 (12.5)	0.047 (0.07)
Hartford	5	45 (4.4)	32 (3.1)	0.016 (0.04)	5	56 (8.9)	53 (1.9)	0.036 (0.10)
Litchfield	5	117 (10.3)	97 (4.1)	0.089 (0.17)	4	38 (7.9)	39 (7.7)	0.062 (0.09)
Middlesex	5	23 (0)	33 (0)	0 (0)	5	20 (5)	47 (0)	0.011 (0.04)
New Haven	5	108 (10.2)	61 (3.3)	0.062 (0.11)	5	40 (15)	91 (2.2)	0.097 (0.18)
New London	5	106 (10.4)	67 (1.5)	0.073 (0.11)	4	69 (5.8)	102 (8.8)	0.108 (0.16)
Tolland	6	68 (4.4)	199 (8.0)	0.097 (0.19)	5	53 (11.3)	96 (2.1)	0.089 (0.10)
Windham	5	57 (3.5)	183 (3.8)	0.050 (0.11)	5	105 (3.8)	93 (3.2)	0.058 (0.12)
Total	41	642 (9.0)	745 (4.7)		38	409 (7.8)	545 (4.2)	

[a]SD, standard deviation.



Source: State of Connecticut, Department of Energy & Environmental Protection, U.S. Geological Survey, National Mapping Program

* Sites (3) that were dragged in 2019, but excluded from dragging in 2020

Fig. 1. Map of the percentage of ticks positive for *A. Phagocytophilum* and the number of ticks collected by site and CT county, 2019–2020.

Table 3. Spearman rank correlation between incidence rate (IR) and acarological risk index (ARI)

Year	Mean IR (SD [a])	Mean ARI (SD [a])	Spearman correlation	*P* value
2019	10.57 (18.41)	0.064 (0.04)	0.595	0.1195
2020	4.76 (5.21)	0.064 (0.03)	0.548	0.1600
2019–2020 (Combined)	7.66 (13.41)	0.064 (0.04)	0.543	0.0297

[a]SD, standard deviation.

County Level Trends

Litchfield County had a 71% decrease in IR from 2019 to 2020 (Table 1 and Fig. 3). In this same period, there was a 30% decrease in ARI (Table 2 and Fig. 3). In addition, Fairfield County saw an IR decrease of approximately 70% from 2019 to 2020 and an ARI decrease of 61%. However, New London County had a 225% increase in IR from 2019 to 2020 and a 48% increase in ARI. Overall, Litchfield and Fairfield Counties had large decreases in both IR and ARI, but still had high proportions of clinical cases of anaplasmosis when compared to other counties. New London County had the most substantial increase in both values from 2019 to 2020.

Discussion

In this study, we identified an association between IR and ARI, at both the state and county levels of analysis. Previously published work supports the significance of active tick-borne pathogen surveillance in conjunction with human disease surveillance in understanding the changing ecology of tickborne pathogens and provide data for risk forecasting of tickborne illnesses, including *B. burgdorferi* (Lyons et al. 2021). Studies have supported a multimodal tick surveillance approach incorporating ATS to determine geographic spread of ticks and identify areas in need of additional surveillance (Lyons et al. 2021). This approach has been well described in understanding the geospatial and temporal changes in the distribution of *B. burgdorferi* (Guillot et al. 2022). Our study supports the utility of this surveillance approach for *A. phagocytophilum* in areas where these organisms are endemic and evaluating the potential spread into new areas.

Important limitations of this analysis should be acknowledged. Many human cases of anaplasmosis may be subclinical and self-limiting and would not be detected through case reporting and passive surveillance, there by underestimating IR. Geographical correlation between IR and ARI could also be inaccurate due to clinical cases seeking care outside of the area where they were infected. Additionally, ATS may detect *I. scapularis* infected with *A. phagoctyophilum* strains that are nonpathogenic to humans. This study was limited to 2 yr of ATS data and the sample size was relatively small, which may have limited the statistical power to identify geographical associations between IR and ARI, and the analysis was limited to a short period of

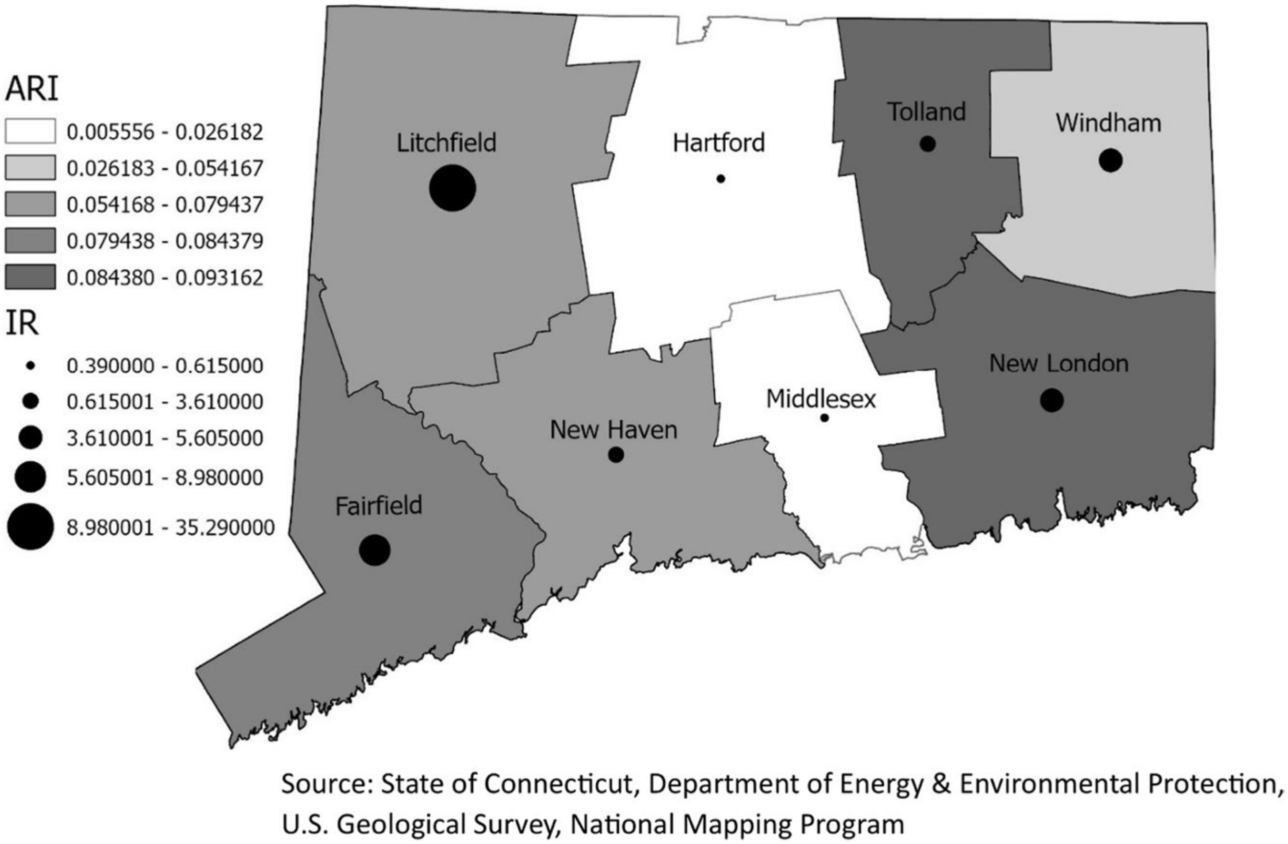


Fig. 2. Map of the average IR per 100,000 population and *A. phagocytophilum* acarological risk index (ARI) index by CT county for 2019–2020.

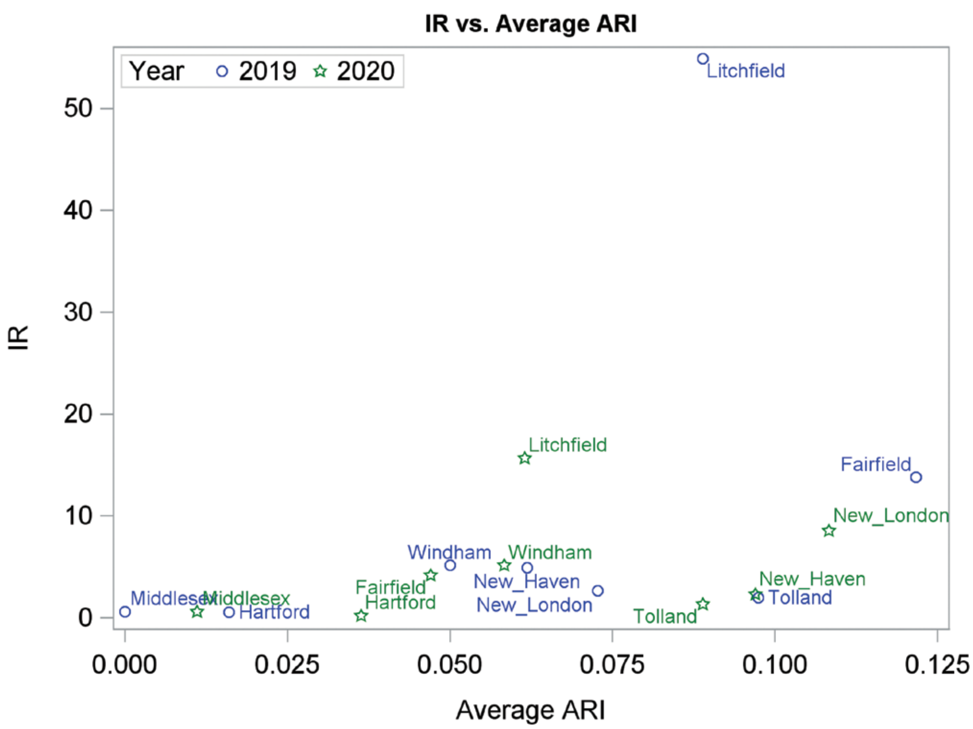


Fig. 3. Graph of anaplasmosis incidence rate (IR) versus *A. phagocytophilum* acarological risk index (ARI) by CT county, 2019 and 2020.

time precluding the ability to assess for temporal trends. Testing was performed by the CAES using established protocols which included a single PCR test thereby limited to the sensitivity and specificity of the test. Despite these limitations, this study demonstrates the correlation between anaplasmosis IR and *A. phagocytophilum* ARI, which has important implications for public health surveillance. Studies of larger sample sizes and data collection over a longer time period will help further understand this correlation and enhance the impact of this surveillance approach in the future.

This study highlights the utility of an ATS program and emphasizes the need to continue conducting surveillance throughout the state to identify temporal and geographic changes in *I. scapularis* abundance and *A. phagocytophilum* prevalence and the need to expand clinical surveillance for anaplasmosis. In 2024, the CTDPH added anaplasmosis as a clinician reportable disease in order to expand detection of clinical cases (CTDPH 2024). With this update, we will be able to identify more cases, including historical asymptomatic cases based on serology testing. The impact of this on the analysis that we performed is unknown, but an area for future potential study. By combining ATS data with human case epidemiology, public health practitioners can understand changing epidemiology and localized areas of increased risk in order to direct targeted clinician and public education on tick-borne infection prevention and management in order to maximize impact and improve public health.

Acknowledgments

The ATSP at the Connecticut Agricultural Experiment Station is majority financially supported by the Epidemiology and Laboratory Capacity for Prevention and Control of Emerging Infectious Diseases (ELC) through the Centers for Disease Control and Prevention and in part by the State of Connecticut General Fund and USDA National Institute of Food and Agriculture funds.

Author contributions

Julia Desiato (Conceptualization [lead], Formal analysis [equal], Investigation [equal], Methodology [equal], Writing—original draft [Lead]), Grace Chan (Conceptualization [supporting], Methodology [supporting], Writing—review & editing [supporting]), Marco Palmeri (Conceptualization [supporting], Writing—original draft [supporting], Writing—review & editing [supporting]), Jamie Cantoni (Conceptualization [equal], Writing—review & editing [equal]), Duncan Cozens (Investigation [equal], Methodology [equal]), Megan Linske (Investigation [equal], Methodology [equal]), Doug Brackney (Investigation [equal], Methodology [equal]), Kirby Stafford (Conceptualization [equal], Writing—review & editing [equal]), and David Banach (Conceptualization [equal], Investigation [equal], Methodology [equal], Writing—original draft [equal], Writing—review & editing [equal])

Conflicts of interest. None declared.

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