2-20-2015

Passive Tick Surveillance for Ixodes scapularis and the Incidence of Lyme Disease in Connecticut

Tsa J. Shelton
University of Connecticut - Storrs, tsa.shelton@yahoo.com

Passive Tick Surveillance for *Ixodes scapularis* and the Incidence of Lyme Disease in Connecticut

Tsa Jaynelle Shelton

B.S., University of Connecticut, 2010

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Public Health

At the

University of Connecticut

2015

APPROVAL PAGE

Masters of Public Health Thesis

Passive Tick Surveillance for *Ixodes scapularis* and the
Incidence of Lyme Disease in Connecticut

Presented by

Tsa Jaynelle Shelton, B.S.

Major Advisor____
Dr. David Gregorio, Ph.D., M.S.

Associate Advisor____
Dr. Richard Stevens, Ph.D

Associate Advisor____
Dr. Sandra Bushmich, M.S., D.V.M.

University of Connecticut

2015

Acknowledgements

I have worked with a great number of people whose contributions in various ways have been instrumental in the successful completion of this thesis project. I must express my apologies to those that I could not mention personally one by one. Your support and involvement in this project has truly nourished and triggered my intellectual maturity for decades to come.

With great appreciation, I acknowledge my advisory committee, Dr. David Gregorio, Dr. Richard Stevens, and Dr. Sandra Bushmich, for their continuous support, guidance and engagement. You have all inspired and enriched my growth as a student.

Special thanks must be given to the Connecticut Veterinary Medical Diagnostic Laboratory and the Connecticut Agricultural Experiment Station for the use of the data analyzed in this thesis.

The most important acknowledgments are reserved for my family. Without each of you, this thesis would not have been possible.

My mother, Janell Shelton, deserves special mention for her unwavering love and support in all that I do and continuing to encourage me to be a better person.

To my Aunt, Geraldine Jones, thank you for helping bedrock my success and always being there when I needed you most. You have fostered my love of learning.

To my "fairy" God-mothers, Colleen and Ronda, thank you both for being proud of everything I do and instilling love, patience, and the pleasure of adventure in me.

To my brothers, Charles and Desmond, thank you both for your sacrifices and being supportive, encouraging and caring siblings. I will continue to prosper and succeed to fulfill one of Desmond's last wishes.

To my niece and nephew, Aisjana and Desmond, thank you for your patience as I spent nights in the library and late nights at school. One day you both will surpass my success.

To my best friend and partner, Cleone Brandy, thank you for being you. Your dedication, love and strong confidence in me has taken a load off my shoulders. You have entrusted your passions, ambitions and intellect to me which has helped me achieve my goals with your encouragement through it all.

I would also like to thank the Brandy family for allowing me to nest amongst their own. Your unwavering support and acceptance has not gone unnoticed.

Lastly but not least, thank you Angela Colantano, Barbara Case and Mike Craven for your efforts and support in the completion of this thesis.

Table of Contents

List of Tables

List of Figures

Abstract

Lyme disease is a tick-borne disease, transmitted to humans via the bite of an *Ixodes scapularis* tick infected with the spirochetal bacterium *Borrelia burgdorferi*. This disease is endemic in Connecticut and has been increasing in prevalence throughout New England for the past 30 years. Data collected from *I. scapularis* ticks submitted by the public to the Connecticut Agricultural Experiment Station and the Connecticut Veterinary Medical Diagnostic Laboratory for *B. burgdorferi* testing from 2002 to 2012 were compared with Lyme disease case data from the Connecticut Department of Public Health to determine the capacity at which passive tick surveillance can be used to assess the risk of acquiring Lyme disease. The cumulative number of passively submitted ticks was moderately correlated with the number of reported Lyme disease cases among all Connecticut towns ($r = 0.488$, $p > 0.0001$, $n = 169$ towns). Passive tick submissions and Lyme disease cases were also correlated using data within the same surveillance year as well as the following surveillance year ($r = 0.473$, $p > 0.0001$ and $r = 0.250$, $p > 0.001$, respectively). The results of this project suggest that passive tick surveillance, using ticks submitted by the public for *B. burgdorferi* testing, may be used to evaluate the spatial and temporal impacts on Lyme disease incidence in Connecticut. However, the results of this study further imply that passive tick surveillance was more strongly correlated to the temporal measures examined in comparison to the spatial predictors examined. Consequently, passive tick surveillance may not be a reliable method for evaluating the spatial aspects of Connecticut's Lyme disease incidence, but it may be a better predictor of Lyme disease incidence from year to year.

Introduction

According to the 2011 Summary of Notifiable Diseases published from the *Morbidity and Mortality Weekly Report*, Lyme disease is the most commonly reported vector-borne disease in the United States, with 33,097 cases reported in that year. [1] This disease is caused by the spirochetal bacterium *Borrelia burgdorferi*, which is transmitted to humans via the bite of an infected *Ixodes scapularis* tick. [2,3] *I. scapularis* feed upon a wide variety of small mammals and birds, but incidental hosts include large mammals such as humans. Nearly 70-80% of people who contract Lyme disease develop a red, radiating rash, known as erythema migrans, which is typically followed by flu-like symptoms of fatigue, headache, stiff neck, joint and muscle aches, and fever. [4] In untreated cases, long-term symptoms, including neurologic, cardiac, or articular complications that develop months after exposure, have been reported. [4] The timely diagnosis of Lyme disease is critical to avoiding the effects of severe disease, which typically requires aggressive treatment. Laboratory diagnosis can be confirmed with a positive culture for *B. burgdorferi,* Polymerase Chain Reaction (PCR), enzyme-linked immunosorbent assay (ELISA) and Western Blot by a positive two-tiered serological test (IgM and IgG immunoglobulin seropositivity). [5,6]

Lyme disease was previously described in Europe, but was first identified as a clinical entity in the United States from a cluster of cases in three Connecticut towns surrounding Lyme, Connecticut, in 1975. [7] Initial symptoms of Lyme disease observed included of recurrent attacks of asymmetric swelling and pain in a few large joints, especially the knee, which at the time was characterized as arthritis. [7] Further investigations suggested that the disease was a late manifestation of a multisystemic,

vector-borne disease caused by ticks. [8] Since initially described in Lyme, Connecticut, the disease has steadily increased in incidence and expanded its geographic range from the eastern U.S to southeastern Canada, resulting in a distinct regional epidemic. [4] *B. burgdorferi* was isolated years after the discovery of the disease in 1981. [3,8]

The incidence of Lyme disease has been associated with the increased density of *I. scapularis* ticks. [2] Research has suggested that the incidence of Lyme disease is not only dependent upon the abundance of host-seeking ticks, but is also significantly correlated with the prevalence of the Lyme disease pathogen, *B. burgdorferi,* in actively collected nymphal ticks. [2,3] These factors may reflect a change in the land-usage practices, the abundance of ticks and the increase in tick host densities. [9] In New England, most cases of Lyme disease appear to be acquired close to or around residential areas, as people begin to build homes in more rural, wooded areas. [9] Lyme disease has become endemic in 14 northeastern and mid-western states (Connecticut, Delaware, New Hampshire, Maine, Maryland, Massachusetts, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia and Wisconsin), [8] and cases continue to rise annually. These 14 states accounted for 95% of the reported Lyme disease cases in 2012. [10] Lyme disease was also the seventh most common reported disease in the U.S. in 2012. [10]

Background

Life Cycle of *I. scapularis* Ticks

The *I. scapularis* tick, also known as the black-legged tick or the deer tick, is the vector for *B. burgdorferi*, the causative agent of Lyme disease. The life cycle of *I. scapularis* takes two years, and includes four life stages: the egg, larva, nymph, and adult. The tick also has three

hosts throughout its life cycle. First, the adult female lays ~2,000 eggs after a blood meal in the spring and the larva feed on small sized mammals, such as the white-footed mouse, during the late summer months. [9] Next, fed larvae molt into nymphs the following spring. Nymphs typically feed on small and medium sized hosts, mostly birds and mammals (incidentally including humans), during the summer months. Only after having a blood meal is the nymph then able to molt into an adult in autumn. Adult ticks feed upon large mammals through the fall and the following winter and spring (Figure 1).

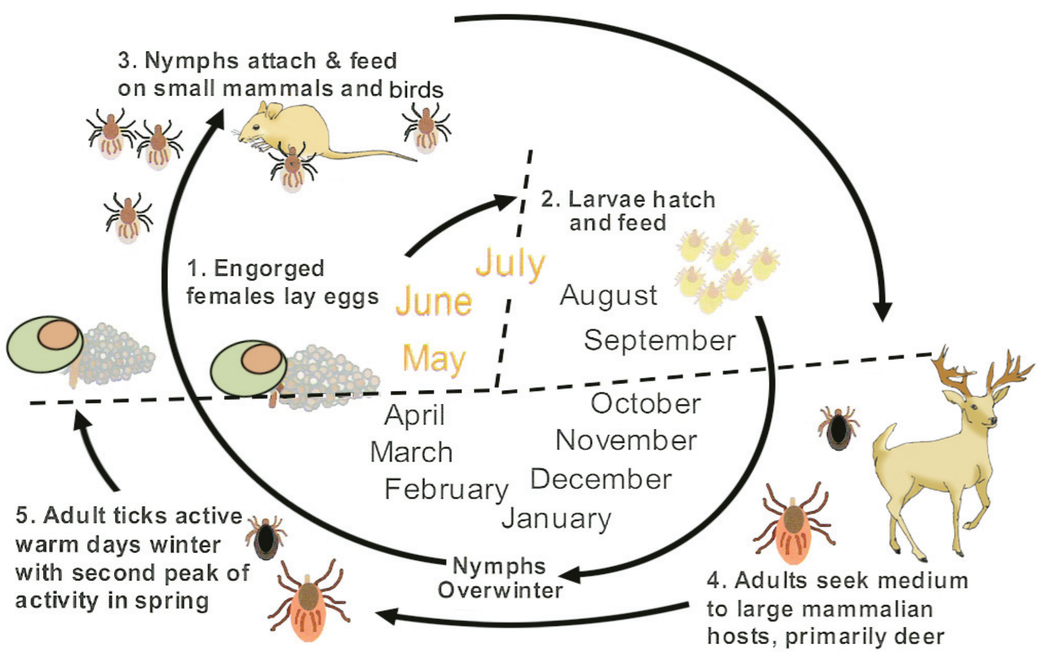

Figure 1: Two year life cycle of *I. scapularis* [9]

Hosts and Reservoirs for *B. burgdorferi*

A single *I. scapularis* tick will feed upon three hosts within its two-year life cycle. The main reservoir host, which serves as a source of infection and as a means of sustaining *B. burgdorferi*, is the white-footed mouse (*Peromyscus leucopus*).[9] The white-tailed deer is not the reservoir host for the spirochete, but acts as the primary host for the adult *I. scapularis* ticks. Tick survival is dependent upon the adult's blood meal, which is essential for egg laying (Figure 2). When tick larvae hatch they are not infected with *B. burgdorferi.* The larvae and nymphs become infected after feeding upon an infected animal, and then may transmit the disease during their next feeding.[9] Adult ticks typically have more frequent exposures to infected hosts than the larva and nymphs, as adult ticks have had two opportunities, as a larva and again as a nymph, to feed on potentially infected hosts.[9] Because ticks have a two-year life cycle, a human may be infected with Lyme disease during any part of the year.[2]

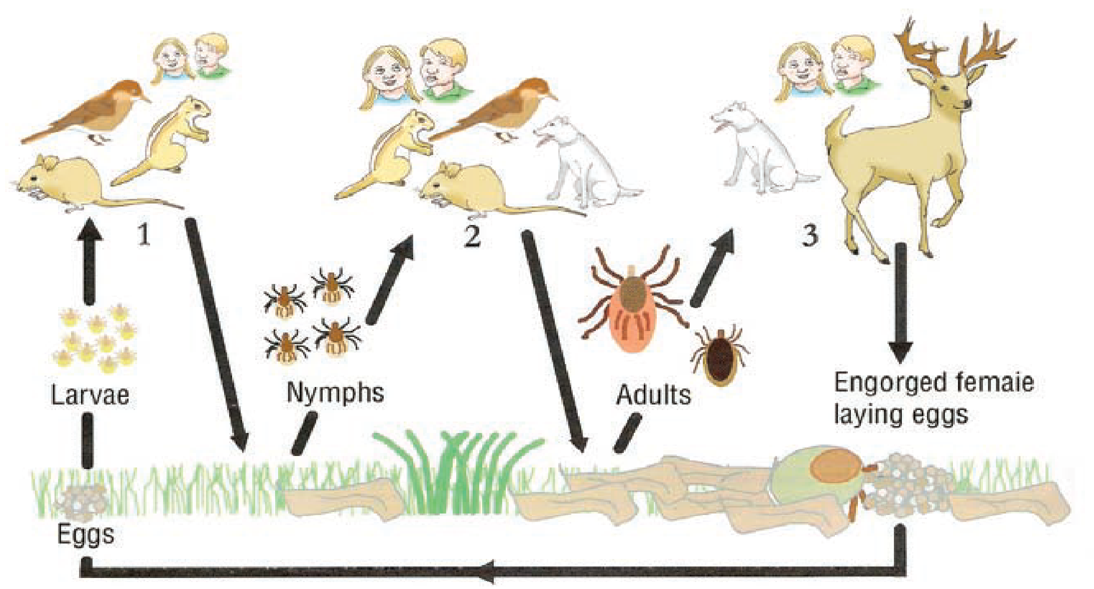

Figure 2: Three host life cycle of ticks[9]

Factors that Increase the Risk of Human Exposure to Infected Ticks

The population of tick vectors and human behavior are the greatest influences in human exposure to infected ticks. Acquiring an infected tick is dependent upon the exposure to the tick. It is suggested that the incidence of Lyme disease is not only dependent upon the abundance of host-seeking ticks, but is also significantly correlated with the prevalence of *B. burgdorferi* in actively collected nymph ticks. [2,3] When measuring the abundance of infected ticks, one must consider evaluating the population density of ticks and the rate of infection with *B. burgdorferi*. The density of *I. scapularis* is highest in densely forested areas, forest-field edges and the lowest in fields. [9] Humans are at risk of exposure to infected ticks when they enter such habitats. When evaluating the domestic environment, fewer ticks are found near ornamental vegetation and lawns, and most of the ticks found in lawns are located within three yards of the its perimeter. [9]

Most Lyme disease infections occur during the spring and throughout the summer. Female adults generally feed in the fall thus laying eggs in the spring. The larvae hatch from eggs in mid to late July, and peak larval feeding activity is in August. [9] After the larvae feed and drop off the host, the larvae molt into nymphs that will mature during the following late spring and summer in May, June, July and August. [9] Nymphs can infect the next generation of animal hosts or humans mainly in June and July if they have fed upon an infected host previously. [9] Human exposure is greatest during the summer months, when ticks are most active and when people spend more time outdoors. Humans are at risk of infection within tick habitats all months of the year, but the risk is greatest from late May to August, which coincides with the nymph-feeding season. [9]

Lyme Disease Surveillance Case Definition

The national surveillance case definition for Lyme disease is not intended for diagnosis, but to define cases that meet the national Lyme disease surveillance inclusion criteria. A *suspected case* has been defined as a patient that experiences one or more erythema migrans without a known tick exposure or a patient with positive laboratory evidence of infection but no clinical symptoms reported.[5] A *probable case* is a case where a physician has diagnosed the disease with laboratory evidence of infection.[5] A *confirmed case* has multiple classifications, which include: 1.) a patient with erythema migrans with a known tick exposure, or 2.) a patient with erythema migrans and laboratory evidence of infection without known exposure, or 3.) a patient with at least one late manifestation of Lyme disease that has laboratory evidence of infection.[5] These reports for Lyme disease are collected and verified by state and local health departments as defined by the national surveillance case definition.

Lyme Disease Surveillance in Connecticut

Surveillance for Lyme disease is an important public health concern, particularly in highly endemic areas.[11] As of January 1, 1991, Lyme disease became a nationally reportable disease in the U.S. The state of Connecticut has been defined under the Lyme disease case definition as being endemic for Lyme disease. Connecticut's endemic status has been declared due to the fact that each county has more than two confirmed, locally acquired cases and/or has an established population of ticks infected with *B. burgdorferi.*[4] Not only is Connecticut defined as endemic on the county level, but nearly all of Connecticut's 169 towns are endemic as well (Figure 3). As mandated by the U.S. Public

Health Service, states collect and report Lyme disease surveillance data to the CDC annually.

According to the Connecticut Department of Public Health (DPH) reports, annual cases of Lyme disease appear to have dramatically decreased from 2002-2004 (Figure 4). This appears to be due to the end of mandatory laboratory surveillance, which was required prior to 2002 by the Connecticut DPH. [12] The number of reported Lyme disease cases decreased by 69.7% in 2003 alone. [12] The number of cases reported annually continued to decrease from 2003-2006, the period with no laboratory surveillance, which was nearly 78.2% less than the 2002 annual reported mean. [12] After 2007, the surveillance requirements changed and laboratory reporting was reinstated for laboratories with electronic reporting capabilities, which caused the number of reports to increase by 228.3%. [12] Following 2007, the number of physician-based surveillance practices noticeably declined which decreased the number of reported cases overall from 2010-2012.

In 2012 alone, 2,657 new cases of Lyme disease were reported in Connecticut at a rate of 46 per 100,000. In that year, 30,831 cases of Lyme disease were reported in the U.S. at a rate of 7.0 per 100,000, which reflects the incidence of Lyme disease in the U.S. as a whole. [10] In 2012, the highest rates (per 100,000) in Connecticut were reported from New London (128.8), Tolland (115.9) and Windham (135.9) counties. [13] Lyme disease in Connecticut is unfortunately under-reported and misdiagnosed, which affects surveillance of this disease. [4] Due to these shortcomings, the Connecticut Department of Public Health has implemented several additional surveillance methods in order to accurately reflect the true incidence of the disease. Lyme disease surveillance in Connecticut utilizes both

passive and active methods using three different surveillance methods that include passive physician, active physician and mandatory laboratory reporting in 169 towns. [12] The human surveillance data reported within this study reflects the cases that meet the case classification definitions as reported to the National Notifiable Disease Surveillance System.

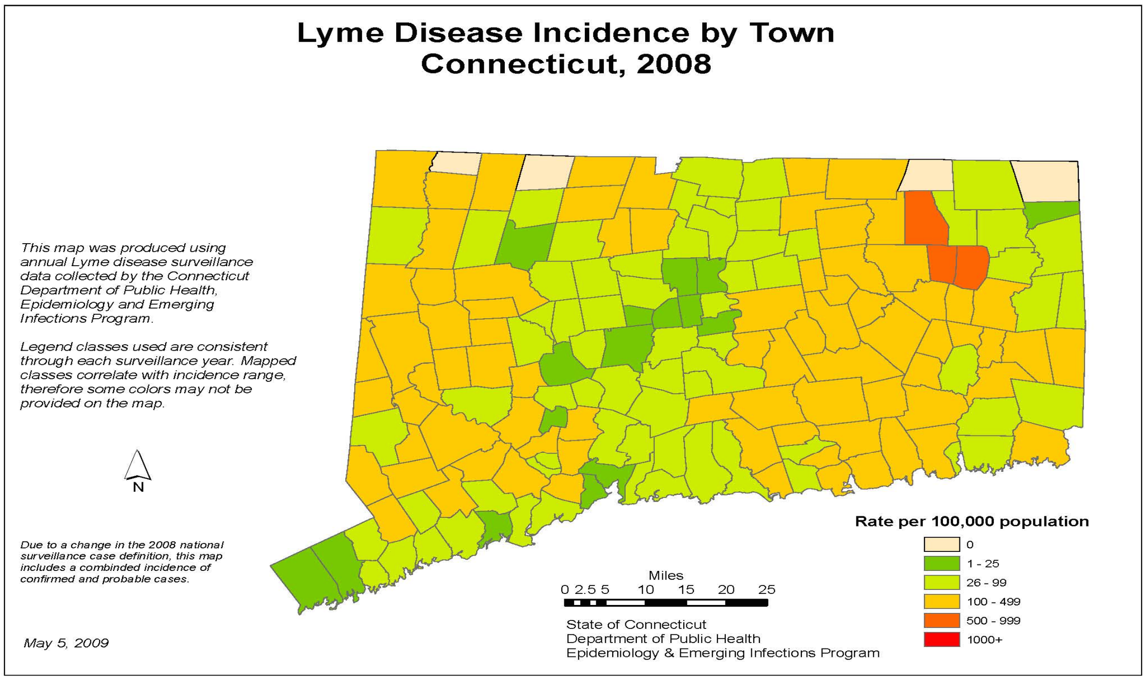

Figure 3: Lyme disease incidence by town as reported to the Connecticut Department of Public Health, 2008 [14]

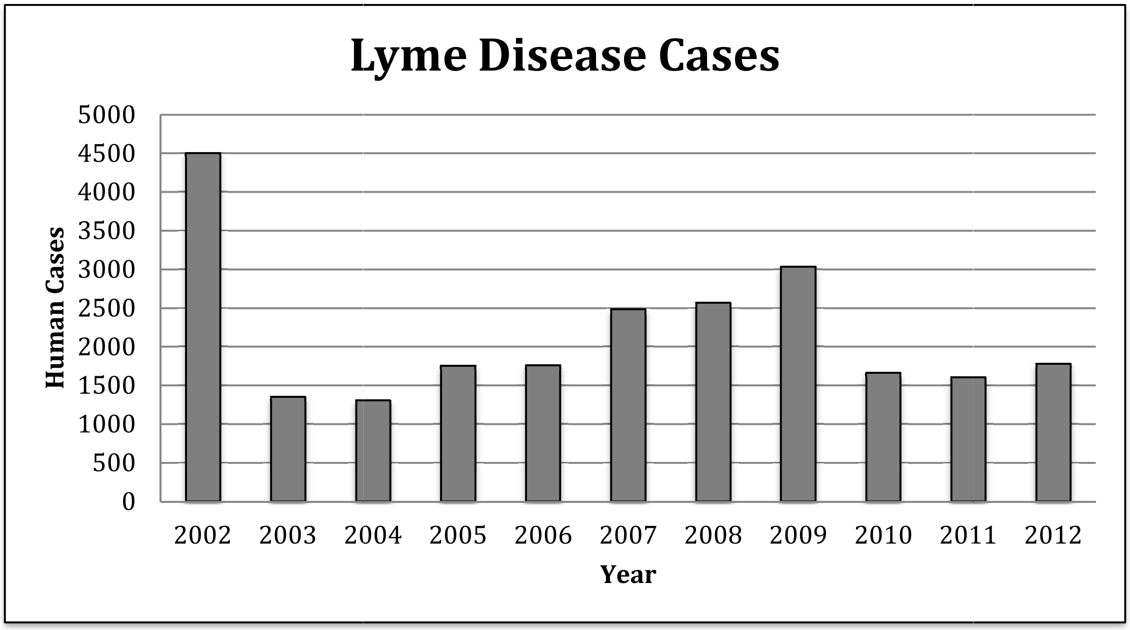

Figure 4: Number of Connecticut acquired cases of Lyme disease as reported to the Connecticut Department of Public Health, 2002-2012

Passive Tick Surveillance in Connecticut

Tick surveillance in Connecticut entails the identification of the tick species, stage of development and the evaluation of the degree of blood engorgement. Ticks are examined to determine the degree in which the tick is engorged, which is evidence of a blood meal; they are then tested for the presence of *B. burgdorferi*. Passive tick surveillance in Connecticut refers to the testing of ticks submitted by residents, typically after being bitten, to the Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL; Storrs, CT) and/or the Connecticut Agriculture Experiment Station (CAES; Hamden, CT). Active tick surveillance is more labor intensive because it involves dragging strips of white cloth mounted on poles through habitats that are suspected of harboring ticks.[9]

The practice of passive tick surveillance is beneficial to public health because the time and financial investment are relatively low, compared to active surveillance. [11] Passive tick surveillance can also be used to measure exposure to infected ticks, allowing public health officials to make informed decisions about implementing additional public health awareness campaigns to stress personal protection practices and potentially allocate public health resources to this activity. [11] Through the use of passive tick surveillance, outcomes can not only provide information regarding the spatial distribution of tick vectors and the prevalence of tick borne pathogens, but it can provide a direct estimate of Lyme disease transmission and risk, too. It is also important to evaluate surveillance practices to define their validity and potential to predict relationships between the density of infected ticks and human disease risk. [3] Most importantly, the use of passive tick surveillance can provide useful information on the spatial and temporal distribution of tick borne pathogens to assess human risk.

Despite the inherent benefits of passive surveillance, there are several notable drawbacks. Passive surveillance is typically considered to have poor sensitivity because it only captures a small subset of the ticks that bite humans and it lacks the specificity to identify the exact location of an established tick population. [3,11,15] However, conducting passive surveillance can provide accurate information on the geographical patterns of tick abundance. To further address the potential use of passive tick surveillance as a cost effective method for assessing Lyme disease risk surveillance, an evaluation of the current passive tick surveillance practices and outcomes are important to assess.

Study Predictions and Goals

Previous studies report that the abundance of ticks and the incidence of Lyme disease appear to be correlated. [2,3,16] This strongly suggests that the number of ticks passively submitted by the public for *B. burgdorferi* testing may be related to the incidence of Lyme disease as well. [2,3] The goal of this study was to characterize the relationship between the numbers of ticks submitted for *B. burgdorferi* testing, the number of ticks infected with *B. burgdorferi*, the prevalence of *B. burgdorferi* within the infected ticks submitted (defined as the tick infection prevalence) and the incidence of Lyme disease on a temporal and spatial scale. This study also sought to determine whether there is a link between the prevalence of *B. burgdorferi* infected *I. scapularis* and Lyme disease cases reported in Connecticut. These findings will help demonstrate whether passive tick surveillance can be used as an effective tool to assess the risk of acquiring Lyme disease in Connecticut. With 10 years of passive tick surveillance data collected in Connecticut (2002-2012), the following predictions were made:

Prediction 1: There is a positive correlation between the number of submitted ticks, from 2002 through 2012, and the number of Lyme disease cases.

Prediction 2: There is a positive correlation between the number of submitted ticks and the number of Lyme disease cases in the same surveillance year and the following year.

Prediction 2a: There is a positive correlation between the number of submitted ticks and the number of Lyme disease cases based upon the number of reported cases of Lyme disease on a town-by-town basis.

Prediction 3. There is a positive correlation between the number of infected ticks

submitted annually and the number of Lyme disease cases in the same surveillance year and the following year.

Prediction 4: There is a positive correlation between the number of submitted ticks and the incidence of Lyme disease.

Prediction 5: There is a positive correlation between the tick infection prevalence and the number of Lyme disease cases, from 2002 through 2012.

Prediction 5a: There is a positive correlation between the tick infection prevalence and the number of Lyme disease cases based on the tick infection prevalence of *B. burgdorferi* in each town.

Prediction 6: There is a positive correlation between the tick infection prevalence and the number of Lyme disease cases in the same surveillance year and the following year.

Methods

Methods of Data Collection

Every year the CAES and the CVMDL test ticks submitted by the public for the presence of *B. burgdorferi* in *I. scapularis.* Both agencies have granted the author permission to use the data gathered on *I. scapularis* to test the predictions of this thesis. Passive tick surveillance data gathered from each town were summarized by the following variables: the number of *I. scapularis* ticks submitted, the year the tick was submitted, the number of ticks submitted, the number of ticks tested, the number of ticks infected with *B. burgdorferi* and the tick infection prevalence of *B. burgdorferi*. The tick infection prevalence was calculated as the proportion of ticks that tested

positive for *B. burgdorferi*. The tick infection prevalence rates were classified as followed: >33 (per 1000); between 23 and 32 (per 1000); and <22 (per 1000).

Lyme disease case data was obtained from annual reports published by the Connecticut DPH, from 2002 through 2012. All Lyme disease cases have met the national case definition for Lyme disease. [5] The spatial distribution of towns included all 169 towns in Connecticut, which were classified based on the cumulative number of Lyme disease cases as follows: >186 cases, (high); 67-185 cases, (low); <66 cases, (rare). The cumulative annual case data for Lyme disease were summarized based on the number of cases reported to the Connecticut DPH. The incidence rate (per 100,000) was determined using decennial census data covering the year of data collection (2000 and 2010). [12]

Statistical Analysis

The independent variables (number of ticks submitted, the number of infected ticks, and the prevalence of *B. burgdorferi* in infected ticks) and the dependent variables (Lyme disease cases and incidence) were interval data. The above hypotheses were evaluated using Pearson's and Spearman's correlation analysis. Because some of the data may not fully meet the assumptions for parametric tests, such as equal variance and normal distribution, a two-tailed Spearman's non-parametric linear regression analysis was performed. Should the Spearman's analysis differ markedly from the more statistically powerful Pearson's results, the results using the Spearman's tests were considered. All statistical analyses were conducted in SAS statistical software.[17] A two-tailed Pearson's analysis was used to evaluate the association in both the positive or

negative direction. This would demonstrate that the findings do not support the predictions. Correlations with $p<0.10$ were also considered, given the fact that analyzing field data could result in a relationship that may be missed if only correlations with $p<0.05$ were considered. The data used does not have a high degree of precision because it was not gathered under controlled conditions, such as in laboratory studies.

Results

I. scapularis ticks were submitted for testing from residents of Connecticut's 169 towns over the course of ten years, from 2002-2012. During each year, the CVMDL and CAES examined the *I. scapularis* ticks for the degree of engorgement, species identification and stage of development (Table 1). A total of 47,721 *I. scapularis* were submitted to the CVMDL and CAES, from 2002 to 2012, and a total of 23,780 cases of Lyme disease were reported to the Connecticut DPH. Of the 35,897 tested ticks, 27.6% (n=10,493) were positive for *B. burgdorferi* (Table 2).

Many of the passive tick surveillance factors evaluated within the current study were positively correlated and statistically significant to the number of reported Lyme disease cases in Connecticut when examined on a temporal scale (Figure 5). Reported Lyme disease cases and the number of ticks submitted were correlated when evaluated statistically ($r=0.488$, $p=0.0001$). This correlated finding implies that the number of ticks passively submitted had a statistically significant relationship with the reported number of Lyme disease cases between 2002 and 2012. This was also true when examining this association in the same surveillance year and the following year (Table 3). The number of ticks submitted and the number of Lyme disease cases also appears

to follow a similar pattern when examined on an annual basis (Figure 6). There was a positive correlation between the tick infection prevalence of *B. burgdorferi* and the number of Lyme disease cases in the same surveillance year and the following year (Table 4). The relationship between the tick infection prevalence of *B. burgdorferi* and the number of Lyme disease cases was statistically significant from 2002 through 2012 (Table 5). On a spatial scale, the number of passively submitted ticks from Connecticut towns appears to have an appreciable correlation to the number of Lyme disease cases in 49.1% of participating towns reporting a low number of cases, between 67 and 185 cases/year, when examined on a town-by-town basis (n=83). This was also supported in towns with rarely reported Lyme disease cases (<67 cases/year).

Although, the number of passively submitted ticks was not positively correlated to the Lyme disease incidence in some Connecticut towns, however these two variables follow a similar temporal trend in the years evaluated for the state as a whole (Figure 7). Only 26% of Connecticut towns had a negative correlation between the number of ticks submitted and a high number of Lyme disease cases (>186 cases/ year, n=44). The tick infection prevalence of *B. burgdorferi* in submitted ticks and the number of Lyme disease cases was not correlated among Connecticut towns with more than 23 submitted ticks (per 1000 submitted) when evaluating the spatial associations between the two variables overall; even still these variables appear to follow a similar temporal trend for the state as a whole (Figure 8). However, this trend was not observed among the towns with a tick infection prevalence of >23 infected ticks (per 1000 submitted) in relation to the number of Lyme disease cases on a year-to-year basis.

Prediction 1: *There is a positive correlation between the number of submitted ticks, from 2002 through 2012, and the number of Lyme disease cases.*

Prediction 1 was supported with a positive and statistically significant correlation ($r=0.488$, $p=0.0001$) between the total number of *I. scapularis* ticks submitted via passive surveillance and the number of reported Lyme disease cases, from 2002 through 2012. The numbers of ticks submitted passively were moderately correlated to the number of reported Lyme disease cases. The number of *I. scapularis* ticks submitted accounted for 23.8% of the variation in Lyme disease cases reported in this time period ($r^2=0.238$). Temporally, these variables appear to follow a similar trend when examining the number of submitted ticks and the number of reported cases annually (Figure 6). This finding was highly statistically significant at $p<0.05$ and both the Pearson's and Spearman's test supported this finding.

Prediction 2: *There is a positive correlation between the number of submitted ticks and the number of Lyme disease cases in the same surveillance year and the following year.*

Prediction 2 was supported by a statistically significant, positive correlation between the number of ticks submitted and the number of Lyme disease cases in the same surveillance year and the subsequent year ($p=0.049$, Table 3). The number of *I. scapularis* ticks submitted appears to account for some of the variation observed in the cases reported among towns in the same surveillance year and the following year. This finding was supported by the Spearman's test.

Prediction 2a: *There is a positive correlation between the number of submitted ticks and the number of Lyme disease cases based upon the number of reported cases of Lyme disease on a town-by-town basis.*

The total number of *I. scapularis* ticks submitted, from 2002 through 2012, in nearly 25% (24.8%) of Connecticut towns that reported a rare number of Lyme disease cases (<67 cases/year) were positively correlated with each other (r=0.466, p=0.002, n=42). The number of *I. scapularis* ticks submitted accounted for nearly 22% of the variation in the number of Lyme disease cases reported among these Connecticut towns with rarely reported cases (r^2=0.217). In other words, the number of passively submitted ticks from towns with rarely reported cases of Lyme disease are positively correlated.

Furthermore, the total number of ticks submitted was positively correlated to nearly half (49.1%) of the Connecticut towns reporting a low number of Lyme disease cases, between 67 and 185 cases/year (r=0.366, p=0.0007, n=83). The number of *I. scapularis* ticks submitted does appear to account for some of the variation in the number of Lyme disease cases reported among towns with a low number of reported cases from 2002-2012, (r^2=0.133). In other words, the number of ticks submitted annually accounted for 13% of the variation observed in towns reporting a low number of Lyme disease cases.

On the other hand, the total number of ticks submitted and the number of reported Lyme disease cases in 26% of Connecticut towns with a high number of reported cases (>186 cases/ year) has a positive correlation, but was not statistically significant, at p<0.10 and 0.05 (r=0.092, p=0.55, n=44). In other words, the number of ticks submitted passively from the 44 Connecticut towns that reported more than 186

Lyme disease cases does not support a statistically significant correlation. Although these correlations were moderately weak, this analysis was supported by both the Pearson's and Spearman's analyses.

Prediction 3. *There is a positive correlation between the number of infected ticks submitted annually and the number of Lyme disease cases in the same surveillance year and the following year.*

Prediction 3 was supported in both the same surveillance year and the following year. There was a statistically significant, positive correlation between the numbers of infected ticks submitted and the number of reported Lyme disease cases in the same surveillance year and the following year (Table 4). In other words, the number of infected ticks in one year is highly predictive of the number of cases in the same and subsequent surveillance year. Due to the fact that the variable was not normally distributed the test for significance was considered under the Spearman's test only.

Prediction 4: *There is a positive correlation between the number of submitted ticks and the incidence of Lyme disease.*

Prediction 4 was not supported. There was a negative correlation ($r=-0.237$, $p=0.0019$) between the total number of *I. scapularis* ticks passively submitted and the incidence of Lyme disease. The number of *I. scapularis* ticks submitted does not appear to have accounted for much of the variation in the Lyme disease incidence reported during this time ($r^2=0.056$). In other words, the number of passively submitted ticks appears to have an inverse effect upon the incidence of Lyme disease. This was

supported by both the Pearson's and Spearman's analyses; however, when evaluating this relationship temporally there appears to be a consistent trend between the number of ticks submitted and the incidence of Lyme disease cases (Figure 7). Temporally, the Lyme disease incidence appears to follow a fairly consistent trend with the number of ticks submitted over time. This finding was statistically significant at $p<0.05$.

Prediction 5: *There is a positive correlation between the tick infection prevalence and the number of Lyme disease cases, from 2002 through 2012.*

Prediction 5 was not supported; however it does appear to be follow a similar temporal trend as shown in Figure 8. There was a negative correlation ($r=-0.096$, $p=0.21$) between the prevalence of *B. burgdorferi* in *I. scapularis* ticks submitted, from 2002 through 2012, and the number of reported Lyme disease cases. This finding was not statistically significant at $p<0.05$ and $p<0.10$. In other words, the tick infection prevalence of *B. burgdorferi* in ticks submitted passively does not positively correlate to the number of Lyme disease cases in Connecticut. This finding was supported by both the Pearson's and Spearman's analysis.

Prediction 5a: *There is a positive correlation between the tick infection prevalence and the number of Lyme disease cases based on the tick infection prevalence of B. burgdorferi in each town.*

Prediction 5a was not fully supported. There was a positive correlation for the towns with a low tick infection prevalence of <22 infected passively submitted *I. scapularis* ticks (per 1000 ticks submitted), from 2002 through 2012, and the reported

number of Lyme disease cases in each town (r=0.551, p =0.0002, n=42). In other words, the low cumulative tick infection prevalence rate of <22 infected ticks (per 1000 ticks submitted) does significantly correlate to the number of Lyme disease cases in Connecticut and may contribute to nearly 30% of the observed variation in the Lyme disease reporting from 25% of the towns evaluated ($r^{2=}$0.302).

In towns that submitted a moderate to high number of infected ticks, more than 23-32 and >33 infected *I. scapularis* ticks (per 1000 ticks submitted), Prediction 5a was not supported. There were negative correlations between the tick infection prevalence of *B. burgdorferi* in submitted *I. scapularis*, from 2002 through 2012, and the number of reported Lyme disease cases (r=-0.014, p=0.90, n=92; r=-0.224, p=0.16, n=35, respectively). In other words, the tick infection prevalence of *B. burgdorferi* in ticks from just over 75% of Connecticut towns submitting more than 23-32 and >33 infected *I. scapularis* ticks (per 1000 ticks submitted) does not appear to account for the variation in the number of reported Lyme disease cases, (r^2=0.0021 and r^2=0.005 respectively). Both the Pearson's and Spearman's test supported this finding.

Prediction 6: *There is a positive correlation between the tick infection prevalence and the number of Lyme disease cases in the same surveillance year and the following year.*

Prediction 6 was supported by a statistically significant, positive correlation between the tick infection prevalence of *B. burgdorferi* in passively submitted *I. scapularis* ticks and the number of reported Lyme disease cases in the same surveillance year and the following year for the respective years examined (Table 5). In other words, the tick infection prevalence among ticks infected with *B. burgdorferi* in one year is

highly predictive of the number of Lyme disease cases in the same year and following surveillance year.

Table 1: Connecticut acquired tick species, in order of frequency, 2002-2012

Tick Species	Year										
	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Ixodes scapularis	6437	5886	4495	6123	4855	2602	3126	3672	4495	2903	1958
Dermacentor variabilis	329	295	326	257	235	159	208	285	326	228	327
Amblyomma americanum	53	56	47	64	67	36	71	67	47	55	70
Amblyomma maculatum	1	0	0	0	0	0	0	0	0	0	0
Ixodes cookei	1	4	3	9	3	5	2	1	3	1	3
Ixodes marxii	1	0	0	3	0	0	0	0	0	0	0
Ixodes pacificus	1	2	0	0	0	0	0	0	0	1	0
Rhipicephalus sanguineus	1	0	1	3	3	2	0	0	1	0	0
Ixodes pacificus	0	2	0	2	0	0	0	0	0	0	0
Ixodes dentatus	0	0	1	0	0	1	0	0	1	3	4
Amblyomma maculatum	0	0	0	0	0	0	2	0	0	0	1
Amblyomma species	0	0	0	0	0	0	2	0	0	0	0
Ixodes ricinus	0	0	0	0	0	0	1	1	0	0	1
Unknown/ not listed	0	27	0	0	0	0	2	3	0	0	0
Total	7153	6272	5199	6718	5398	2964	3622	4314	5199	3419	2691

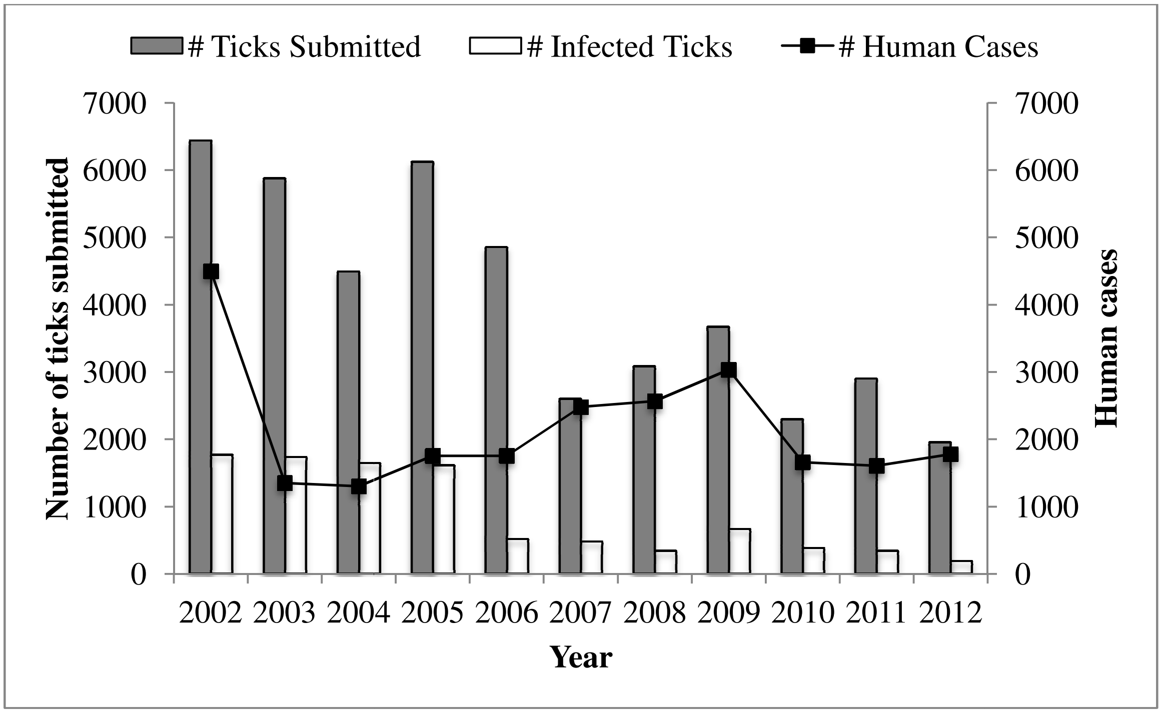

Figure 5: Number of ticks submitted, number of positive ticks and number of Lyme disease cases in Connecticut, 2002-2012.

Table 2: Percent of *I. scapularis* ticks collected by passive surveillance infected with *B. burgdorferi*

Year	Percent infected	Number tested
2002	28%	6539
2003	30%	6023
2004	37%	4543
2005	27%	6267
2006	22%	2576
2007	34%	1707
2008	13%	1887
2009	37%	2095
2010	30%	1434
2011	13%	1708
2012	21%	1118

Table 3: Number of ticks passively submitted versus Lyme disease cases

	Same Year	Next Year
2002	r=0.473	r=0.250
	p>0.0001	p=0.0010
2003	r=0.232	r=0.249
	p=0.0024	p=0.0010
2004	r=0.295	r=0.308
	p=0.001	p>0.0001
2005	r=0.359	r=0.0332
	p>0.0001	p>0.0001
2006	r=0.216	r=0.359
	p=0.0047	p=0.0001
2007	r=0.451	r=0.533
	p=0.0001	p=0.0001
2008	r=0.561	r=0.524
	p=0.0001	p=0.0001
2009	r=0.503	r=0.442
	p=0.0001	p=0.0001
2010	r=0.383	r=0.364
	p=0.0001	p=0.0001
2011	r=0.454	r=0.449
	p=0.0001	p=0.0001
2012	r=0.350	-------
	p=0.0001	

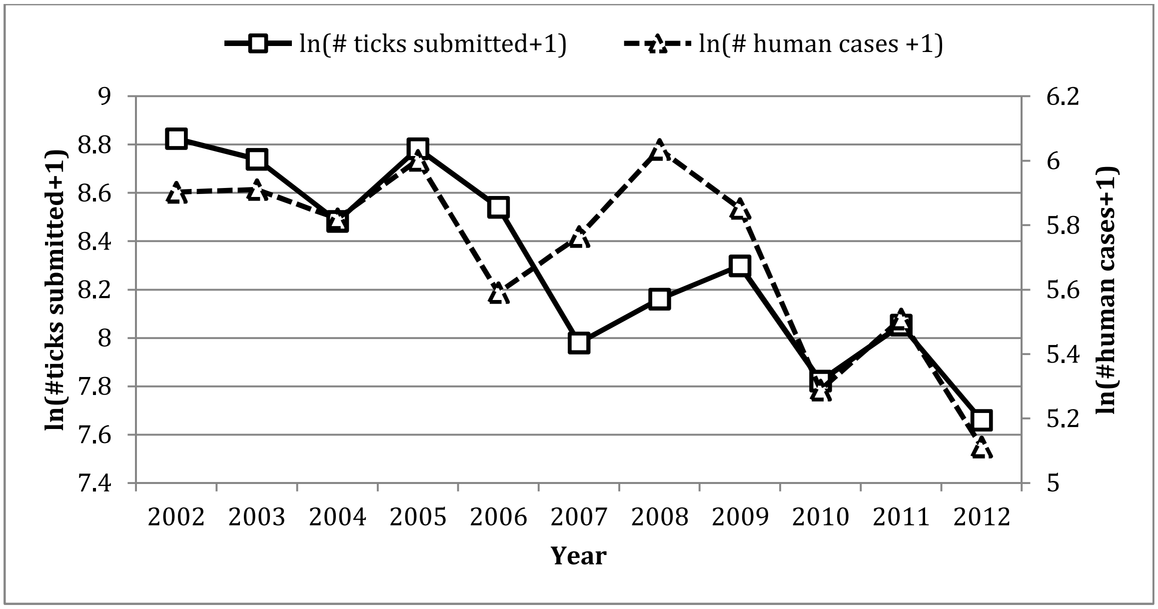

Figure 6: Connecticut annual *I. scapularis* submissions versus the number of reported Lyme disease cases, 2002-2012

Table 4: Tick infection prevalence versus Lyme disease cases.

	Same Year	Next Year
2002	r=0.261	r=0.130
	p=0.0006	p=0.047
2003	r=0.128	r=0.129
	p=0.09	p=0.09
2004	r=0.085	r=0.159
	p=0.273	p=0.038
2005	r=0.308	r=0.298
	p>0.0001	p=0.0001
2006	r=0.145	r=0.208
	p=0.059	p=0.007
2007	r=0.332	r=0.295
	p=0.0001	p=0.0001
2008	r=0.288	r=0.305
	p>0.0001	p=0.0001
2009	r=0.230	r=0.186
	p=0.0025	p=0.0151
2010	r=0.168	r=0.166
	p=0.029	p=0.031
2011	r=0.297	r=0.244
	p=0.0001	p=0.001
2012	r=0.166	-------
	p=0.03	

Table 5: Number of positive ticks vs. Lyme disease cases

	Same Year	**Next Year**
2002	r=0.461	r=0.2895
	p=0.0001	p=0.013
2003	r=0.134	r=0.305
	p=0.005	p=0.006
2004	r=0.339	r=0.272
	p=0.004	p=0.022
2005	r=0.259	r=0.294
	p=0.026	p=0.01
2006	r=0.212	r=0.257
	p=0.088	p=0.04
2007	r=0.280	r=0.145
	p=0.023	p=0.246
2008	r=0.092	r=0.193
	p=0.46	p=0.12
2009	r=0.341	r=0.291
	p=0.002	p=0.008
2010	r=0.291	r=0.275
	p=0.008	p=0.01
2011	r=0.192	r=0.282
	p=0.109	p=0.022
2012	r=0.299	-------
	p=0.025	

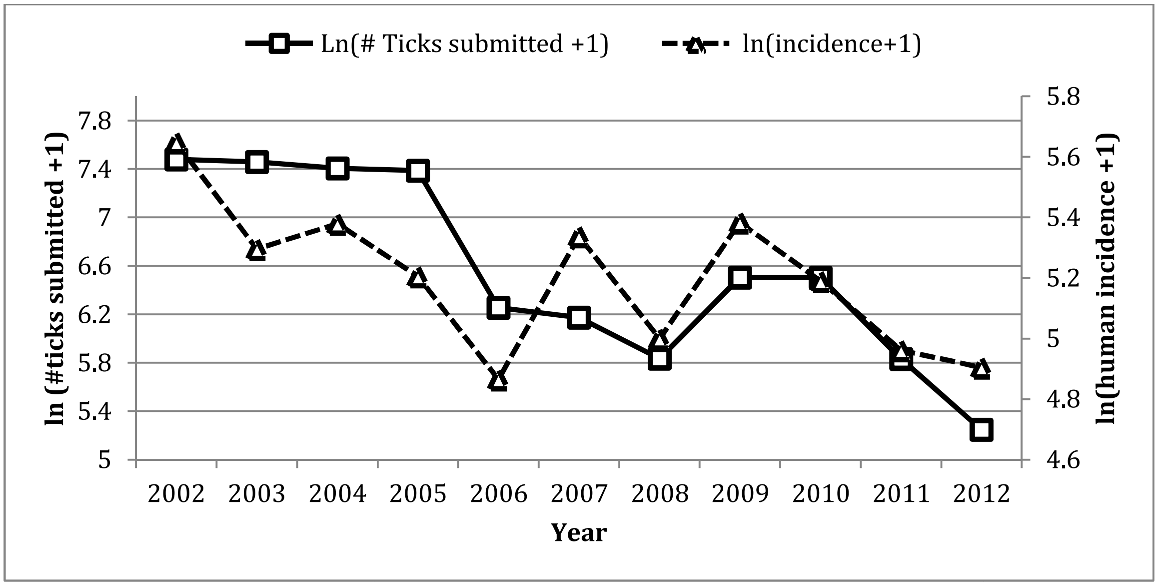

Figure 7: Connecticut annual *I. scapularis* submissions, versus the incidence rates for Lyme disease, 2002-2012

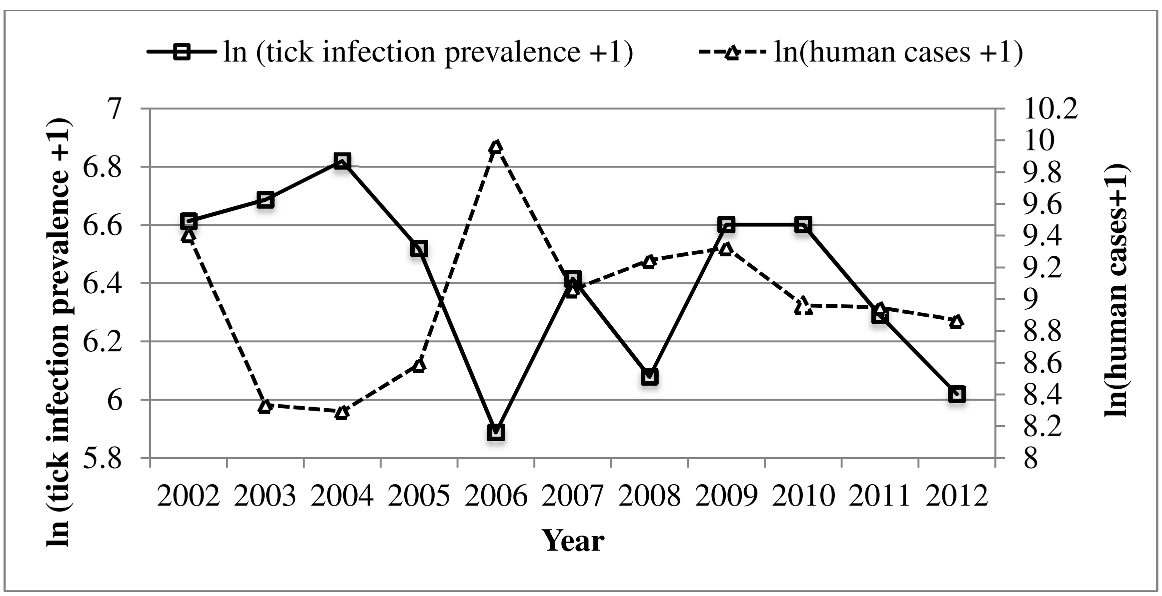

Figure 8: Tick infection prevalence for *B. burgdorferi* versus the number of reported Lyme disease cases, 2002-2012

Discussion

This study evaluated the effectiveness of passive tick surveillance to predict the risk of acquiring Lyme disease on a spatial and temporal scale. This research has examined the number of passively submitted ticks, the number of ticks infected with *B. burgdorferi*, the tick infection prevalence of *B. burgdorferi* within these ticks, and their relationship to the incidence of Lyme disease in Connecticut from 2002-2012. As reported by previous research, the density of ticks collected from a specific location appears to correlate with the incidence of Lyme disease, which in turn can suggests that the number of ticks submitted by the public for *B. burgdorferi* testing can possibly be used to predict the incidence of reported Lyme disease cases. [2,3] This study has shown that there is a moderate association between the data collected from passive tick surveillance and the reported number of Lyme disease cases in Connecticut. These results validate the hypothesis that passive tick surveillance can be a valuable epidemiological tool in assessing the risk of acquiring Lyme disease on a spatial and temporal scale. The number of Connecticut acquired *I. scapularis* submitted over the 10 years evaluated provides moderate temporal and spatial correlations to the reported cases of Lyme disease. Although the correlations presented were moderate, the data does support previous research findings.

A study conducted by Rand et al[16] suggested that there is a relationship among passively collected ticks and the number of Lyme disease cases. The current study provides supportive evidence for the conclusions described by Rand et al[16]. The number of ticks passively submitted and the number of Lyme disease cases included in this study were positively correlated and statistically significant. When considering that the ticks

submitted by individuals may have been acquired in their town of residence, the tick population appears to correspond with the number of cases of Lyme disease in each town. [2] This finding was supported by the variations observed in the number of passively submitted ticks and the number of reported Lyme disease cases in the current study. This finding was also likely attributed to the fact that this study examined this relationship in all of Connecticut's 169 towns. According to a study conducted by Pepin et al[3], the density of ticks within a region can explain a statistically significant amount of variation in the human incidence of Lyme disease. The current study does suggest that the number of infected ticks submitted was statistically significant and positively correlated to the number of reported Lyme disease cases, from 2002 through 2012, in Connecticut. Rand et al[16] also supports this finding within their study, which indicated that there is a close association between the number of *I. scapularis* nymphs submitted and the reported Lyme disease cases not only within Maine, but also within New England. [16] Johnson et al[11] also reported that the number of ticks submitted each year and the number of positive ticks submitted were correlated with the number of Lyme disease cases over time.

Pepin et al[3] reported supportive evidence for their hypothesis that the density of *I. scapularis* translates to the incidence of Lyme disease; however, this finding was not supported in the current study. This study has shown that the density of *I. scapularis* ticks (in reference to the number of passively submitted ticks) does not have a statistically significant correlation to the incidence of reported Lyme disease cases in Connecticut, as concluded by Pepin et al[3]. As investigated within the current study, the number of ticks submitted passively was comprised of data on all of the submitted *I.*

scapularis ticks from 2002 through 2012 on a town-by-town basis. These data included adult, nymph and larval ticks that were submitted passively from residents of Connecticut on a random basis. The present study suggests that there is a negative relationship between these two variables, which could imply that the number of ticks submitted has an inverse relationship upon the incidence of Lyme disease, suggestive of a protective effect. The potential protective affect of passive tick surveillance may be explained by that fact that a person bitten by a tick they submitted for *B. burgdorferi* testing, which was reported to them as being infected, may request treatment for Lyme disease before they exhibit any symptoms of the disease. The examination of medications used for Lyme disease treatment may shed more light on this potential phenomenon.

Pepin et al[3] further implied that when their analysis was restricted to the low and high incidence areas studied, they found a relationship between the density of ticks and the incidence of Lyme disease. This result was comparable to the evidence described within this study for passively collected ticks. This finding was supported in the current study through evaluating the number of ticks submitted from towns that have high, low or rarely reported incidence of Lyme disease. Specifically, this study found a statistical significance and positive correlation between the number of ticks passively submitted among towns with high and rarely reported cases of Lyme disease. The towns that reported a low number of Lyme disease cases had a positive correlation, but the correlation was not statistically significant. This variation may be due to the current studies sample size (n=169 towns) in comparison to identifying the exact location where the tick was acquired in comparison to Pepin et al[3] (n=2,411). There is also the potential

that ticks were acquired in non-residential areas. Another caveat that should be noted when interpreting these results are geographical differences in Lyme disease control efforts at the residential or individual level that could contribute to the observed variation. Pepin et al[3] conducted a sub-analysis on states with a high incidence of Lyme disease and determined that Connecticut does not have a statistically significant relationship between the density of infected ticks and the incidence of Lyme disease. Within their study it was determined that the lack of correlation was due to the small number of counties in Connecticut (n=8) in comparison to states with a large number of counties. For the current study this may not be the case. One factor which may be influencing this study, that is difficult to account for, is the potential that people residing in highly endemic areas may automatically assume that ticks are infected or positive for *B. burgdorferi* and as a result they may seek treatment immediately without testing the tick. Another factor that is unaccountable in this study is the individual's knowledge of tick testing services offered in Connecticut and to what degree the advertisement reaches the publics view for these services within all Connecticut towns.

The tick infection prevalence of *B. burgdorferi* in actively collected ticks was associated with the Lyme disease incidence on a local level according to Stafford et al. [2] This finding was supported in the current study when evaluating the tick infection prevalence of *B. burgdorferi* in passively collected ticks and the number of reported Lyme disease cases. Not only was this finding positively correlated, it was also statistically significant among towns with high and rarely reported Lyme disease cases. Pepin et al [3] also found that the densities of infected *I. scapularis* nymphs were positively correlated to the Lyme disease incidence on a regional scale. Within the current study it was found that

the number of infected ticks and the number of Lyme disease cases had a positive and statistically significant association not only in the same surveillance year but in the following year as well. However, the overall tick infection prevalence of *B. burgdorferi* within passively submitted ticks did not positively correlate to the number of reported Lyme disease cases in the current study. This was further investigated on three levels by evaluating the tick infection prevalence in towns with a high, low and rare tick infection prevalence rate of *B. burgdorferi,* all of which had a negative correlation. Only towns with <22 per 1000 passively submitted ticks were statistically significant. Influence by the public's participation in passive tick surveillance in Connecticut is a likely contributor to these results. Generally speaking, an individual who sent in a tick was familiar with the availability of tick testing, whether it was from an individual or a physician, but this does not mean that all patients shared the same knowledge of such programs. It is also possible that patients that had a tick attached to their bodies had a physician remove the tick but the physician did not submit the tick for testing, instead they had the individual tested and treated for Lyme disease. These practices likely contributed to a tick infection prevalence that was not entirely representative of the true tick population.

Over time, the current study evaluated the number of ticks passively submitted annually along with the tick infection prevalence of *B. burgdorferi* and the number of infected ticks submitted. All three variables were found to be positively correlated and statistically significant when evaluating relationships in the same surveillance year and the following year. The tick infection prevalence of *B. burgdorferi* in submitted ticks was related to the incidence of new cases of Lyme disease on an annual basis, which had consistently positive and statistically significant correlations in the same surveillance year

as well as the following surveillance year (Table 3). The findings presented within the current study suggest that the number of infected ticks submitted in one year can be used to estimate the number of Lyme disease cases in the same year and following surveillance year. Although this study did not evaluate the density of actively collected ticks, this study did examine the density of passively collected ticks, which were submitted on a random basis. Based upon the research conducted, this study is one of the few studies to examine the relationship between the number of passively submitted ticks and the Lyme disease cases on a temporal level. Temporal correlations were statistically more significant in comparison to the spatial predictions examined.

The fact that the correlations presented within this study were not strong is surprising given that studies conducted by Pepin et al [3] and Stafford et al [2] had correlations that were much more robust when evaluating tick abundance and Lyme disease cases. In contrast, the Rand et al [16] study did yield more modest correlations. The current study addressed the number of ticks submitted passively, from 2002 through 2012, and the number of reported Lyme disease cases rather than the nymph density of a 12-town area, as in the Stafford et al [2] study. or within 2,411 counties, as evaluated by Pepin et al. [3] One would expect any association among the 169 Connecticut towns to be stronger than reported but it is likely that there was a lot of "noise" in the data. For one, the tick submissions were measured by counting the number of ticks that an individual found upon their body and submitted them with the town of residence when the tick may have been acquired from another location outside the town of residence. Even if the individual could recount the exact location the tick was acquired, this individual may not have correctly filled out the tick submission questionnaire to provide accurate information. In addition to

this, not all of the ticks that were submitted to the CAES were tested within the years evaluated. For example, if the tick was not partially or fully engorged with blood then the tick was not tested, thus eliminating ticks that could have been evaluated in this analysis. Additionally, not every individual with Lyme disease can recount if they were ever bitten by a tick and if they are able to it is unknown if they would have submitted the tick for testing to the CVMDL or the CAES. [8] As stated previously in regards to the use of the town of residence, the cases reported to the Connecticut DPH are reported based on the town of residence, but it is highly possible that the acquisition of a tick occurred outside the town of residence if a person recently travelled. [3] There is a possibility that there was some spatial and temporal bias in people submitting ticks and physicians reporting cases which were not accounted for in this study. Nevertheless, the reported correlations within this study were statistically significant for the years evaluated.

Another factor not considered in this study that may have affected the number of ticks tested and the results, was the method of testing for the presence of *B. burgdorferi*. At one point (before 2006), the ticks collected for this study were tested using a direct florescence antibody (FA) staining method instead of Polymerase Chain Reaction (PCR). The sensitivity and specificity of PCR is significantly greater than FA staining. In addition to this, only certain ticks can be tested using FA staining. For example, if the tick is too dehydrated or desiccated it cannot be tested. [6] Other factors influencing the number of ticks submitted that could not be directly considered in this study include the effect of cost and time on the number of ticks submitted to and tested by the CVMDL and the CAES. For example, the CVMDL acquires ticks from the public and the Connecticut Pathology Lab. These ticks are tested on a fee-for-service basis, allowing the CVMDL the ability to

provide a relatively rapid response regarding the infection status of the submitted tick. In contrast, the CAES is a free service provided by the state of Connecticut to the public, which results in a relative constant submission rate from the public, but at the indirect cost of time. Awareness of the programs available for tick testing may be relatively limited based upon public knowledge of such programs. This factor is not easily measured, but should be mentioned as a potential confounder.

Conclusion

The current study supports previous research that suggests that the number of passively submitted *I. scapularis* ticks may play an important role in predicting the emergence of new Lyme disease cases.[2,3,] Although this finding is typically supported by active tick surveillance, the use of passive tick surveillance, as shown by this study, provides practical information on the Lyme disease temporal and spatial distributions in Connecticut. Data collected on Lyme disease and tick vectors can be studied using cost-effective tools like passive tick surveillance for tracking the distribution of ticks and tick-borne diseases; in addition to this, passive tick surveillance can contribute to the surveillance of Lyme disease and help direct-targeted Lyme disease prevention programs. The goal of this study was to characterize the spatial and temporal relationships between the passive tick surveillance and the number of reported Lyme disease cases in Connecticut. The findings provided within this study deliver evidence supporting the hypothesis that passive tick surveillance has significant public health implications for assessing the risk of acquiring Lyme disease. This study has also shown that the passive tick surveillance data may be a useful and cost effective tool when used to predict the risk

of encountering an infected *I. scapularis* ticks by evaluating the temporal and spatial trends of *B. burgdorferi* infected *I. scapularis* ticks.

This study has addressed two main objectives; the first objective being to determine if the tick infection prevalence of *B. burgdorferi* in passively collected *I. scapularis* ticks has an association to the reported Lyme disease incidence and cases was met. The second objective of this study illustrated the use of passive tick surveillance as an effective measure of assessing Lyme disease risk. This objective was not fully met because this study reports that the temporal correlations examined were stronger than the spatial measures examined. Thus said, passive tick surveillance may not reliably predict Lyme disease risk in Connecticut towns during the 10 years evaluated when examined on a town by town basis. The current findings within this study were supported when evaluating the number of *B. burgdorferi* infected ticks with the number of Lyme disease cases in the same surveillance year and the following year. These results suggest that, the use of passive tick surveillance may be a cost effective tool for evaluating the temporal risk ticks pose to humans on an annual basis. This further suggests that people who live in towns actively engaging in a passive tick surveillance program may benefit from the data collected by evaluating whether they are at an increased risk of acquiring Lyme disease and by developing targeted prevention initiatives.

Connecticut has proven to be an ideal location for conducting this evaluation because of the specificity in identifying towns whose residents submitted ticks for testing contributed directly to Connecticut's passive tick surveillance program. This project has had the advantage of a large sampling pool that included not only reported Lyme disease cases but Connecticut acquired ticks as well. Despite the disadvantages of using passively

collected data, this project has statistically significant and positively correlated trends between the number of tick submissions and the number of Lyme disease cases on both a spatial and temporal scale. The use of data over a decade has also proven to be a major advantage, which supports the suggestions of others. [2,3,9,16] The data presented suggests that people who reside in a town with a high number of Lyme disease cases likely live within a town containing ticks with higher tick infection prevalence.

Analyzing the spatial and temporal trends seen between Connecticut's passive tick surveillance and Connecticut's Lyme disease surveillance data can be used to target Lyme disease prevention programs as well as tick reduction planning within Connecticut and potentially all of New England. During the course of this study, further knowledge was gained on the role of passive tick surveillance in identifying the tick infection prevalence of *B. burgdorferi* infected ticks and the impact this may have upon the spread of Lyme disease on both a temporal and spatial scale. Moving forward from this, passive tick surveillance can also be useful in evaluating the epidemiology and ecology of existing and emerging tick-borne diseases. More research should be conducted to evaluate the effectiveness of passive tick surveillance as a potential cost effective method for measuring the spread and risk of tick-borne diseases for future targeted prevention and other public health initiatives.

References

1. Adams DA, Gallagher KM, Jajosky RA, et al. Summary of notifiable diseases - united states, 2011. *MMWR Morb Mortal Wkly Rep*. 2013;60(53):1-117.

2. Stafford KC,3rd, Cartter ML, Magnarelli LA, Ertel SH, Mshar PA. Temporal correlations between tick abundance and prevalence of ticks infected with borrelia burgdorferi and increasing incidence of lyme disease. *J Clin Microbiol*. 1998;36(5):1240-1244.

3. Pepin KM, Eisen RJ, Mead PS, et al. Geographic variation in the relationship between human lyme disease incidence and density of infected host-seeking ixodes scapularis nymphs in the eastern united states. *Am J Trop Med Hyg*. 2012;86(6):1062-1071.

4. Diuk-Wasser MA, Hoen AG, Cislo P, et al. Human risk of infection with borrelia burgdorferi, the lyme disease agent, in eastern united states. *Am J Trop Med Hyg*. 2012;86(2):320-327.

5. Centers for Disease Control and Prevention. 2011 lyme disease (borellia burgdorferi) case definition. http://wwwn.cdc.gov/NNDSS/script/casedef.aspx?CondYrID=752&DatePub=1/1/2011%2012:00:00%20AM. Updated 2013. Accessed 10/6, 2013.

6. Gaumond G, Tyropolis A, Grodzicki S, Bushmich S. Comparison of direct fluorescent antibody staining and real-time polymerase chain reaction for the detection of borrelia burgdorferi in ixodes scapularis ticks. *J Vet Diagn Invest*. 2006;18(6):583-586.

7. Steere AC, Malawista SE, Snydman DR, et al. Lyme arthritis: An epidemic of oligoarticular arthritis in children and adults in three connecticut communities. *Arthritis Rheum*. 1977;20(1):7-17.

8. Bacon RM, Kugeler KJ, Mead PS, Centers for Disease Control and Prevention (CDC). Surveillance for lyme disease--united states, 1992-2006. *MMWR Surveill Summ*. 2008;57(10):1-9.

9. Stafford KC,3rd. *Tick managment handbook.* New Haven: Connecticut Agriculture Experiment Station; 2004.

10. Centers for Disease Control and Prevention. Reported cases of lyme disease by state or locality, 2003- 2012. http://www.cdc.gov/lyme/stats/chartstables/reportedcases_statelocality.html. Updated 2012. Accessed October 3, 2012.

11. Johnson JL, Ginsberg HS, Zhioua E, et al. Passive tick surveillance, dog seropositivity, and incidence of human lyme disease. *Vector Borne Zoonotic Dis*. 2004;4(2):137-142.

12. Ertel SH, Nelson RS, Cartter ML. Effect of surveillance method on reported characteristics of lyme disease, connecticut, 1996-2007. *Emerg Infect Dis*. 2012;18(2):242-247.

13. Connecticut Department of Public Health. Lyme disease cases and rate by town and county - 2011. . 2012.

14. Connecticut Department of Public Health. Lyme disease statistics for connecticut. http://www.ct.gov/dph/cwp/view.asp?a=3136&q=399694. Accessed 03/14, 2013.

15. Koffi JK, Leighton PA, Pelcat Y, et al. Passive surveillance for I. scapularis ticks: Enhanced analysis for early detection of emerging lyme disease risk. *J Med Entomol*. 2012;49(2):400-409.

16. Rand PW, Lacombe EH, Dearborn R, et al. Passive surveillance in maine, an area emergent for tick-borne diseases. *J Med Entomol*. 2007;44(6):1118-1129.

17. *The data analysis for this paper was generated using SAS software, version 9.3 of the SAS System for windows. copyright © 2002-2010 SAS Institute inc. SAS and all other SAS Institute inc. product or service names are registered trademarks or trademarks of SAS Institute inc., cary, NC, USA.. .*