DISSERTATION

EPIDEMIOLOGIC STUDIES OF HARD TICK-ASSOCIATED ILLNESS IN THE UNITED STATES

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY RENDI MURPHREE ENTITLED "EPIDEMIOLOGIC STUDIES OF HARD TICK-ASSOCIATED ILLNESS IN THE UNITED STATES" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

EPIDEMIOLOGIC STUDIES OF HARD TICK-ASSOCIATED ILLNESS

IN THE UNITED STATES

This dissertation describes three epidemiologic studies of hard tick-associated illness in the United States. The first is the prospective health assessment of Fort Campbell, Kentucky patrons bitten by ticks during 2004 – 2006. The study was designed to determine the frequency, clinical characteristics, and etiology of *Amblyomma americanum*-associated illness and to identify associated risk factors. *Amblyomma americanum* is an aggressive human biting tick associated with a Lyme disease-like illness of unknown etiology. Study findings suggested that a variety of symptoms were temporally associated with tick bite but data provided no clear evidence that symptoms were caused by an infectious process. Removing ticks by hand or being bitten on a limb may have been risk factors for illness. The second examines 248,074 cases of Lyme disease reported to the Centers for Disease Control during 1992–2006 using descriptive and inferential statistics. In the United States, Lyme disease is caused by *Borrelia burgdorferi* sensu stricto, a spirochete transmitted to humans by infected *Ixodes scapularis* and *I. pacificus* ticks. During the 15-year study period, the number of cases reported annually increased 101% and the majority of cases occurred in northeastern and north-central states. An increasing trend in the number of counties reporting at least one case annually was observed in Minnesota, Rhode Island, and Wisconsin. A disproportionate increasing trend in reported cases was observed in children and young males compared with other demographic groups. The third study is a pilot ecologic

analysis of human social or economic factors affecting, or resulting from, Lyme disease emergence. The objectives were to identify space-time clusters of increased Lyme disease risk and determine if risk could be partially explained using existing data on environment, socioeconomics, and healthcare. As expected, *Ixodes* tick distribution was a significant predictor of counties with increased risk. Measures of socioeconomic status surfaced as predictors of ecologic risk, and it appeared that persons of high SES lived where ticks were reported in northeastern states and persons of low SES lived where ticks were reported in the north-central states.

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CHAPTER 1

1. INTRODUCTION

The studies described in this dissertation were each designed to reduce gaps in existing knowledge about the epidemiology of the most important hard tick-associated illness in the United States, Lyme disease, and a clinically similar condition for which no etiology has been identified. The topics are related but the study design and methods employed are diverse, providing a rich lesson in epidemiologic concepts. Three studies are reported in four chapters. Each chapter contains an introduction, methods, results, discussion, tables, and figures section.

Chapter 2 describes the prospective health assessment of Fort Campbell, Kentucky patrons bitten by ticks. This study was designed to determine the frequency, clinical characteristics, and etiology of *Amblyomma americanum*-associated illness and we made few *a priori* assumptions about the scope of illness or possible etiologies. The value of this approach has increased over time because microbiologic studies of Lyme disease-like rash have shown almost no association with the once implicated etiologic agent, *Borrelia lonestari*. Our efforts were rewarded during the peer-review process when anonymous Military Medicine reviewers commented on the importance of the subject matter, offered

useful suggestions for improvement, and added that "The authors are to be congratulated on their work."

The second study on trends in Lyme disease cases reported 1992 – 2006 is presented in two chapters. A comprehensive review of Lyme disease cases reported in the United States was last published in 2000 when Orloski et al. analyzed data for 1992 – 1998. We added eight years of surveillance data, described trends in a similar manner for consistency, and presented new findings in Chapter 3. In Chapter 4 we extended our analyses of reported Lyme disease cases beyond that traditionally conducted by the Centers for Disease Control and Prevention by using generalized linear models and inferential statistics to confirm the observed trends reported in Chapter 3.

In Chapter 5 we describe a pilot ecologic analysis of human socioeconomic factors affecting, or resulting from, Lyme disease emergence. Two components of this study were particularly interesting to the primary author. First was the identification of space-time clusters of reported Lyme disease cases using scan statistic software called SaTScan™. Cluster analysis is a useful epidemiologic tool for public health practitioners and health departments can easily download and use SaTScan™. Second was realizing the payback on the immense effort spent searching for existing county-level measures potentially associated with increased Lyme disease risk. After spending more than a year acquiring and formatting data, we were pleased to see the benefits of using secondary data to generate hypotheses about the association between measures of county socioeconomics and reported cases of Lyme disease.

Finally in Chapter 6, we discuss how the findings of this research might reduce illness resulting from, or associated with, human exposure to hard ticks in the United States. References for the entire dissertation follow Chapter 6.

CHAPTER 2

2. PROSPECTIVE HEALTH ASSESSMENT OF FORT CAMPBELL, KY PATRONS BITTEN BY TICKS

A shortened version of this chapter has been accepted for publication in Military Medicine, the international journal of the Association of Military Surgeons of the United States. A proof of the manuscript is provided in Appendix A.

2.1. INTRODUCTION

Amblyomma americanum (the lone star tick) is an aggressive human biting tick distributed throughout the southeastern United States (U.S.) and along the eastern coast as far north as Maine (Figure 2.7.1) (1). This species is capable of transmitting numerous human bacterial pathogens including *Francisella tularensis*, *Ehrlichia chaffeensis* and *Rickettsia* spp (2) (Table 2.6.1). Although *A. americanum* does not transmit the agent of Lyme disease, *Borrelia burgdorferi* (3-6), it has been associated with a Lyme disease-like rash illness of unknown etiology (7-11). Inability to distinguish *A. americanum*-associated illness from Lyme disease hinders management of patients with suspected tick-borne illness, causes public confusion and misunderstanding of Lyme disease risk and complicates national surveillance efforts.

In 1996, a bacterium named *Borrelia lonestari* was suggested as a possible cause of rash illness following *A. americanum* bite (12). Since then, there has been little evidence in support of this hypothesis (13) except for one case report where *B. lonestari* DNA was found in the rash biopsy specimen and attached tick of a patient (9). Therefore, other explanations for reports of *Amblyomma*-associated illness, such as transmission of unknown microbes, hypersensitivity reaction to tick bite, or misdiagnosis of rash illness, should be explored.

With the support of the Centers for Disease Control and Prevention (CDC) and Colorado State University (CSU), colleagues at the U. S. Army Center for Health Promotion and Preventive Medicine (CHPPM) were approached with the idea of launching a prospective study to better define the frequency and public health significance of illness in persons bitten by *A. americanum* ticks. CHPPM has conducted a Human Tick Test Kit Program at U.S. Army and other military health clinics throughout the U.S. since 1999. In this program, ticks removed from active duty military, reservists, guardsmen, retirees, dependents and civilian employees are submitted to CHPPM where they are identified and tested for pathogens that may cause rash illness, including *B. lonestari*, *B. burgdorferi*, *E. chaffeensis* and *Rickettsia* spp. (14). However, the program did not capture information on other risk factors or health outcomes for participants bitten by ticks. Closing this gap became the basis for the collaborative epidemiologic study TickPro described here.

TickPro was a prospective study of adult tick-bite victims attending the Fort Campbell, Kentucky Environmental Health Clinic (EHC). Briefly, consenting adults completed an enrollment survey providing information about demographics, tick exposure and general health. Their tick was tested under the existing CHPPM Human Tick Test Kit Program. Volunteers were contacted 30-45 days following enrollment to obtain information on acute illness and clinical management. Results from CHPPM tick identification and pathogen testing were linked to epidemiologic data to determine the burden, characteristics and correlates of *Amblyomma*-associated illness.

TickPro was a collaboration between: (1) CDC (Rendi Bacon, Principal Investigator (PI)) who led study design, site coordination and logistics, training, data collection and analyses, writing of summaries and publications and obtained approval for human subjects research from CDC, US Army and CSU, (2) CHPPM (Ellen Stromdahl, Co-PI) who contributed to study design and site coordination and logistics, provided tick identification and conducted tick pathogen testing, and (3) Fort Campbell EHC (Nita Campbell, Collaborator) who was responsible for participant recruitment and enrollment, including informed consent, and tick collection and submission.

2.1.1. Planned Potential Use of Study Findings

Information gained from this study may help CDC and the public health community: (1) design targeted strategies to prevent *Amblyomma*-associated illness, (2) tailor education programs to at-risk populations, (3) provide physicians with a more detailed description of *A. americanum*-associated illness, (4) provide information for clinical treatment guidelines and (5) improve the specificity of tick-borne disease surveillance systems.

2.1.2. Objectives

Our primary objectives were to: (1) determine the frequency and clinical characteristics of *A. americanum*-associated illness, (2) determine if the occurrence of *A. americanum*-associated illness is related to tick characteristics, host traits, or other risk factors and (3) evaluate the risk of *A. americanum*-associated illness following the bite of ticks containing *B. lonestari*, *B. burgdorferi*, *E. chaffeensis*, or *Rickettsia* spp.

2.2. LITERATURE REVIEW

2.2.1. North American Hard Tick Vectors of Human Bacterial Illness

Worldwide there are 13 known genera of the Ixodidae family ticks (15). Those known to serve as vectors of human bacterial illness in North America are *Ixodes*, *Amblyomma* and *Dermacenter* (15-18). Ixodidae are often called hard ticks because they possess a shell-like dorsal plate in all life stages (larvae, nymph and adult). Hard ticks are similar in appearance throughout their stages, except that larvae have only six legs (compared to eight for nymphs and adults) and the adult males and females look different (Figure 2.7.2). Their mouthparts (capitulum) are attached to their anterior and visible from the dorsal view (Figure 2.7.2).

Hard ticks are ectoparasites and survive solely on the blood of vertebrate hosts (15). Blood meals are their only source of nutrients and are required to initiate development through life stages, including reproduction (15-17). Some hard ticks complete their development by feeding on one or two hosts, but 90% have a three-host cycle. Eggs hatch into larva that feed and become nymphs by casting off their outer shell and growing

a new larger one (molt). Nymphs feed and molt into sexually dimorphic adults. Males may take in several small blood meals or none at all while searching for suitable mates. After mating, females require a blood meal for the production of eggs. Due to cold temperatures in winter months, most North American hard tick experience reduced metabolism, physical activity and feeding causing a delay in their developmental or reproductive cycle. Therefore a life cycle generally takes two years to complete.

Hard ticks live in brushy, wooded, or weedy areas that contain ample vertebrate hosts and provide protection from adverse weather conditions (high temperature, wind and low humidity) that could lead to fatal desiccation (15-18). Transitional areas, like the interface between forest and lawn, are also suitable habitats for hard ticks. Tick density in these areas, however, is often unevenly distributed since survival depends on proper microclimate and host availability.

When seeking a blood meal, hard ticks linger on vegetation with their barbed legs outstretched allowing them to clutch a suitable host as they pass by (quest) (16-18). Immature ticks frequently rest on low lying vegetations where smaller vertebrates forage. Adults may ascend a meter or more on brushy vegetation to wait for larger vertebrates. Once onboard a host, they search for a suitable feed location, cut a small hole in the host epidermis with their outer mouth parts (chelicerae) and insert the inner mouth parts (hypostome) into their host. Over time, many ticks will produce a cement cone around their capitulum and host skin making tick removal difficult. Secure attachment and anticoagulants released by tick salivary glands permit slow continuous uptake of blood

over one to several days. During this feeding period ticks may take in pathogens from a vertebrate host, or pass bacteria to a vertebrate host. After feeding is complete, the tick drops off the host, molts to the next life stage and goes on to complete its life cycle.

The cycle of tick-borne bacterial transmission requires infected vertebrate hosts, ticks capable of acquiring and transmitting the pathogen among hosts and susceptible vertebrate hosts (17-19). In North America, the hard tick species known to vector medically important agents of human bacterial illness are *I. scapularis* and *I. pacificus* (transmit the agents of Lyme disease, anaplasmosis and babesiosis), *A. americanum* (transmits the agent of human monocytic ehrlichiosis and tularemia), and *D. variabilis* and *D. andersoni* (transmit the agents of Rocky Mountain spotted fever and tularemia) (16, 19). Features unique to these tick species and brief descriptions of the diseases, agents and transmission cycles are presented in the following sections.

Avoiding tick bite is the best protection from tick-borne infections. Other preventive measures aimed at reducing exposure to ticks include using repellent, wearing long sleeve shirts, tucking pants into socks and checking daily for ticks (20). Tick abundance can be reduced around private home and in recreational areas by removing brush and leaf litter, creating a buffer zone of wood chips or gravel between forests and lawn, applying acaracides, or excluding hosts. Tick-borne illness can be mitigated by prompt and proper tick removal and by recognizing and seeking treatment for early signs of illness (11, 20, 21). Antibiotic prophylaxis following tick bite has shown efficacy in reducing the risk of

Lyme disease in certain circumstances (22), although widespread use of antibiotic therapy following tick bite is not recommended (11).

2.2.1.1. *Ixodes* species

Ixodes scapularis and *I. pacificus* firmly attach to vertebrates, including humans, as larvae, nymphs, or adults (17). Both ticks are known to transmit the agent of Lyme disease, *Borrelia burgdorferi*, to humans (Table 2.6.1) but their geographic range and transmission cycles are quite different (16, 23). In contrast to other hard ticks, they lack eyes and chain-like marking on the posterior dorsal plate (festoons) and adults have no white markings on their back (Figure 2.7.1).

Ixodes scapularis is commonly known as the blacklegged tick or deer tick (15-17). It is endemic in parts of northeast, upper mid-west and southern United States (Figure 2.7.1). Adults are most active in fall, winter and spring and prefer to feed on white-tailed deer, *Odocoileus virginianus*, but will bite humans. Sub-adults are most active in spring and summer when they feed on rodents, other small mammals, birds and humans when encountered. However, nymphs in the south frequently feed on lizards and rarely bite humans. These two factors contribute to the rare occurrence of Lyme disease in southern states (19, 24).

Ixodes pacificus, the western blacklegged tick, is found along the pacific coastal margins of the U.S. and in a few areas in Arizona, Nevada and Utah (Figure 2.7.1) (15-17). Adults are active from fall to late spring when they feed on mule deer, *Odocoileus hemionus*. Sub-adults are active in spring and summer and prefer small mammals, lizards

and birds. As with *I. scapularis* in southern states, feeding patterns of *I. pacificus* ticks contribute to the rare occurrence of Lyme disease in western states (25).

2.2.1.2. *Amblyomma americanum*

Amblyomma americanum aggressively bite humans as larvae, nymphs, or adults. The agents of tularemia and human monocytic ehrlichiosis are the most frequently reported bacteria transmitted to humans during the bite of *A. americanum* (Table 2.6.1), but rare transmission of *Ehrlichia ewingii* and potential transmission of *Rickettsia* spp. and *Coxiella burnetii* to humans has also been reported (2, 26-28). The adult female has single white spot on her back giving rise to the common name for this species, the lone star tick (Figure 2.7.2).

Amblyomma americanum is found in the southeastern United States and along east coastal areas as far north as Maine (Figure 2.7.1) and are known as the most aggressive human-biting ticks. Adults and nymphs are active from early spring through summer and larvae from late summer through early fall (15-18). They prefer feeding on a variety of mid-sized vertebrates, but prefer deer. They bite rodents less frequently than *Ixodes* spp. ticks and, therefore, are less likely to vector rodent-associated pathogens.

2.2.1.3. *Dermacenter* species

Dermacenter variabilis and *D. andersoni* ticks attach lightly to humans only in the adult stage (15-18). Both ticks are known to transmit the agents of Rocky Mountain spotted fever and tularemia (Table 2.6.1). Like *Ixodes* and *Amblyomma* spp. they encounter hosts passively. However they may travel great distances in response to carbon dioxide and are often abundant along roadways or trails.

Dermacenter variabilis, the American dog tick, is found in most areas east of the Rocky Mountains and along the pacific west coast (16, 23). Larvae are active early spring through mid summer, nymphs from midsummer to early fall and adults late spring through early fall (15-18). Sub-adults prefer rodents but will feed on other small mammals. Adults favor feeding on dogs but will readily bite humans when encountered. If unfed at any life stage, dog ticks may live from 15 – 30 months or longer.

Dermacenter andersoni, the Rocky Mountain wood tick, is found in and around the Rocky Mountains (16, 23). Climate conditions in their geographic range restrict activity of larvae, nymphs and adults to late spring and summer months and shorten the life span of unfed ticks. Sub-adults prefer to feed on small mammals (chipmunks and squirrels) and adults feed on large mammals (cattle, sheep, deer) and humans.

2.2.2. Bacterial Agents Vectored by North American Hard Ticks

2.2.2.1. Agents Transmitted by *Ixodes* species

Lyme disease occurs when humans are infected with *B. burgdorferi* from the bite of an *I. scapularis or I. pacificus* tick (Table 2.6.1) (24, 29, 30). Lyme disease is geographically focused in regions that support a natural zoonotic cycle for transmission of the bacterial spirochete *B. burgdorferi*. Mice, squirrels and other small vertebrates serve as natural reservoirs for *B. burgdorferi*. *Ixodes* spp. ticks become infected while feeding on the blood of reservoir hosts. The rate of *B. burgdorferi* infection is generally higher in *I. scapularis* (~25%) than in *I. pacificus* nymphs (~2%) (31). Infected ticks transmit *B. burgdorferi* to the blood or skin of incidental hosts, like humans, after feeding for at least

24 hours (32). This transmission cycle is well established in the ten states that account for 90% of all Lyme disease cases reported nationally (Connecticut, Delaware, Maryland, Massachusetts, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island, Wisconsin) and along the Pacific Coast in northern California and southern Oregon (33).

Lyme disease is a systemic illness resulting in mild to life-threatening dermatologic, rheumatologic, neurologic and/or cardiac abnormalities (11, 29, 30). In nearly 80% of cases, a red, expanding rash with central clearing (erythema migrans or EM) is observed within 30 days of exposure to *B. burgdorferi*. This rash may be accompanied by other acute symptoms, particularly fatigue, fever, headache, mild stiff neck, arthralgia or myalgia. Early disseminated infection infrequently causes neurologic symptoms (meningitis, radiculopathy, facial palsy) and cardiac abnormalities (atrioventricular heart block, carditis). If infection persists, patients may continue to experience neurologic and cardiac symptoms and may also experience persistent or intermittent arthritis in large bone joints.

The diagnosis of Lyme disease is primarily based on a patient's clinical manifestations and a history of exposure to vector ticks in a Lyme disease endemic area (11, 30, 34). No laboratory tests are recommended for patients with a characteristic EM and exposure to ticks where Lyme disease is common. For those with later stages of infection, a two-tiered serologic test has been recommended by the Association of Public Health Laboratories and the CDC since 1995 (35). In this approach, serum is first tested by an enzyme-linked immunosorbent assay (ELISA) or immunofluorescent assay (IFA).

Positive or equivocal samples are evaluated by a second test, a standardized immunoblot. Serologic testing is not recommended for persons lacking exposure to ticks in an area where Lyme disease is endemic (34).

When recognized in the early stages of infection, Lyme disease can be treated with an oral course of antibiotics (doxycycline, amoxicillin, or cefuroxime axetil) (11, 30). Persons with persistent symptoms may require a second round of oral antibiotics. Those with neurologic or cardiac symptoms may require intravenous antibiotic treatment with ceftriaxone or penicillin. A single dose of doxycycline is recommended for prophylaxis of Lyme disease in persons older than seven who have been bitten by a nymph or adult blacklegged tick in an area where at least 20% of ticks are thought to be infected with *B. burgdorferi* (11). The tick must have been attached for more than 36 hours and prophylaxis taken within 72 hours of tick removal.

National surveillance for Lyme disease began in 1991. For surveillance purposes, the Council for State and Territorial Epidemiologist and CDC define a reportable case of Lyme disease as 1) physician-diagnosed EM equal to or great than 5 cm across its largest diameter in persons with known exposure to tick habitat in a county where Lyme disease is endemic, 2) physician-diagnosed EM with laboratory evidence of *B. burgdorferi* infection in persons without known exposure, or 3) at least one late manifestation (musculoskeletal, nervous, or cardiac system involvement) with laboratory evidence of *B. burgdorferi* infection (36). Since national surveillance began, the number of Lyme disease cases reported to the CDC has gradually risen from 9,470 cases reported in 1991

(37) to 19,931 cases in 2006 (38). More than 90% of all Lyme disease cases are reported from ten states (Connecticut, Delaware, Maryland, Massachusetts, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island and Wisconsin). Males account for a slight majority of cases, greater than 95% are white, and age follows a bimodal distribution with peaks in children aged 5 – 14 years and adults aged 45 – 54 years. The onset of illness is most frequently reported as May, June, July, or August when ticks are most actively seeking a blood meal.

Anaplasma phagocytophilum is another bacterial pathogen vectored by *I. scapularis* and *I. pacificus* ticks (39-41). First described in humans in 1994 (42), it belongs to the Anaplasmataceae family of obligate intracellular pathogens which also includes the *Ehrlichia* spp vectored by *A. americanum* ticks described below and is found in less than 10% of ticks tested (43, 44). Ticks become infected with *A. phagocytophilum* after feeding on reservoir rodents, deer, or elk.

A. phagocytophilum primarily infects granulocytes (i.e., neutrophils) and rarely eosinophils (39-41). Illness is characterized by acute and usually self-limiting fever, headache, malaise and muscle aches. Thrombocytopenia, leucopenia and increased hepatic transaminases are also characteristic. Nausea, vomiting, diarrhea, cough, joint pains and confusion are reported less frequently. Rash and meningoencephalitis are rare. There is no standardized case definition or diagnostic test recommended, however, examination of peripheral blood smear, results of complete blood cell count and comprehensive metabolic panel might be useful. Infection clears after a few days of

doxycycline or some other tetracycline-class antibiotic but treatment for 10 – 14 days is recommended to provide therapy for possible co-infection with *B. burgdorferi*. From 1997 – 2001 654 confirmed cases were reported with the highest incidence reported by Connecticut, Minnesota, New York and Rhode Island (45).

2.2.2.2. Agents Transmitted by *Amblyomma americanum*

Known causes of illness in persons bitten by *A. americanum* include *Francisella tularensis*, *E. chaffeensis*, *E. ewingii* and *Rickettsia* spp. (2). *Francisella tularensis* is a gram negative nonmotile coccobacillus that is easily aerosolized, has a low infectious dose and can survive inside host macrophages. Tick infection prevalence is less than 1% (2) but the bacterium is usually passed among ticks and more than 100 species of wild mammals and other vertebrates including humans (46-49). Voles, beaver, muskrats, mice, hares and rabbits are the most important reservoirs for transmission cycle maintenance. Human exposure also occurs through deer fly bite, ingestion or contact with contaminated water, food, or soil and inhalation. Human to human transmission does not occur.

Tularemia is characterized by sudden onset of flu-like symptoms including fever, chills, headache, malaise, sore throat and cough (46-49) . Skin ulcers at the initial infection site with regional glandular swelling are characteristic of tick or fly bites. If ingested, pharyngitis abdominal pain, vomiting and diarrhea are typical. Inhalation of 10 or more aerosolized bacteria may lead to infection causing primary pneumonia. In all cases, severe disease may result in shortness of breath, sepsis leading to shock or secondary pneumonia. Clinical diagnosis can be supported by a rise in serum antibody titer,

detection of DNA, or by culture. Infection with *F. tularensis* is effectively eliminated with streptomycin, gentamicin, or ciprofloxacin, although streptomycin is preferred and shown effective in 97% of patients. In the U.S. from 1990 – 2000, 1,368 cases of tularemia were reported (50). Most of these cases occurred in mid-central states of Arkansas, Missouri, Oklahoma and Kansas; South Dakota and Montana; and Massachusetts. Seventy percent of the cases are reported in May through August.

Disease from infection by *Ehrlichia* spp was recognized in humans in 1986 (51). *E. chaffeensis* causes acute febrile illness accompanied by headache, myalgia, rigors and/or malaise (52). Macular, papular or petechiae rash is common among children (53), but infrequent among adults. The prevalence of *E. chaffeensis* in *A. americanum* ranges from 1 to 15% depending on the tick sampling and testing methods and location (2). Infection by *Ehrlichia ewingii* causes an ehrlichiosis that is clinically similar to HME. Most patients, however, have preexisting immunosuppressive medical conditions and infrequently report rash. *E. ewingii* has been found in approximately 5% of *A. americanum* ticks tested (2).

A spotted fever group bacterium, *Rickettsia parkeri* has been shown to cause febrile illness accompanied by multiple eschars and maculopapular eruption in one adult (54). *R. parkeri* has long been associated with *Amblyomma maculatum* ticks (55, 56) and was experimentally introduced to lab reared *A. americanum* (57). Another spotted fever group *Rickettsia* of unknown pathogenicity, *Rickettsia amblyommii*, has high infection prevalence among *A. americanum* ticks (58-60) and was found in the tick of one patient

who developed a 5 inch macular rash at the bite site three days after tick removal (26). Researchers speculate that low virulent *Rickettsia spp.* will likely explain more cases of mild illness in persons with *Amblyomma* tick bite (40).

Although isolated from *A. americanum* ticks in Texas and Mississippi and therefore potentially vectored to humans, transmission of *C. burnetii* is thought to be confined to transmission among non-human mammals and other wildlife vertebrates (2).

2.2.2.3. Disease Agents Transmitted by *Dermacenter* species

Dermacenter variabilis and *D. andersoni* are the primary vectors of *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever (RMSF) (18, 40, 61-63). *Rickettsia rickettsii* are gram-negative coccobacillus and obligate intracellular bacteria. Ticks acquire the bacteria when feeding on small mammals such squirrels, voles, chipmunks and snowshoe hares which serve as natural reservoirs.

RMSF is characterized by rapid onset of fever with the followed by the development of a maculopapular rash that erupts on the extremities within a few days and gradually expands to cover the entire body (40, 62, 63). Other acute symptoms include chills, headache, deep muscle pain and gastrointestinal symptoms in children. Serologic tests are unreliable in acute infections, however, immunoassays, molecular tests, or culture may eventually provide laboratory confirmation of clinical diagnoses. Therefore, the classic triad of RMSF, fever, rash and history of tick bite, are enough reason to initiate antibiotic therapy since nearly all infections can be eliminated with 5 – 10 days of treatment with tetracyclines (e.g., doxycycline and chloramphenicol). During 1997 –

2002 the average annual incidence was 2.2 per million population with 56% of cases reported from North Carolina, South Carolina, Tennessee, Oklahoma and Arkansas (64), although cases have been reported from 46 of the 48 contiguous states.

D. variabilis and *D. andersoni* are also known to transmit *Francisella tularensis* to humans causing tularemia (46-49). Human tularemia disease was described in detail in a previous section.

2.2.3. *Amblyomma americanum*-associated Lyme disease-like Illness of Unknown Etiology

Each year, several hundred cases of Lyme disease are reported in areas that do not support the known zoonotic cycle for *B. burgdorferi*. Retrospective studies have shown that these cases are associated with the bite of *A. americanum*, rather than *Ixodes spp.*, ticks (7, 8, 10). In these analyses, evidence of exposure to *B. burgdorferi* could not be confirmed by culture of rash biopsy specimens or by serologic testing of patient serum samples, leaving the authors to conclude that illness resulted from an *A. americanum*-associated etiology. Additional research has shown that *A. americanum* are not competent vectors for transmitting *B. burgdorferi* to laboratory mice (3-6), adding strength to the argument that Lyme disease is not the explanation for *A. americanum*-associated illness. The illness or syndrome has also been called Lyme disease-like illness due to its clinical similarity with acute Lyme disease, southern tick associated rash illness (STARI) due to its association with *A. americanum* the most aggressive human biting tick in the southern U.S., or Master's disease after the physician, Edwin Masters, who has described Lyme disease-like illness in his Missouri patients since the late 1980s (65). To

be precise, *A. americanum*-associated Lyme disease-like illness (AALDI) will be used from this point forward.

2.2.3.1. Etiology and Transmission of AALDI

In 1996, a spirochete (named *B. lonestari species novum*) was reported in *A. americanum,* providing a possible etiology of AALDI in regions where true Lyme disease is unlikely (66). Using polymerase chain reaction (PCR), the presence of *B. lonestari* DNA has been reported in: (1) 1-12% of *A. americanum* ticks from Alabama, Arkansas, Delaware, Georgia, Kansas, Kentucky, Maryland, Missouri, New Jersey, New York, North Carolina, South Carolina, Tennessee, Texas and Virginia (44, 59, 67-73), (2) blood samples from white-tailed deer, *O. virginianus*, in Arkansas, Georgia, North Carolina and South Carolina (74), and (3) the skin biopsy sample and attached *A. americanum* from one human patient with possible exposure in Maryland or North Carolina (9).

Understanding the natural history and transmission cycle for *B. lonestari* in ticks has been complicated by the difficulty in detecting this organism and growing it in laboratory settings. Because white-tailed deer are the preferred host of *A. americanum*, a few studies have examined the role of deer in supporting a *B. lonestari* transmission cycle. Moore and colleagues tested blood collected from deer in eight states and found evidence of *B. lonestari* DNA in seven of 80 deer tested (8.7%). The positive animals were from South Carolina, Arkansas, North Carolina and Georgia (74). Later, Varela-Stokes documented transmission of *B. lonestari* from wild-caught *A. americanum* ticks to three captive reared white-tailed deer. Following controlled exposure to ticks, *B. lonestari*

DNA was detected in the blood of all three deer and one of the deer seroconverted (75). Experimental infection of white-tailed deer following needle inoculation was shown by Moyer and colleagues, however, mice, calves and dogs were not susceptible to infection (76). These studies are cohesive in their conclusion that white-tailed deer may serve as reservoir hosts in a natural cycle of *B. lonestari* transmission by *A. americanum*.

Reports of AALDI associated with other etiologies vectored by lone star ticks are rare. A recent report by Billeter and colleagues described a woman from North Carolina who developed a 5-inch macular rash that appeared at the site of a tick bite (26). The implicated partially engorged *A. americanum* female tick had been removed three days before rash onset and saved. The tick contained *Rickettsia amblyommii* DNA and tested negative for *Borrelia*, *Anaplasma*, *Ehrlichia*, *Babesia* and *Bartonella* DNA. Unfortunately, no human specimens were obtained to determine if infection with *R. amblyommii* could be documented in the patient.

Most case reports and studies of persons with AALDI describe failure to detect the DNA of known pathogens in human samples and/or a lack of serologic response to known pathogens, including *B. lonestari* (7-10, 13, 77, 78). These studies are described in more detail in the next section.

2.2.3.2. AALDI Symptoms, Diagnosis and Treatment

CDC conducted a retrospective case-control study of 45 Missouri patients with onset of erythema migrans in 1990 – 1991 and 45 controls matched by residential area (7). The median age of case-patients was 37 years (range 3 – 84 years), 58% were male and 98%

were white. The mean age of controls was 40 years (range 4 – 85 years), 33% were male and 93% were white. Among those with available data, the median maximal rash diameter was 10 cm (range, 1 – 38). Redness and itching were reported more often than burning or pain. Seventeen patients (38%) reported anatomical site of tick bite as the torso (trunk, groin, or axilla), ten (22%) were bitten on the hip or upper thigh and 18 (40%) were bitten on the extremities. Fatigue was the most commonly reported secondary symptom followed by headache, stiff neck, myalgia and arthralgia. Twenty-nine percent reported fever. Twenty percent or less reported photophobia, sore throat, chills, cough, dizziness, vomiting and diarrhea. Thirteen patients reported no symptoms. The authors examined serum from 22 patients and, although diagnostic criteria for *B. burgdorferi* infection were not met, greater than 80% had serum antibodies that reacted with the *B. burgdorferi* flagellin B antigen. This finding strengthened speculation that AALDI was likely caused by infection with a *Borrelia* spp. even though flagellin B antigen is found in many motile bacteria (e.g., *Helicobacter*, *Bacillus*, *Clostridium*, *Salmonella*, *Campylobacter*, *Pseudomonas* and *Leptospira* spp.). Attempts to culture borreliae from 25 rash biopsy specimens were negative. There was no serologic evidence of acute infection with *F. tularensis*, *Rickettsia typhi*, or any virus tested (mosquito-borne agents of western equine encephalomyelitis, St. Louis encephalitis, La Crosse, and Cache Valley and tick-borne agents of Powassan and Colorado tick fever). One patient had stable reactive antibody to *E. chaffeensis* and *R. rickettsii*. The authors concluded this was likely due to previous exposure to these or related agents. One patient seroconverted to *C. burnetii* concurrent with development of rash. He reported no symptoms other than rash and responded well to treatment with azithromycin. Patients were treated with a

variety of oral antimicrobials (ampicillin, doxycycline and macrolides) from 10 – 30 days. The authors had knowledge of only two case patients whose symptoms persisted one year after initial treatment (one with arthralgia and one with fatigue).

A prospective study of persons with EM or EM-like rash among residents and staff of an outdoor camp for females in North Carolina during the summers of 1994 and 1995 was conducted and reported by Kirkland and colleagues (10). Fourteen patients were evaluated. Thirteen were female. Median age was 15 years (range, 13 – 49 years). Patient rashes ranged in size from 3 x 5 cm to 6 x 7 cm. Several were reportedly tender or pruritic. Lesions were located on the extremities of 11 case patients. The rest were found on the torso. Ten patients reported mild systemic illness (10 headache, 8 musculoskeletal pain, 7 fatigue and 6 nausea). Only one patient had mild fever at the time of examination. Rash biopsy specimens were culture negative in Barbour-Stoenner-Kelly II (BSK II) medium. No serologic evidence of *B. burgdorferi* infection was found. Of those tested, none had serologic evidence of *R. rickettsii* infection. Serology for one case patient indicated a prior infection with *E. chaffeensis*. All patients received a 10-day course of doxycycline and all recovered.

In 1998, Masters and colleagues described seventeen patients with erythema migrans following known *A. americanum* tick exposure who presented to his clinic in Cape Girardeau, Missouri from May, 1990 to September 1993 (79). In these patients, the median rash diameter was 7.5 cm. No rashes were reportedly itchy or painful. Sixteen patients reported anatomical site of tick bite as the torso (back, abdomen, or groin) and

one was bitten on the leg. Five patients had mild flu-like symptoms which were not described in detail by the authors. Rash biopsy specimens were obtained for all patients and were culture negative using BSK II medium, the preferred medium for growth of *B. burgdorferi*. Eight of 17 had Lyme disease serology results that the investigators interpreted as "suggestive of a borreliosis". Testing for other unspecified etiologies was negative except for one patient who tested positive for *C. burnetii*, the agent of Q fever. All patients were treated with amoxicillin or doxycycline lasting at least 20 days and all had resolution of symptoms.

From June 1991 – June 1994, Felz and colleagues conducted a prospective evaluation of 23 patients from Georgia and South Carolina with physician diagnosed EM rash exceeding 5 cm and exposure to ticks in the preceding month (8). Their median age was 47 years (range 25 – 69 years), 14 patients (61%) were male and 22 (96%) were white. Lesions averaged 9.6 cm in maximal diameter (range, 2 – 20), 8 patients (35%) reported pruritis and one reported pain. Fifteen patients reported rash location on the torso (back, shoulder, abdomen and chest) and eight occurred on the leg. Six patients had flu-like symptoms (malaise, headache, fever, arthralgia, myalgia, sore neck, sore throat, or chills). Serologic data for these patients did not suggest infection with *B. burgdorferi*. Multiple tests for the presence of *B. burgdorferi* DNA (flagellin B and outer surface protein A genes) in rash biopsy specimens were conducted. The flagellin B gene was detected in five specimens but no specimens contained ospA. Only one patient had culture evidence of borreliae infection and this culture was later identified as a European strain, *Borrelia garinii*. The authors were unsure whether this patient acquired infection in the U.S. or

Europe. All 23 patients were treated with 21 days of doxycycline. Rash in all but one patient resolved within seven days of therapy initiation. Persistence of symptoms beyond 21 days was not known to occur in any patient.

In 2001, James and colleagues published a case report suggesting that *B. lonestari* may be associated with AALDI (9). The patient was a 74 year old black man who presented with one 11 x 19 cm EM rash and one 3 x 4 cm EM rash that had been present for about four days. An attached engorged *A. americanum* female was found within the margin of the largest rash and removed by the physician for laboratory analysis. The patient was a resident of Westchester County, New York but had been walking in grassy areas in Maryland and North Carolina in prior weeks. The patient also reported fatigue, cough and right shoulder discomfort lasting for about one week. Using *Borrelia* genus-wide primers for the flagellin B gene, *B. lonestari* DNA sequence was obtained in a skin biopsy from the leading edge of the largest rash and in the attached *A. americanum* tick. No evidence of *B. burgdorferi* infection was detected by culture, PCR, or serology. Patient serum did contain antibodies that reacted with *B. burgdorferi* flagellin B antigen. After a 14-day course of doxycycline, the patient returned to normal health.

Armstrong and colleagues took a different approach to learning more about AALDI. Between 1994 and 1996, he surveyed 74% of 335 people who lived on Gibson Island, Maryland (77). In this community 15% of residents reported a prior diagnosis of Lyme disease despite the fact that only 3% of 1,556 ticks submitted by residents were *I. scapularis* and only about 20% of questing deer ticks collected on the island were

infected with *B. burgdorferi*. In contrast 95% of ticks submitted by residents were *A. americanum* and 1- 2% of questing lone star ticks collected on the island were infected with *B. lonestari*. Among 37 residents with history of diagnosed Lyme disease, 65% reported rash, 35% muscle aches, 32% fever, 24% arthritis and 19% fatigue. Five percent or less reported joint swelling, severe headache, night sweats, facial paralysis, visual disturbances, stiff neck and lymphoedema. The authors reported that serum from residents reporting Lyme disease lacked evidence of recent infection with *B. burgdorferi*, *E. chaffeensis*, *A. phagocytophila*, *R. rickettsii*, *R. typhi*, *C. burnetii*, *F. tularensis* or *B. microti*. However, in a serosurvey of 167 seronegative individuals that were monitored during the study period, two seroconverted to *B. burgdorferi*. The authors concluded that residents of Gibson Island have an exaggerated perception of Lyme disease risk and they suggested that illness following *A. americanum* bite may bias Lyme disease surveillance in this population.

The CDC Division of Vector Borne Infectious Diseases has been collecting skin biopsy specimens from volunteers living outside of Lyme disease endemic areas but with EM or EM-like rash and recent tick bite or potential exposure to *A. americanum* since 1997 (Pilgard and Johnson personal communication). Rash biopsy specimens from 20 patients were obtained and tested by PCR. One specimen produced an equivocal result when tested for the presence of *B. lonestari* DNA. Patients reported tick exposure in Arkansas, Georgia, Missouri, Nebraska, North Carolina, Tennessee and Virginia.

The most comprehensive clinical evaluation of patients with EM or EM-like rash was conducted by Wormser and colleagues at New York Medical College (11). In this study, 21 rashes observed on Missouri patients referred by Dr. Edwin Masters were compared with 101 rashes on patients presenting for evaluation at Westchester Medical Center, Valhalla, NY. Demographic features between the two groups were not significantly different. However many statistically significant difference in other factors were noted. Peak incidence for Missouri case patients was May and June compared with June and July for New York. Compared with New York cases, Missouri cases were more likely to report: (1) tick bite at the skin lesion site, (2) shorter time interval from tick detachment until onset of skin lesion, (3) fewer symptoms, (4) fewer reports of stiff neck, fatigue, concentration or memory problems, joint pain, dizziness, loss of appetite and headache, (5) multiple skin lesions or regional lymphadenopathy in proximity to the lesion, (6) smaller mean diameter of the skin lesion. Fatigue was the most common symptoms reporting among both groups. Fever was reported by 14% of Missouri patients and 28% of New York patients. Compared to rashes of New York patients, the rashes of Missouri patients were more likely to be (1) found on the torso, (2), less uniform in color, (3) less tender, (4) less pruritic and (5) have central clearing. Symptoms resolved in all Missouri cases within 21 days of initiation of antibiotic therapy and in all but three of the New York cases. Missouri case patients were less likely to report subjective symptoms such as arthralgia and fatigue in those who complied with 3 month follow-up visits. This finding suggests that patients with AALDI do not suffer lingering sequelae that are often reported by Lyme disease patients. However, data from long term clinical studies of persons with AALDI are needed to support this hypothesis.

In a companion study, rash biopsy specimens obtained from these 21 Missouri patients plus 7 more from the same geographic area were evaluated by culture and polymerase chain reaction targeting several borreliae and eubacterial genes and patient serum samples were tested for antibodies against *B. burgdorferi* (13). Neither *B. lonestari* nor *B. burgdorferi* was detected by PCR nor were any borreliae cultured from these samples. Serologic testing for the presence of antibodies to *B. burgdorferi* was uniformly negative.

2.2.3.3. AALDI Epidemiology

The lack of a uniform case definition, known etiology and diagnostic criteria make it difficult to conduct epidemiologic studies or surveillance for AALDI. There is overwhelming consensus in the published literature that this illness or syndrome is associated with exposure to *A. americanum* ticks. There are only two published analytic epidemiologic studies of persons with AALDI in areas where *A. americanum* is the predominant human biting tick and true Lyme disease is rare (7, 77). Of risk factors evaluated in a retrospective case-control study, Campbell and colleagues found that when compared with controls, case patients were statistically more like to live near a pond or lake, recall recent chigger bite, hunt and be male (7). In the prospective study of residents living in coastal Maryland where 95% of ticks submitted were *A. americanum*, Armstrong found that those with a history of Lyme disease diagnosis were significantly more likely to garden, have more than one tick bite per week, use personal protection measures and reside on Gibson Island for more than five consecutive summers when compared to residents lacking a history of Lyme disease diagnosis.

We can glean epidemiologic insight into the demographics of AALDI by analyzing Lyme disease case reports from regions where true Lyme disease is unlikely and *A. americanum* is the predominant human-biting tick. In a report of national surveillance for Lyme Disease in the U.S. in 2001-2002 CDC reported that among 38 states with below-average incidence, the modal age of patients was 44 years and 47% were male compared with data from 12 states with above-average incidence where modal age was 6 years and 54% were male (80).

2.3. METHODS

2.3.1. Study Population

The TickPro study population included military personnel, retirees, dependents and civilian employees eligible for health services at Fort Campbell, Kentucky. Fort Campbell is located on the Kentucky-Tennessee border between Hopkinsville, KY and Clarksville, TN (Figure 2.7.3). It is well within the geographic range of *A. americanum* ticks (Figure 2.7.1) and the risk of locally acquired Lyme disease is rare. Any person aged 18 or older of any sex or race was eligible for enrollment. Participants must have recently removed an embedded tick from their skin and the tick must have been submitted to the Human Tick Test Kit Program concurrent with enrollment. Therefore, the entire study population was at risk for *A. americanum*-associated illness. Public notice that CDC, CHPPM and Fort Campbell EHC were recruiting adults with tick bite was given through newsletters, local newspapers and posters placed in high traffic areas (Appendix A).

Fort Campbell, KY is home to the U.S. Army's only air assault division, the 101st Airborne Screaming Eagles (www.campbell.army.mil). The Fort was opened in 1942 and boasts 105,068 acres (164 square miles). Although nearly two-thirds of the acreage is located in Tennessee, the post office is in Kentucky. Approximately 12,000 acres are used for troop accommodations and support while the remaining lands are dedicated to training and firing ranges. From October 1, 2005 to September 30, 2006 (fiscal year 2006), Fort Campbell employed 30,334 active duty service members and 4,388 civilians. The Fort also supported 56,537 family members (10, 537 of whom lived on post) and 135,108 retirees, retiree family members and members of reserve components. The healthcare system reported an average of 2,192 outpatient visits daily during fiscal year 2006.

2.3.2. Study Design and Implementation

TickPro, a prospective health assessment of persons with tick bite in a geographic area where *A. americanum* is the dominant human-biting tick vector, was designed to define the frequency and spectrum of AALDI and to develop new hypotheses as to its etiology. A prospective design is appropriate when sampling disease free subjects from an at-risk population, when exposure is rare and when multiple or undefined outcomes are possible (81).

Adults submitting ticks to Fort Campbell EHC were asked to read informed consent documents that describe the study objectives and outlined the obligations and rights of research subjects (Appendix A). Prospective volunteers were invited to ask questions and were given an opportunity to discuss any part of the study with enrolling clinic

collaborators before giving their consent to join the study. EHC has participated in the Human Tick Test Kit Program since 2000 and were experienced in the collection and transport of tick removed from humans. Detailed instructions for the consensual enrollment of human research subjects were provided to EHC staff in writing, by telephone and by onsite visit from the CDC PI. Special attention was given to the informed consent process, tick specimen form and shipping instructions.

TickPro had three points of data collection: (1) enrollment survey, (2) 30-day follow-up survey, and (3) CHPPM tick identification and pathogen testing. Surveys and the CHPPM tick submission form are provided in Appendix A.

(1) On the day of enrollment, adult volunteers with tick bite submitted: (a) informed consent, (b) contact information for collection of follow-up data, (c) enrollment survey to collect demographic, tick exposure and existing health information, and (d) their attached or recently removed tick.

(2) Approximately 30-45 days after enrollment, each volunteer was contacted by the CDC PI to complete a 30-day follow-up survey providing information on acute symptoms, healthcare and subsequent tick bites.

(3) CHPPM identified ticks and tested for known pathogens.

All data were merged by unique identifier at the end of each study year by the CDC PI.

In keeping with existing practices, CHPPM continued to notify Fort Campbell EHC if a tick was found to contain *B. lonestari*, *B. burgdorferi*, *E. chaffeensis*, *E. ewingii*, or *Rickettsia spp.* CHPPM provided the guidelines for the use of Human Tick Test Kit

Program results. These explained that PCR evidence of bacteria in ticks is not in itself justification for treatment or prophylaxis of tick borne disease. Clinical management of patients with tick bite should generally be based on clinical symptoms of illness one to four weeks following tick bite. Health care providers then decided whether to communicate CHPPM tick test results to tick bite victims. Collaborators at Fort Campbell EHC indicated that prescribing antibiotic therapy solely on the basis of CHPPM tick test results was not a standard treatment protocol.

Educational materials were provided to all volunteers and Fort Campbell EHC collaborators to help them recognize the signs and symptoms of tick-borne disease (Appendix A). Persons with tick bite were advised to seek prompt medical attention if they experienced any symptoms of tick-borne illness.

The study was reviewed and approved by the CDC Human Research Protection Office Institutional Review Board and CSU Research Integrity and Compliance Review Office Human Research Committee to ensure compliance with the HHS Policy for Protection of Human Research Subjects codified in the Code of Federal Regulations at 45 CFR part 46.

2.3.3. Data Collection and Management

For each volunteer, one tick collection vial and enrollment forms were pre-labeled with a unique identification number and supplied to Fort Campbell EHC in a single packet. This packet included: (1) informed consent, (2) volunteer contact info sheet, (3) enrollment survey, (4) Human Tick Test Kit Program specimen vial and tick submission form, and (5) participant education flyers (Appendix A).

Only the CDC PI had access to all study documents including personal identifiers. Data were maintained electronically in a secure and password protected database throughout the study. At the end of each study year and after the data entries were validated, tick identification numbers were replaced with random unique numbers. Data were then made available to co-collaborators upon request.

2.3.3.1. Exposure Assessment

The self-administered surveys were designed to collect data for the following independent variables related to the: (1) index tick bite (e.g., crawling or embedded, date of removal, method of removal, length of attachment and location of tick bite), (2) host traits (e.g., age, sex, race, military status, previous diagnosis of tick-borne illness or chronic disease, antibiotic or immunosuppressive drug therapy and tick bite history, and (3) space and time (e.g., exposure location and month or year of tick bite).

CHPPM determined the species, sex, life stage, engorgement level and viability of all ticks removed from humans. In addition tick DNA was extracted using standard research methods (14) and tested for the presence of specific pathogen DNA using the following PCR methods:

1) *B. lonestari* DNA was detected using PCR with melting curve analysis of a portion of the glycerophosphodiester phosphodiesterase (glpQ) gene (82).

2) *B. burgdorferi* DNA was detected using PCR amplification of the OspA gene (83).

3) *E. chaffeensis* and *E. ewingii* DNA was detected by PCR using melting curve analysis of amplification of the groESL gene (84).

4) Spotted fever group *Rickettsia* spp. were detected by PCR using primers for the OmpB gene (85) and confirmed using amplification of the OmpA gene (Rr190.70p and Rr190.602n) (86).

2.3.3.2. Assessment of Health after Tick Bite

Using a standardized questionnaire, the CDC PI contacted participants thirty to forty-five days after enrollment by mail, email and/or telephone to inquire about selected signs and symptoms of acute illness including rash, joint pain, joint swelling, swollen lymph nodes/glands, headache, stiff neck, paralysis, generalized weakness, fever, chills, fatigue, impaired memory, confusion, abdominal pain, nausea, vomiting, diarrhea, cough, difficulty breathing, jaundice and photosensitivity. Participants were also asked about physician visits, diagnoses, prescribed therapy, clinical outcome and additional tick exposures during the month after tick bite.

2.3.4. Potential for Biases

Several opportunities for bias were considered during the design of this study. Study and survey design and implementation were modified to reduce known or potential causes of systematic bias.

2.3.4.1. Reducing Information Bias

The enrollment and follow-up surveys were designed to be self-administered by the volunteer to avoid interviewer bias. Both surveys were pilot tested with 10 lay persons to identify needs for clarification.

EHC collaborators were given strict instructions to avoid interaction with the volunteer while they were completing the enrollment survey. The 30-day follow-up survey was designed to be self-administered by the volunteers (via mail or email) and every attempt was made to reduce the number of follow-up surveys administered by telephone. When used, telephone surveys were administered by the CDC PI.

2.3.4.2. Reducing Selection Bias

Self-selection bias could have occurred if tick bite victims that volunteered for the study were more likely have other factors related to rash illness than those who did not volunteer. The age and sex distribution of study participants was compared with that of persons who submitted a tick to the Fort Campbell EHC during the same time period but choose not to participate in TickPro. Comparisons with other predictor variables, however, were not possible because additional data sources for the study population were not available.

2.3.4.3. Exposure Misclassification and Confounding

To reduce exposure misclassification, the study population was restricted to a geographic area with few *I. scapularis* ticks and little to no transmission of *B. burgdorferi*. Participants were also asked about factors that might bias any apparent relationship between tick and pathogen exposures and AADLI (e.g., antibiotic use,

immunosuppression, hypersensitivity). Evaluation and description of potential confounding bias using was not possible as planned due to small sample and inability to conduct stratified analyses. The influence of unknown or unmeasured confounders could not be eliminated or estimated.

2.3.5. Sample Size and Power

While designing the current study, sample size and power were calculated as follows. A background proportion of rash illness of 1.0 % and a prevalence of rash illness in persons with tick bite of 3.0% (observed prevalence of rash illness in persons with *I. scapularis* tick bite reported by Nadelman and colleagues) were assumed. For comparing sample proportions and when anticipating a small sample size a 2-sided exact binomial hypothesis test is preferred. With a target significance level of 0.05 a sample of 378 was needed to achieve 80% power to detect a difference of 2.0% between the null hypothesis proportion of 1.0% and the alternative hypothesis proportion of 3.0%.

2.3.6. Tests of Statistical Inference

Small sample size led to sparse cells and inability to detect associations between many exposures (e.g., *B. lonestari* infected tick bite (n=0)) and most outcomes (e.g., self-reported EM rash (n=2)) in univariate analyses. Therefore, a dichotomized outcome variable was created by classifying illness based on the report of no symptom after tick bite (no disease) or at least one symptom reported after tick bite (disease). For each risk factor or exposure evaluated, the difference in sample proportions and 95% confidence intervals with a continuity correction to adjust for the difference between the normal approximation and the discrete binomial distribution was calculated as the primary statistical measure of inference (exposure among those without disease compared to

exposure among those with disease). Categorical variables were compared in 2 x 2 tables or 3 x 2 tables with a Fisher's exact test. For continuous variables, the difference between sample means was tested using a Student's t-test. All p-values were two-tailed.

Multivariable logistic regression analysis to evaluate predictors of rash illness and other clinical outcomes was planned. However, this analysis was not possible due to small sample size and lack of statistical power.

2.4. RESULTS

2.4.1. Demographics, Exposure Location and Seasonality

Forty two adult participants enrolled in TickPro. Fifteen (36%) reported that their tick bite was acquired on the Fort Campbell Military Reservation (Figure 2.7.4). Eighteen (50%) reported exposure locations in Montgomery County (n=14), Christian County (n=2), Robertson County (n=1) or Davidson County (n=1), Tennessee. Three reported exposure in Stewart County, Kentucky. Six participants (14%) reported an unknown exposure location.

The average age of participants was 41 years (range 20-79 years). Twenty three (55%) were male, 25 (60%) were white, 9 (21%) black, 2 (5%) Native Hawaiian or Pacific Islander, 1 (2%) Hispanic and 1 (2%) were American Indian or Alaskan Native (Table 2.6.2). Thirteen participants (30%) were active duty military, 10 (24%) retired, 10 (24%) military dependents, 5 (12%) civilian employees and 3 (6%) were another status (National Guard, Reserves, or other).

Date of tick removal was available for all participants. Half enrolled in 2006 (Table 2.6.2). Month of tick bite appeared to correlate well with known tick activity patterns (with the exception of July which may be an artifact of troop movement in 2005-2006 and a long hot and dry spell in 2007) (Figure 2.7.5). Participants were most likely to experience tick bite during April, May and June (n=30, 71 %). No persons with tick bite were enrolled from November to February.

There were no significant differences (probability value ≤ 0.05) in demographic features or seasonality of tick bite between participants reporting no and at least one symptom after tick bite (Table 2.6.2). Results suggest, however, that persons bitten during late spring or early summer may have increased risk of having at least one symptoms compared with those bitten in late summer (Figure 2.7.6).

2.4.2. Previous Health of Participants

Patients were asked about prior and existing health conditions or symptoms that may confound or modify the effect of tick bite (Table 2.6.3). On the day of enrollment (generally the same day the embedded tick was noticed), six participants (14%) reported symptoms of Lyme disease or Lyme disease-like illness in the 12 months before enrollment but these were not removed from the study to maintain sample size and prior illness was evaluated as a risk factor for prospective illness (results provided in the next paragraph). Seventeen (41%) reported ever having severe allergic reactions or hypersensitivity, 15 (36%) reported skin conditions, four (10%) reported joint or muscle fatigue or weakness and one (2%) reported a previous tick-borne infection (Lyme

disease). At enrollment, two participants (5%) reported current use of steroid or other immunosuppressive therapy. Five (12%) reported antibiotic use in the four weeks prior to enrollment. Thirteen participants (31%) reported removing more than the index tick from their body in the 30 days before enrollment (Table 2.6.3).

There were no significant differences in the occurrence of tick-borne disease symptoms in the 12 months before study enrollment or reported lifetime health history between participants reporting no and at least one symptom after tick bite (Table 2.6.3). The proportion of immunosuppressive or antibacterial therapy in the month before enrollment or during the study period did not differ significantly between these two groups. Although the data hint that the proportion of antibacterial therapy in the month before tick bite is higher among those with disease (p=0.14), exposure or disease misclassification of a single participant could change this interpretation.

2.4.3. Tick Bite Characteristics

Participants were bitten by either *A. americanum* (n=36, 86%) or *D. variabilis* (n=6, 14%) (Table 2.6.4). They reported removing embedded ticks with tweezers (n=18, 43%) or by hand (n=24, 57%). Anatomical location of tick bite was available for 36 participants (90%) reporting a single tick bite (Table 2.6.4). Among these, nineteen (53%) were bitten on the torso, 12 (33%) on a limb, 5 (14%) on the head or neck.

On the day of enrollment (generally the same day the embedded tick was noticed), participants were asked if they had a skin rash or lesion at the tick bite site. Most did not (n=24, 57%) (Table 2.6.4). Among 16 participants that did, seven indicated that the

lesion was less than 1 inch wide and nine reported 1-3 inch lesion. Twelve reported pain or itch at the bite site, nine reported the rash was raised or bumpy and six reported redness (data not shown).

The difference in proportion of many risk factors related to tick and tick bite characteristics were evaluated (Table 2.6.4). The proportion of participants who removed the index tick by hand (as opposed to tweezers or some other method) and the proportion who were reportedly bitten on a limb was significantly higher among those with at least one symptoms as compared to those with no symptom ($p=0.03$ and $p=0.02$ respectively). However, when these variable were analyzed using a 2 x 2 table, a strong association was evident (Fishers Exact p-value = 0.02) (data not shown). That is, most participants who removed the index tick by hand were bitten on a limb.

When comparing groups of participants reporting no and at least one symptom after tick bite, no statistically significant differences were noted in the proportion of participants with *A. americanum* tick bite and more than one index tick bite (Table 2.6.4). Geographical location of tick exposure and whether or not the tick bite resulted from occupational exposure were not statistically different. The proportion of participants who received additional tick bites during the study period was similar between these groups.

2.4.4. Characteristics of Ticks Removed from Participants

Characteristics of ticks removed from participants with one index tick bite (n=34) are shown in Table 2.6.5. The remainder were bitten by two (n=4), four (n=2), seven (n=1), or 12 (n=1) ticks (data not shown).

Among those bitten by a single *A. americanum* (n=29), 19 (65%) were bitten by adults (11 female, 8 male) and 10 (34%) by a nymph (Table 2.6.5). Twenty-four (83%) participants removed and submitted unengorged *A. americanum*, four (14%) partially engorged tick and one (3%) fully engorged tick. Upon arrival at the CHPPM laboratory, 27 *A. americanum* (93%) were dead and 2 (7%) were alive.

Laboratory tests for the presence of *B. lonestari* and *B. burgdorferi* in *A. americanum* were negative. *E. chaffeensis* DNA was detected in the tick removed from one participant. The tick of one participant was too small for DNA extraction and, therefore, was not tested by PCR.

Five participants were bitten by one adult *D. variabilis* (3 female, 2 male) (Table 2.6.5). All (n=5, 100%) *D. variabilis* were unengorged, three (60%) were dead and two (40%) were alive upon arrival at the CHPPM laboratory. Laboratory tests for the presence of *R. rickettsii* in *D. variabilis* were negative.

2.4.5. Frequency of Symptoms After Tick Bite

Among all enrolled participants (n=42), nine were lost to follow-up and 33 (79%) completed follow-up surveys. Among the 33 for whom follow-up data were available (Table 2.6.6), 14 (42%) reported at least one symptom (range 1 – 7). Fatigue was reported most often (n=6, 18%), followed by headache/stiff neck (n=5, 15%), cough (n=4, 12%), sore throat (n=3, 9%), joint swelling (n=2, 6%), erythema migrans rash (n=2, 6%) and other rash (n=2, 6%). Diarrhea, joint pain, numbness/paralysis, chills,

confusion, vomiting, difficulty breathing and light sensitivity were each reported once (3% each). No volunteer reported fever, skin ulcer, jaundice, or impaired memory/difficulty concentrating.

2.4.6. Description of Participants Reporting Rash After Tick Bite

Four participants reported rash after tick bite. A brief description of each is given here.

One 53 year old black female military dependent (#59) reported expanding circular red skin rash three or more inches in diameter, unexplained chills, unexplained fatigue, confusion, vomiting, diarrhea, cough and sore throat for which she sought medical evaluation. She submitted a partially engorged *A. americanum* nymph that was PCR negative. She reported no prior history of health conditions, antibiotic use, or immunosuppressive therapy.

One 23 year old black female military dependent (#79) reported expanding circular red skin rash three or more inches in diameter, severe headache or stiff neck and numbness or paralysis of the face, arms, or legs for which she sought medical evaluation. She was reportedly diagnosed with Lyme disease and was prescribed doxycycline. She submitted a partially engorged *A. americanum* nymph that was PCR negative for pathogens tested. She reported no prior history of health conditions, antibiotic use, or immunosuppressive therapy.

One 34 year old female active duty service member (#33) reported red bumps around the site of tick bite for which she sought medical evaluation and was prescribed

hydrocortisone cream. She submitted one unengorged female *A. americanum* that was too small for DNA extraction and PCR testing. She reported a history of allergies for which she took medicine.

One 58 year old white male retiree (#04) reported recurrent rash on wrists, ankles, or elbows and unexplained fatigue for which he sought medical evaluation. Symptoms had not resolved at follow-up. He submitted four unengorged male *A. americanum* that were PCR negative for pathogens tested, reported taking doxycycline in the month before to enrollment and in the month after tick bite and had a previous history of rheumatoid arthritis and reported severe joint pain or swelling of the knees in the year prior to enrollment.

2.4.7. Medical Follow-up Among Participants Reporting Symptoms After Tick Bite

Of 14 participants reporting at least one symptom within 30 days of tick bite, eight sought medical attention for their symptoms (Table 2.6.7). Three were told their symptoms were related to tick bite (one was diagnosed with Lyme disease) and therapy was administered or prescribed for five (antibiotics, steroid cream, or injectable headache relief). Six participants were prescribed antibiotics or reported taking them during the study period. On the date of follow-up survey, four reported persistent symptoms.

2.5. DISCUSSION

2.5.1. Findings

The proportion of participants who removed their embedded tick by hand was 42% higher among those reporting disease compared with those reporting no disease. This observation supports recommendations by CDC and others who suggest tick removal by grasping the tick with tweezers very close to human skin (11, 20, 21, 33). This presumably reduces chance that tick midgut contents (where pathogens may reside) are forced into the host due to grasping, squeezing, or crushing tick with your fingers (33, 87).

The proportion of participants who reported anatomical site of tick bite as the limb (arm, hand, upper leg, lower leg, or foot) was 40% higher among those with disease as compared to those with no disease. This finding is consistent with the prospective study conducted by Kirkland and colleagues (10) but contrary to studies published by Campbell and colleagues, Felz and colleagues and Wormser and colleagues (7, 8, 11) who reported that most patients were bitten on the torso. These were studies of patients with EM or EM-like rash which presumably requires a longer tick feeding period and, therefore, a bias against ticks more easily noticed on the extremities could have occurred.

A few other findings are worth noting even though they lack statistical significance. Sixty percent of TickPro participants were white. This contrast sharply with known demographics for persons with Lyme disease where >95% are white and may simply

reflect diversity in the Fort Campbell sample population rather than suggest a biological difference in zoonotic risk between persons exposed to *A. americanum* and those exposed to *I. scapularis*. At enrollment (presumably within a day or two of tick removal), almost half of participants (43%) reported the development of a skin lesion at the tick bite site. This swift reaction was likely caused by a local inflammatory response to tick bite rather than infection by a bacterial or viral etiologic agent. All PCR tests were negative except for tick removed from an asymptomatic participant that contained *E. chaffeensis* DNA. This finding is not unexpected considering the small number of participants and low infection rates for the agents tested. It is important to note that no participants were bitten by *Ixodes* and all *A. americanum* tested negative for *B. burgdorferi* providing additional support for the notion that true Lyme disease is rare in this region.

It appears that participants accurately recalled and reported length of tick attachment because 88% self-reported length of tick attachment as less than one to 3 days and 86% of ticks were identified as unengorged by CHPPM (data not shown). This is consistent with feeding habits of *A. americanum* as they take in very little blood during the first 3 – 4 days of feeding and then take in a "big sip" during the last 12 hours of attachment (J. Piesman personal communication).

Comparing the frequency of symptoms observed among TickPro participants to published reports of persons exposed to *A. americanum* bite is difficult because TickPro participants were enrolled based on exposure and previous studies enrolled patients based on a strict definition of disease (EM or EM-like rash) (7, 8, 10, 11). In previous studies,

100% of participants had EM or EM-like rash compared with 5% of TickPro participants. Therefore comparisons in the frequency of other symptoms should be made with caution. In previous studies and in TickPro, reports of fatigue, headache and/or stiff neck and malaise or musculoskeletal pains were most frequent. Unfortunately these influenza-like symptoms are common to many infectious diseases including most known tick-borne diseases and do not provide much unique data on which to draw new hypotheses about the etiology of STARI. In contrast to most patient cohorts diagnosed with tick-borne infectious diseases, however, TickPro participants did not report fever. This finding is somewhat at odds with other STARI investigations where 5% – 29% of patients reported fever (n=1 to n=11 patients with fever) (7, 8, 10, 11, 77). Even so, fever does not appear to be a distinctive feature of STARI suggesting the lack of infection by known viral or bacterial agents.

EM-like rash was reported by two participants. They had very similar profiles but were bitten in different years. Both were black, female, military dependents bitten by partially engorged *A. americanum* nymphs.

2.5.2. Study Limitations

Small sample size and study limitations reduced the extent to which study objectives were met and weakened the usefulness of study findings. Several limitations were identified prior to data collection. Characteristics of the sample population likely differed from the larger population of persons who experience tick bite and, therefore, reduce the extent to which findings could be generalized. For example, the TickPro study population was expected to be comprised of young, healthy and active military

servicemen and women that were not representative of the general at-risk population. But after considering many alternatives, this group was chosen because of the large size of the population, their opportunity for exposure to *A. americanum* bites through recreational and occupational activities and because collaborators at CHPPM and Fort Campbell were willing to participate in TickPro. Tick density, activity and infection rates are often spatially and/or temporally focused due to variance in reservoir host availability and micro climate conditions. This may have reduced the number of persons subject to tick bite (e.g., when climate conditions are unsuitable for ticks) or reduce the potential for exposure to agents in ticks (e.g., tick infections rates are low when few reservoirs hosts are available for tick feeding). Advertising the study in order to recruit tick bite victims may have caused people to check for ticks more frequently and therefore reduce the amount of time that a tick has fed on volunteers. Lyme disease research indicates that a tick needs to feed on humans for more than 24 hours in order to transmit *B. burgdorferi*, but the feeding time needed to transmit *B. lonestari, E. chaffeensis, E. ewingii and Rickettsia* spp. is unknown. Most 30-day follow-up surveys were self-administered but the CDC PI administered several by telephone. Although the CDC PI was blinded to the results of tick identification and pathogen testing, differences in the survey collection method could have caused non-differential misclassification of disease if, for example, participants were more reluctant to report clinical signs and symptoms to the CDC PI in person than participants responding anonymously using a self-administered survey. Volunteers may have had difficulty accurately recalling prior illnesses or tick encounters resulting in misclassification of exposure. However, since participants did not know their disease status at the time of exposure data collection, the

chance of misclassification is likely random (non-differential). In fact, considering that the outcome was defined as having reported at least one symptom, there is almost no chance of differential misclassification of disease status. Non-differential misclassification is a conservative bias that can lead to an underestimation of the true risk. Signs of symptoms of disease were self-reported by study participants and were not corroborated by a health care provider or with standardized diagnostic testing. Although physician diagnosed illness with laboratory support of infection is a gold standard in epidemiologic studies, achieving this was not possible in our study because there is no standard case definition for STARI, the illness is mild and may not require a physician visit, there are no standard serologic assays for *B. lonestari and* PCR testing for *B. lonestari* in human specimens is complex.

Several limitations were recognized after the study began and during data analyses. Forty-two adult participants enrolled in TickPro during 2005 - 2007. This is approximately 24% of all persons who submitted ticks to EHC during the same time period. Many were less than 18 years old and therefore not eligible for enrollment in TickPro. The EHC Collaborator reported few refusals when she was available for recruitment. In her absence, active duty service members working in the EHC accepted ticks and may not have adequately described the study to tick bite victims. Among TickPro participants who were lost to follow-up (n=9), seven were male and 2 were female. This uneven distribution is likely due to troop movement in support of Operation Iraqi Freedom. Thirteen participants (31%) reported removing more than the index tick from their body in the 30 days before enrollment, increasing the chance of exposure

misclassification. However, among those with complete data, the proportion of participants with more than the index tick bite was not dissimilar among those reporting at least one symptom as compared to those reporting no acute symptoms. Therefore it appears that multiple tick exposures did not influence the relationship between index tick bite and disease although the influence of this bias could not be properly examined due to small sample size. The average number of days between tick removal and laboratory receipt was 13 (median 10, range 5 – 53) and 93% of ticks were dead upon arrival at the laboratory generating concern about the chance of false negative PCR test results. When reporting results to healthcare providers, CHPPM warns that "*tests performed on live ticks are the most accurate and that negative test results from dead ticks can be unreliable (i.e., false negative) because the DNA of pathogenic organisms begins to degrade once the tick dies*". EHC staff notified all participants of tick test results within days of receiving the information from CHPPM. The CDC PI was unaware of this practice during study design and implementation. There is no doubt that knowing tick results prior to data collection could have caused systematic bias in self-reported information (e.g., persons bitten by ticks containing pathogens may have been more likely to recall symptoms). But ticks removed from all but one patient were PCR negative eliminating the potential for diagnostic bias in this study.

2.5.3. Study Strengths

One of the novel components of this study, compared with other epidemiologic studies of STARI, was recruitment of participants with known tick bite coupled with laboratory identification and PCR testing of the index tick(s) and prospective evaluation of a variety of acute symptoms. Previous retrospective studies used a strict definition of rash (i.e.,

EM described for Lyme disease surveillance purposes) to recruit patients and may have limited observation of a spectrum of clinical outcomes following *A. americanum* bite (7, 8, 10, 11, 77). Perhaps the most important strength of this study is the long term storage of DNA extracted from ticks removed from TickPro participants allowing the opportunity to test for newly suspected etiologies when they are identified in the future.

2.6. TABLES

2.6.1. North American Hard Tick Vectors of Common Human Bacterial Illness

Vector	Bacterial Agent	Illness	Found in the TickPro Study Area (Fort Campbell, KY)
Ixodes scapularis	*Borrelia burgdorferi*	Lyme disease	Rarely
	Anaplasma phagocytophilum	anaplasmosis	Rarely
Ixodes pacificus		Lyme disease	No
	Borrelia burgdorferi	anaplasmosis	No
	Anaplasma phagocytophilum		
Amblyomma americanum		tularemia	Yes
		human monocytic	Yes
	Francisella tularensis	ehrlichiosis	Yes
	Ehrlichia chaffeensis	ehrlichiosis	
	Ehrlichia ewingii		
			Yes
Dermacenter variabilis		Rocky Mountain	Yes
	Rickettsia rickettsii	spotted fever	
	Francisella tularensis	tularemia	No
			No
Dermacenter andersoni	*Rickettsia rickettsii*	Rocky Mountain	
	Francisella tularensis	spotted fever	
		tularemia	

2.6.2. Demographics of participants, seasonality of tick bite and presence or absence of reported symptoms

	All n=42 (%)	No acute symptoms n=19 (%)	=>1 acute symptom n=14 (%)	Difference between sample proportions (95% CI)	p-value
age					
mean	41	41	47		0.2018[1]
range	20 – 79	20 – 59	23 – 79		
male	23 (55%)	9 (47%)	7 (50%)	-3% (-4%, 4%)	1.0000[2]
race					
White	25 (60%)	12 (63%)	10 (71%)	-8% (-47%, 30%)	0.0910[2]
Black	9 (21%)	2 (11%)	4 (29%)	-18% (-52%, 16%)	
other	8 (19%)	5 (26%)	0 (0%)	26% (0%, 52%)	
active duty	13 (31%)	4 (21%)	3 (21%)	0% (-35%, 34%)	1.0000[2]
year of enrollment					
2005	8 (19%)	4 (21%)	2 (14%)	7% (-25%, 39%)	1.0000[2]
2006	21 (50%)	10 (53%)	8 (57%)	-5% (-45%, 36%)	
2007	13 (31%)	5 (12%)	4 (29%)	-3% (-39%, 35%)	
season of enrollment					
Mar - May	20 (48%)	6 (32%)	9 (64%)	-33% (-72%, 6%)	0.0853[2]
Jun – Sep	22 (52%)	13 (68%)	5 (36%)	33% (-6%, 72%)	

[1] p-value for two sided t test

[2] p-value for Fisher's exact test

2.6.3. Participant health and presence or absence of reported symptoms

	All n=42 (%)	No acute symptoms n=19 (%)	=>1 acute symptom n=14 (%)	Difference between sample proportions (95% CI)	p-value[1]
symptom of tick-borne disease in past 12 months	6 (14%)	1 (5%)	3 (21%)	-16% (-46%, 14%)	0.2882
lifetime health history					
any condition	27 (64%)	11 (58%)	9 (64%)	-6% (-46%, 33%)	1.0000
allergy	17 (40%)	4 (21%)	6 (43%)	-22% (-60%, 16%)	0.2569
skin condition	15 (33%)	9 (47%)	3 (21%)	26% (-11%, 63%)	0.1604
joint/muscle disorder	4 (10%)	1 (5%)	3 (21%)	-16% (-46%, 14%)	0.2882
tick-borne disease	1 (2%)	1 (5%)	0 (0%)	5% (-11%, 22%)	1.0000
therapy in month before tick bite					
immunosuppressive	2 (5%)	0 (0%)	1 (7%)	-8% (-29%, 13%)	0.4062
antibacterial	5 (12%)	1 (5%)	4 (29%)	-23% (-55%, 9%)	0.1420
therapy during study period					
immunosuppressive	1 (2%)	0 (0%)	1 (7%)	-7% (-27%, 13%)	0.4242
antibacterial	6 (14%)	2 (11%)	4 (29%)	-17% (-52%, 17%)	0.3649
tick bite in month before study enrollment	13 (31%)	6 (32%)	4 (29%)	3% (-35%, 41%)	1.0000

[1] p-value for Fisher's exact test

2.6.4. Tick bite characteristics and presence or absence of reported symptoms

	All n=42 (%)	No acute symptoms n=19 (%)	=>1 acute symptom n=14 (%)	Difference between sample proportions (95% CI)	p-value[1]
species					
A. americanum	36 (86%)	15 (79%)	13 (93%)	-14% (-43%, 15%)	0.3662
D. variabilis	6 (14%)	4 (21%)	1 (7%)	14% (-15%, 44%)	
more than one tick bite at enrollment	8 (19%)	4 (21%)	2 (14%)	7% (-25%, 39%)	1.0000
removed tick(s) by hand	24 (57%)	7 (37%)	11 (79%)	-42% (-78%, -5%)	0.0329
skin reaction at bite site	6 (14%)	5 (26%)	7 (54%)	-28% (-68%, 13%)	0.1502
anatomical location of bite					
torso	19 (48%)	10 (55%)	5 (36%)	17% (-23%, 57%)	0.4824
limb	12 (30%)	2 (11%)	7 (50%)	-40% (-75%, -4%)	0.0191
head or neck	5 (13%)	4 (22%)	1 (7%)	14% (-15%, 43%)	0.3662
other unspecified	4 (10%)	2 (11%)	1 (7%)	3% (-22%, 29%)	1.0000
exposure location on base	15 (36%)	4 (25%)	5 (39%)	-13% (-54%, 27%)	0.6822
occupational exposure	7 (17%)	2 (11%)	2 (14%)	-4% (-33%, 25%)	1.0000
additional tick bites during study period	6 (14%)	4 (21%)	2 (14%)	7% (-27%, 41%)	1.0000

[1]p-value for a Fisher's exact test

2.6.5. Characteristics of ticks removed from participants with one tick bite

	A. americanum n=29	*D. variabilis* n=5
stage		
nymph	10 (34%)	0 (0%)
adult	19 (65%)	5 (100%)
engorgement		
flat	24 (83%)	5 (100%)
partial	4 (14%)	0 (0%)
full	1 (3%)	0 (0%)
condition		
alive	2 (7%)	2 (40%)
dead	27 (93%)	3 (60%)
pathogen		
B. lonestari	0 (0%)	not done
B. burgdorferi	0 (0%)	not done
E. chaffeensis	1 (3%)	not done
R. rickettsii	not done	0 (0%)

2.6.6. Frequency of symptoms reported by humans after *Amblyomma americanum* bite

Symptom	Frequency	% of all participants n=33	% of participants reporting => 1 symptom n=14
fatigue	6	18	43
headache	5	15	36
cough	4	12	29
sore throat	3	9	21
erythema migrans rash	2	6	14
other rash	2	6	14
diarrhea	2	6	14
joint pain	1	3	7
numbness/paralysis	1	3	7
chills	1	3	7
confusion	1	3	7
vomiting	1	3	7
difficulty breathing	1	3	7
light sensitivity	1	3	7

2.6.7. Medical follow-up among participant reporting at least one symptom (n=14)

	Number	%
sought medical evaluation	8	57
received tick-related diagnosis	3	21
physician prescribed therapy	5	36
took antibiotics during study period	6	43
reported persistent symptoms	4	29

2.7. FIGURES

2.7.1. Approximate distribution of *Ixodes scapularis*, *Ixodes pacificus* and *Amblyomma americanum* ticks in the United States.

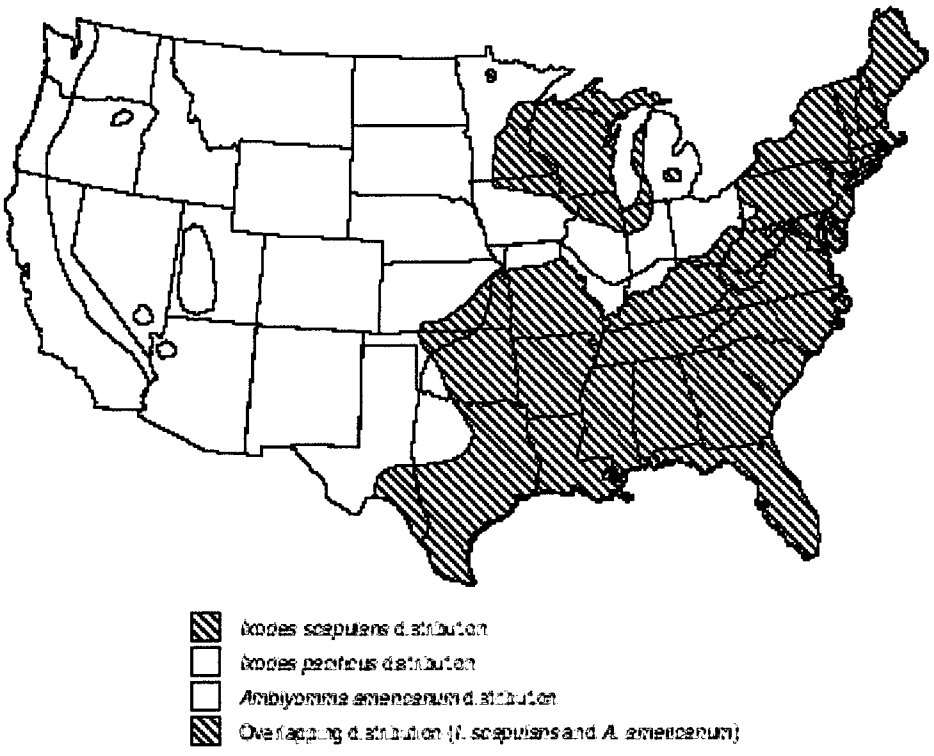


Image provided by Centers for Disease Control and Prevention, Division of Viral and Rickettsial Diseases (http://www.cdc.gov/mmwR/preview/mmwrhtml/rr5504a1.htm).

2.7.2. Appearance and relative size of North American hard tick vectors of human bacterial pathogens.

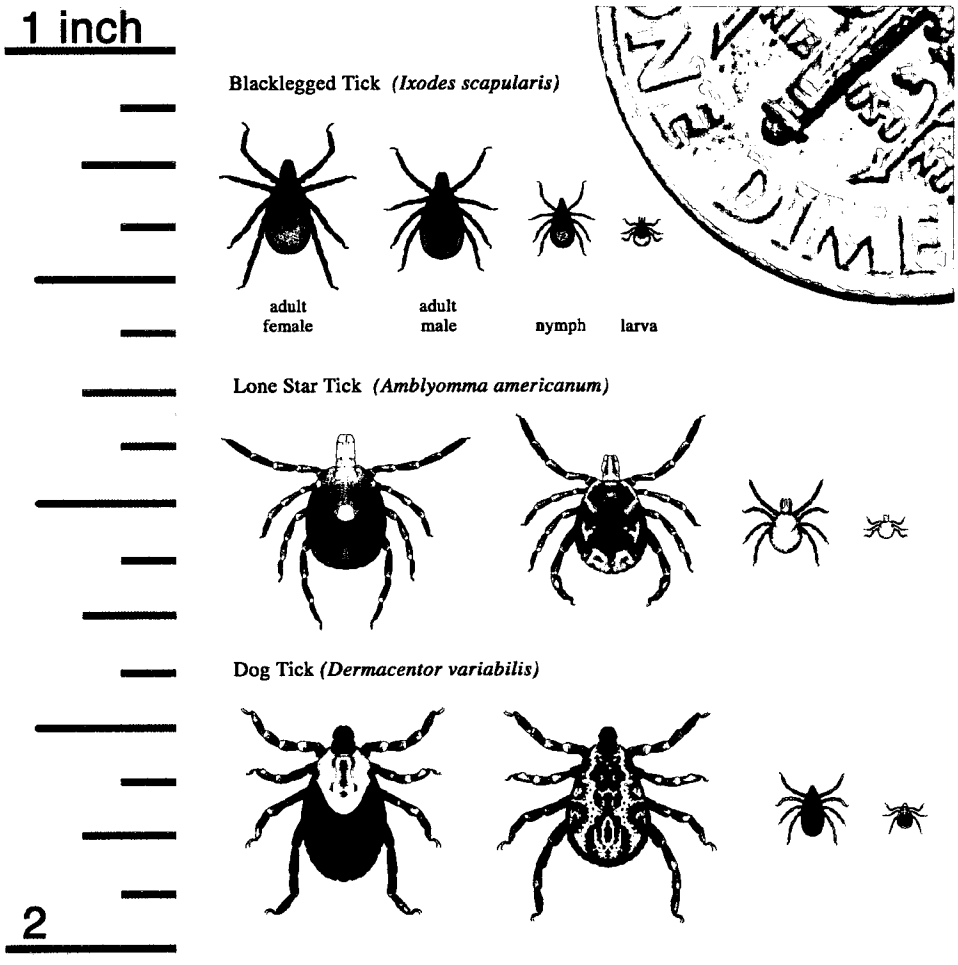
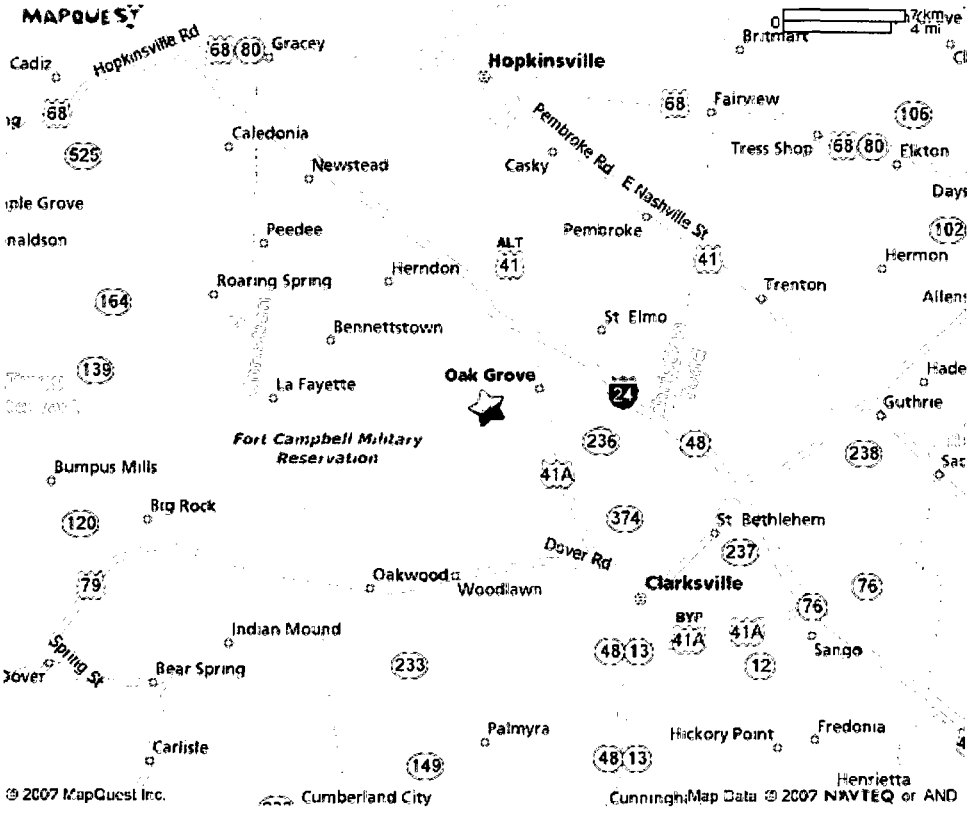
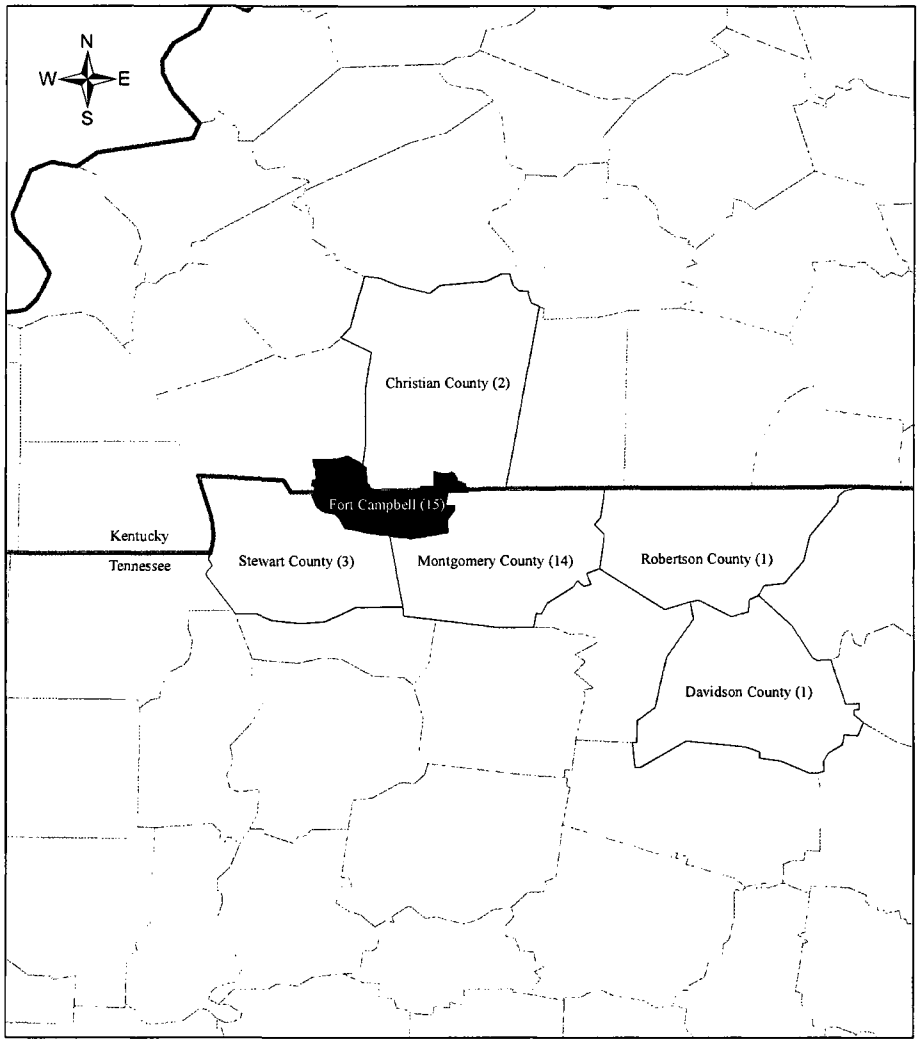


Image provided by Centers for Disease Control and Prevention, Division of Vector Borne Infectious Diseases (http://www.cdc.gov/ncidod/dvbid/lyme/ld_transmission.htm).

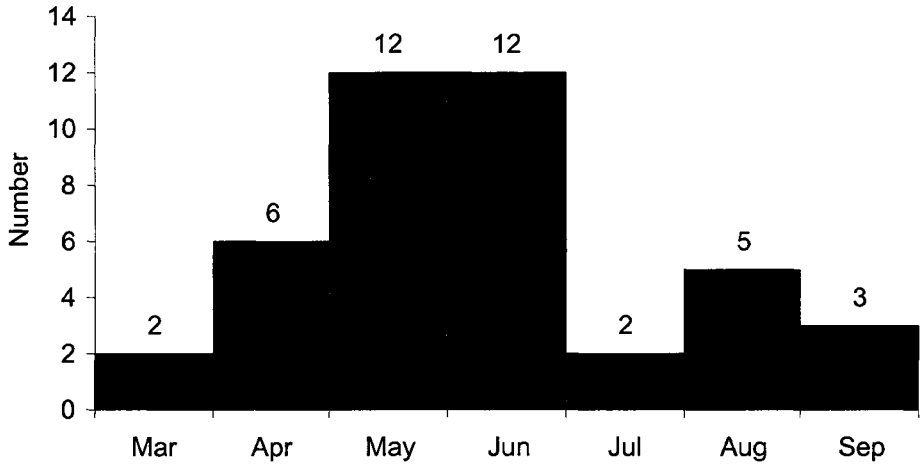
2.7.3. Map Locating Fort Campbell, Kentucky Military Reservation



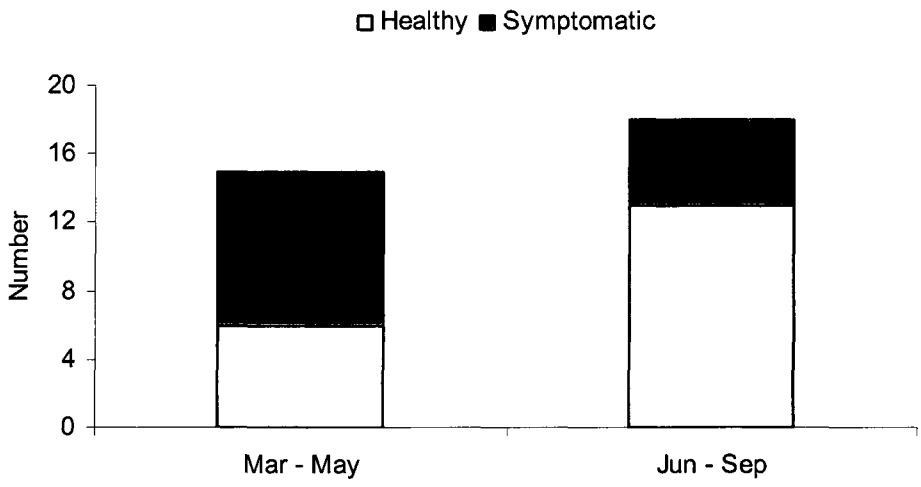
2.7.4. County of exposure (number) of participant with known exposure to index tick bite.



2.7.5. Month of tick bite reported by all participants



2.7.6. Season of tick bite and presence or absence of symptoms



CHAPTER 3

3. TRENDS IN LYME DISEASE CASES REPORTED 1992 - 2006

This chapter was published as "Surveillance for Lyme disease – United States, 1992 – 2006" in Morbidity and Mortality Weekly Report Surveillance Summaries, on October 3, 2008, volume 57, number SS-10 and is provided in Appendix B.

3.1. ABSTRACT

Problem/Condition: Lyme disease is a multisystem disease that occurs in North America, Europe, and Asia. In the United States, the etiologic agent is *Borrelia burgdorferi sensu stricto*, a spirochete transmitted to humans by infected *Ixodes scapularis* and *I. pacificus* ticks. The majority of patients with Lyme disease develop a characteristic rash, erythema migrans [EM], accompanied by symptoms of fever, malaise, fatigue, headache, myalgia, or arthralgia. Other manifestations of infection can include arthritis, carditis, and neurologic deficits. Lyme disease can be treated successfully with standard antibiotic regimens.

Reporting Period: 1992–2006.

Description of System: U.S. health departments report cases of Lyme disease voluntarily to CDC as part of the National Notifiable Disease Surveillance System. Variables collected include patient age, sex, race, county and state of residence, date of illness onset, and reported signs and symptoms.

Results: During 1992–2006, a total of 248,074 cases of Lyme disease were reported to CDC by health departments, and the annual count increased 101%, from 9,908 cases in 1992 to 19,931 cases in 2006. During this 15-year period, 93% of cases were reported from 10 states (Connecticut, Delaware, Massachusetts, Maryland, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island, and Wisconsin). Incidence was highest among children aged 5–14 years, and 53% of all reported cases occurred among males. More than 65% of patients with EM had illness onset in June and July, compared with 37% of patients with arthritis.

Interpretation: Lyme disease is the most commonly reported vectorborne illness in the United States. The geographic distribution of cases is highly focused, with the majority of reported cases occurring in the northeastern and north-central states. During 1992–2006, the number of reported cases more than doubled. A disproportionate increasing trend was observed in children and in young males compared with other demographic groups.

Public Health Action: The results presented in this report underscore the continued emergence of Lyme disease and the need for tick avoidance and early treatment

interventions. Public health practitioners can use the data presented in this report to target prevention campaigns to populations with increasing incidence (i.e., children and young males).

3.2. INTRODUCTION

Lyme disease was first described in 1977 following investigation of a cluster of arthritis cases among children living near Lyme, Connecticut (88). Further study indicated that arthritis was a late manifestation of a multisystem, tick-transmitted disease. In 1981, a bacterial spirochete, *Borrelia burgdorferi*, was identified in *Ixodes scapularis* (89) and later demonstrated to be the etiologic agent of Lyme disease (90, 91).

Borrelia burgdorferi occurs naturally in reservoir hosts, including mice, squirrels, shrews, and other small vertebrates (19). *Ixodes scapularis* and *I. pacificus* (also referred to as blacklegged or deer ticks) become infected with *B. burgdorferi* while feeding on the blood of natural reservoir hosts. During subsequent blood meals, the ticks can transmit infection among reservoir hosts or to incidental hosts, including humans. Although deer are not infected with *B. burgdorferi*, they play a role in transporting ticks and maintaining tick populations.

In humans, infection with *B. burgdorferi* can result in dermatologic, musculoskeletal, neurologic, or cardiac abnormalities (11, 29, 30). In approximately 70%–80% of cases, patients develop a characteristic rash, erythema migrans (EM), within 30 days of infection with *B. burgdorferi*. EM is a red expanding rash, with or without central

clearing, which often is accompanied by symptoms of fatigue, fever, headache, mild stiff neck, arthralgia, or myalgia. Within days or weeks, untreated infection can spread to other parts of the body, causing more serious neurologic conditions (e.g., meningitis, radiculopathy, and facial palsy) or cardiac abnormalities (e.g., carditis with atrioventricular heart block). Over a period of months or years, untreated infection can lead to mono- or oligoarticular arthritis, peripheral neuropathy, or encephalopathy.

Lyme disease is diagnosed on the basis of physician-observed clinical manifestations and a history of probable exposure to infected ticks (11). Laboratory tests are neither suggested nor required to confirm diagnosis for patients with recent onset (2–3 weeks) of a characteristic EM rash (34). However, positive results of recommended two-tiered serologic testing (35) can provide confirmation of infection in patients with musculoskeletal, neurologic, or cardiac symptoms. Testing methods that have not been adequately validated can be misleading (92) and are not recommended (93).

The majority of infections can be cured with use of recommended antimicrobials. Patients with physician-diagnosed EM can be treated with oral doxycycline, amoxicillin, or cefuroxime axetil (11, 30). Patients with other manifestations of Lyme disease are treated with either oral or intravenous antimicrobials (e.g., ceftriaxone), depending on the specific clinical condition.

Measures to prevent Lyme disease and other tickborne infections include avoiding tick-infested areas when possible, using insect repellents containing 20%–30% DEET (*N,N*-

diethyl-*m*-toluamide) on exposed skin and clothing, and performing daily self-examination for ticks (20). Tick abundance can be reduced around private homes and in recreational areas by removing brush and leaf litter, creating a buffer zone of wood chips or gravel between forests and lawn, applying acaricides, and excluding deer (20, 94). Tickborne illness can be mitigated by prompt and proper tick removal and by recognizing and seeking treatment for early signs of illness (11, 21, 32). A single dose of doxycycline should be considered for prophylaxis of Lyme disease in persons aged ≥8 years who have been bitten by a nymph or adult *I. scapularis* or *I. pacificus* tick in an area in which at least 20% of ticks are thought to be infected with *B. burgdorferi* (11). The tick must have been attached for ≥36 hours and prophylactic antibiotic administered within 72 hours of tick removal.

With the cooperation of state and local health departments, CDC initiated surveillance for Lyme disease in 1980; the first summary of 226 cases was published in 1981 (95). Before 1991, Lyme disease surveillance case definitions and reporting practices varied among states and between states and CDC. Standardized surveillance and reporting for Lyme disease began in 1991 after the Council of State and Territorial Epidemiologists (CSTE) designated Lyme disease as a nationally notifiable disease and published a standardized surveillance case definition‡ (96). This report describes the characteristics and distribution of Lyme disease cases reported in the United States during 1992–2006, providing 15-year trends and the frequency of reported symptoms. In addition, it details differences between cases reported from within and outside of the 10 states (Connecticut, Delaware, Maryland, Massachusetts, Minnesota, New Jersey, New York, Pennsylvania,

Rhode Island, and Wisconsin) in which Lyme disease is highly endemic* (97). These results underscore the continued emergence of Lyme disease and provide a basis for targeting prevention campaigns to populations with increasing incidence.

3.3. METHODS

3.3.1. Surveillance Case Definitions

During 1991–1996, a case of Lyme disease was defined for national surveillance purposes as 1) physician-diagnosed EM of ≥5 cm in diameter or 2) at least one objective late manifestation (i.e. musculoskeletal, cardiovascular, or neurologic) with laboratory confirmation of infection with *B. burgdorferi* (19). Laboratory confirmation required 1) isolation of *B. burgdorferi* from clinical specimens, 2) demonstration of diagnostic levels of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies to *B. burgdorferi* in serum or cerebrospinal fluid (CSF), or 3) significant change in IgM or IgG antibody response in paired serum samples. In 1997, CSTE and CDC implemented a revised surveillance case definition on the basis of the availability of improved serologic testing (36). Clinical criteria were not changed; however, laboratory confirmation was modified to require 1) isolation of *B. burgdorferi* from a clinical specimen or 2) demonstration of diagnostic levels of IgM or IgG antibodies to *B. burgdorferi* in serum or CSF. A two-test approach (a sensitive enzyme immunoassay or immunofluorescence antibody assay followed by Western blot) was recommended but not required (CDC 1995).

3.3.2. Data Sources

U.S. state and territorial health departments report cases of Lyme disease voluntarily to CDC as part of the National Notifiable Disease Surveillance System (NNDSS).

Provisional data are transmitted to CDC weekly using the National Electronic Telecommunications System for Surveillance, and final data are published annually in CDC's Summary of Notifiable Diseases. State or local health departments are responsible for ensuring that cases reported to CDC meet the case definition.

This report is based on data for all Lyme disease cases reported to CDC for 1992–2006.† During this 15-year period, state health officials used various methods to ascertain cases, including provider-initiated passive surveillance, laboratory-based surveillance, and enhanced or active surveillance. Basic demographic data (e.g., age, sex, race, and county of residence) were available for >90% of reported cases; however, information specific to Lyme disease (e.g., county of exposure, symptoms and signs, antibiotic treatment, and laboratory results) was incomplete. For example, only 61% of case reports contained data for reported signs and symptoms.

3.3.3. Analyses

Annual U.S.-, state-, county-, sex-, and age group–specific incidence rates per 100,000 population were calculated using U.S. Census Bureau population estimates for July 1 for each year of the reporting period (1992–2006). Analyses of symptom data were restricted to case reports for which at least one symptom was coded as "yes" (n = 150,829 records). Characteristics of cases reported from the 10 HP2010 reference states were compared with cases reported from all other (non-HP2010) states and territories.

3.4. RESULTS

3.4.1. U.S. Case Counts and Rates

During 1992–2006, a total of 248,074 Lyme disease cases were reported to CDC. Although annual counts fluctuated by as much as 57% from year to year, the overall trend indicates a steady increase in the number of reported cases (Figure 3.9.1). During the 15-year study period, the number of cases reported increased 101%, from 9,908 cases in 1992 to 19,931 cases in 2006.

3.4.2. State Rates

The 15-year mean annual rate for all states ranged from <0.01 cases per 100,000 population in Montana and Colorado to 73.6 cases per 100,000 population in Connecticut (median: 0.5 cases) (Table 3.8.1). The 10 HP2010 reference states accounted for 229,782 cases, representing 92.6% of overall cases and at least 88% of cases reported in any single year. Reported annual rates for seven HP2010 reference states (Maryland, Massachusetts, Minnesota, New Jersey, New York, Pennsylvania, and Wisconsin) were relatively stable during 1992–2006. Annual rates were more variable in three states (Connecticut, Delaware, and Rhode Island), in part because of changes in surveillance practices. In Connecticut, annual rates per 100,000 population increased from 53.7 cases in 1992 to 133.9 cases in 2002; in 2003, the rate decreased to 40.3 cases. In Delaware, the number of cases increased from 339 in 2004 to 646 in 2005, boosting the annual rate per 100,000 population from 40.9 to 76.7 cases. The annual rate per 100,000 population reported in Rhode Island increased from 27.5 cases in 1992 to 68.5 cases in 2003, then declined to 23.1 cases in 2004 and 3.6 cases in 2005; 28.9 cases were reported in 2006.

3.4.3. County Rates

County of residence was provided for 243,430 (98.1%) cases. The mean number of counties reporting at least one case of Lyme disease was 714 (range: 625–796). In all years, the percentage of counties reporting at least one case was >75% in six states (Connecticut, Delaware, Massachusetts, Maryland, New Jersey, and Rhode Island). In contrast, during 1992–2006, the percentage of counties reporting at least one case increased from 33% to 74% in Minnesota, from 79% to 97% in Pennsylvania, and from 76% to 97% in Wisconsin. In New York, the percentage of counties reporting at least one case ranged from 61% to 85%, with no obvious increasing or decreasing temporal trend.

The 15-year average county-specific rate for counties reporting at least one case during 1992 – 2006 ranged from <0.01 case per 100,000 population in Honolulu County, Hawaii, to 595.1 cases per 100,000 population in Nantucket County, Massachusetts (median: 0.7 cases per 100,000 population) (Figure 3.9.2). Counties with the highest average county-specific rate for three 5-year periods during the 15-year reporting period (1992–1996, 1997–2001, and 2002–2006) are presented in Table 3.8.2. Five counties ranked among the top 10 incidence counties for each 5-year period: Windham County, Connecticut; Nantucket County, Massachusetts; Hunterdon County, New Jersey; Dutchess County, New York; and Putnam County, New York. The only counties outside the northeast to rank among the top 10 counties for any 5-year period were Washburn County and Burnett County, Wisconsin. Because of marked differences in population

size across counties, a high rate does not necessarily indicate a substantial number of reported cases.

3.4.4. Selected Demographics

Information regarding age was available for 241,931 (97.5%) reported cases. Reported ages ranged from <1–106 years and were bimodal in distribution (Figure 3.9.3). Average annual rates peaked among children aged 5–9 years (8.6 cases per 100,000 population) and adults aged 55–59 years (7.8 cases per 100,000 population). The lowest rate was reported among adults aged 20–24 years (3.0 cases per 100,000 population).

Information about sex was available for 243,564 (99.1%) reported cases. Of these, 129,349 (53.1%) occurred among males, yielding an average annual rate per 100,000 population of 6.3 cases for males and 5.4 cases for females. During 1992–2006, rates increased disproportionately among males compared with females (Figure 3.9.4). This trend was most pronounced among persons aged 5–19 years; rates per 100,000 population in this age group increased 194% in males, from 3.5 cases in 1992 to 10.3 cases in 2006, and 114% in females, from 2.9 cases in 1992 to 6.2 cases in 2006.

Information regarding race was available for 166,194 (70.0%) reported cases. Of these, 156,346 (94.1%) patients were identified as white, 2,765 (1.7%) as black, 1,299 (0.8 %) as Asian/Pacific Islander, and 452 (0.3%) as American Indian/Alaska Native. Age and sex of persons with Lyme disease differed among the 10 HP2010 reference states compared with other states. In the reference states, the modal age was 7 years, and males

accounted for 120,369 (53.4%) reported cases. In the remaining states, the modal age was 44 years, and males accounted for 8,890 (49.4%) cases.

3.4.5. Seasonality

Month of disease onset was available for 188,340 (75.9%) reported cases (Figure 3.9.5). Although cases occurred in all months of the year; the majority of patients had onset in June (48,413 [25.7%]), July (56,507 [30.0%]), or August (22,867 [12.1%]), the 3 months in which ticks actively seek mammalian hosts and human outdoor activity is greatest. In the HP2010 reference states, 99,762 (56.5%) cases had onset during June or July, compared with 5,518 (44.2%) among non-HP2010 reference states. Among 150,829 cases with reported clinical features, seasonal variation was most pronounced for cases with EM (Figure 3.9.6). Approximately 67% of patients with EM had onset in June and July, compared with 37% of those with arthritis.

3.4.6. Clinical Features

Information on clinical features of illness was available for 150,829 (60.8%) cases. Among these, EM was reported for 104,387 (69.2%) cases, arthritis characterized by brief attacks of joint swelling for 48,272 (32.0%) cases, neurologic symptoms (facial palsy or cranial neuritis, radiculoneuropathy, lymphocytic meningitis, encephalitis, or encephalomyelitis) for 18,157 (12.0%) cases, and second- or third-degree atrioventricular block for 1,222 (0.8%) cases. More than one clinical manifestation was reported for 19,321 (12.8%) cases. Data on clinical features of cases from all states was representative of data on clinical features of cases from the HP2010 reference states. By comparison, among 7,745 cases reported from non-HP2010 states EM was reported less frequently (4,887 cases [63.0%], and musculoskeletal, neurologic, and cardiac

manifestations were reported more frequently (3,285 cases [42.4%], 1,442 cases [18.6%], and 100 cases [1.3%], respectively).

Temporal trends in national data indicate that the overall frequency of reported clinical features were generally stable over time (Figure 3.9.8). However, the frequency of reported symptoms was highly variable across the youngest age categories (Figure 3.9.9) and among HP2010 reference states (Table 3.8.3).

3.5. DISCUSSION

During 1992–2006, the annual number of Lyme disease cases reported to CDC increased considerably, while remaining highly focused in northeastern and north-central states. Multiple reasons might explain this increase, including a true increase in the number of infections, enhanced surveillance, increased awareness among health-care professionals and the public, misdiagnosis, and reporting errors (98-100). In six HP2010 reference states (Connecticut, Delaware, Massachusetts, Maryland, New Jersey, and Rhode Island) in which the majority of counties regularly reported cases, a true increase in transmission might have resulted from greater tick densities and encroachment of human development into rural and suburban areas. In other HP2010 reference states, particularly Minnesota, Pennsylvania, and Wisconsin, the number of counties reporting cases increased appreciably, suggesting an additional role for geographic expansion of reservoir mammals and vector ticks into new areas. In certain states, especially those in the southeastern United States, Lyme disease surveillance is complicated by the occurrence of southern tick-associated rash illness, a condition that can resemble early Lyme disease

but is not caused by *B. burgdorferi* (7, 13, 78). Overall, features of reported cases changed little over time. Peak rates were reported among children, males, and whites in each year throughout the 15-year period. However, rates increased disproportionately among young males compared with young females; the reasons for this difference are not known. The proportion of cases with EM and arthritis, the most commonly reported symptoms, has been relatively stable since 1993. However, across age categories, the frequency of reported symptoms varied widely among persons aged <20 years, with the lowest percentage of EM (58.2%) and the highest percentage of arthritis (38.7%) reported for children aged 10–14 years. These findings provide a basis for targeting prevention campaigns to populations with increasing incidence.

The findings in this report highlight both the benefits of infectious disease surveillance and the opportunity for improvement. Detailed analysis of reported cases enables public health authorities to define the demographics and distribution of disease and to survey trends. However, growing case counts and the implementation of electronic laboratory reporting have created a substantial reporting burden on certain state and local health departments as they attempt to verify compliance with the surveillance case definition (101, 102). This burden has caused certain states to curtail or modify portions of their surveillance system, resulting in fluctuations in case tallies. In 2007, CSTE revised the national surveillance case definition for Lyme disease with the twin goals of reducing the burden of reporting while potentially enhancing the system's ability to capture a broader range of clinical manifestations. The revised case definition, which was implemented in January 2008, specifies required laboratory evidence in more detail than previous

iterations and allows reporting of confirmed and, for the first time, probable cases of Lyme disease to CDC (103).

3.5.1. Limitations

The findings in this report are subject to at least three limitations. First, an unknown portion of all Lyme disease cases are reported; cases probably are underreported in areas in which the disease is endemic and overreported in areas in which the disease is not endemic. Misdiagnosis and overreporting from areas in which the disease is not endemic might explain the demographic differences noted between cases reported from HP2010 and non-HP2010 reference states. Second, variation in reporting practices and adherence to the surveillance case definition occurs among states, in part because states invest unequally in infrastructure for Lyme disease surveillance. As a result, Lyme disease–specific variables for cases reported by certain states are incomplete, unavailable, or not transmitted to CDC. Finally, cases are reported on the basis of the patient's state of residence rather than on the state in which the exposure occurred. Therefore, Lyme disease in a traveler returning from an area in which the disease is highly endemic cannot be construed as evidence of local transmission.

3.5.2. Conclusion

The number of reported cases of Lyme disease continues to increase, underscoring the need for targeted prevention strategies, early disease recognition and treatment, and a sustainable surveillance system. During the 15-year study period, incidence increased disproportionately among children, particularly males. Geographic expansion was apparent in Minnesota, Pennsylvania, and Wisconsin. Differences in the features of cases reported from HP2010 reference states and all other states suggest either aberrant

reporting or fundamental differences in the epidemiology of Lyme disease in areas in which the disease is not endemic. The percentage of cases for which signs of disseminated infection were reported did not decrease during the reporting period, underscoring the need for continued education about early disease recognition and treatment. Despite the limitations of national surveillance data, these findings are useful in defining demographics, distribution, and trends in Lyme disease cases. Intensive surveillance methodologies, such as active population-based surveillance and the use of nonhuman data (e.g., serologic testing of dogs and surveillance for vectors), could be used to augment these data and provide a better understanding of this emerging infectious disease.

3.6. ACKNOWLEDGEMENTS

The data provided in this report were collected and reported by state, territorial, and local health departments, health-care providers, and laboratories.

3.7. FOOTNOTES

* In 2000, these 10 states were defined as Healthy People 2010 (HP2010) Lyme disease reference states. A Healthy People 2010 goal (objective no. 14-8) is to reduce Lyme disease to 9.7 new cases per 100,000 population in the 10 HP2010 reference states (US Department of Health and Human Services, 2000) through the implementation of community-based prevention programs, host-targeted acaricides to reduce the numbers of vector ticks, and appropriate use of Lyme disease vaccine. However, the only vaccine approved by the Food and Drug Administration for use against Lyme disease in humans

was removed from sale by the manufacturer in February 2002 citing low demand, greatly reducing the possibility of achieving this objective.

† Although data for 1991 were available, these data were excluded from the analysis because certain states reported aggregate case counts rather than information for individual case reports.

‡ The Lyme disease surveillance case definition was developed to standardize national public health surveillance and reporting of Lyme disease cases; it is not meant to be used as absolute criteria for clinical diagnosis.

3.8. TABLES

3.8.1. Annual rate* of Lyme disease, by state/area and year — United States, 1992–2006

State/Area	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	Average
Alabama	0.2	0.1	0.1	0.3	0.2	0.3	0.6	0.5	0.1	0.2	0.3	0.2	0.1	0.1	0.2	0.2
Alaska	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.0	0.3	0.3	0.5	0.5	0.5	0.6	0.5	0.2
Arizona	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.2	0.2	0.2	0.1
Arkansas	0.8	0.3	0.6	0.4	1.1	1.1	0.3	0.3	0.3	0.2	0.1	0.0	0.0	0.0	0.0	0.4
California	0.8	0.4	0.2	0.3	0.2	0.5	0.4	0.4	0.3	0.3	0.3	0.2	0.1	0.3	0.2	0.3
Colorado	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Connecticut†	53.7	41.3	62.1	47.4	95.0	70.3	104.9	98.0	110.6	104.8	133.9	40.3	38.6	51.7	51.0	73.6
Delaware†	31.7	20.4	15.0	7.8	23.8	14.8	10.4	22.2	21.2	19.1	24.1	26.0	40.9	76.8	56.5	27.4
District of Columbia	0.5	0.4	1.6	0.5	0.6	1.9	1.5	1.2	1.9	2.9	4.3	2.4	2.8	1.7	10.7	2.3
Florida	0.2	0.2	0.2	0.1	0.4	0.4	0.5	0.4	0.3	0.3	0.5	0.3	0.3	0.3	0.2	0.3
Georgia	0.7	0.6	1.8	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.3
Hawaii	0.2	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Idaho	0.2	0.2	0.3	0.0	0.2	0.3	0.6	0.2	0.3	0.4	0.3	0.2	0.4	0.1	0.5	0.3
Illinois	0.4	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.3	0.3	0.4	0.6	0.7	1.0	0.9	0.4
Indiana	0.4	0.6	0.3	0.3	0.6	0.6	0.7	0.4	0.4	0.4	0.3	0.4	0.5	0.5	0.4	0.5
Iowa	1.2	0.3	0.6	0.6	0.7	0.3	0.9	0.8	1.2	1.2	1.4	2.0	1.7	3.0	3.3	1.3
Kansas	0.7	2.1	0.7	0.9	1.4	0.2	0.5	0.6	0.6	0.1	0.3	0.2	0.1	0.1	0.1	0.6
Kentucky	0.8	0.4	0.6	0.4	0.7	0.5	0.7	0.5	0.3	0.6	0.6	0.4	0.4	0.1	0.2	0.5
Louisiana	0.2	0.1	0.1	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.0	0.2
Maine	1.3	1.5	2.7	3.6	5.1	2.7	6.3	3.3	5.6	8.4	16.9	13.4	17.1	18.7	25.6	8.8
Maryland†	3.7	3.6	6.8	9.0	8.8	9.7	12.9	17.4	13.0	11.3	13.6	12.6	16.1	22.1	22.2	12.2
Massachusetts†	3.7	2.5	4.1	3.1	5.3	4.8	11.4	12.8	18.2	18.2	28.1	23.8	23.8	36.3	22.3	14.5
Michigan	0.4	0.2	0.3	0.1	0.3	0.3	0.2	0.1	0.2	0.2	0.3	0.1	0.3	0.6	0.6	0.3
Minnesota†	4.4	3.1	4.6	4.5	5.4	5.5	5.5	5.9	9.4	9.3	17.3	9.4	20.1	17.9	17.7	9.3
Mississippi	0.0	0.0	0.0	0.6	0.9	1.0	0.6	0.1	0.1	0.3	0.4	0.8	0.0	0.0	0.1	0.3
Missouri	2.9	2.1	1.9	1.0	1.0	0.5	0.2	1.3	0.9	0.7	0.7	1.2	0.4	0.3	0.1	1.0
Montana	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0

State/Area	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	Average
Nebraska	1.4	0.4	0.2	0.4	0.3	0.1	0.2	0.7	0.3	0.2	0.4	0.1	0.1	0.1	0.6	0.4
Nevada	0.1	0.4	0.1	0.4	0.1	0.1	0.3	0.1	0.2	0.2	0.1	0.1	0.0	0.1	0.2	0.2
New Hampshire	4.0	1.3	2.7	2.4	4.1	3.3	3.8	2.3	6.8	10.3	20.5	14.8	17.4	20.3	46.9	10.7
New Jersey†	8.8	10.0	19.4	21.4	27.3	25.3	23.6	21.1	29.2	23.8	27.4	33.4	31.1	38.6	27.9	24.6
New Mexico	0.1	0.1	0.3	0.1	0.1	0.1	0.2	0.1	0.0	0.1	0.1	0.1	0.1	0.2	0.2	0.1
New York†	19.1	15.5	28.6	24.5	29.2	18.3	25.6	24.2	22.8	21.4	28.9	28.1	26.4	28.8	23.1	24.3
North Carolina	1.0	1.2	1.1	1.2	0.9	0.5	0.8	1.0	0.6	0.5	1.7	1.9	1.4	0.6	0.4	1.0
North Dakota	0.2	0.3	0.0	0.0	0.3	0.0	0.0	0.2	0.3	0.0	0.2	0.0	0.0	0.5	1.1	0.2
Ohio	0.3	0.3	0.4	0.3	0.3	0.4	0.4	0.4	0.5	0.4	0.7	0.6	0.4	0.5	0.4	0.4
Oklahoma	0.8	0.6	3.1	1.9	1.3	1.4	0.4	0.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.7
Oregon	0.4	0.3	0.2	0.6	0.6	0.6	0.6	0.5	0.4	0.4	0.3	0.5	0.3	0.1	0.2	0.4
Pennsylvania†	9.8	9.0	11.9	13.0	23.4	18.2	23.0	23.2	19.1	22.8	32.4	46.4‡	32.2	34.6	26.1	23.0
Rhode Island†	27.5	27.3	47.4	34.9	54.1	44.8	79.9	55.1	64.2	48.2	79.7	68.5	23.1	3.6	28.9	45.8
South Carolina	0.1	0.3	0.2	0.5	0.2	0.1	0.2	0.2	0.6	0.2	0.6	0.4	0.5	0.4	0.5	0.3
South Dakota	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.3	0.1	0.1	0.3	0.1	0.1
Tennessee	0.6	0.4	0.3	0.5	0.5	0.8	0.9	1.1	0.5	0.5	0.5	0.3	0.3	0.1	0.3	0.5
Texas	0.6	0.3	0.3	0.4	0.5	0.3	0.2	0.4	0.4	0.4	0.6	0.4	0.4	0.3	0.1	0.4
Utah	0.3	0.1	0.2	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.2	0.1	0.0	0.1	0.2	0.1
Vermont	1.6	2.1	2.8	1.5	4.4	1.4	1.9	4.4	6.6	2.9	6.0	7.0	8.1	8.7	16.8	5.1
Virginia	1.9	1.5	2.0	0.8	0.9	1.0	1.1	1.8	2.1	2.2	3.6	2.6	2.9	3.6	4.7	2.2
Washington	0.3	0.2	0.1	0.2	0.3	0.2	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.2
West Virginia	0.8	2.8	1.6	1.4	0.7	0.6	0.7	1.1	1.9	0.9	1.4	1.7	2.1	3.4	1.5	1.5
Wisconsin†	10.5	7.9	8.0	7.2	7.7	9.2	12.6	9.3	11.7	11.1	20.0	13.5	20.8	26.4	26.4	13.5
Wyoming	1.1	1.9	1.1	0.8	0.6	0.6	0.2	0.6	0.6	0.2	0.4	0.4	0.8	0.6	0.2	0.7

*Per 100,000 population using U.S. Census Bureau population estimates for July 1 for each year of the reporting period (1992–2006).

†*Healthy People 2010* Lyme disease reference state in which the disease is endemic.

‡Includes 4,722 confirmed and 1,008 suspected cases.

3.8.2. Average rate* and number of cases of Lyme disease, by county and 5-year period — United States, 1992–2006

Rank	1992–1996			1997–2001			2002–2006		
		Rate	No. cases		Rate	No. cases		Rate	No. cases
1	Nantucket County, MA	755	(55)	Nantucket County, MA	669	(60)	Columbia County, NY	962	(609)
2	Hunterdon County, NJ	337	(385)	Columbia County, NY	639	(403)	Dutchess County, NY	439	(1281)
3	Dutchess County, NY	337	(899)	Dutchess County, NY	445	(1234)	Nantucket County, MA	361	(36)
4	Putnam County, NY	278	(248)	Hunterdon County, NJ	443	(535)	Dukes County, MA	337	(52)
5	Washington County, RI	227	(262)	Windham County, CT	304	(330)	Hunterdon County, NJ	276	(356)
6	Middlesex County, CT	197	(290)	Washington County, RI	296	(361)	Greene County, NY	271	(133)
7	Washburn County, WI	182	(27)	Putnam County, NY	222	(211)	Cameron County, PA	239	(14)
8	Burnett County, WI	161	(23)	Dukes County, MA	201	(30)	Washburn County, WI	238	(39)
9	New London County, CT	156	(400)	Litchfield County, CT	195	(355)	Windham County, CT	220	(249)
10	Windham County, CT	130	(137)	New London County, CT	183	(472)	Putnam County, NY	219	(219)

*Per 100,000 population.

3.8.3. Number and percentage* of reported symptoms among Lyme disease patients, by state[†] — *Healthy People 2010* reference states, 1992–2006

State	EM[§]		Arthritis		Neurologic		Cardiac	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Connecticut	25,538	(74)	7,845	(23)	3,305	(10)	171	(0.5)
Delaware	846	(51)	828	(50)	263	(16)	14	(0.9)
Massachusetts	8,196	(68)	3,948	(33)	1,849	(15)	179	(1.5)
Maryland	4,908	(60)	2,919	(36)	1,738	(21)	80	(1.0)
Minnesota[‡]	218	(87)	48	(19)	15	(6)	1	(0.4)
New York	33,024	(74)	10,953	(25)	4,047	(9)	362	(0.8)
Pennsylvania	17,014	(61)	13,093	(47)	4,040	(15)	215	(0.8)
Rhode Island	4,189	(65)	2,375	(37)	599	(9)	45	(0.7)
Wisconsin	5,567	(70)	2,978	(37)	859	(11)	55	(0.7)

*Total percentages exceed 100% because some patients had multiple symptoms.

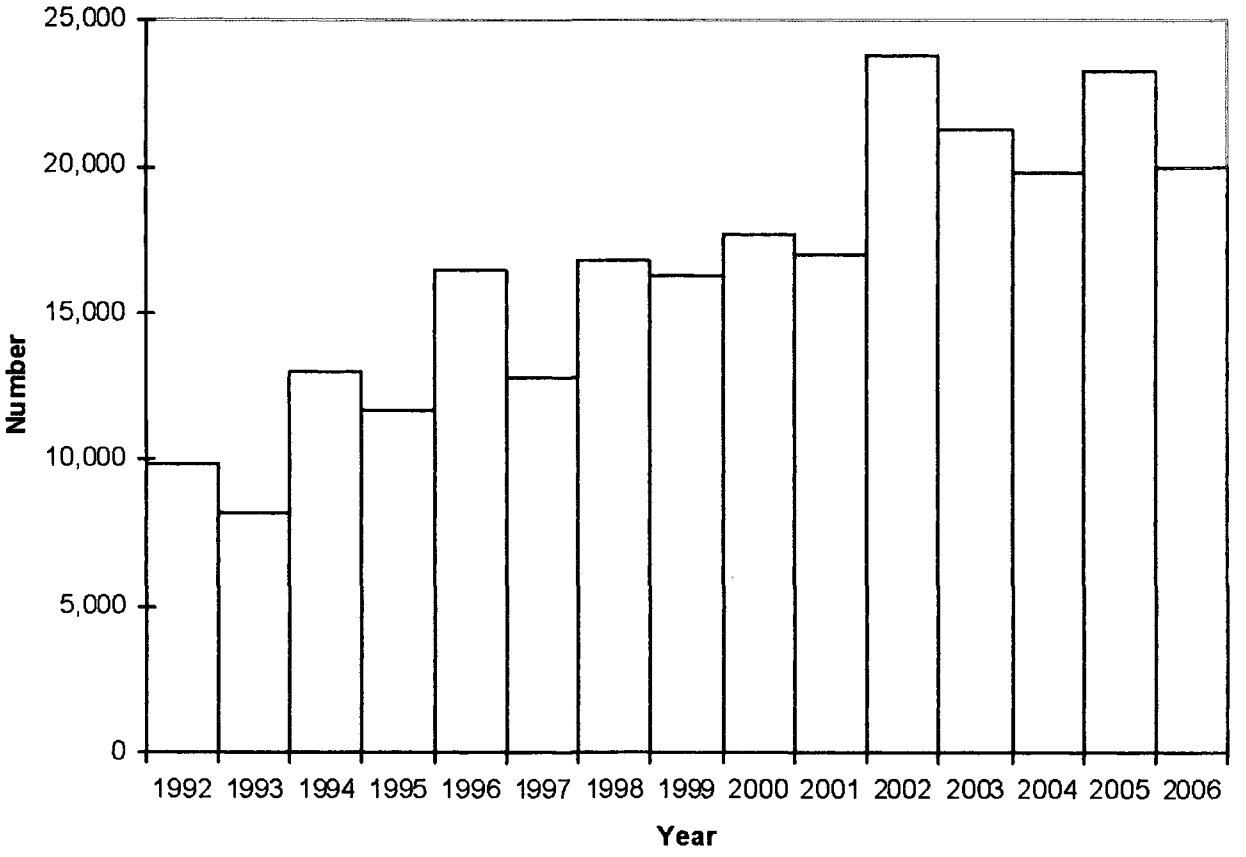
[†] Data represent approximately 60% of reported cases from HP2010 reference states. States did not report data on symptoms for all years during the 15-year study period, and one state (New Jersey) did not report any data on symptoms.

[§] Erythema migrans.

[‡] Data regarding symptoms reported only for 1996.

3.9. FIGURES

3.9.1. Number* of reported Lyme disease cases, by year — United States, 1992–2006



* N = 248,074

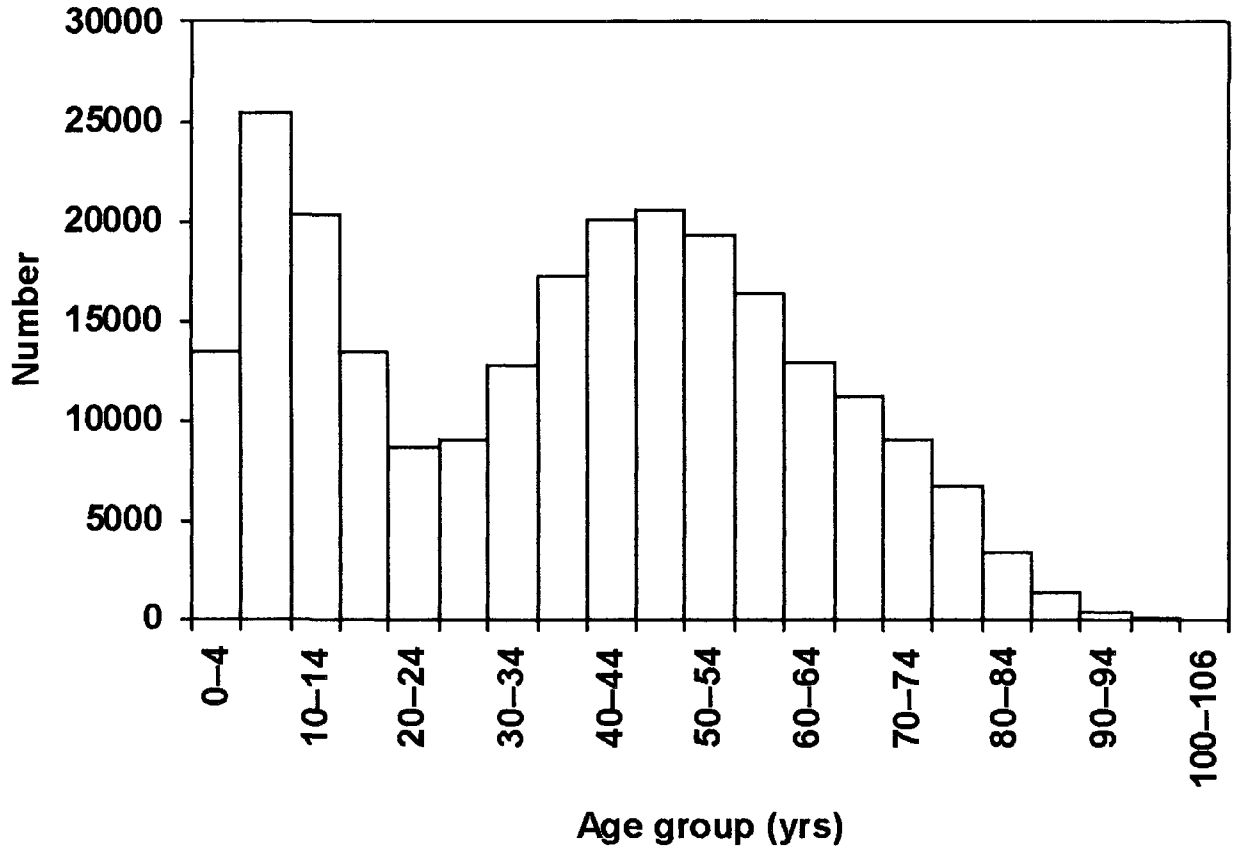
3.9.2. Average rate* of Lyme disease, by county† — United States, 1992–2006‡

* Per 100,000 population.

† County of residence was available for 98.1% of cases reported during 1992 – 2006.

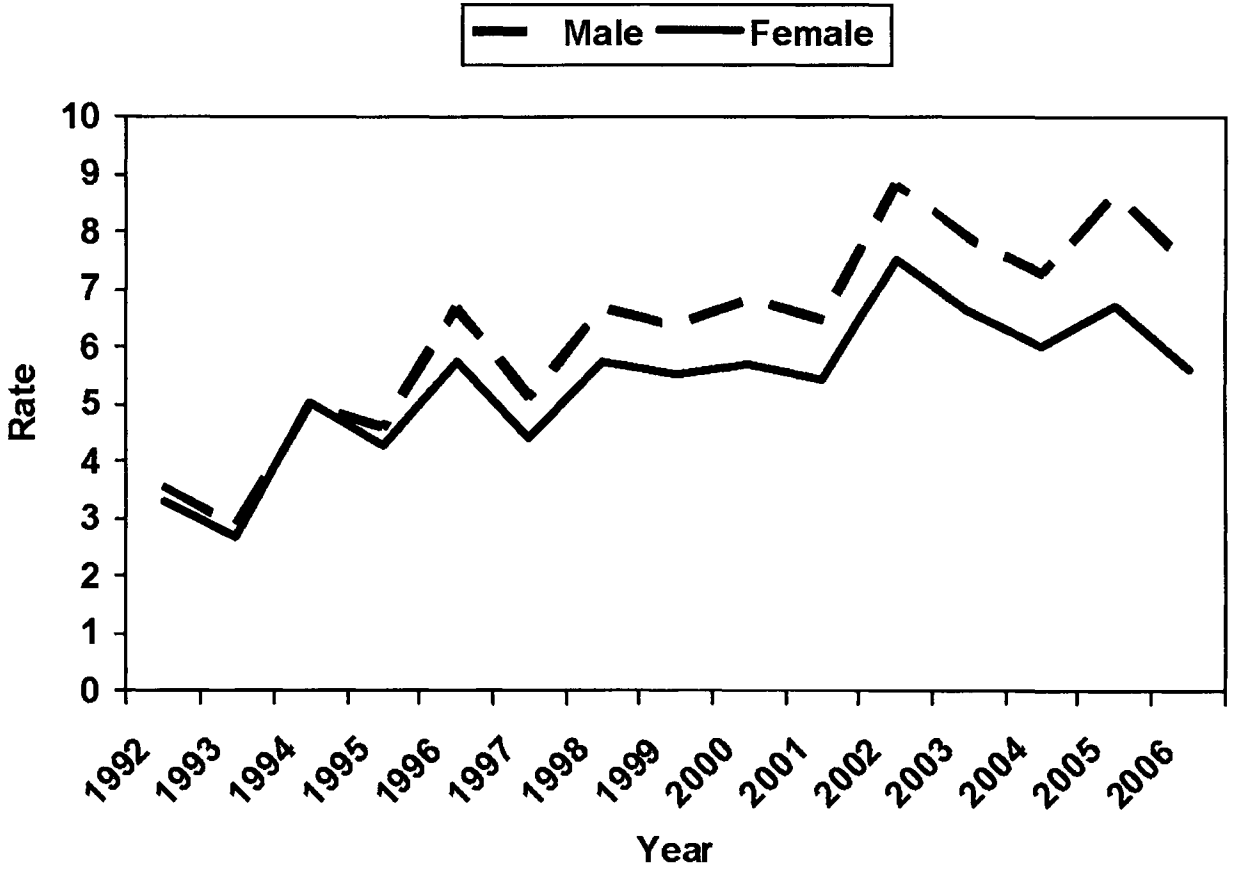
‡ During 2003, Pennsylvania reported 4,722 confirmed cases and 1,008 suspected cases

3.9.3. Number* of reported Lyme disease cases, by age group — United States, 1992–2006



* N = 241,931

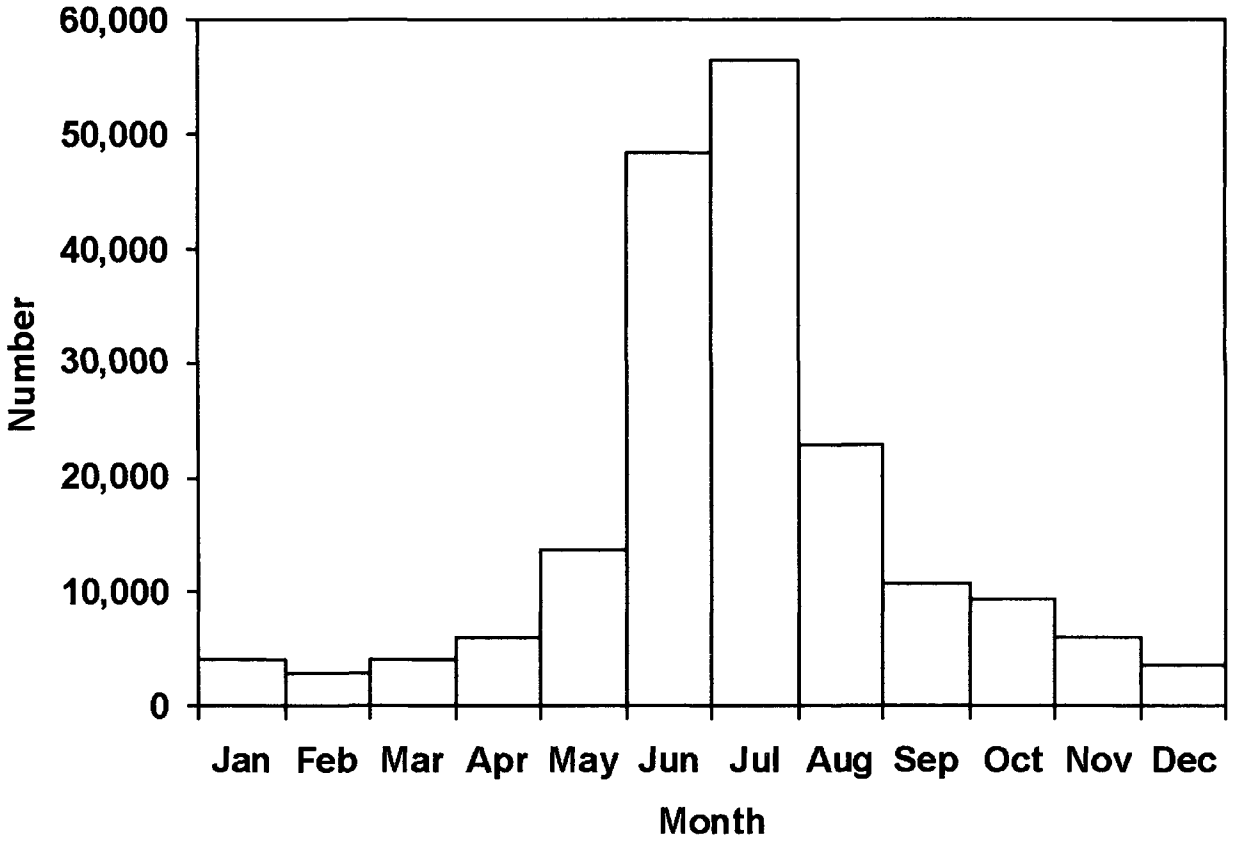
3.9.4. Rate* of Lyme disease†, by year and sex — United States, 1992–2006



* Per 100,000 population.

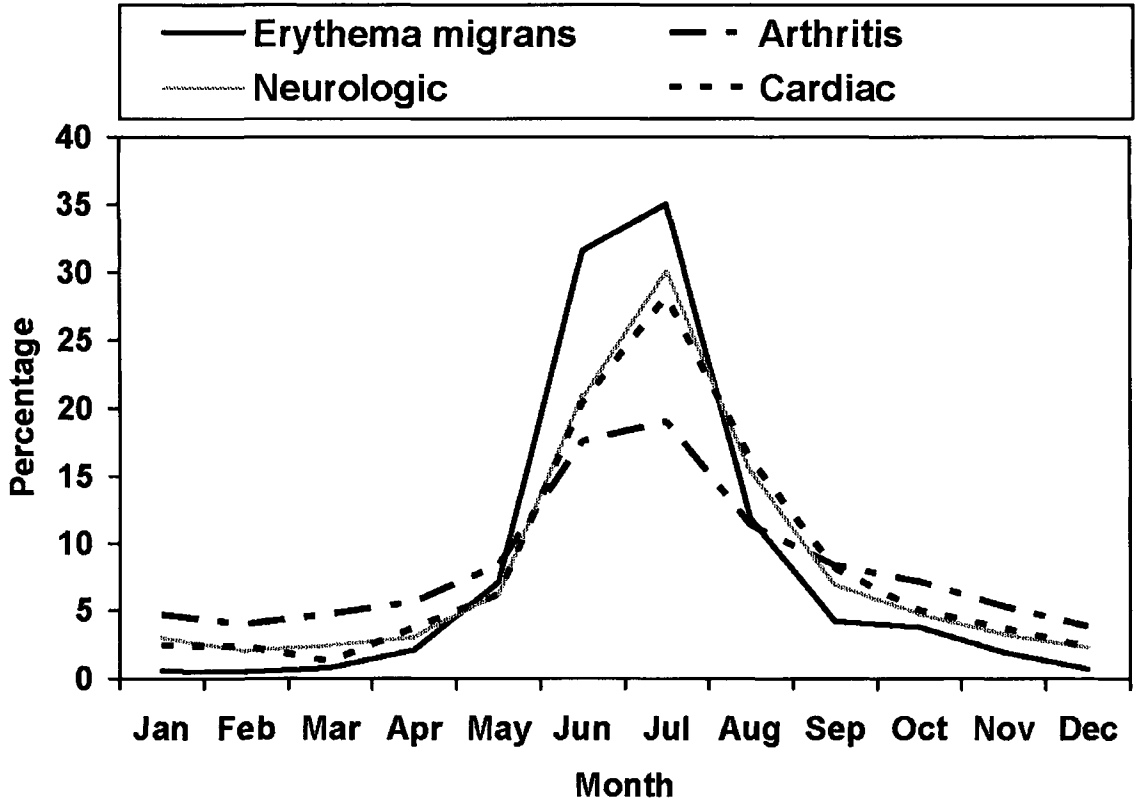
† N = 243,564

3.9.5. Number* of newly reported Lyme disease cases, by month of illness onset — United States, 1992–2006



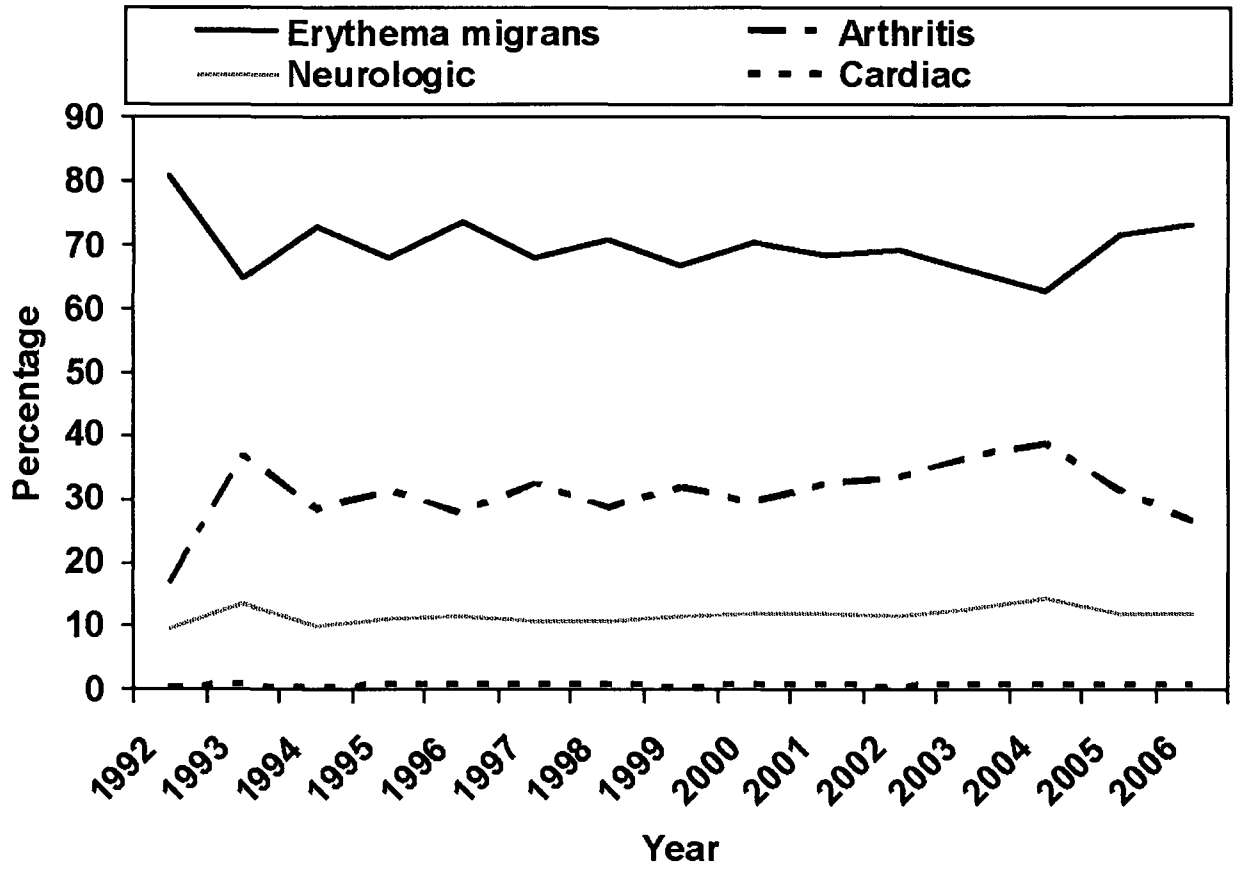
* N = 188,340.

3.9.6. Percentage of symptoms reported among Lyme disease patients,* by month of illness onset — United States, 1992–2006



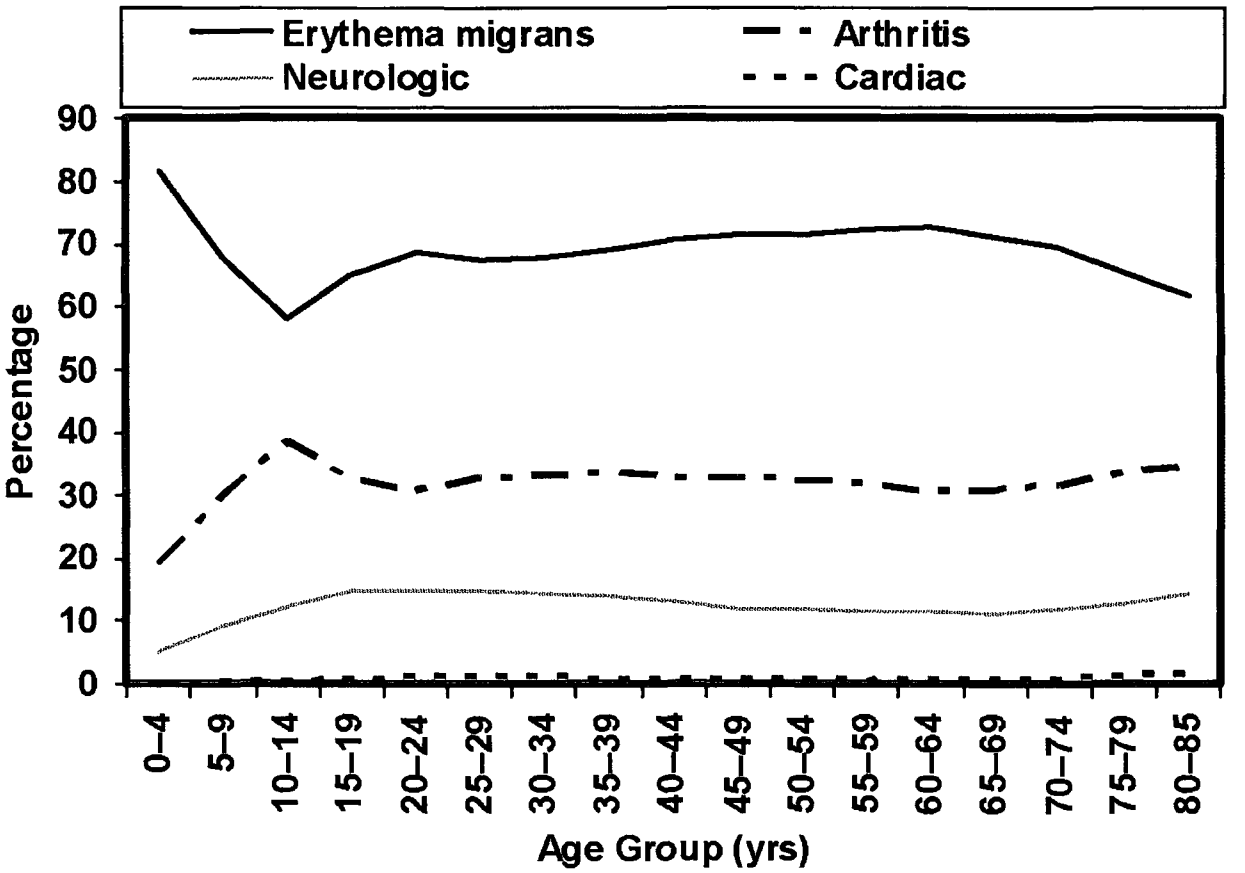
* N = 150,829.

3.9.7. Percentage of symptoms reported among Lyme disease patients,* by year — United States, 1992–2006



* N = 150,829.

3.9.8. Symptoms reported among Lyme disease patients, by age group — United States, 1992–2006



* N = 148,899.

CHAPTER 4

4. STATISTICAL ANALYSIS OF TRENDS IN LYME DISEASE CASES REPORTED 1992 – 2006

4.1. INTRODUCTION

This chapter is an extension of the descriptive analysis of Lyme disease cases reported to the Centers for Disease Control and Prevention (CDC) during 1992 – 2006, published in Morbidity and Mortality Weekly Report Surveillance Summaries (104) and reprinted as Chapter 3 of this dissertation. CDC traditionally does not apply analytic epidemiologic methods to examine, or draw statistical inference from, surveillance data because not all cases of Lyme disease are reported and, more importantly, those reported may not constitute an unbiased sample of the entire population with Lyme disease.

Nevertheless, there is precedence and value in using objective statistical measures to corroborate observations made using simple descriptive analyses of Lyme disease cases reported by individual states. The most common analytic methods utilized in previous studies were chi square ($\chi 2$) tests to compare sample proportions and test for linear trend, odds ratios and relative risks (101, 102, 105-107). Sometimes, a Student's *t* test was used

to compare two sample means (106) and a Z statistic calculated to compare equality of rates (105).

4.1.1. Objectives

The primary objective of this study was to use methods of analytic epidemiology to confirm and strengthen observations drawn from simple descriptive analysis of Lyme disease cases reported to CDC during 1992 – 2006.

4.2. METHODS

The following statistical methods were used to support conclusions drawn from descriptive analyses of 248,074 cases of Lyme disease reported to CDC by health departments during 1992–2006 as reported by Bacon and colleagues (104).

4.2.1. Temporal Trend Analyses

A generalized linear model (GLM) was used to evaluate the influence of year on the following response variables: total number of reported cases; number of counties reporting at least one case; number of counties reporting at least one case from each of the 10 states where Lyme disease is highly endemic (Connecticut, Delaware, Maryland, Massachusetts, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island and Wisconsin); proportion male; proportion of cases reporting onset of illness during summer (Jun, July, or August); proportion with erythema migrans rash (EM); proportion with arthritis and proportion in each age category.

GLM allows the mean of a population to depend on a linear predictor through a nonlinear link function and, therefore, a model can fit response variables with any probability

distribution from the exponential family. This approach was preferred over a $\chi 2$ test for linear trend and other traditional linear methods for several reasons. First, GLM allowed the author to specify the most appropriate response probability distribution (e.g., Poisson, negative binomial, or binomial). Second, a user-specified link function was employed to transform the non-linear response data to a linear scale providing improved estimates of the response distribution mean. Finally the model parameters provided a measure for the influence of explanatory variables on the response. GLM also permitted the evaluation of interaction among explanatory variables and control for possible confounding, although, these features were not needed for the current analyses.

Poisson regression with logarithm link function was used to model the effect of year on count data (number of cases reported nationally, number of counties reporting at least one case nationally, number of counties reporting at least one case in Connecticut, Delaware, Maryland, Massachusetts, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island and Wisconsin). If the Poisson assumption holds (i.e., independent observations occurred randomly over time as evidenced by a response distribution with mean equal to its variance), the model dispersion parameter (or scale) is equal to 1. For these analyses, the dispersion parameter was estimated from the deviance of the fitted model (SAS option scale=D) allowing for adjustment of inferential statistics if overdispersion (scale >1) or underdispersion (scale<1) was detected. This approach is reasonable when data are underdispersed or when overdispersion is modest (108). The dispersion parameter and unscaled deviance statistic provided a measure of dispersion and model fit.

When overdispersion clearly violated assumptions of the Poisson model (i.e., mean and variance were unequal), a negative binomial distribution was used to model count data (number of cases reported nationally, number of counties reporting at least one case nationally) (108). Gardner describes this approach as a form of Poisson regression because a negative binomial model retains the assumption that data were created by a Poisson process (i.e., memoryless) but introduces a random variable to account for unexplained overdispersion (a factor missing from the Poisson distribution). The dispersion parameter for negative binomial models was also estimated from the deviance of the fitted model (SAS option scale=D) to allow for adjustment of inferential statistics when overdispersion was detected. Although overdispersion in negative binomial models also indicates imperfect model fit, the fit is better than with an overdispersed Poisson model (108).

Logistic regression was used to model the effect of year on binomial proportions (number of 0/1 events over a fixed number of trials) (illness onset in June, July, or August, male, reported erythema migrans, reported arthritis, age category). Although normal linear and Poisson regression models can be used as approximations when certain conditions are met, we used a binomial model because it is most appropriate for these data. Model fit was assessed qualitatively by viewing plots of the Studentized deviance residuals by predicted residual values and plots of actual by predicted counts. The degree to which logistic regression models fit the data was evaluated using the Pearson $\chi 2$ and deviance test statistic.

The association between year and the outcome of interest was modeled using Poisson, negative binomial, or logistic regression in SAS Version 9.1 (SAS Institute, Cary, NC) Proc GENMOD. Model coefficients for the independent variables were exponentiated to obtain the single unit average % change in the dependent outcome. Plots used to assess the fit of binomial models were generated using JMP Version 7.0 (SAS Institute, Cary NC).

4.2.2. Comparing Sample Proportions

Among all cases reported 1992 – 2006, a $\chi 2$ goodness-of-fit statistic and two-tailed p-value was calculated using SAS to determine if, under the null hypothesis of equality, the following binary proportions were unequal: the proportion male compared with female; proportion white compared with all other races; and proportion with illness onset during summer (June, July, August) compared with all other months were tested.

Among all cases reported 1992 – 2006, the overall Pearson $\chi 2$ and two-tailed p-value was calculated using SAS to determine if, under the null hypothesis of independence, there were differences in the observed and expected cell frequencies in cross-classification tables with two or more categorical explanatory or response variables. When analyzing cross tabulated data, SAS provides a $\chi 2$ and p-value for the entire table, but only a $\chi 2$ value for each cell of the table. Therefore, the cell-specific $\chi 2$ values were used to identify cells with the largest disparity between expected (row total * column total / total number of reported cases) and observed frequencies. The following tables were evaluated: erythema migrans by summer illness onset; arthritis by summer illness onset;

age category by arthritis; age category among males by arthritis; age category among females; and arthritis by sex.

4.2.3. Comparing Sample Medians

The median age of cases reported from the 10 states where Lyme disease is most common (n=223,880) was compared with the median age of cases reported from all other states and territories (n=18,051) using a non-parametric test (Kruskal-Wallis) in SAS. A non-parametric test was used since the age distribution among reported cases is bi-modal not normal.

4.2.4. Estimates of Risk

Among all cases reported during 1992 – 2006 (n=248,074), the relative risk of reporting arthritis among children 0-4 (n=8,712), children 10-14 (n=13,117), males, 10-14 males (n=8,223) and 10-14 females (n=4,850) was calculated using OpenEpi (www.openepi.com).

4.3. RESULTS

4.3.1. Temporal Trends

The parameter estimate and confidence interval for model coefficients described in this section are provided in Table 4.5.1 for reference. The text below provides the statistical significance of the coefficient followed by an interpretation of the exponentiated coefficient.

The number of cases reported during 1992 – 2006 increased by year of report ($p<0.0001$). On average, a one year increase in year of report yielded a 6% increase in the estimated

mean number of cases reported. Year of report did not affect the number of counties reporting at least one case in the United States (p=0.1369). The number of counties reporting at least one case from each of the 10 states where Lyme disease is highly endemic increased by year of report in Massachusetts (p=0.0218), Minnesota (<0.0001), Pennsylvania (<0.0001) and Wisconsin (<0.0001) and decreased by year of report in Rhode Island (p=0.0114). A 5-year increase in year of report resulted in 37% increase in Minnesota, a 10% increase in Pennsylvania and a 10% increase in Wisconsin. The results for Massachusetts and Rhode Island were disregarded because, although the p-values were significant, a check of the observed versus predicted values revealed an ill fitting model. These states have few counties (15 in Massachusetts and 5 in Rhode Island) and the annual trend in the number of counties reporting at least one case during the 15-year time period was excessively influenced by minor changes.

Year of report had a modest effect on the proportion of cases reported reporting onset of symptoms during June, July, or August (p<0.0001) and the proportion among males (p<0.0001). Among males, a 5-year increase in year of report resulted in an average increase of 9% in the proportion of reported cases (and conversely a 9% decrease in the proportion female). When cases were restricted to those with onset of symptoms during June, July, or August, a 5-year increase in year of report resulted in an average increase of 6% in the proportion of reported cases. Among cases for which at least one symptoms was reported as "yes", the proportion of cases reporting EM (p<0.0001) or arthritis (p<0.0001) was influenced by year of report. A 5-year increase in time resulted in an

average decrease of 3% in the proportion of cases with EM and an average 8% increase in the proportion of cases with arthritis.

The effect of year of report on the estimated mean proportion of reported cases in each 5-year age-group is summarized in Figure 4.6.1. Note that the sum of proportions for all age categories must equal 100%. Therefore, an increase in one or more age groups naturally results in a decrease in others. A one year increase in year of report resulted in a statistically significant ($p<0.05$) increased log odds among persons aged 5 – 19 and 45 – 64 and a significantly ($p<0.05$) decreased log odds among persons aged 25 – 44 and 65 – 74. The average change in the mid-year population estimate by age category during 2000 – 2006 is also provided in Figure 4.6.1.

4.3.2. Proportions

Among all cases reported during 1992 – 2006, a greater proportion of cases were reported among males (53%) than females (47%) ($p=<0.0001$) and an overwhelmingly greater proportion of reported cases were white (94%) ($p=<0.0001$). The majority of cases reported onset of illness during June, July, or August (68%) compared with all other months combined ($p=<0.0001$). Among cases with information on clinical features of illness, 78% of cases with EM reported onset of illness during June, July or August ($p=<0.0001$). Among cases with arthritis, the majority of cases (52%) reported onset of illness during non-summer (August through May) ($p=<0.0001$).

Cases with information on clinical features of illness were tabulated by age category and reported arthritis. The greatest disparity between observed and expected cases of arthritis

occurred in the 0-4 and 10-14 age groups (Table 4.5.2). The number of cases reporting arthritis was less than expected among persons age 0-4 years (1,673 observed; 2,800 expected) and the number was greater than expected among persons age 10-14 (5,072 observed; 4,215 expected). Slightly more than the expected number of cases with arthritis was reported among persons age 35-39 (3,409 observed; 3,222 expected).

Cases tabulated by age category and arthritis were further stratified by sex (Table 4.5.2). Among males, the greatest disparity between observed and expected cases of arthritis occurred in the 0-4 (899 observed; 1,422 expected) and 10-14 (3,242 observed; 2,598 expected) age groups. Among females, the greatest disparity between observed and expected cases of arthritis occurred in the 0-4 (observed 765; expected 1,367), 5-9 (1,955 observed; 2,240 expected), 10-14 (1,809 observed; 1,587 expected), 30-34 (1,296 observed; 1,133 expected) and 35-39 (1,848 observed; 1,593 expected) age groups.

When age category was ignored and reported cases tabulated by sex and arthritis, the overall $\chi 2$ was significant ($p<0.0001$) but the number of observed verses expected cases of arthritis among males and among females differed by less than 2% each.

4.3.3. Medians

The Kruskal-Wallis test indicated a statistically significant difference ($p=0.0446$) in the sample median of cases reported from the 10 states where Lyme disease is highly endemic (median age 40) compared with the mean age of cases reported from all other states (median age 39). However, the p-value was just under the traditional threshold for significance and was not a biologically meaningful finding.

4.3.4. Risk Estimates

The probability of reporting arthritis is 42% lower for children aged 0-4 compared to persons from all other age groups (95% CI 0.558, 0.609) and 3% lower for males compared to females (95% CI 0.967, 0.987). Elevated risk of reporting arthritis was noted among persons aged 10-14 (RR 1.227; CI 1.200, 1.256), 10-14 year old males (RR 1.284; CI 1.248, 1.322) and 10-14 year old females (RR 1.152; CI 1.109, 1.197).

4.4. DISCUSSION

4.4.1. Findings

During 1992 – 2006, the estimated mean number of reported cases increased an average of 6% per year despite large year to year fluctuations in the actual number of cases reported annually (from 3% to 58%).

There was a male predominance among reported cases and the difference increased during the 15-year reporting period. Increased proportion among males might result from increased exposure to tick habitat, reluctance to wear repellent or employ other prevention measures, or hesitation to seek treatment for early signs of illness. An extremely high proportion of whites has been attributed to higher socioeconomic status (109), home or land ownership leading to increased peridomestic exposure to tick habitat (109-112) and ease in rash diagnosis due to light skin color (113). In addition, there may be some unknown host trait among persons of color that protect them against tick bite or reduce the chance of bacterial transmission during tick bite or they may seek health care less frequently although this has not been reported in the peer-reviewed literature.

Further studies are needed to better understand the factors associated with the high proportion of white reported cases. An ecologic study of the relationship between area-based social, economic and health care measures and counties with higher than expected Lyme disease risk may be useful in generating rational hypotheses. Towards this end, a pilot ecologic study has been conducted and the findings are reported in Chapter 5.

Overall, a significantly larger proportion of cases were reported in the summer months (June, July and August) when ticks actively seek mammalian hosts and human outdoor activity is greatest. Although slight, the proportion of cases reported in summer months increased by year during 1992 – 2006.

Among cases reporting at least one symptom, a slight but statistically significant decreasing temporal trend in the proportion of EM and increasing temporal trend in the proportion of arthritis was detected. This refuted our hypothesis that, over time, more cases of Lyme disease were diagnosed and reported during the acute phase of illness when a short course of oral antibiotics was therapeutic. The decreasing trend in the proportion of reported cases with EM might have resulted from failure of patients to seek treatment for EM, misdiagnosis, or an increase in the proportion of patients with unrecognized acute disease (e.g., patients may be asymptomatic or lack EM). The increasing trend in the number of cases with reported arthritis was probably influenced by increased laboratory-based surveillance in some states since the results of serologic tests are required for reported cases of Lyme arthritis but not for EM. However, inherent bias in national surveillance limits interpretation of these results. There is great variability in

the frequency of symptoms reported by states and not all states consistently transmit symptom data to CDC (104). In addition, cases are reported through a mix of physician- and lab-based reporting and enhanced or active surveillance and the quality of symptom data varies depending on the source of the report. Regardless, there is a clear need for targeting prevention campaigns to help the general public recognize and seek treatment for early signs of Lyme disease and to improve physician knowledge about the signs and treatment for early Lyme disease.

Bimodal age distribution has been a characteristic of reported Lyme disease cases since national surveillance began in 1991 (114). The peak in children has been attributed to increased outdoor activity and underutilization of personal protective measures among children (115, 116). Children may also be more likely to develop symptoms after infection (117), although the reasons for this finding are unclear. An explanation for the peak in adults is also speculative. It is biologically plausible that frequent exposure to ticks during childhood years provides some transient protective immunity during early adult years that wanes by mid-life putting persons aged 45 – 59 years at increased risk. Regardless of the explanation, bimodal age distribution is a consistent characteristic of reported Lyme disease cases and provides a clear opportunity for targeted prevention campaigns.

Using trend analysis, we showed that the proportion of cases reported among persons aged 5 – 19 years and among persons aged 50 – 59 years increased by year of report. However, it is important to remember that trends in age group presented in this report

were based on modeling the proportion of cases reported annually in each age group, not the age-adjusted rate per 100,000 for each age group. Therefore, our findings could simply reflect a demographic shift in the U.S. population over the same time period. U.S. Census population estimates by age group during 2000 – 2006 show that trends for some groups, but not all, mirror those observed in reported Lyme disease cases (Figure 4.6.1.B.). Interestingly, when the age distribution of reported Lyme disease cases are tabulated by 5-year time period, it appears that the bimodal peak in adults is shifting to older age groups over time (PS Mead, unpublished data). A closer examination of how changes in population demographics influence the age distribution of reported Lyme disease cases is needed.

Most cases with EM were reported during summer months. This was expected for three reasons. First, nymphal ticks actively seek hosts in early summer when human outdoor activity greatest (17). Nymphal ticks are very small and, therefore, often go unnoticed for several days increasing the opportunity for transmitting *Borrelia burgdorferi*. Finally, onset of rash occurs 3 – 30 days following infection (11). By comparison, most cases with arthritis were reported in non-summer months. This is consistent with the fact that arthritis results from disseminated infection that can occur weeks, months, or years following infection with *B. burgdorferi* (11).

Based the report by Bacon and colleagues that more arthritis was reported among children than other age groups (104), we examined the proportion of arthritis by age group and by sex. We found the greatest disparity between the observed and expected

number of cases reporting arthritis among persons aged 0-4 years (fewer reported cases than expected) and among persons aged 10-14 year (more reported cases than expected). A convincing argument can be made that arthritis was likely under-diagnosed in infants or toddlers. The finding of greater than expected arthritis among youths has been reported before (102, 107) but no explanation for this finding was given. Combined with the fact that Lyme disease was first recognized as arthritis among children living near Lyme, Connecticut, we argue that a biologically relevant explanation for this finding should be explored. For example, perhaps the process of rapid bone and joint development in youths makes it easier for *B. burgdorferi* to invade these spaces causing arthritis. However, if the 0 – 4 age group was removed from the analyses because of negative diagnostic bias, the disparity between the observed and expected number of cases with arthritis in the 10 – 14 age group would be greatly reduced thereby diminishing the significance and significance of this finding.

When data were tabulated by arthritis, age group and sex, interesting differences were noted. Overall, interpretation for young males and females was almost identical to results for both sexes combined (e.g., fewer reported cases than expected among 0 – 4 and more reported cases than expected among 10 - 14). However, a greater than expected number of females aged 30 – 34 years and 35 – 39 years reported arthritis. This might be attributed to diagnostic bias in post-partum or pre-menopausal women (e.g., myalgia and arthralgia common during these life stages may be misdiagnosed as Lyme arthritis). Or, since the preferred antibiotic for treatment of early Lyme disease (i.e., doxycycline) is contraindicated in women who are pregnant or plan to become pregnant, it is possible

that women of child bearing years were less likely to receive antibiotic therapy for signs of early Lyme disease contributing to disseminated infection causing arthritis. If the latter is true, it also reflects a bias in under-reporting cases of Lyme disease for which therapy is not prescribed or a bias towards reporting of more severe illness.

It is important to remember that the Lyme disease surveillance case definition places constraints on reporting of symptom data and interpreting findings of data analyses. For a case of Lyme disease to be reported through the National Notifiable Disease Surveillance System, the patient must have EM or at least one sign or symptom of disseminated infection (i.e., arthritis, neurologic manifestations, or cardiac manifestations). Of the disseminated symptoms, arthritis is most commonly reported and only 9% of cases reported both EM and arthritis during 1992 – 2006 as shown in Figure 4.6.2. As a result, the frequency of cases reporting EM is almost the exact reciprocal of the frequency of cases reporting arthritis. Therefore, a discussion of trends or frequency of reporting arthritis should include a parallel discussion of reporting EM.

In summary, temporal trends observed in the simple descriptive analyses of Lyme disease cases reported during 1992 – 2006 were confirmed using statistical estimates from generalized linear models (i.e., the number of cases reported and number of counties reporting at least one case in Minnesota, Pennsylvania and Wisconsin) and we detected previously unobserved temporal trends in the frequency of reported symptoms (i.e., EM and arthritis). The observed male and white preponderance was supported with a significant $\chi 2$ statistic as was the proportion of cases with reported illness onset during

the summer months of June, July and August. The suggestion that arthritis is reported more frequently (and conversely EM less frequently) among children age 10 – 14 years was supported with statistical analyses and we further show a greater than expected number of cases with arthritis (and conversely less than expected EM) reported among women aged 30 – 39 years.

4.4.2. Limitations

The limitations of the surveillance data used in this analysis were described previously by Bacon and colleagues (104). This study was limited to examination of a few observations noted previously rather than a systematic approach to data analysis and unexpected but important findings may remain undiscovered.

4.4.3. Strengths

The dataset used in this analysis represents the largest collection of reported Lyme disease cases ever analyzed and provides more than enough power to calculate meaningful statistics. The findings presented here are concordant with observations drawn from the descriptive analyses adding strength and cohesion to the conclusions.

4.5. TABLES

4.5.1. Results of temporal trend analyses using generalized linear models using a negative binomial[1], Poisson[2], or binomial distribution[3]

	Coeff.	Coeff. 95% LCL, UCL	Coeff. $\chi 2$	Coeff. $\chi 2$ p-value
Number cases by year[1]	0.0614	0.0440, 0.0798	47.58	<0.0001
Number US counties by year[1]	0.0050	-0.0016, 0.0116	2.21	0.1369
Number CT counties by year[2]	0.0000	-0.0414, 0.0414	0.00	1.0000
Number DE counties by year[2]	0.0000	-0.0676, 0.0676	0.00	1.0000
Number MD counties by year[2]	0.0043	-0.0009, 0.0095	2.60	0.1071
Number MA counties by year[2]	0.0055	0.0008, 0.0102	5.26	0.0218
Number MN counties by year[2]	0.0624	0.0489, 0.0759	81.83	<0.0001
Number NJ counties by year[2]	0.0010	-0.0004, 0.0024	2.04	0.1529
Number NY counties by year[2]	0.0016	-0.0093, 0.0124	0.08	0.7782
Number PA counties by year[2]	0.0189	0.0126, 0.0252	34.27	<0.0001
Number RI counties by year[2]	-0.0095	-0.0169, -0.0021	6.40	0.0114
Number WI counties by year[2]	0.0194	0.0128, 0.0260	33.29	<0.0001
Proportion summer onset by year[3]	0.0115	0.0091, 0.0139	87.68	<0.0001
Proportion male by year[3]	0.0165	0.0146, 0.0185	272.64	<0.0001
Proportion EM by year[3]	-0.0056	-0.0088, -0.0025	12.25	0.0005
Proportion arthritis by year[3]	0.0147	0.0115, 0.0178	84.04	<0.0001

[1]generalized linear models using negative binomial distribution and log link function

[2]generalized linear models using Poisson distribution, log link function and scale=deviance

[3]generalized linear models using binomial distribution and logit link function

4.5.2. Chi-square test of proportions among Lyme disease cases reporting arthritis, - United States, 1992 – 2006

No. cases observed No. cases expected Cell Chi-Square	Arthritis[1]	Arthritis among males[2]	Arthritis among females[3]
0 – 4	1673 2800 453.4	899 1422 192.4	765 1367 265.1
5 – 9	4965 5269 17.5	2993 3003 <0.1	1955 2240 36.3
10 – 14	5072 4215 174.2	3242 2598 159.7	1809 1587 31.0
15 – 19	2771 2727 0.7	1629 1692 2.4	1133 1016 13.4
20 – 24	1631 1694 2.4	914 1014 9.8	713 671 2.6
25 – 29	1712 1667 1.2	899 912 0.2	810 751 4.7
30 – 34	2396 2313 3.0	1094 1173 5.3	1296 1133 23.4
35 – 39	3409 3222 10.9	1549 1622 3.3	1848 1593 40.9
40 – 44	3988 3879 3.1	1965 1993 0.4	2008 1874 9.7
50 – 54	3921 3859 1.0	1915 1923 <0.1	1992 1926 2.3
55 – 59	3279 3291 <0.1	1655 1634 0.3	1609 1645 0.8

No. cases observed No. cases expected Cell Chi-Square	Arthritis[1]	Arthritis among males[2]	Arthritis among females[3]
60 – 64	2430 2537 4.5	1212 1256 1.5	1208 1273 3.4
65 – 69	2124 2200 2.6	1108 1069 1.4	998 1122 13.7
70 – 74	1776 1789 0.1	916 856 4.1	854 927 5.8
75 – 79	1431 1357 4.0	708 618 13.0	718 737 0.5
80 – 84	768 710 4.8	369 313 10.0	397 395 <0.1
85 - 89	323 284 5.4	138 121 2.3	185 163 3.0
90 – 94	90 67 7.8	30 20 4.7	59 46 3.6
95 – 99	24 17 2.5	9 8 0.2	15 9 3.2
>100	3 4 0.2	1 2 0.4	2 2 <0.1

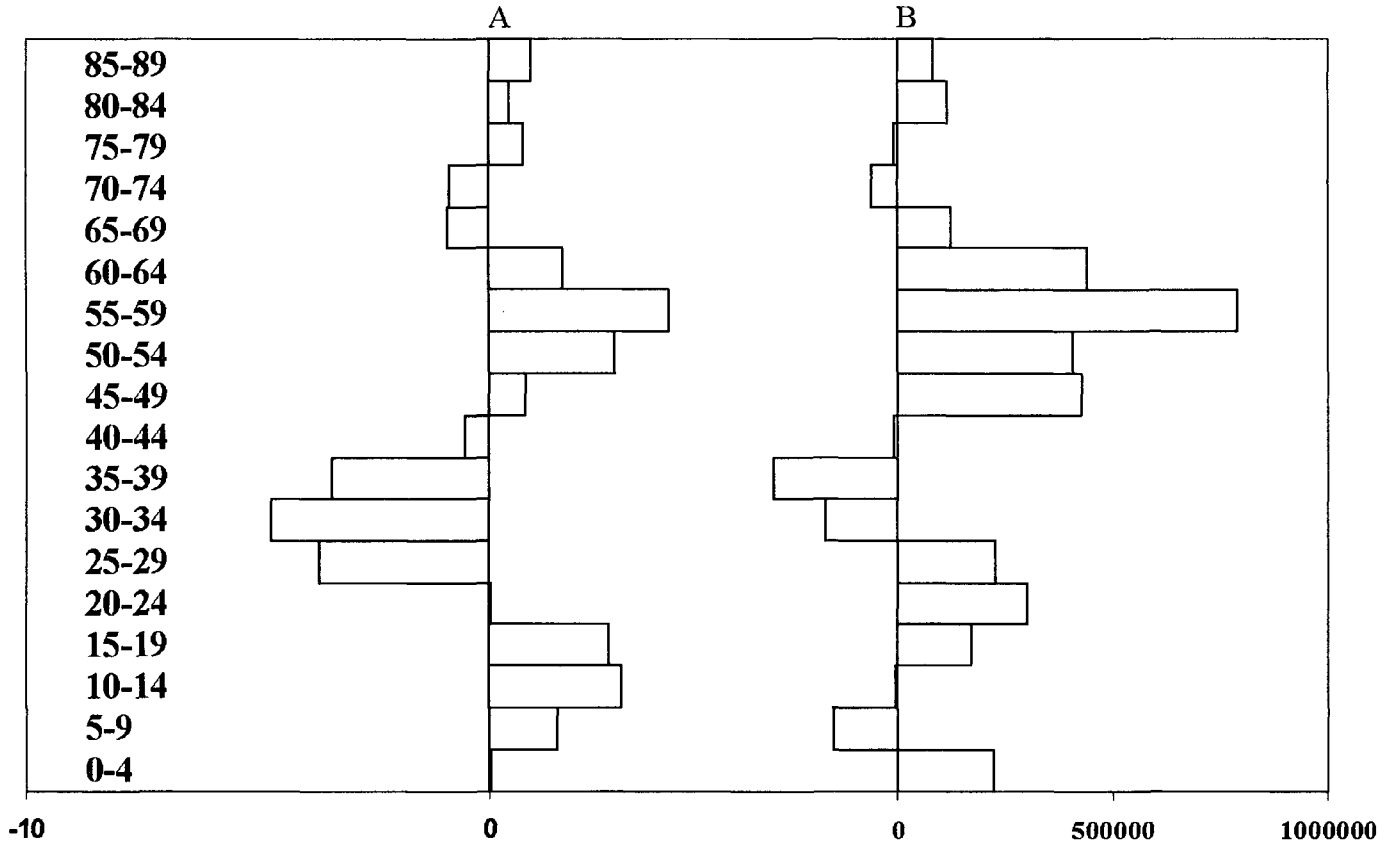
[1] N=47,937 cases reporting arthritis among 149,174 with at least one symptom reported

[2] N=25,324 cases reporting arthritis among 80,158 males with at least one symptom reported

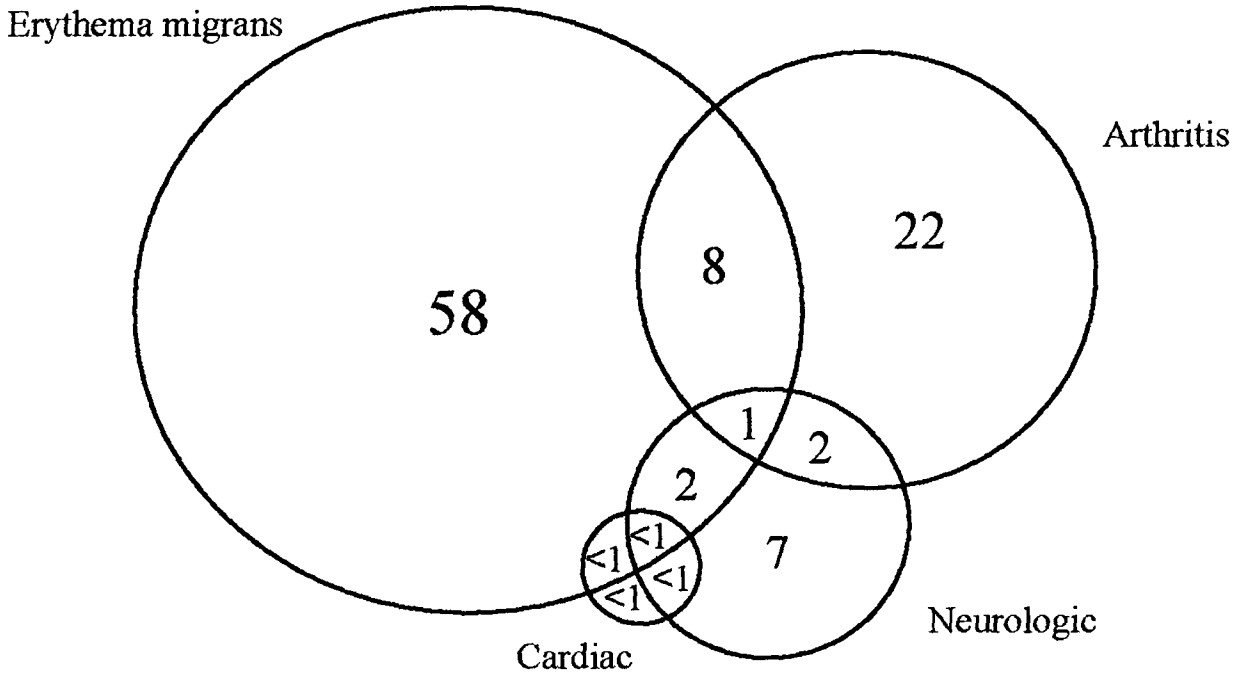
[3] N=22,427 cases reporting arthritis among 68,544 females with at least one symptom reported

4.6. FIGURES

4.6.1. A. Average one-year percent change in the log odds for each age category among reported Lyme disease cases, - United States 1992 – 2006. B. Average one-year change in the U.S. Census mid-year population estimate, by age group - United States 2000 – 2006.



4.6.2. Percentage of symptoms reported among Lyme disease patients*, by symptom† — United States, 1992–2006



*N = 150,829 cases reporting at least one symptom

†arthritis and cardiac ≤1% (not shown)

CHAPTER 5

5. ECOLOGIC STUDY OF SOCIAL AND ECONOMIC RISK FACTORS FOR LYME DISEASE

5.1. INTRODUCTION

In a 1993 manuscript published in *Science*, Drs. Alan Barbour and Durland Fish wrote on "*The Biological and Social Phenomenon of Lyme Disease*" (118). These experts reminded readers that characterizing a zoonosis requires understanding microbiology of the agent, ecology of the vector and its reservoir hosts, and the epidemiology of human disease. In addition, they emphasized that a full understanding of Lyme disease would require consideration of poorly understood behavioral and economic factors affecting, or resulting from, its emergence. In the 15 years since this publication, our understanding of the biological factors required for propagation of Lyme disease has greatly improved through extensive scientific research. However, gaps exist in our understanding of the socioeconomic factors that affect the occurrence of Lyme disease. The current study is a first step towards increasing knowledge in this area.

The zoonotic cycle for Lyme disease is well-documented for most endemic areas in the United States (19). Factors influencing the distribution, density and infection prevalence of *Ixodes* have received extensive study within small geographic foci using primary data

and on larger spatial scales by combing intelligence on the local scale with existing data on the environment, climate, and vegetation (119). The latter has been facilitated by the increased availability of geocoded data and improvements in geospatial information technology over the past few decades.

The demographics of Lyme disease cases reported in the United States have also been described in detail (104, 120). Analytic studies of human risk primarily focused on individual practices that affected human exposure to ticks or suitable tick habitat (110, 114, 121-125). However, few authors included measures of socioeconomics when examining the relationship between reported Lyme disease cases and characteristics of their behaviors or physical environment. Although national surveillance for Lyme disease has been conducted since 1991 (96, 126), no published studies that examined the relationship between cases reported nationally and social or economic data that are also available for the entire United States were found.

Here we describe a pilot study that explored the use of existing data to identify unknown correlates of reported Lyme disease incidence. Although variables related to tick distribution or habitat were included in the analyses, our primary interest was on other characteristics (e.g., social and economic factors and indicators of health care availability). An ecologic study design was employed since available data were grouped at the county level.

5.1.1. Potential Use of Study Findings

This study will stimulate discussion about the use of existing county-level data to identify risk factors for Lyme disease and other nationally notifiable diseases. Our research provides an example of how an epidemiologic study of national Lyme disease surveillance data can move beyond simple descriptive analysis by combining existing data on area-based measures of potential risk with information on reported cases. Result interpretation generates hypotheses about the possible influence of socioeconomic factors on reported Lyme disease risk and provide a platform on which additional studies can be built. Further, we demonstrate that studies of entomologic or human risk for Lyme disease should be conducted separately for northeastern and north central United States.

5.1.2. Objectives

This study was designed to achieve three specific objectives: 1) to identify spatial and temporal clusters of increased Lyme disease risk in the United States using a method that was not restricted by state lines; 2) find existing and available data on area-based measures for risk factors potentially associated with Lyme disease risk; 3) determine if increased Lyme disease risk could be partially explained using existing data on environment, socioeconomics, and health care.

5.2. LITERATURE REVIEW

The Lyme disease zoonotic cycle (19), national surveillance and reporting practices (96, 103, 120), and a descriptive summary of cases reported during 1992 – 2006 (104) were provided in previous chapters and, therefore, will not be reviewed here. It is worth

restating, however, that available data on the number of Lyme disease cases reported in the United States were obtained from the National Notifiable Diseases Surveillance System (38). Since it is unknown whether cases reported through this surveillance system constitute an unbiased sample of all Lyme disease cases, it is important to note that the current study investigates risk factors associated with a reported case of Lyme disease. Also note that throughout this manuscript "Lyme disease zoonotic cycle" will be used to describe the collective biology of Lyme disease including the ecology of ticks, their habitat, and hosts.

The literature reviewed below includes investigations to identify correlates of the Lyme disease zoonotic cycle and risk factors for human disease. Methodologies used in the current analyses will also be reviewed including the use of the SaTScan™ spatial scan statistics for identifying excess disease risk (or "clusters"), the use of existing data for epidemiologic studies of disease risk, and the appropriateness of an ecologic study design with logistic regression analyses for producing hypotheses-generating data.

5.2.1. Correlates of the Lyme Disease Zoonotic Cycle

In the United States, Lyme disease is caused by the spirochete *Borrelia burgdorferi* when transmitted to humans by the bite of an infected *Ixodes* spp. tick (19). Therefore, it seems that defining the geographic risk for Lyme disease might be as simple as defining the distribution of *B. burgdorferi*-infection *Ixodes*. But tick collections are labor intensive and populations are known to fluctuate as a result of changes within their microclimate requiring repeated sampling to establish valid entomologic indices. In 1998, Dennis and colleagues reported on the distribution of *Ixodes* in the United States using data reported

in 182 manuscripts published during 1966 - 1996, 108 records available in the National Tick Collection database of the National Museum of Natural History from 1907- 1995, 99 questionnaires completed by public health officials, entomologists, and Lyme disease investigators, and 27 unpublished academic papers or local bulletins (127). Counties were given an arbitrary designation of "established" *Ixodes* distribution if at least 6 ticks or 2 of the 3 life stages had been identified in a single collection period within the county. *Ixodes* distribution was considered "reported" if at least 1 tick of any life stage had been identified or reportedly collected at any time in a county. Though the validity of their methods can be questioned, the immense effort towards creating the first *Ixodes* distribution map for the United States is commendable.

In the last decade or so, much work has gone into defining the correlates of *Ixodes* distribution and density in the hopes of defining or predicting entomologic risk for Lyme disease (119). A thorough review of literature relating spatial distribution and density of *Ixodes* to characteristics of their environment was published by Killilea and colleagues in June 2008 (119). Killilea reported that the environmental variable consistently associated with increased entomologic risk was the presence of forests, but that the underlying reasons behind this association requires further study. Other factors potentially associated with entomologic risk were temperature, saturation deficit (a measure of humidity), latitude, soil type, and deer density. However, drawing conclusions about the influence of factors on entomologic risk is difficult because published studies differ in regard to spatial scale, methods for establishing tick density (i.e., tick collection methods), and explanatory variable measurement. In addition, results of studies are

sometimes discordant. For example, deer density was associated with larval density on coastal islands of Massachusetts, but others have found weak, no, or negative associations between ticks and deer.

In an attempt to use new methodologies to improve on the efforts of Dennis and colleagues (127), and recognizing that the increasing range of *I. scapularis* suggested that not all suitable habitats were occupied, Brownstein and colleagues published an *I. scapularis* distribution map for the United States in 2003 (128). The distribution was based on a climate model built to predict the probability that a given geographic cell could support *I. scapularis* populations. Spatial distribution maps like these have been relied upon, perhaps too heavily, to define the risk for human Lyme disease. In fact, Brownstein and colleagues later reported that their studies in southern Connecticut support the concept that the occurrence of Lyme disease is driven more by human behaviors than entomologic risk (129). Others have argued for the use of epidemiologic data (combined with vector data) when modeling the risk of human exposure to vector-borne pathogens because of the potential for finding associations between human disease and socioeconomic factors, rather than just environmental factors influencing entomologic risk (130).

5.2.2. Risk Factors for Lyme Disease in Humans

Studies that evaluated risk factors for human Lyme disease can be grouped in two categories. First we consider traditional epidemiologic studies that evaluated risk factors for human Lyme disease using data on individuals, their behaviors and environment. The results of analytic studies have found that the presence of ticks or deer, rural residence,

woodlands on or near residence, brush or leaf litter on residential property, and various outdoor activities or occupations increase the risk of disease (110, 114, 121-125). These individual-level studies either did not evaluate measures of socioeconomics or access to healthcare, or the results were not reported. Second we consider spatial studies using group-level environmental predictors with rare inclusion of factors related to population demographics or socioeconomics (119). Killilea and colleagues summarized the published literatures relating the spatial distribution of Lyme disease cases to environmental factors as proxy for tick distribution and again noted that forests (or a surrogate measure for forests) are the only factor consistently associated with human risk. Other environmental factors with inconsistent or weak associations were low-density residential developments, population density, percentage of land-cover edge, small-mammal abundance and climate. Median household income was the only socioeconomic data included in the literature reviewed by Killilea and colleagues It was significantly associated with 2,137 Lyme disease cases grouped by 514 geographic regions (bound by roads) (109, 131).

5.2.3. Excess Disease Risk Using SaTScan™

SaTScan™ version 7.0 software utilizes a scan statistic to detect statistically significant space, time, or space-time clusters of events (132). SaTScan™ is a trademark of Martin Kulldorff who described the statistical theory used in the software in 1997 (133). SaTScan™ software was developed under the joint auspices of (i) Martin Kulldorff, (ii) the National Cancer Institute, and (iii) Farzad Mostashari of the New York City Department of Health and Mental Hygiene. It can be applied to surveillance data collected under a Poisson process (134). Features that make SaTScan™ suitable for

surveillance data include (i) automatic adjustment for uneven population density, (ii) user specified adjustment for categorical covariates, temporal trends, known space-time clusters, and missing data, (iii) elimination of pre-selection bias since clusters are identified without specifying their size and location, (iv) adjustment for multiple testing, (v) p-value based hypothesis testing, and (vi) identification of cluster location and size when the null hypothesis is rejected (135). The scan statistic itself is described in more detail in the methods section below.

Although previous reports of studies using SaTScan™ to identify excess risk among reported Lyme disease cases could not be found, it has been used to identify other infectious diseases clusters in dozens of published studies, including a few involving zoonotic or vector-borne infectious diseases. For example, Lian and colleagues used SaTScan™ to retrospectively detect nine space-time aggregates and five high-risk areas within the 2002 outbreak of equine West Nile virus infection in Texas (136). Mostashari and colleagues detected active spatial clustering of dead bird reports in New York City in 2000 using SaTScan™ and considered the usefulness of results in providing an early warning system for West Nile virus activity the following year (137). A high-risk spatial cluster of human granulocytic ehrlichiosis cases was identified near Lyme, Connecticut using the residential address for 245 confirmed cases during 1997 – 2000 (138). This was the same, area around the mouth of the Connecticut River, identified as having increased risk using a spatial filtering (smoothing) method in the same study. Recuenco and colleagues reported on the retrospective evaluation of space time patterns of raccoon rabies in census tracts of New York State during 1997 – 2003 (139). Using SaTScan™

they conducted cluster analyses without and with adjustment for covariates in an attempt to observe the affect of these factors on spatial clustering. Covariates evaluated included land use type, elevation, population density, the presence of major roads and rivers/lakes, and a summary measure of county, latitude and ecoregion designed to account for large scale geographical variation. The inclusion of covariates allowed the authors to consider the potential influence of these factors on raccoon rabies and provided insight to the possible reasons for the highest risk areas.

5.2.4. Using Existing Data to Explain Excess Disease Risk

Many epidemiologic studies use at least some secondary data (140). For instance, the use of existing disease registry and health care administrative data has been keystone in the field of health care research (141). Grady and Wallston point out that *post hoc* analysis of existing data should not be considered a replacement for designed epidemiologic studies, but rather a good starting point for a new research project and they offer the following suggestions for improving the usefulness of examining secondary data. The first suggestion is to develop a question and seek data to help answer that question. The second is to carefully consider the source and purpose of the initial data collection, including the potential for bias, and learn everything you can about how the data were collected (e.g., subjects recruitment, questionnaires, chart extraction, and data entry). The final suggestion is to design a follow-up study to check the validity of your findings (141).

Several published studies using existing data to learn about risk factors potentially associated with disease risk contributed to the conceptual framework for the current

study. The most influential was a cross-sectional ecologic study by MacKinnon and colleagues where case reports were obtained from a disease registry, the Florida Cancer Data System. SaTScan™ was used to identify Florida census blocks with an excess of late stage breast cancer (142). Explanatory data on socioeconomics was obtained from the U.S. Census Bureau (as a ratio of income to poverty), on urbanization using Urban/Rural Continuum Codes, on mammography prevalence from the Behavioral Risk Factor Surveillance System for the State of Florida, and on insurance status from the Florida Cancer Data System. Using multivariate logistic regression to control for urbanization, mammography, and insurance, the authors found that women living in neighborhoods of severe and near poverty were more likely to live in areas of higher-than-expected incidence of late state breast cancer when compared with women living in non-poverty. MacKinnon noted that individual-level measures of socioeconomics are not available in most surveillance systems and that existing U.S. Census-derived measures are meaningful indicators of the socioeconomic context of an area and not mere proxies for individual-level data.

A county-level ecologic study reported by Carozza and colleagues measured the association between childhood cancer incidence (obtained from the North American Association of Central Cancer Registries during 1995 – 2001) and U.S. Census-based agricultural data while controlling for county-level age and sex using logistic regression (143). The authors compared the results of logistic regression with those from Poisson regression and noted that they were about the same so they only published results of the logistic models.

Although many studies of Lyme disease utilize existing data on reported cases and environmental factors as previously described, only one published study that included a socioeconomic indicator could be found. To control for socioeconomic differences in case reporting among 12 Maryland counties, Jackson and colleagues included median annual household income in a spatial risk analysis of Lyme disease and forest-edge habitat (109, 131). They found that three variables together (percent herbaceous edge adjacent to forest, percent herbaceous cover, and median annual household income) explained the most variation in Lyme disease incidence. A December 2008 search of PubMed using the terms Lyme and income, Lyme and poverty, Lyme and insurance, and Lyme and healthcare produced no other relevant research reports. PubMed is the search engine for the MEDLINE database that contains life science articles published since 1950 and is maintained by the National Libraries of Medicine and the National Institutes of Health (http://www.ncbi.nlm.nih.gov/pubmed/).

5.2.5. Ecologic Study Design and Justification for Use

Ecologic studies evaluate the characteristics of groups rather than individuals and they often utilize secondary data (144). This study design is generally used by epidemiologist to look for spatial or temporal patterns of disease in the hopes of identifying a possible etiology (i.e., exploratory) or to assess the association between an ecologically measured exposure level and rate of disease among groups (i.e., etiologic). Ecologic studies permit the application of analytic statistical methods to existing data, but compared to other analytic studies, are generally less expensive and more practical to complete. However, the ability to assess causality is greatly diminished because of several important

limitations of the ecologic study design. Perhaps the most important is the possibility that exposure-outcome associations at the group level do not relate to a biologic effect at the individual level (also known as ecologic fallacy or ecologic bias). Second, confounding and effect modification (i.e., interaction) are different at the group and individual levels and, therefore, require special consideration. In ecologic studies, confounding bias may occur if the background rate of disease in the unexposed population varies across groups. In this case, the potential confounding exposure can be assessed and bias may be reduced by including it as a covariate in regression analyses. Within group confounding bias is harder to detect and reduce. Bias can be assessed and reasonably controlled if the exposure is homogenous within each group. However, if the confounding exposure is heterogeneous within each group, it can not be controlled without information on individual exposures or exposure variability within the group (Wakefield 2008). Furthermore and in contrast to individual-level epidemiologic studies, adjusting for confounders may overinflate estimates of ecologic risk. The assessment of interaction between variables is also troublesome in ecologic studies because the combined effect of two group exposures may result in an ecologic risk estimate greater than would be observed at the individual level. Again, inability to assess the effects at the individual level severely limits the interpretation of results. Other limitations include undefined temporality of exposure and outcome, strong collinearity among some explanatory variables, migration across groups contributing to misclassification, and incomplete or unavailable data on the exposures or behaviors of interest.

Despite these drawbacks, the ecologic study design was chosen for the current project because: 1) exposure data for individual Lyme disease cases were unavailable; 2) county of residence was provided for 98% of Lyme disease cases reported during the study period; and 3) little was known about the associations between socioeconomic characteristics and reported Lyme disease incidence and, therefore, evaluating ecologic risk was an appropriate starting point for this new area of research. Furthermore, the use of existing data eliminated the need for primary data collection reducing investments of time and resources required for this pilot study.

Morgenstern reported that effect estimation in ecologic studies is determined by regressing the group-specific disease rates (a continuous scale) on the group-specific exposure prevalence using linear or log-linear models (144). Using hypothetical analyses, Bjork and Stromberg noted that linear (for continuous outcomes) and logistic (for binary outcomes) regression models performed similarly when the effect of exposure was modest (e.g., resulted in small odds ratios) (145). Several additional studies using logistic regression to evaluate associations between ecologic risk factors and binary outcomes have been reported in the peer-review literature and shaped the design of the current analyses (139, 142, 143). Importantly, Carozza and colleagues used experimental data to demonstrate that results of logistic regression models and log-linear (Poisson) models were comparable (143).

5.3. METHODS

5.3.1. Lyme disease Cases Reported 1992 – 2006

During 1992–2006, a total of 248,074 cases of Lyme disease were reported to CDC by the 50 states, the District of Columbia, and Guam as part of the National Notifiable Disease Surveillance System (104). The number of reported cases increased 101% from 9,908 cases in 1992 to 19,931 cases in 2006. Greater than 90% of cases are reported from ten states, Connecticut, Delaware, Massachusetts, Maryland, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island, and Wisconsin. Each case report contained data on patient age, sex, race, county and state of residence, date of illness onset, and reported signs and symptoms. For these analyses individual cases were aggregated by county of residence and year of report.

5.3.2. SaTScan™ Software and Cluster Detection

SaTScan™ was used to detect spatial-temporal high risk clusters among U.S. Lyme disease cases reported during 1992 – 2006. To accomplish this, SaTScan™ gradually scanned the data with a cylindrical window. The cylinder radius related to geographic space and the cylinder height related to time. As it continuously scanned, the cylinder evaluated space-time combinations varying in size from zero up to 50% of the U.S. population and 50% of the 15-year study period. In SaTScan™, count data could be analyzed using a Poisson, Bernoulli, or space-time permutation model. We reported in Chapter 4 that the Lyme disease surveillance data are temporally over dispersed, however, of the choices available in SaTScan™ the Poisson probability distribution most closely fit our data. Under a Poisson assumption the number of observed cases and

expected cases within each cylinder was noted and compared with the number of observed and expected cases outside the cylinder. A likelihood ratio test statistic was calculated based on this comparison (135). The cylinder with the maximum likelihood ratio was named the most likely high risk cluster (the least likely to occur by chance). For cylinders sharing a common geographic center, only the cluster with the highest likelihood ratio was reported by SaTScan™ (i.e., clusters that overlap the most likely cluster were not reported). Instead, geographic and temporally distinct secondary clusters that were statistically significant on their own strength were reported.

To obtain the p-value for a likelihood ratio test statistic, its distribution was obtained using Monte Carlo hypothesis testing. In this process, 999 random replications of the data set were generated and the test statistics from the real data were ranked among the test statistics for the random data sets. If the rank was among the 5 percent highest likelihood test statistics, then the test was considered significant at the alpha=0.05 level and the null hypothesis that no clusters were present was rejected.

The following SaTScan™ settings for basic and advanced features were employed to identify space-time clusters where the number of reported cases was in greatest excess. The input case file included the number of Lyme disease cases reported by county of residence and year during 1992 - 2006. Latitude and longitude for the geographic center of each county was specified in a coordinates file. The U.S. Census population estimate for 1992 was provided in the population file and the data for following years were estimated by SaTScan™ using a linear interpolation (132). Retrospective space-time

analysis facilitated the identification of 'alive clusters' (those lasting until the end of the study period) or 'historic clusters' (those that end before 2006). The maximum spatial size was set to 50% of the U.S. population and the maximum temporal size was set to vary from one to 7 years (up to 50% of the study period). These are SaTScan™ default settings recommended for initial studies ((132).

SaTScan™ calculated a risk estimate for each space-time cluster and each geocoded location (i.e., county) within a cluster:

$$\frac{\text{cluster observed / cluster expected}}{\text{(total cases – cluster observed) / (total cases – cluster expected)}}$$

It can be shown that the SaTScan™ risk estimate is equivalent to a standard relative risk (computation by A. Bachand not shown):

$$\frac{\text{probability of disease among the exposed}}{\text{probability of disease among unexposed}}$$

5.3.3. Outcome Variables

SaTScan™ produced the following statistics for each cluster identified: geographical coordinates and time period; expected and observed number of cases per 100,000 population, relative risk, likelihood ratio, Monte Carlo p-value. We created a national outcome variable based on whether a U.S. county was located within a most likely active cluster or not. This categorization is described in detail in the results section below.

SaTScan™ also provided the number of cases, expected cases, and relative risk for each county within an individual active cluster. The county-specific relative risks were used to

create a multinomial outcome variable for regression analysis within the active high risk clusters. This categorization is described in detail in the results section below.

5.3.4. Explanatory Variables

Existing data on the characteristics of counties and their residential populations were obtained for use as explanatory variables in regression analyses. Hundreds of area-based measures were identified. Those chosen for analysis and the source from which they were obtained are described briefly here. They fall into three broad categories; those describing the county physical environment, socioeconomics of the county or its residents and the availability of health care services within the county.

5.3.4.1. Physical Environment

Ixodes scapularis and *I. pacificus* are the only ticks known to transmit *B. burgdorferi*, the agent of Lyme disease in the United States (19). Data on the reported distribution of these ticks were reconstructed from the 1998 report by Dennis and colleagues (127). Data from the table listing the counties with reported or established tick populations were cross-checked with the map of reported distribution by county. When data conflicted, the map data were used for the reconstructed data layer.

Bailey's Ecoregions and Subregions of the United States, Puerto Rico, and the U.S. Virgin Islands were used as an indicator of county physical environment and, perhaps, as proxy for habitat that support the natural zoonotic cycle for Lyme disease. This classification was developed by Robert G. Bailey, U.S. Department of Agriculture, Forest Service, Fort Collins, Colorado to define areas that share common climate, vegetation, geology, soils, water and natural communities. Bailey mapped three hierarchical

subdivision (domains, divisions, and provinces) in 1976 and then updated the subdivisions in 1995 (www.fs.fed.us/land/ecosysmgmt). Division classifications were used for the current analyses because it appeared the best scale for county-level analyses. The map layer was downloaded at www.nationalatlas.gov and the division covering the majority of each county was extracted using the zonal statistics function in ArcMap version 9.0 (Redlands, CA).

Mean elevation for each county was extracted from the North America shaded relief map available from the Environmental Systems Research Institute, Inc. (ESRI, Redlands, CA). The grid data were derived from the global digital elevation model from the U.S. Geological Survey's Earth Resources Observation Center Data Center Distributed Active Archive Center. Mean elevation for each county was obtained using the zonal statistics function in ArcMap version 9.3 (Redlands, CA) as previously described.

The U.S. Geological Survey and the U.S. Department of Agriculture Forest Service who collaborate to collect forest cover information from satellite images. The Forest Cover Types image showing the distribution of 25 classes of general forest cover as well as water and non-forest land, in the United States and Puerto Rico was downloaded at www.nationalatlas.gov. The image was produced using Advanced Very High Resolution Radiometer composite images and Landsat Thematic Mapper data from the 1991 growing season. The class covering the majority of each county was extracted using the zonal statistics function in ArcMap version 9.0 (Redlands, CA) as described in section 4.3.6.

5.3.4.2. Social and Economic Characteristics

Murder was selected to represent county-level crime. The U.S. Crimes Database contains data and statistics for eight crimes reported during 1994 – 2002 (murder, forcible rape, robbery, aggravated assault, burglary, larceny, motor vehicle theft, and arson) were downloaded from www.nationalatlas.gov. The data are collected by the Federal Bureau of Investigation Uniform Crime Reporting Program. More than 18,500 law-enforcement agencies representing more than 89% of the U.S. population, participate in this program. The data are the number of murders for a given year normalized by the population for the same year (i.e., number of murders per person by county). Murder was defined as the willful (non-negligent) killing of one human being by another, including non-negligent manslaughter.

Per capita personal income was selected as a measure of income for county residents. Income and employment data has been collected by the U.S. Department of Commerce Bureau of Economic Analysis since 1979. County-level data on total personal income, number of jobs, average wage per job, per capita personal income, and per capita number of jobs during 1992 – 2003 were downloaded at www.nationalatlas.gov. The data are not adjusted for annual inflation. Per capita calculations were achieved using U.S. Census annual midyear population estimates. Per capita person income is calculated as the total annual personal income of county residents minus contributions to social insurance (Social Security, Medicare, Medicaid, etc) divided by the resident population.

The annual average unemployment rate was used as a measure of county labor economics. The U.S. Department of Labor, Bureau of Labor Statistics collects information on labor economics and statistics. Data for total labor force the number of employed and unemployed, and the unemployment rate for 2000 – 2004 were downloaded at www.nationalatlas.gov. The unemployment rate was defined as the ratio of unemployed persons to the civilian labor force expressed as a percent [i.e., 100 times (unemployed/labor force)].

A measure of urbanicity for each county was assigned one of 12 Urban Influence Codes developed by the U.S. Department of Agriculture, Economic Research Service in 2003. The 12 groups are based on population and commuting data from the 2000 Census for metropolitan counties (determined by the Office of management and Budget) and adjacency to metropolitan counties in the case of nonmetropolitan counties. The Urban Influence Codes were obtained as part of the Area Resource File 2006 Release compiled and maintained by the U.S. Department of Health and Human Services, Health Resources and Services Administration (www.arfsys.com).

The percent of county population aged 25 years or more and with four or more years of college was determined by the 2000 Census of Population and Housing Demographic Profile and obtained from the Area Resource File 2006 release.

Data on the percent persons in poverty by county during 1997 – 2004 were determined by the U.S. Census Bureau Small Area Income Poverty Estimates and were obtained from

the Area Resource File 2006 release. These data are constructed from statistical models based, in part, on summary data from 1997 – 2004 federal income tax returns, Food Stamp program participation data, and census data.

The numbers of whites residing in counties during 1996 – 2004 were estimated by the U.S. Census Bureau midyear county population estimates (2004), county characteristics estimates (2000 – 2003) and estimates of the population by age, sex, race, and Hispanic origin (1996 – 1999). The data were obtained from the Area Resource File 2006 Release.

Housing unit density per square mile was determined by the U.S Census Bureau Census 2000 and obtained from the Area Resource File release 2006. The total number of housing units includes each house, apartment, mobile home, group of rooms, or single room that is occupied (or intended for occupancy) as a separate living quarter.

5.3.4.3. Health Care Services

Data on the number of medical doctors by county during 1992 – 2005 was compiled by the American Medical Association and obtained from the Area Resource File 2006 release. For the current analyses, data on the number of non-federal doctors seeing patients in an office-based general practice or family medicine practice was used as a county health care indicator.

The total number of visits (emergency, clinics, and referred) to county hospitals (short term general and non-general hospitals, and long term hospitals) during 1995 – 2004 was

determined in the American Hospital Association Annual Survey of Hospitals and obtained from the Area Resource File 2006 release.

The percent of persons without health insurance by county in 2000 was estimated by the U.S. Census Bureau Small Area Health Insurance Estimates and obtained from the Area Resource File 2006 release. The county estimates were produced using models that combine results from the Annual Social and Economic Supplement to the Current Population survey, food stamp participation records, Medicaid participation records, aggregated federal tax return data and demographic population estimates.

5.3.5. ArcMap

ArcMap software was used for two important study components. The first was to extract county-level values for downloaded explanatory variable data and the second was to create spatial maps for visual inspection when forming initial opinions about ecologic risk factors of greatest interest in logistic regression analyses.

First, county-level values for explanatory variable data were downloaded as shape, geo.tiff, raster, or grid files superimpose with county boundary files of identical projection. This activity required the use of an ArcMap version 9.3 (Redlands, CA) Spatial Analyst extension tool called "zonal statistics". Briefly, the available variable data files were downloaded and manipulated for importation into ArcMap. Once in ArcMap a U.S. county shape file of similar projection was located and joined to the variable data. Simply stated, the county shape file projection had to match the Earth surface dimension or curvature scale on which the data were originally collected. Then,

the ArcMap zonal statistics tool was used to extract summary measures for raster data grouped by county polygon. When necessary, features were converted to raster data prior to extraction. Ms. Anna Winters graciously converted three data layers downloaded by the authors as geo.tiff files in a similar manner.

Second, ArcMap version 9.0 was used to create maps showing the spatial distribution of each individual risk factor and county relative risk within actives clusters identified by SaTScan™. Spatial maps were also created to show the relationship between measures of socioeconomics (poverty and income) and the distribution of *Ixodes*.

5.3.6. Logistic Regression using SAS

SAS Version 9.1 (SAS Institute, Cary, NC) was used to combine explanatory and response variables into one database for cleaning prior to analyses and for all subsequent analyses. Categorical variables were evaluated using individual frequency tables and by cross tabulation with the multinomial outcome variable (NatlOut). The scale of continuous variables was evaluated by regressing continuous data binned by quartile on binomial outcome variables and examining plots of the coefficients versus the quartile midpoints (i.e., checked for linearity in the logit).

Generalized, cumulative, or binary logistic regression was used to model the effect of explanatory variables on response variables using SAS Proc Logistic. The type of regression employed was determined by the structure of the response variable (i.e., generalized logistic used for nominal multinomial outcomes, cumulative logistic used for ordinal multinomial outcomes, and binary logistic used for binomial outcomes). The

relationship between explanatory variables and each response variable was first evaluated in univariate logistic regression. Those with a Wald statistic p-value <=0.250 were later evaluated in multivariate logistic regression. Using purposeful selection the environmental, socioeconomic, and health care indicator assumed to have to strongest on reported Lyme disease risk were evaluated first. These were chosen based on knowledge gained from univariate analyses, visualization of spatial maps, evaluation of collinearity among explanatory covariates, biological relevance, the effect on model parameters (e.g., odds ratio and confidence interval) as an indicator of confounding, and the statistical significance of their multivariate model coefficient. Those with a Wald statistic p-value <=0.100 or deemed to be biologically necessary were retained as covariates in the main effects model. Once the most parsimonious main effects model was obtained, each rejected covariate was added back individually to double-check apparent insignificance. The scale of continuous covariates retained in the main effects model was re-evaluated as previously described. After final decisions were made on covariates for the main effects model, the statistical significance of biologically plausible interaction terms was evaluated using the same process.

The degree to which regression models fit the estimated probabilities for the covariate patterns was evaluated using the Pearson chi-square statistic ($\chi 2$) and deviance test. The ability of regression models to discriminate or predict the outcome for each county was evaluated by measuring the area under the receiver operator characteristics (ROC) curve (*c*). Model residuals and extreme covariate patterns (i.e., outliers) were checked visually using SAS influence plots except when the outcome variable had more than two levels.

5.4. RESULTS

5.4.1. SaTScan™ Cluster Detection and County Risk

SaTScan™ identified seven significant space-time clusters (p=0.001) among Lyme disease cases reported in the United States during 1992 – 2006 (Figure 5.7.1). The two most likely clusters (cluster 1, RR 10.251; cluster 2, RR 4.798) were active (i.e., they included the last year of the reporting period. Other clusters were identified during one or two years in the 1990s and are of little interest for the current analyses.

Active cluster 1 included 154 counties from 11 northeastern states (Connecticut (n=8), Delaware (n=3), Maine (n=1), Maryland (n=14), Massachusetts (n=14), New Hampshire (n=9), New Jersey (n=21), New York (n=39), Pennsylvania (34), Rhode Island (n=5), and Vermont (n=6)) and included years 2000 – 2006. The relative risk for cluster 1 compared with the rest of the United States was 10.251 and the relative risk for each county within cluster 1 compared to other counties within cluster 1 ranged from 0.136 to 155.511 (median 3.368; mean 9.289) (Figure 5.7.2; Figure 5.7.3).

Active cluster 2 included 99 counties from four states in north central states (Iowa (n=6), Michigan (n=3), Minnesota (n=47), and Wisconsin (n=43)) and included years 2000 – 2006. The relative risk for cluster 2 compared with the rest of the United States was 4.798 and the relative risk for each county within cluster 2 compared with other counties within cluster 2 ranged from 0.126 to 40.341 (median 4.742; mean 6.739) (Figure 5.7.2;

Figure 5.7.4). Two Iowa and two Michigan counties captured within cluster 2 reported no cases during the seven year time period and, therefore, had a zero relative risk.

5.4.2. Categorizing Outcome Variables using SaTScan™ Results

SaTScan™ results were used to create three outcome variables for regression analyses. A brief description of each is provided in Table 5.6.1 and detailed in the following subsections.

5.4.2.1. Nominal Multinomial Outcome Variable NatlOut

A nominal outcome variable (NatlOut) was created by categorizing all U.S. counties based on their inclusion in active cluster 1 (northeastern) or 2 (north central) (Figure 5.7.1):

- 0=county not located within active cluster 1 or 2
- 1=county located in active cluster 1 (northeastern)
- 2=county located in active cluster 2 (north central)

5.4.2.2. Ordinal Multinomial Outcome Variable ClusOut

An ordinal outcome variable for counties located within the active clusters (ClusOut) was created by categorizing counties based on the quartiles of county relative risk (Figure 5.7.2).

- 0=low risk (25^{th} percentile; 0<RR<=1.158)
- 1=moderate risk (50^{th} percentile; 1.158<RR<=3.634)
- 3=elevated risk (75^{th} percentile; 3.634<RR<=9.340)
- 4=high risk (100^{th} percentile; 9.340<RR<=155.511)

5.4.2.3. Binary Outcome Variable RedOut

A binary outcome variable (RedOut) was created by categorizing counties above the 75th percentile (Figure 5.7.2) and below the 75th percentile of county-level RR.

- 0=low to elevated risk (75th percentile; 0<RR<=9.340)
- 1=high risk (100th percentile; RR>9.340)

5.4.3. Description and Characteristics of Explanatory Variables

Decisions about categorizing continuous explanatory variables were made by reviewing descriptive statistics generated by SAS PROC Univariate (measures of central tendency, range, and extreme values) and considering whether or not they appeared linear in the logit. Briefly, continuous data were grouped by quartile and regressed on BiNatl (0=county not within an active cluster, 1= county within active clusters) and BiCounty (0=50th percentile of risk for counties within active clusters, 1=100th percentile of risk for counties within active clusters). Model coefficients were plotted by quartile midpoint to assess linearity. If data appeared non-linear, decisions to categorize were made by considering coefficients and their p-values. Final variable names and general characteristics of data are summarized in Table 5.6.1 and described in detail below.

Categorical explanatory variable data were evaluated using individual frequency tables and by cross tabulation with the multinomial outcome variable, NatlOut. Final variable names and general characteristics of data are described in detail below and summarized in Table 5.6.1.

5.4.3.1. Physical Environment

Data on the reported distribution of *Ixodes* were collapsed to create a dichotomized explanatory variable called tickdi. Counties lacking reported or established *Ixodes* were coded tickdi=0; counties with reported or established *Ixodes* were coded tickdi=1 (127). Figure 5.7.5 illustrates greater homogeneity of *Ixodes* in the northeastern as compared with north central states based on this measure of tick distribution.

Bailey's Ecoregion divisions (n=24 categories) resulted in sparse data in many cells when cross tabulated with binomial NatlOut and ClusOut outcome variables. Using Bailey's Ecoregion domains (n=4 categories) led to complete separation of the data because both cluster 1 and 2 are located in the same domain (data not shown). For these reasons, ecoregion was not evaluated in regression analyses.

Mean elevation was not linear in the logit. Therefore, data were dichotomized at the 75% percentile. Counties with mean elevation <= 486.823 feet were coded elevdi=0; counties >486.823 were coded elevdi=1). Figure 5.7.6 illustrates that most counties in cluster 1 (northeastern) and 2 (north central) states were classified as having low elevation.

Data on forest type (n=23 categories) resulted in sparse data in many cells when cross tabulated with binomial NatlOut and ClusOut outcome variables. Greater than 70% of U.S. counties fell in a single category (non-forest). Based on these observations and considering that a vegetative index as proxy for tick distribution was less important than

data on tick distribution, forest type was eliminated from regression analyses in favor of real data on tick distribution.

5.4.3.2. Social and Economic Characteristics

The number of murders was not linear in the logit. Therefore, data were dichotomized at the 50th percentile. Counties with <=0.005 per capita murders were coded murderdi=0; those with >0.005 murders per capita were coded murderdi=1. Data for 318 counties were missing. Figure 5.7.7 shows that most counties in cluster 1 (northeastern) and 2 (north central) states were classified as having a low murder rate. In northeastern states, higher murder rate appeared somewhat related to highly urban areas (e.g., Boston, MA, New York City, NY, Philadelphia, PA, and Washington, D.C.) but this observation was not the case in north central states.

Data on per capita income was not linear in the logit. Therefore data were dichotomized at the 50th percentile. Counties with <=$22,238 per capita income were coded incomedi=0; those with >$22,238 per capita income were coded incomedi=1. Figure 5.7.8 illustrates that high income was homogenous throughout most counties within cluster 1 (northeast) except for a few counties on the western edge of the cluster. However, in cluster 2 (north central states) high income was clustered in counties along and south west of the Mississippi river (rivers shown in Figure 5.7.4).

The average unemployment rate for each county was not linear in the logit. Therefore data were dichotomized at the 50th percentile. Counties with <=4% unemployment rate were coded unempdi=0; those with >4% average unemployment rate were coded

unempdi=1. Figure 5.7.9 shows an apparent relationship between high unemployment and counties with lower risk of reported Lyme disease in the west regions of cluster 1 (northeast states). In contrast, high unemployment seemed spatially related to higher risk counties in cluster 1 (north central states).

Urban Influence Code data contained 12 categories causing sparsely populated cells when cross-tabulated by outcome variable. Therefore this variable was not evaluated in regression analyses.

Data on the percent of county population at least 25 years old with at least four years of college for each county was linear in the logit. Therefore, data were evaluated as a continuous variable in regression analyses. The data ranged 4.9% to 63.70% (mean 16.5%). The variable was renamed collper. Figure 5.7.10 illustrates the distribution of percent college education categorized based on Jenks Natural Breaks in ArcMap. On average, counties in the northeast had a higher percentage of college educated population than counties in north central states. In both clusters, counties with a high percentage of their population with college education overlapped with large urban centers, but this was not always the case as many suburban counties also had a high percentage of educated population (particularly in the northeast).

Data on the percent persons in poverty for each county was linear in the logit. Therefore, data were evaluated as a continuous variable in regression analyses. The data ranged from 0% - 42.2% (mean 13.3%). The variable was renamed povper. Figure 5.7.11

illustrates the distribution of percent poverty categorized based on Jenks Natural Breaks in ArcMap. For the most part, counties with higher poverty had lower reported Lyme disease risk in the northeast and higher reported Lyme disease risk in north central states.

Data on the number of white residents for each county was adjusted using the county population. Data on the percent of white residents for each county were not linear in the logit. Therefore, data were dichotomized at the 50th percentile. Counties with the percent white population <= 94.737 were coded whiperdi=0; those with percent white population >94.737 were coded whiperdi=1. Figure 5.7.12 shows that counties with a higher percent of white population had a lower risk of reported Lyme disease in the northeast. In north central states, most counties were classified as having higher percent white.

Data on housing density per square mile for each county was adjusted using the county population. The housing density per 100,000 county population (housing density rate) was not linear in the logit. Therefore, data were dichotomized at the 75th percentile. Counties with housing density rate <=101.163 units per 100,000 population were coded hdratedi=0; those >101.163 units per 100,000 population were coded hdratedi=1. Figure 5.7.13 illustrates the data for this dichotomized variable. There was no apparent relationship between housing density and reported Lyme disease risk.

5.4.3.3. Health Care Services

The number of physicians for each county was adjusted using the county population. The number of physicians per 100,000 county population was not linear in the logit. Therefore, data were dichotomized at the 75th percentile. Counties with <=34.983

physicians per 100,000 county population were coded mdratedi=0; those with >34.983 physicians per 100,000 county population were coded mdratedi =1. Figure 5.7.14 illustrates data for this dichotomized variable. There was no apparent relationship between the number of physicians and reported Lyme disease risk.

The number of outpatient visits for each county was adjusted using the county population. The number of outpatient visits per person (outpatient visit ratio) was not linear in the logit. Therefore, data were dichotomized at the 75th percentile. Counties with <=2.485 out patient visits per persons were coded ovratiodi=0; those with >2.485 out patient visits were coded ovratiodi=1. Figure 5.7.15 illustrates data for this dichotomized variable. There was no apparent relationship between the number of physicians and reported Lyme disease risk.

Data on the percent persons without health insurance were linear in the logit. Therefore, data were evaluated as a continuous variable in regression analyses. The data ranged 3.8% - 38.0% (mean 14.8%). The variable was renamed insurper. Figure 5.7.16 illustrates the distribution of percent uninsured categorized based on Jenks Natural Breaks in ArcMap. The data were distributed with heterogeneity in the northeast and although many in north central states counties with the highest percentage of uninsured also had high reported Lyme disease case, this was not always the case.

5.4.4. Univariate Logistic Regression

Separate univariate logistic regression models were used to evaluate the effect of each explanatory variable on three outcome variables (i.e., NatlOut, ClusOut, and RedOut).

See Table 5.6.1 for a brief description of these. NatlOut was a nominal multinomial outcome variable and, therefore, univariate analyses were conducted using a generalized logistic regression model with NatlOut=0 as the reference level. ClusOut was an ordinal multinomial outcome variable and required a cumulative logistic regression model with ClusOut=0 as the reference level. The binomial outcome RedOut was modeled using standard binary logistic regression with RedOut=0 as the reference level.

5.4.4.1. Results for Active Clusters (NatlOut)

The results of univariate generalized logistic regression with each explanatory variable and the ordinal outcome variable NatlOut are presented in Table 5.6.2. It is worth noting that had we not used a generalized logistic regression model the important differences among these nominal outcome levels would have been missed because the default analyses for multinomial outcomes in SAS is cumulative regression.

The presence of *Ixodes* and lower elevation was significantly associated ($p<=0.05$) with active cluster 1 (northeast) when compared to the referent category (all counties not located within an active cluster). Compared to the referent, high socioeconomic status (income and college education) was more likely in cluster 1 as were housing density and out patient visits. Measures of low socioeconomic status (murder, unemployment, poverty, and no insurance) and the number of physicians were less likely in cluster 1 as compared to the referent.

The presence of *Ixodes* and lower elevation was also significantly associated ($p<=0.05$) with active cluster 2 (north central states) when compared to the referent category (all

counties not located within an active cluster). Compared to the referent, high socioeconomic status (income) was more likely in cluster 2 as were the number of physicians and percent white. Measures of low socioeconomic status (murder, unemployment, poverty, and no insurance) and housing density were less likely in cluster 2 as compared to the referent.

5.4.4.2. Results for County Risk Categorized by Quartile (ClusOut)

The results of univariate cumulative logistic regression with each explanatory variable and the ordinal outcome variable ClusOut are presented in Table 5.6.3. Note that separate regression analyses were conducted within each active cluster.

The presence of *Ixodes* and high socioeconomic status (income and education) were more likely ($p<=0.05$) among counties in one category of reported Lyme disease risk when compared to those in the next highest category of reported Lyme disease risk within cluster 1. Measures of low socioeconomic status (murder, unemployment, poverty, and no insurance), percent white, and number of out patient visits were less likely among counties in one category of reported Lyme disease risk when compared to those in the next highest category of reported Lyme disease risk within cluster 1. In summary, *Ixodes* and high socioeconomic measures (income and education) were more likely as the county risk level increased within cluster 1; low socioeconomic measures (murder, unemployment, poverty, and no insurance), the percent white population, and the number of outpatient visits per person were less likely as the county risk level increased within cluster 1.

The presence of *Ixodes* and high unemployment were more likely ($p<=0.05$) among counties in one category of reported Lyme disease risk when compared to those in the next highest category of reported Lyme disease risk within cluster 2. High income was less likely among counties in one category of reported Lyme disease risk when compared to those in the next highest category of reported Lyme disease risk within cluster 2 although this finding was not statistically significant ($p=0.0574$). Although unemployment was the only indicator of low socioeconomic status with a significant p-value within cluster 2, the poverty and uninsured were also more likely as the county risk level increased. In summary, the presence of *Ixodes* and low socioeconomics (unemployment) were more likely and high socioeconomics (income) was less likely as the county risk level increased within cluster 2.

5.4.4.3. Results for County Risk Dichotomized at the 75th Percentile (RedOut)

The results of univariate binary logistic regression with each explanatory variable and the binary outcome variable RedOut are presented in Table 5.6.3. Note that separate regression analyses were conducted within each active cluster.

When comparing counties with highest risk of Lyme disease to the referent category (all other counties) within cluster 1, the presence of *Ixodes*, elevation, and income caused quasi-complete separation of the data and unreliable estimates. A measure of high socioeconomic status (college education) was more likely in counties with the highest risk of Lyme disease as compared to the referent. Measures of low socioeconomic status (unemployment, poverty, and no insurance) were less likely among counties with the

highest risk of Lyme disease as compared to the referent. In summary, high socioeconomic measure (education) was more likely and low socioeconomic measures (unemployment, poverty, no insurance) were less likely among counties with highest risk of Lyme disease as compared to all other counties within cluster 1.

The presence of *Ixodes* and measures of low socioeconomic status (unemployment and poverty) were more likely ($p<=0.05$) among counties with the highest risk of Lyme disease as compared to the referent category (all other counties) within cluster 2. High socioeconomic status (college education) was less likely in counties with the highest relative Lyme disease risk as compared to the referent within cluster 2. In summary, *Ixodes* and low socioeconomic measures (unemployment and poverty) were more likely and high socioeconomics (education) were less likely among counties with increased risk of Lyme disease as compared to all other counties within cluster 2.

5.4.5. Multivariate Logistic Regression

Multivariate logistic regression models were used to evaluate the combined effect of explanatory variables on three outcome variables. NatlOut was modeled using generalized logistic regression, ClusOut for each active cluster was modeled using cumulative logistic regression, and RedOut for each active cluster was modeled using binary logistic regression. Except where noted, the Pearson $\chi 2$ and deviance test statistics provided evidence of final model fitness ($p>0.05$) and the area under the ROC curve (AUC) supported acceptable discrimination (>0.7000) for all main effects models presented.

5.4.5.1. Results for Active Clusters (NatlOut)

The results of a multivariate generalized logistic regression with the nominal outcome variable NatlOut and backward stepwise selection of explanatory variables are presented in Table 5.6.5. The Wald $\chi 2$ p-values for percent white population and murder rate were greater than 0.250 and they were removed from the generalized logistic regression model.

As seen in univariate analyses, the presence of *Ixodes* and lower elevation was significantly associated with cluster 1 when compared with the referent category (all counties not located within an active cluster). High income, education, housing density, and number of out patient visits were more likely and the number of physicians and percent uninsured were less likely in cluster 1 as compared to the referent category. Unemployment, poverty, and biologically plausible interactions terms were not significantly associated with county risk within cluster 1.

As seen in univariate analyses, the presence of *Ixodes* and lower elevation was significantly associated with cluster 2 when compared with the referent category (all counties not located within an active cluster). High unemployment and number of physicians were more likely and high income, education, housing density, uninsured, and poverty were less likely in cluster 2 as compared to the referent. Out patient visits and biologically plausible interactions terms were not significantly associated with county risk within cluster 2. The main effects model for NatlOut shown in Table 5.6.5 produced a deviance statistic of 1311.7563 ($p=1.0000$) indicating model goodness of fit however, the Pearson $\chi 2$ statistics was 33981.1483 ($p<0.0001$) which supported lack of model fit.

The model included ten variables resulting in as many covariate patterns as there were observations. In this situation the Hosmer-Lemeshow statistic is a preferred test for goodness of fit but cannot be calculated when the outcome variable has more than two levels (recall that NatlOut has three levels). The AUC was at least 0.9000 for each outcome level indicating excellent discrimination between risk and non-risk counties. However, a model with ten explanatory variables, discordant goodness-of-fit statistics, and near perfect discrimination is probably over fit and unreliable. Therefore, parameters should be interpreted with caution until they can be evaluated further or confirmed in future studies.

5.4.5.2. Results for County Risk Categorized by Quartile (ClusOut)

The results of a multivariate generalized logistic regression with the ordinal outcome variable ClusOut and purposeful selection of explanatory variables are presented in Table 5.6.6. Note that separate analyses were conducted within each active cluster. Many factors were considered when choosing final model covariates including their biological relevance, the statistical significance of their model coefficient, and their relationship with other explanatory variables (e.g., spatial maps and checks of collinearity).

Within cluster 1, the presence of *Ixodes* was more likely and high poverty was less likely among counties in one category of reported Lyme disease risk as compared with the next higher category of reported Lyme disease risk (Table 5.6.6, Cluster 1, Model A). The odds ratio for poverty did not change when the analysis was restricted to counties for which the presence of *Ixodes* was established or reported, although the confidence interval widened slightly (Table 5.6.6, Cluster 1, Model B*). In summary, high poverty

was less likely among counties in one category of reported Lyme disease risk as compared with those in the next highest category.

Within cluster 2, the presence of *Ixodes* and high poverty or unemployment were more likely among counties in one category of reported Lyme disease risk as compared with the next higher category of reported Lyme disease risk (Table 5.6.6, Cluster 2, Model A and B, respectively). Although statistically unemployment was more strongly associated with increasing county risk than poverty, these two explanatory variables are highly collinear (F ratio 1900.897). Notice that the odds ratio for poverty changed only slightly when the analysis was restricted to counties for which the presence of *Ixodes* was established or reported, as did the confidence interval (Table 5.6.6, Cluster 2, Model C). In summary, a measure of low socioeconomics (poverty or unemployment) was more likely among counties in one category of reported Lyme disease risk as compared with the next higher category of reported Lyme disease risk. However, the model produced a deviance statistic of 96.2794 (p=0.8702) indicating model goodness of fit but the Pearson $\chi 2$ statistics was 158.0912 (p=0.0033) which supported lack of model fit. The AUC was less than 0.7000 for each outcome level (ClusOut = 1, AUC=0.4202; ClusOut=2, AUC=0.5670; ClusOut=3, AUC=0.6677) indicating no or poor ability to discriminate between risk and non-risk counties.

5.4.5.3. Results for County Risk Dichotomized at 75th Percentile (RedOut)

The results of binary logistic regression modeling using purposeful selection to explore the relationship between explanatory variables and the ordinal outcome variable RedOut in each active cluster are presented in Table 5.6.7. Based on previous results, model

building began with poverty and then other variables and relevant interaction terms were added to the model to evaluate their relationship with RedOut and assess their potential influence on poverty.

Within cluster 1, poverty alone was associated with counties with the highest risk of reported Lyme disease as compare to the referent (all other counties). Conducting the analyses with or without restriction based on the distribution of *Ixodes* resulted in minor differences between the odds ratios and confidence intervals. In summary, high poverty was less likely among counties with the highest reported Lyme disease risk as compared with the referent.

Within cluster 2, the distribution of *Ixodes* and poverty were most strongly associated with increasing levels of county risk. When restricted to counties with *Ixodes*, poverty alone was associated with highest risk counties although the odds ratios and confidence intervals are quite similar. In summary, high poverty was more likely among counties with the highest reported Lyme disease risk as compared with all other counties within cluster 2.

5.4.6. Additional Comment on Regression Analyses

To further strengthen our findings, we regressed a continuous measure of poverty and income on a continuous measure of county relative risk within each active cluster using normal linear models (data not shown). The overall associations were consistent with results of logistic regression analyses. That is, income increases and poverty decreases as

county relative risk increases in the northeast. Income decreases and poverty increases as county relative risk increases in north central states.

5.4.7. Additional Comment on Spatial Maps

As described in previous sections, spatial maps were reviewed to form opinions about ecologic risk for variables evaluated in logistic regression analyses. After regression analyses were complete, a few additional maps were created to assist in the interpretation of results. These include data on county ecoregion (Figure 5.7.17), forest type (Figure 5.7.18), and urban influence code (Figure 5.7.19). We also found that illustrating *Ixodes* distribution with poverty (Figure 5.7.20) and income (Income 21), in addition to reported Lyme disease risk (Figure 5.7.5) was helpful when interpreting ecologic risks.

5.5. DISCUSSION

5.5.1. Findings

Here we contribute to the body of literature suggesting that Lyme disease is continuing to emerge in northeastern and north central United States (146, 147) using the SatScan™ scan statistic to identify the most likely space-time clusters of cases reported during 1992 – 2006. In addition to the many advantages described previously, this method was preferred because of its ease of use and fact that analyses was not restricted by state line or *a priori* assumptions about the locations of excess disease risk. In this first application of SatScan™ to investigate excess risk among reported Lyme disease cases, we reported results using program default settings and note that much more can be learned about excess risk by restricting analyses to the two active high-risk clusters, customizing programmable features and including other covariates for adjustment in future studies.

Obtaining existing explanatory data of interest that were available for all U.S. counties posed great challenges. Data on climate and vegetation were available for most of the world but stored in formats most suitable for viewing or manipulation in geographic information system software (e.g., raster, grid, or shape files). Downloading the data, projecting them onto county polygons, and extracting county-level summary measures (e.g., minimum, mean, and maximum) required a great deal of manipulation for most variables. Furthermore, extraction of nominal data often resulted in too few or too many categories for use in the current analyses (e.g., ecoregion and forest type). Surprisingly, accessible data on the national distribution of *Ixodes* or their hosts was not found. A national map showing the density of white-tailed deer was published by the Quality Deer Management Association of Bogart, Georgia (www.qdma.org). However a personal visit revealed that once the effort of obtaining these data from state wildlife and agricultural managers was completed, graphic illustrators manually shaded a national map rather than creating a geocoded data layer. With this information and news that data on the distribution of *Ixodes* was no longer available in electronic format (J. Piesman, personal communication), we chose to re-create the data using the table and map presented in the original 1998 publication on the reported distribution of *Ixodes* in the United States by Dennis and colleagues (127). County-level measures summarizing the socioeconomics of residents and health care infrastructure of counties were abundant but the latter were generally only available for decennial Census years. The larger concern was selecting the most appropriate measures of ecologic risk among dozens under consideration. For example, eight county-level crime statistics were obtained for this study. Per capita murder was chosen to represent crime because it was presumed to be an unambiguous

and accurate statistic. However, data were missing for 318 counties (about 10%) and murder was a rather rare event. Therefore we suggest evaluating other measures of county-level crime in future studies. Many sources of data on healthcare, human behaviors and recreation were evaluated for their usefulness (e.g., USDA Forest Service "National Survey on Recreation and the Environment", CDC "National Health and Nutrition Examination Survey', USGS "National Land Cover Dataset", and the CDC "National Health Interview Survey", "Behavioral Risk Factor Surveillance System", National Ambulatory Medical Care Survey, and National Hospital Discharge Survey). However, statistics from these studies were based on fairly small surveys (often less than 100,000 persons or households surveyed nationwide) and data were either not available to the public or grouped at the national, state, or multi-county level to protect the privacy of human subjects or reduce the chance of bias due to small sample size. Several sources of data provided query tools to facilitate review of summary statistics prior to purchase or download (e.g., DHHS Agency for Healthcare Research and Quality "Health Care Utilization Project" and "Medical Expenditure Panel Survey" and Data Ferret, a product of the Census Bureau's Data Integration Division). Public health practitioners gathering information or justifying the need for epidemiologic study (e.g., in a grant proposal) would find these tools helpful.

Possible collinearity between explanatory variables, results of univariate logistic regression analyses, and information gained from spatial maps informed decisions made during multivariate logistic regression (i.e., which covariate(s) might be associated with the outcome of interest). Evidence of a directly proportional relationship (i.e.,

collinearity) was found between measures of low socioeconomic status (poverty, uninsured, and unemployed), measures of high socioeconomic status (income and education) and measures of health care (the number of physicians and outpatient visits). For the most part, measures of low socioeconomics were inversely proportional to measures of high socioeconomics. Percent white population was somewhat related to higher income and lower poverty but should be examined more closely in future studies.

Overall, the results of univariate logistic regression and multivariate logistic regression analyses (with and without restriction on the presence of *Ixodes*) were consistent. As expected for an infection vectored by *Ixodes*, the reported or established presence of ticks was strongly associated with areas of higher reported Lyme disease risk. In the northeast, measures of high socioeconomic status were more likely in areas with higher Lyme disease risk and measures of low socioeconomic status were less likely. Exactly the opposite is true in north central states where high socioeconomic status was less likely in areas with higher Lyme disease risk and low socioeconomic status was more likely. To further consider *Ixodes* and socioeconomic status (particularly income and poverty) as ecologic risk factors for increased incidence of reported Lyme disease risk, we again examined spatial map of explanatory data plotted with categories of county risk within the two active clusters identified by SatScan™.

Within cluster 1 (northeast), the presence of *Ixodes* and high income were homogenous throughout most counties reducing the extent to which within cluster comparisons could be made (recall that both *Ixodes* and income caused quasi-complete data separation when

comparing the highest risk counties (RedOut=1) to others within cluster 1)). However, percent poverty was more heterogeneous and lower in counties where reported Lyme disease risk was high. It was not unexpected that *Ixodes* was a strong ecologic risk factor in the northeast. The area had been the focus of Lyme disease research since the recognition of the disease in the early 1970's and the zoonotic cycle required to support the prorogation of human Lyme disease is well documented. It is likely that high income and low poverty were characteristics of the population who resided in areas with the greatest opportunity for exposure to *Ixodes* and reported Lyme disease. This supports the notion that Lyme disease among residents of the northeast results from peridomestic exposure. However, further studies conducted on smaller geographic scale (e.g., census block, neighborhood, or individual level) are required to determine if these ecologic factors influence risk below the county level.

Within cluster 2 (north central), *Ixodes* were reported or established in only 52% of the counties. Whether this is the truth or the result of under-reporting or misclassification is unknown, but these data allowed a better estimation of the influence of *Ixodes* on reported Lyme disease risk (i.e., *Ixodes* remained a main effects model covariate). Spatial maps showed a cluster of counties lacking *Ixodes* and with low risk of reported Lyme disease south of the Mississippi river. These counties also reported high average income and low to moderate poverty among county residents. These points taken together could have influenced the finding that low socioeconomic status (e.g., high poverty and unemployment) was a risk factor for increased reported Lyme disease risk in the north central states. It is likely that high poverty and low income were characteristics

of the population who resided in areas with the greatest opportunity for exposure to *Ixodes* and reported Lyme disease. A possible explanation is that human exposure to *Ixodes* among residents of north central states occurs during recreation activities, rather than domestic, and there is at least one published report that may be the case (146). Still, there are no doubt other factors influencing the finding low socioeconomic status is a characteristic of counties with high reported Lyme diseases risk in north central United States and these deserve further study to determine if these ecologic factors influence risk below the county level.

A few important lessons were learned from these analyses and offer possibilities for improving future studies of reported Lyme disease cases and entomologic risk. The global ecologic measures evaluated (e.g., ecoregion and forest) were poor substitutes for *Ixodes* distribution in areas with greater than expected reported Lyme disease. It is curious that few studies of entomologic risk used the data reported by Dennis and colleagues as either a foundation on which to build or test their models of entomologic risk. The finding described here indicated that *Ixodes* data correlated well with reported Lyme disease risk. Recall that decisions to leave variables continuous or to dichotomize were made using outcome data for both clusters combined for simplicity and ease of interpretation. However, these findings show that the two regions have distinct environmental features and the characteristics of counties and residential populations may be diverse enough to require separate categorization of explanatory variable data. Accordingly, inferences drawn on the distribution and determinants of Lyme disease risk in the northeast should not be applied to north central states. Furthermore, differences in

the socioeconomic characteristics of these two geographic groups argue the need for customized prevention measures and messages.

5.5.2. Study Limitations

As reviewed previously, the ecologic study design limited the scope of this study and interpretation of our findings. To weaken the effect of residual confounding in future studies, we recommend obtaining data on the joint distribution (individual-level or census-tract level) within data aggregated by county as suggested by others (130, 144, 148, 149). County of residence was used to group reported Lyme disease cases since exposure data for reported Lyme disease cases was either unknown or unreliable. Measures of county socioeconomics and healthcare were chosen with few *a prior* assumptions about the primary risk factor of interest other than *Ixodes* distribution but information gained in these analyses will inform better choices when selecting measures of crime, housing, and urbanicity in future studies. As with most studies, information may have been lost due to categorization of explanatory data. Collinearity among similar measures made multivariate model building and evaluation of interaction challenging.

5.5.3. Study Strengths

The current study benefitted from an exhaustive search of available existing data on exposures related to Lyme disease risk that were, or could be, aggregated at the county-level. Several dozen data sources and hundreds of variables were considered for this study. In addition, the authors were very familiar with the compiled Lyme disease surveillance data which minimized the possibility of misinterpreting these. SatScan™ identified space-time clusters of greater than expected risk with no *a priori* assumptions about cluster size and location greatly reducing the potential for selection bias. This is

the first epidemiologic study to examine the relationship between group-level indicators of socioeconomics and reported Lyme disease risk. We provided data to support the assumption that high income is a possible risk factor for increased risk in the northeast and we generated new hypotheses about the relationship between risk and socioeconomic status in north central states. We hoped to reduce effect of bias in ecologic studies and the chance of misinterpretation of findings by making statements about increased or decreased ecologic risk when they existed rather than using a literal interpretation of odds ratios. The overall findings of logistic regression were strengthened by regressing a continuous measure of poverty and income on a continuous measure of county relative risk within active cluster using normal linear models. Finally, spatial maps were extremely helpful to the authors in interpreting the findings of logistic regression analyses and they have the added value of being easily understood by diverse audiences.

5.6. TABLES

5.6.1. Description of outcome and explanatory variables used in logistic regression analyses.

Variable	Description	Code/Value	Name
	County geocode	FIPS	FIPS
Outcome 1	Active U.S. clusters	0=Not in a cluster 1=Northeastern cluster 2=North central cluster	NatlOut
Outcome 2	Relative risk for counties within an active cluster categorized by quartile	0=RR<1.158 1=1.158<RR<=3.634 2=3.634<RR<=9.340 3=9.340<RR<=155.511	ClusOut
Outcome 3	Relative risk for counties within an active cluster dichotomized	0=RR<=9.340 1=RR>9.340	RedOut
Explanatory 1	Ixodes ticks	0=Not established or reported 1=Established or reported	Tick
Explanatory 2	Mean elevation	0=le 75th percentile 1=gt 75th percentile	Elevation
Explanatory 3	Murder rate	0=le 50th percentile 1=gt 50th percentile	Murder
Explanatory 4	Per capita annual income	0=le 50th percentile 1=gt 50th percentile	Income
Explanatory 5	Annual unemployment rate	0=le 50th percentile 1=gt 50th percentile	Unemployment
Explanatory 6	Percent =>25 years old with =>4 years of college	Range 4.9 – 63.70; continuous and linear in the logit.	Education
Explanatory 7	Percent in poverty	Range 0 – 42.2; continuous and linear in the logit.	Poverty
Explanatory 8	Percent white	0=le 50th percentile 1=gt 50th percentile	White
Explanatory 9	Housing density per 100,00 population	0=le 75th percentile 1=gt 75th percentile	Housing Density
Explanatory 10	Number of physicians per 100,000 population	0=le 75th percentile 1=gt 75th percentile	Physicians

Explanatory 11	Number outpatient visits per person	0=le 75th percentile 1=gt 75th percentile	Out Patient Visits
Explanatory 12	Percent without health insurance	Range 3.8 – 38.0; continuous and linear in the logit.	Uninsured

le = less than or equal to
gt = greater than

5.6.2. Results of fitting univariate generalized logistic regression models for national clusters (NatlOut)

NatlOut 1 v 0 NatlOut 2 v 0	Coefficient	Wald $\chi 2$	p-value	OR
Tick	1.4008	127.3104	<0.0001	16.471
	0.4510	19.3315	<0.0001	2.465
Elevation	-1.1958	27.4340	<0.0001	0.091
	-1.4391	16.1829	<0.0001	0.056
Murder	-0.5695	17.0975	<0.0001	0.320
	-0.4471	8.2531	0.0041	0.409
Income	1.2238	74.9632	<0.0001	11.560
	0.3527	11.0438	0.0009	2.024
Unemployment	-04887	28.3895	<0.0001	0.376
	-0.2512	5.7236	0.0167	0.605
Education*	0.0921	145.9668	<0.0001	1.096
	0.0213	2.8608	0.0908	1.022
Poverty*	-0.2670	109.3593	<0.0001	0.766
	-0.3040	85.3760	<0.0001	0.738
White	-0.1307	2.4484	0.1176	0.770
	0.7000	30.6056	<0.0001	4.055
Housing Density	0.3148	13.5186	0.0002	1.877
	-0.6627	12.7442	0.0004	0.266
Physicians	-0.4451	12.2303	0.0005	0.411
	0.8459	62.8626	<0.0001	5.429
Out Patient Visits	0.3251	14.4039	0.0001	1.916
	-0.1555	1.4442	0.2295	0.733
Uninsured*	-0.2977	117.2159	<0.0001	0.743
	-0.3909	103.7705	<0.0001	0.676

*covariates with continuous data; coefficients were calculated for each 1% change

5.6.3. Results of fitting univariate cumulative logistic regression models for county relative risks (ClusOut) within each active cluster

ClusOut within cluster 1 ClusOut within cluster 2	Coefficient	Wald $\chi 2$	p-value	OR
Tick	0.8267	12.2244	0.0005	5.330
	1.1912	30.5117	<0.0001	10.831
Elevation	-0.5327	1.5919	0.2071	0.345
	0.7148	1.0430	0.3071	4.177
Murder	-0.6090	5.6976	0.0170	0.296
	-0.0861	0.0970	0.7554	0.842
Income	1.0066	12.1528	0.0008	7.488
	-0.3621	3.6121	0.0574	0.485
Unemployment	-0.8876	24.9082	<0.0001	0.169
	0.5063	0.0082	0.0082	2.753
Education*	0.0512	9.2828	0.0023	1.053
	-0.0318	1.2323	0.2670	0.969
Poverty*	-0.3274	34.4054	<0.0001	0.721
	0.1101	2.7858	0.0951	1.1166
White	-0.9878	7.1281	0.0076	0.451
	-0.1047	0.2171	0.6413	0.811
Housing Density	-0.0781	0.2764	0.5991	0.855
	-0.1123	0.1150	0.7345	0.799
Physicians	-0.2711	1.4291	0.2319	0.581
	0.1902	1.0210	0.3123	1.463
Out Patient Visits	-0.2970	3.9069	0.0481	0.552
	0.0407	0.0316	0.8588	1.085
Uninsured*	-0.1755	10.5060	0.0012	0.839
	0.1226	2.9883	0.0839	1.130

*covariates with continuous data; coefficients were calculated for each 1% change

5.6.4. Results of fitting univariate binary logistic regression models for county relative risks dichotomized at the 75th percentile (RedOut)

RedOut within cluster 1 RedOut within cluster 2	Coefficient	Wald $\chi 2$	p-value	OR
Tick	6.8115	0.0016	0.9690	>999.999
	1.2557	14.5068	0.0001	12.321
Elevation	-6.2448	0.0010	0.9751	<0.001
	0.5289	0.5448	0.4605	2.880
Murder	-0.3914	0.9961	0.3183	0.457
	0.1952	0.3495	0.5544	1.477
Income	6.7866	0.0012	0.9726	>999.999
	-0.3883	2.7746	0.0958	0.460
Unemployment	-1.5018	8.4723	0.0036	0.050
	0.5433	5.3420	0.0208	2.964
Education*	0.0529	6.2965	0.0212	1.054
	-0.0977	3.8751	0.0490	0.907
Poverty*	-0.3558	16.7200	<0.001	0.701
	0.1940	4.510	0.0325	1.214
White	-0.2899	2.1315	0.1443	0.560
	0.2170	0.5017	0.4787	1.543
Housing Density	-0.0132	0.0046	0.9459	0.974
	-6.3060	0.0017	0.9672	<0.001
Physicians	-0.0573	0.0363	0.8489	0.892
	0.1682	0.4746	0.4909	1.400
Out Patient Visits	-0.0132	0.0046	0.9459	0.974
	0.4592	2.9322	0.0868	2.505
Uninsured*	-0.2099	6.0111	0.0142	0.811
	0.1584	3.3207	0.0684	1.172

*covariates with continuous data; coefficients were calculated for each 1% change

5.6.5. Multivariate main effects model using generalized logistic regression model for national clusters (NatlOut) with backward stepwise selection†

	Coeff.	Wald $\chi 2$	p-value	OR	OR 95% LCL,UCL
NatlOut=1					
Tick	2.6847	100.3320	<0.0001	14.654	8.666, 24.779
Elevation	-0.8279	2.8429	0.0918	0.437	0.167, 1.144
Income	0.9358	7.5887	0.0059	2.549	1.310, 4.961
Unemployment	0.1686	0.4477	0.5034	1.184	0.722, 1.940
Education*	0.0584	25.0585	<0.0001	1.060	1.036, 1.085
Poverty*	0.0046	0.0084	0.9270	1.005	0.910, 1.109
Housing Density	1.0025	22.3110	<0.0001	2.725	1.798, 4.131
Physicians	-0.8752	9.3146	0.0023	0.417	0.238, 0.731
Outpatient Visits	0.5452	6.5275	0.0106	1.725	1.135, 2.620
Uninsured*	-0.2339	21.5401	<0.0001	0.791	0.717, 0.874
NatlOut=2					
Tick	0.9137	14.3717	0.0002	2.494	1.555, 3.999
Elevation	-2.2360	9.2451	0.0024	0.107	0.025, 0.452
Income	-1.4702	22.1882	<0.0001	0.230	0.125, 0.424
Unemployment	0.9414	10.5985	0.0011	2.563	1.454, 4.518
Education*	-0.0525	7.0315	0.0080	0.949	0.913, 0.986
Poverty*	-0.3594	24.3755	<0.0001	0.698	0.605, 0.805
Housing Density	-1.3617	11.8397	0.0006	0.256	0.118, 0.557
Physicians	1.8260	58.5490	<0.0001	6.209	3.889, 9.911
Outpatient Visits	-0.3849	1.7278	0.1887	0.681	0.383, 1.208
Uninsured*	-0.2506	14.2120	0.0002	0.778	0.717, 0.874

†univariate p-value for entry <=0.250, multivariate p-value for removal <=0.05

*covariates with continuous data; coefficients were calculated for each 1% change

5.6.6. Multivariate main effects model using cumulative logistic regression models and purposeful selection for counties within active clusters (ClusOut)

	Coeff.	Wald $\chi 2$	p-value	OR	OR 95% LCL,UCL
Within Cluster 1					
Model A					
Tick	1.2525	6.1570	0.0131	3.499	1.301, 9.411
Poverty*	-0.3057	29.3640	<0.0001	0.737	0.660, 0.823
Model Deviance			0.9642		
Model Pearson $\chi 2$			0.9618		
Model B†					
Poverty*	-0.3054	26.4658	<0.0001	0.737	0.656, 0.828
Model Deviance			0.8648		
Model Pearson $\chi 2$			0.8910		
Within Cluster 2					
Model A					
Tick	2.4161	30.8305	<0.0001	11.202	4.774, 26.283
Poverty*	0.1185	3.2400	0.0719	1.126	0.990, 1.281
Model Deviance			0.9351		
Model Pearson $\chi 2$			0.1343		
Model B					
Tick	2.3869	30.1680	<0.0001	10.879	4.642, 25.498
Unemployment	1.0296	6.6947	0.0097	2.800	1.284, 6.108
Model Deviance			0.4567		
Model Pearson $\chi 2$			0.6104		
Model C†					
Poverty*	0.1903	3.5525	0.0595	1.210	0.992. 1.474
Model Deviance			0.8702		
Model Pearson $\chi 2$			0.0033		

†analyses restricted to counties for which tickdi=1

*covariates with continuous data; coefficients were calculated for each 1% change

5.6.7. Multivariate main effects model using binary logistic regression models and purposeful selection for counties within active clusters (RedOut)

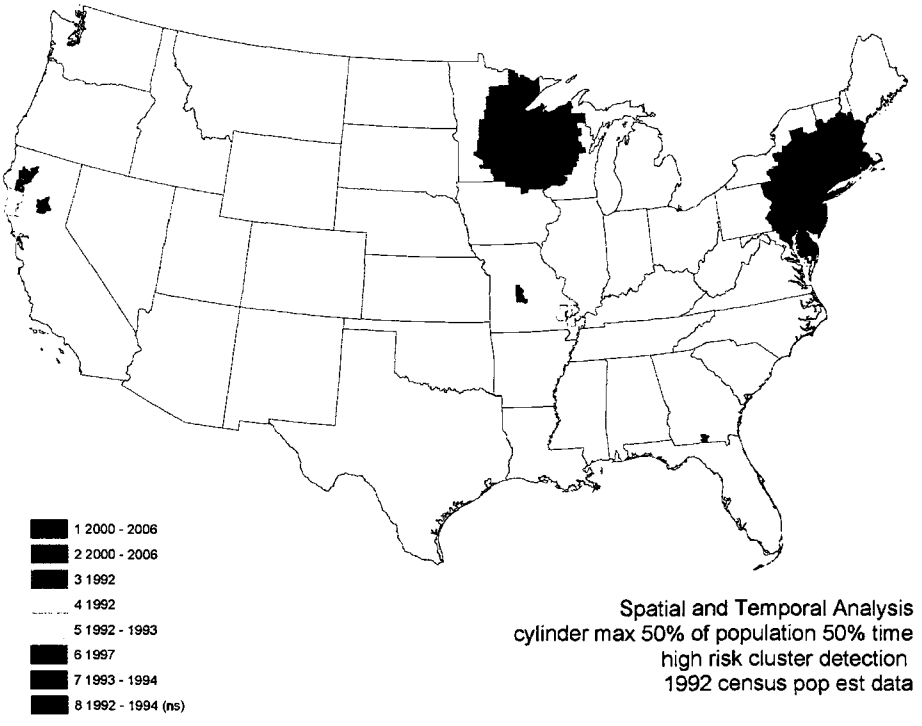
	Coeff.	Wald $\chi 2$	p-value	OR	OR 95% LCL,UCL
Within Cluster 1					
Model A					
Poverty*	-0.2558	16.720	<0.0001	0.701	0.591, 0.831
Model Deviance			0.3348		
Model Pearson $\chi 2$			0.5030		
Model B†					
Poverty*	-0.3231	13.590	0.0002	0.724	0.610, 0.860
Model Deviance			0.2387		
Model Pearson $\chi 2$			0.5280		
Within Cluster 2					
Model A					
Tick	2.6102	14.0004	0.0002	13.602	3.466, 53.380
Poverty*	0.1930	5.2035	0.0225	1.213	1.028, 1.432
Model Deviance			0.7031		
Model Pearson $\chi 2$			0.5935		
Model B†					
Poverty*	0.2643	4.5610	0.0310	1.302	1.024, 1.656
Model Deviance			0.1036		
Model Pearson $\chi 2$			0.4547		

†analyses restricted to counties for which tickdi=1

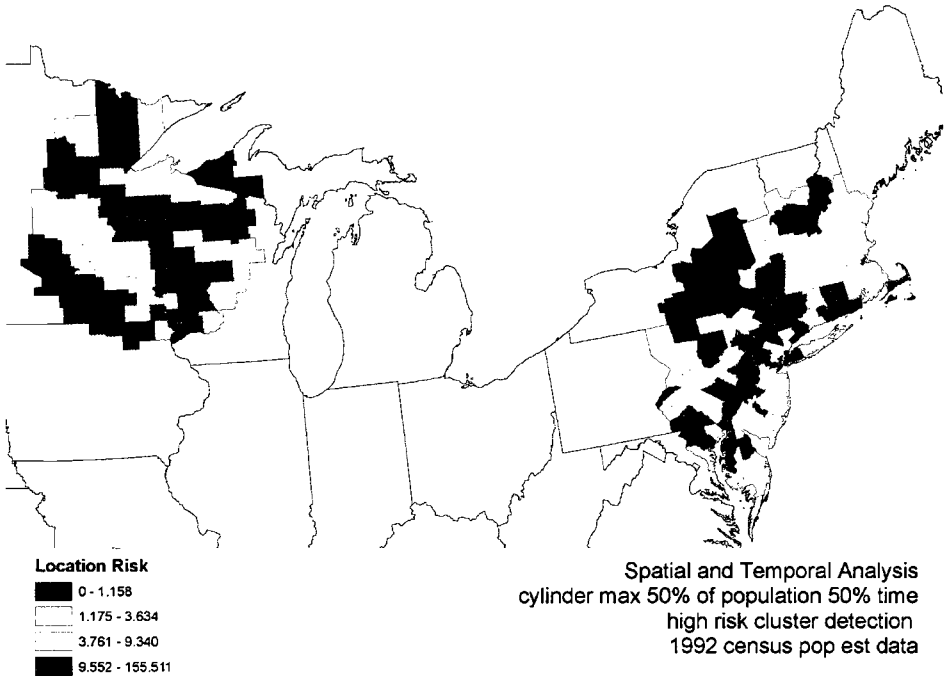
*covariates with continuous data; coefficients were calculated for each 1% change

5.7. FIGURES

5.7.1. Clusters of reported Lyme disease cases during 1992 – 2006 identified by SaTScan™ as least likely to have occurred by chance

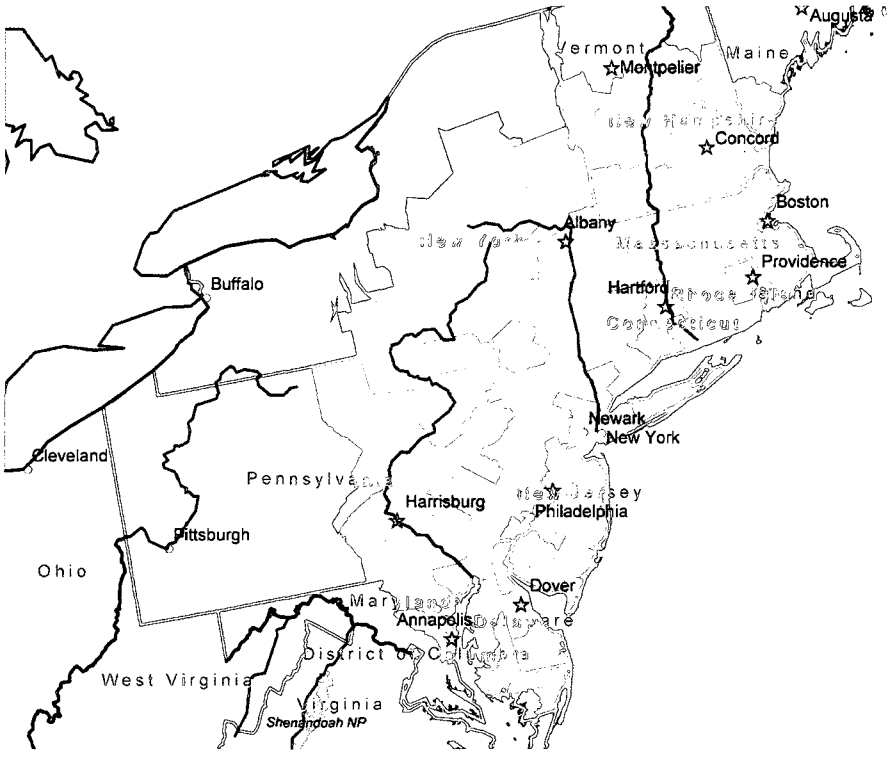


5.7.2. Counties* within active clusters of reported Lyme disease cases during 1992 – 2006 identified by SaTScan™ as least likely to have occurred by chance



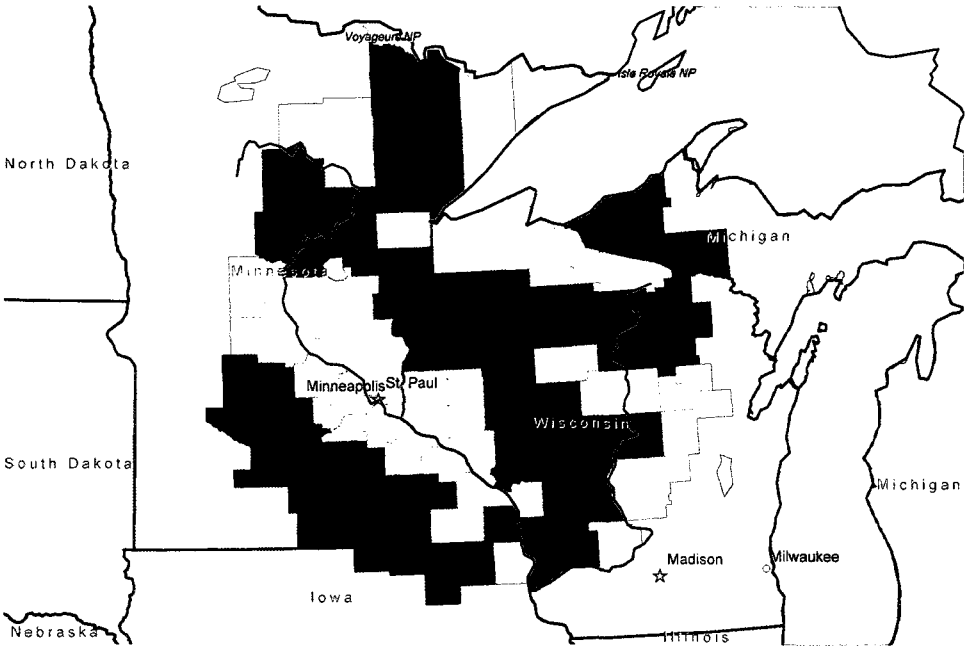
*counties are shaded by county relative risk categorized by quartile

5.7.3. Geography of counties* located within cluster 1



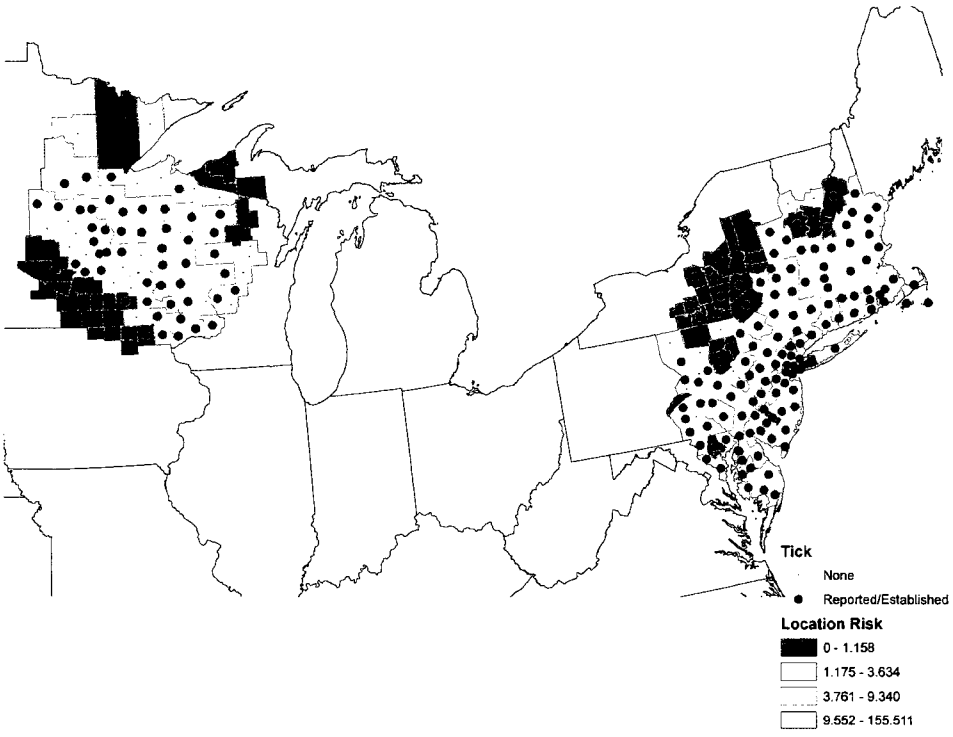
*counties are shaded by county relative risk categorized by quartile as shown in Figure 5.7.2

5.7.4. Geography of counties* located within cluster 2



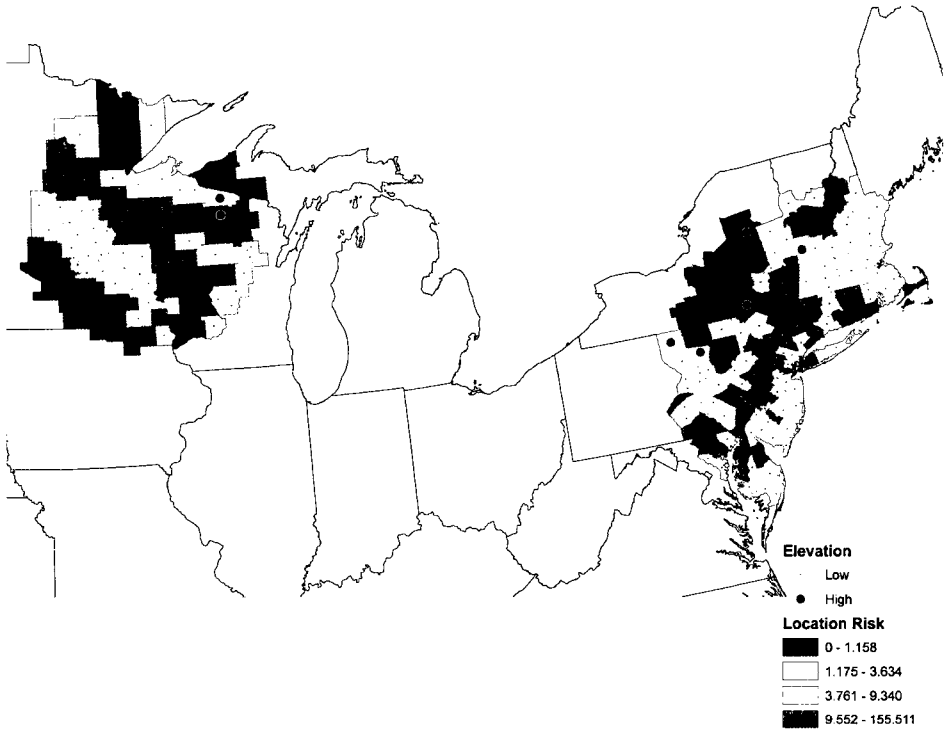
*counties are shaded by county relative risk categorized by quartile as shown in Figure 5.7.2

5.7.5. *Ixodes* * and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006



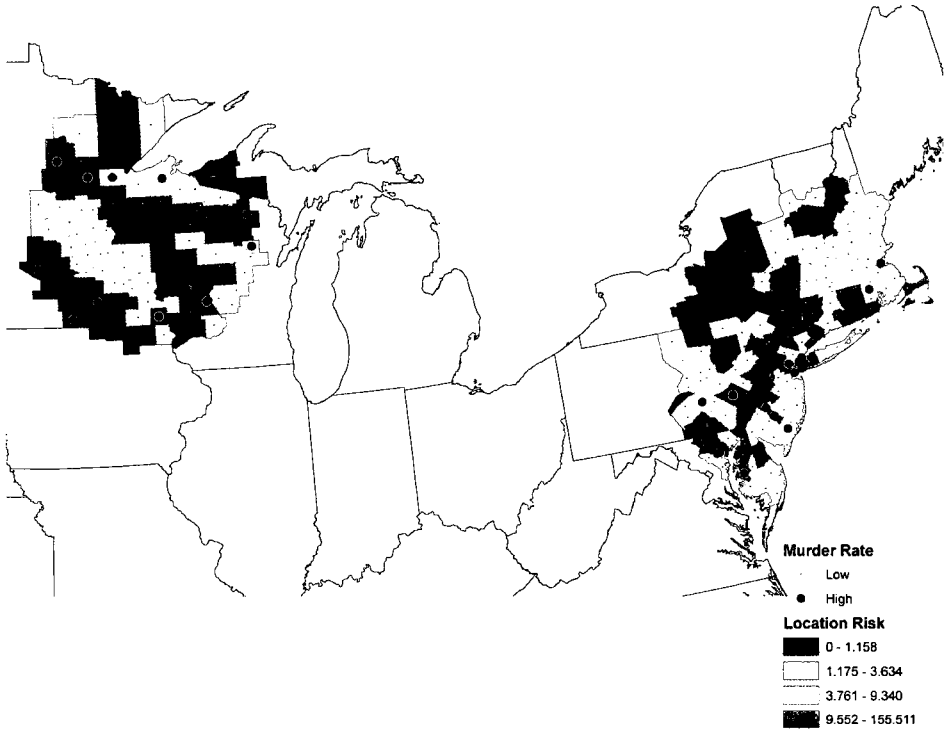
*additional information on this outcome variable was provided in Table 5.6.1

5.7.6. Elevation* and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006



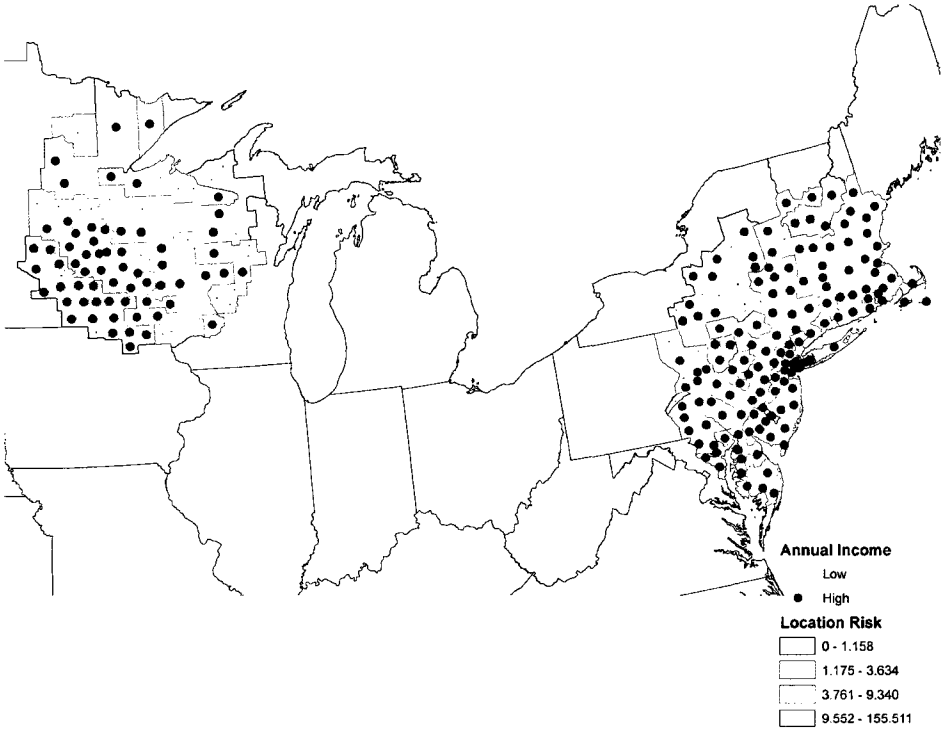
*additional information on this outcome variable was provided in Table 5.6.1

5.7.7. Murder* and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006



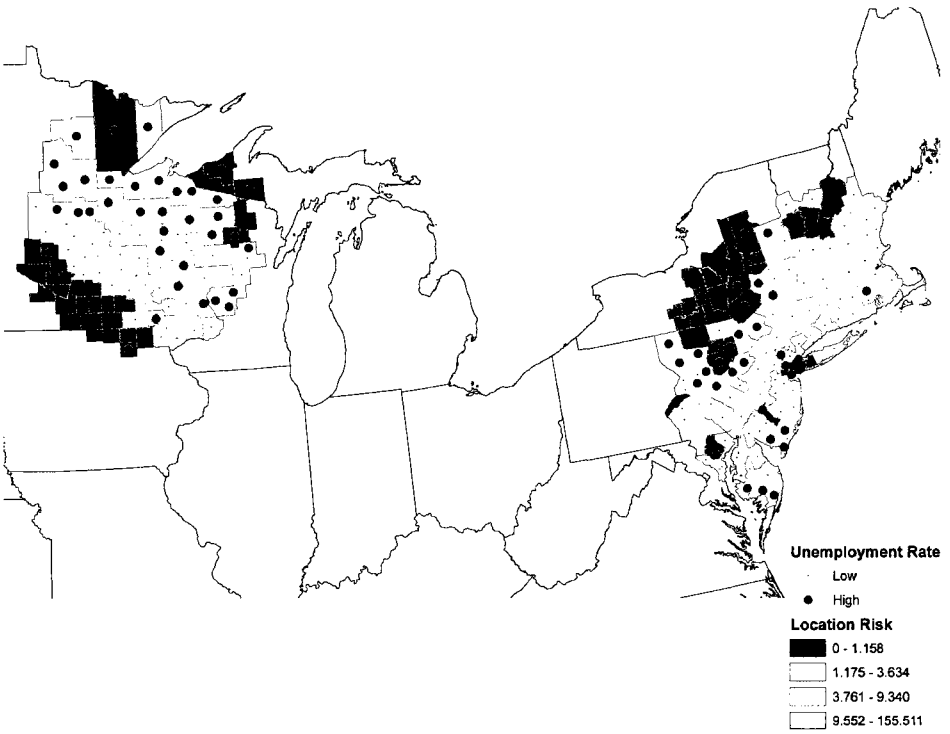
*additional information on this outcome variable was provided in Table 5.6.1

5.7.8. Income* and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006



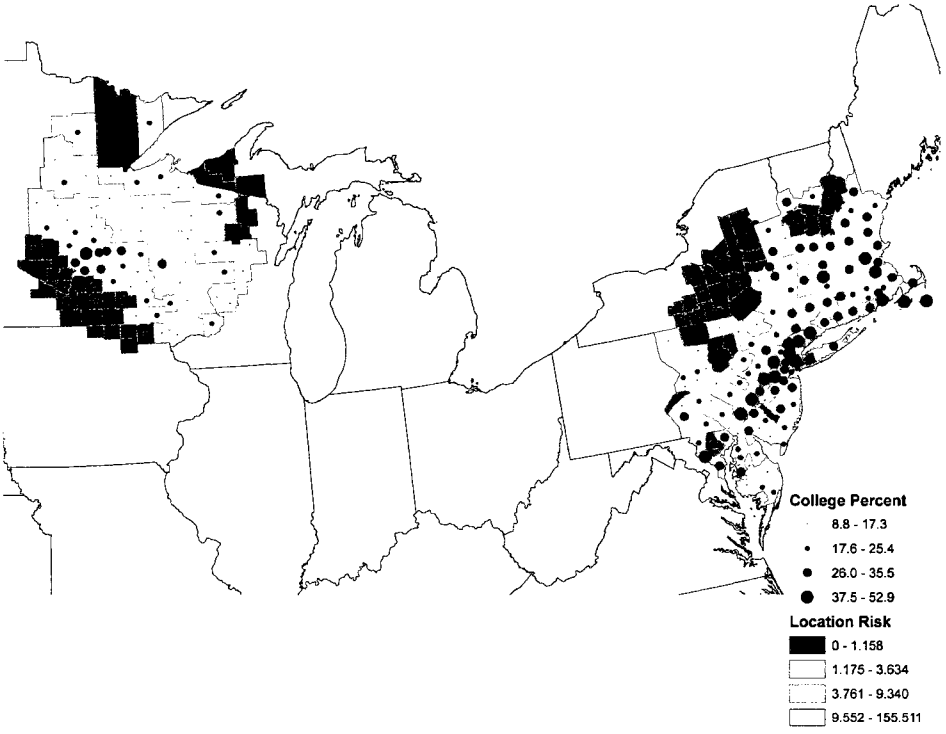
*additional information on this outcome variable was provided in Table 5.6.1

5.7.9. Unemployment* and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006



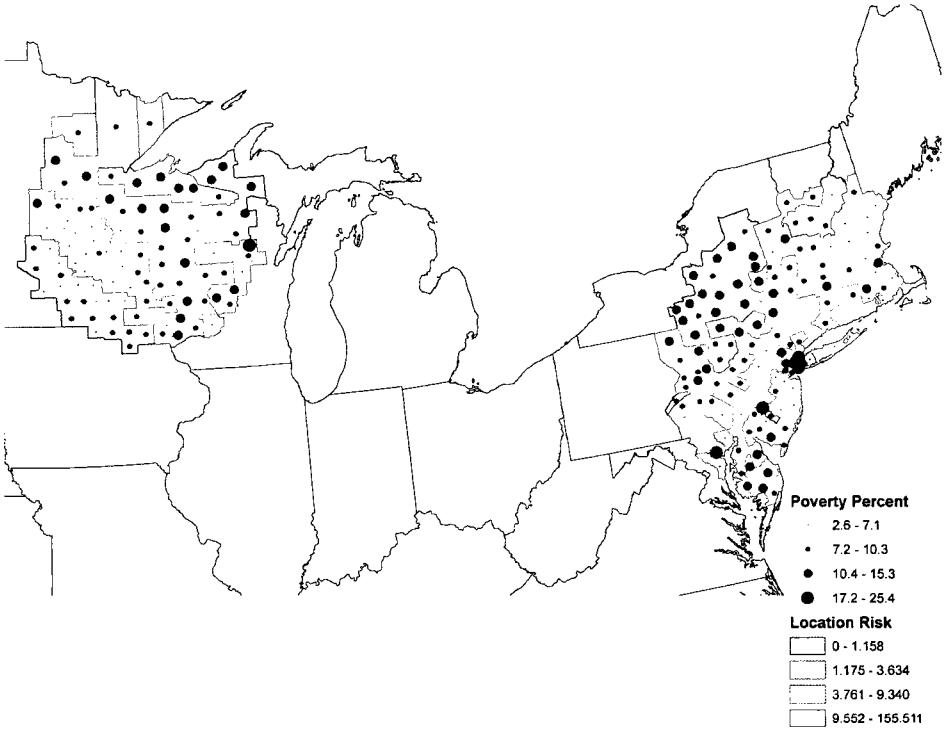
*additional information on this outcome variable was provided in Table 5.6.1

5.7.10. Education* and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006



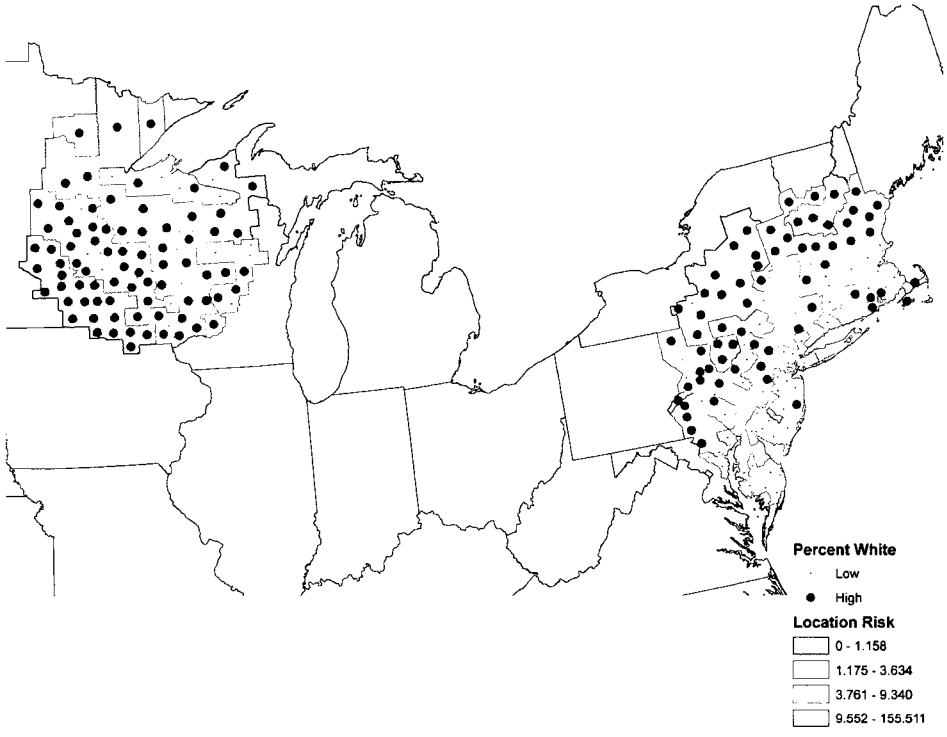
*additional information on this outcome variable was provided in Table 5.6.1

5.7.11. Poverty* and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006



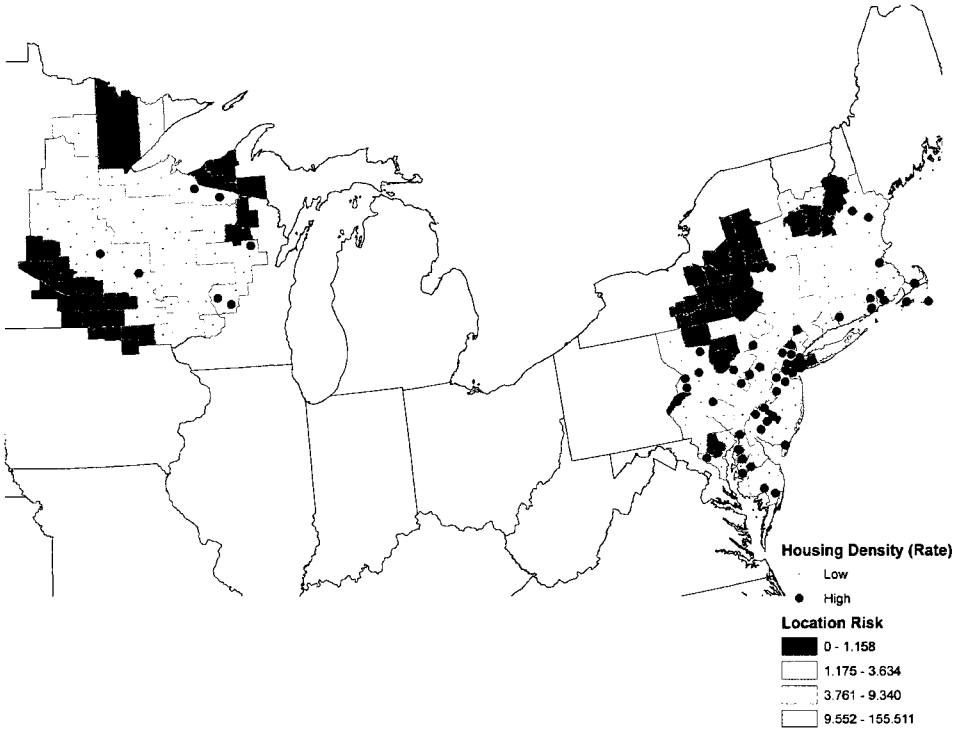
*additional information on this outcome variable was provided in Table 5.6.1

5.7.12. Percent white* and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006



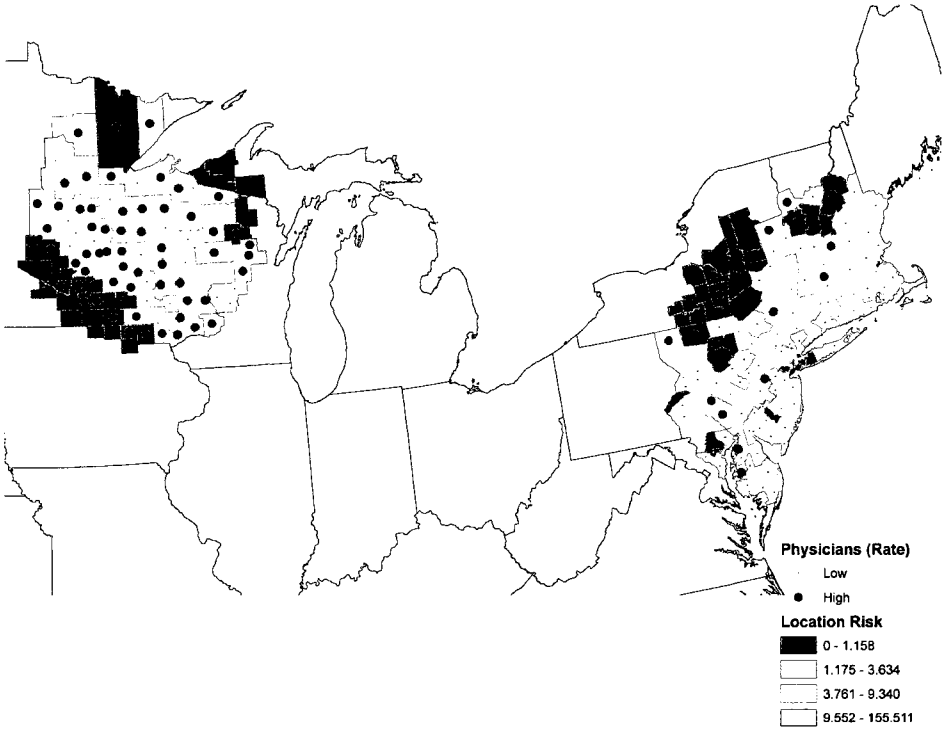
*additional information on this outcome variable was provided in Table 5.6.1

5.7.13. Housing density* and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006



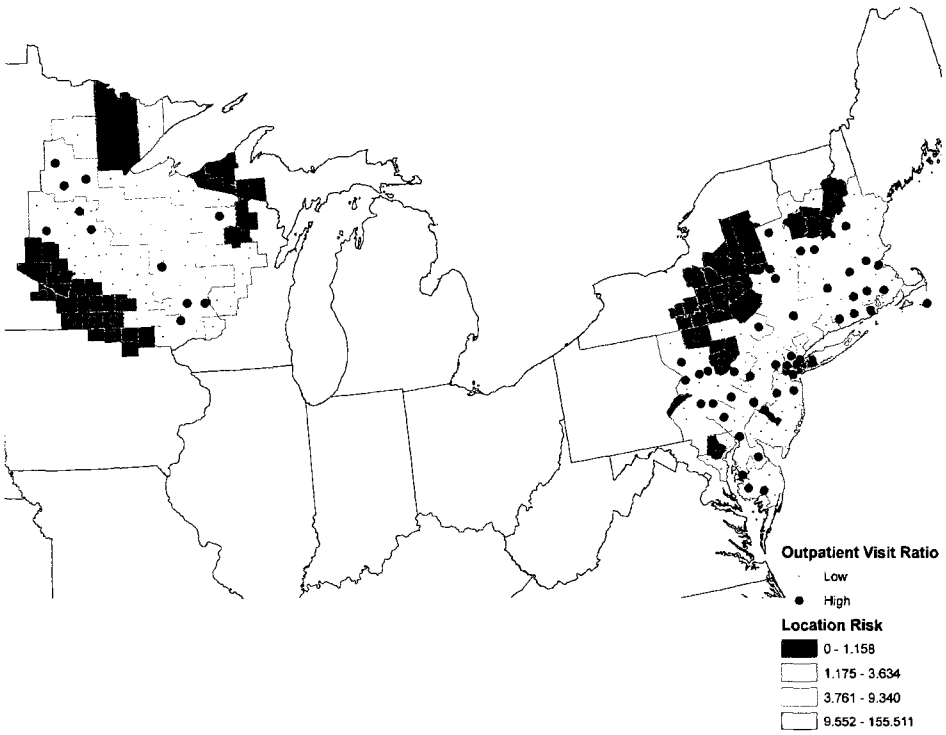
*additional information on this outcome variable was provided in Table 5.6.1

5.7.14. Physician* and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006



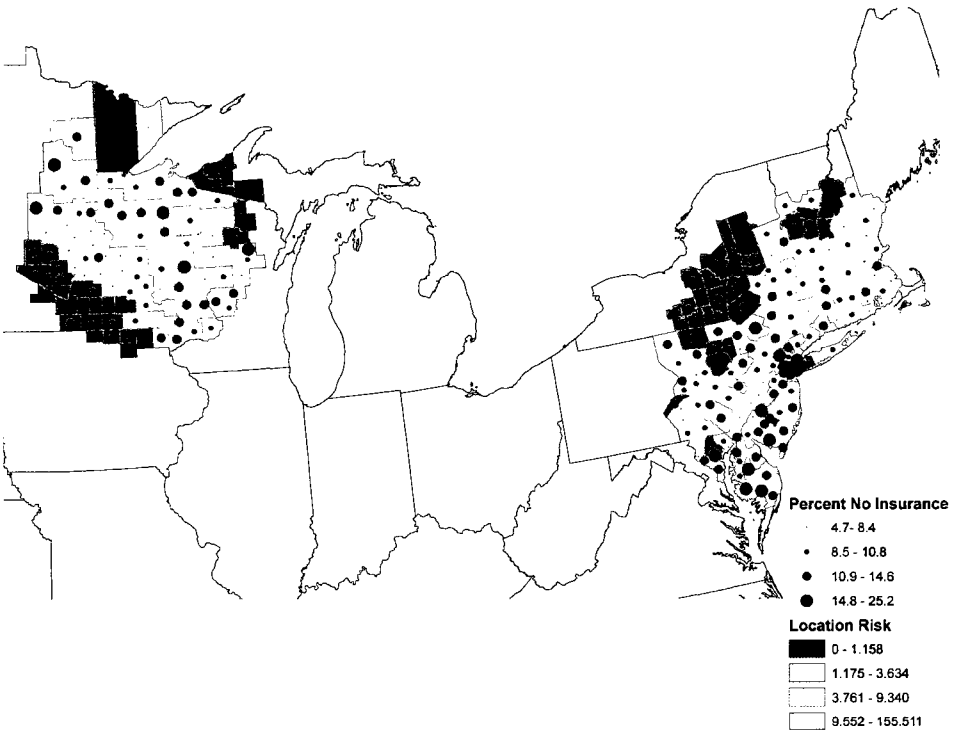
*additional information on this outcome variable was provided in Table 5.6.1

5.7.15. Outpatient visits* and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006



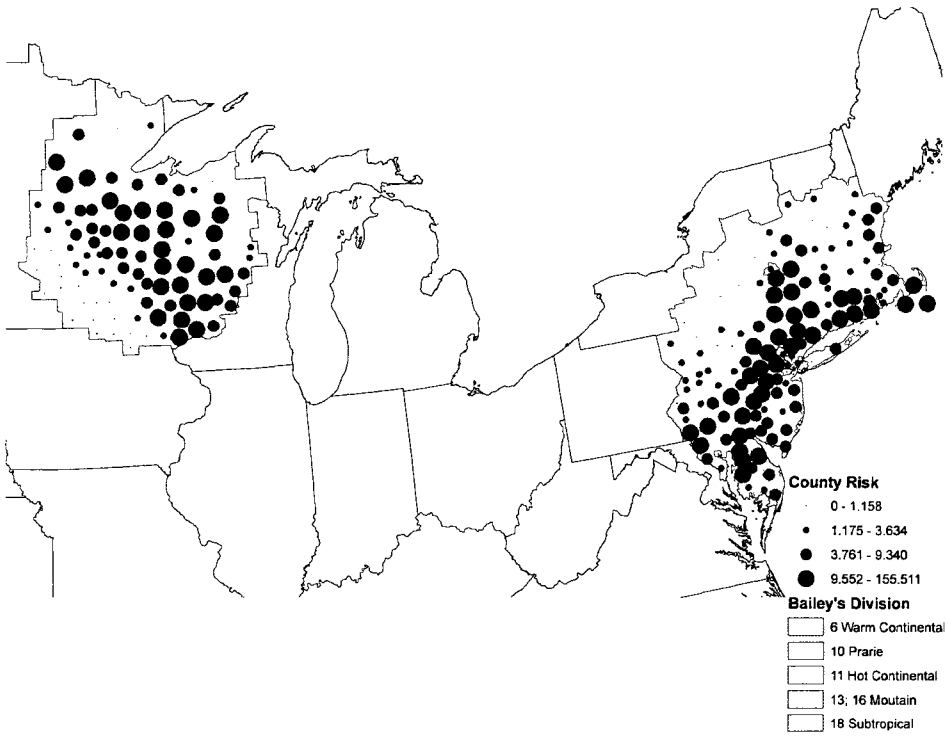
*additional information on this outcome variable was provided in Table 5.6.1

5.7.16. Uninsured* and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006

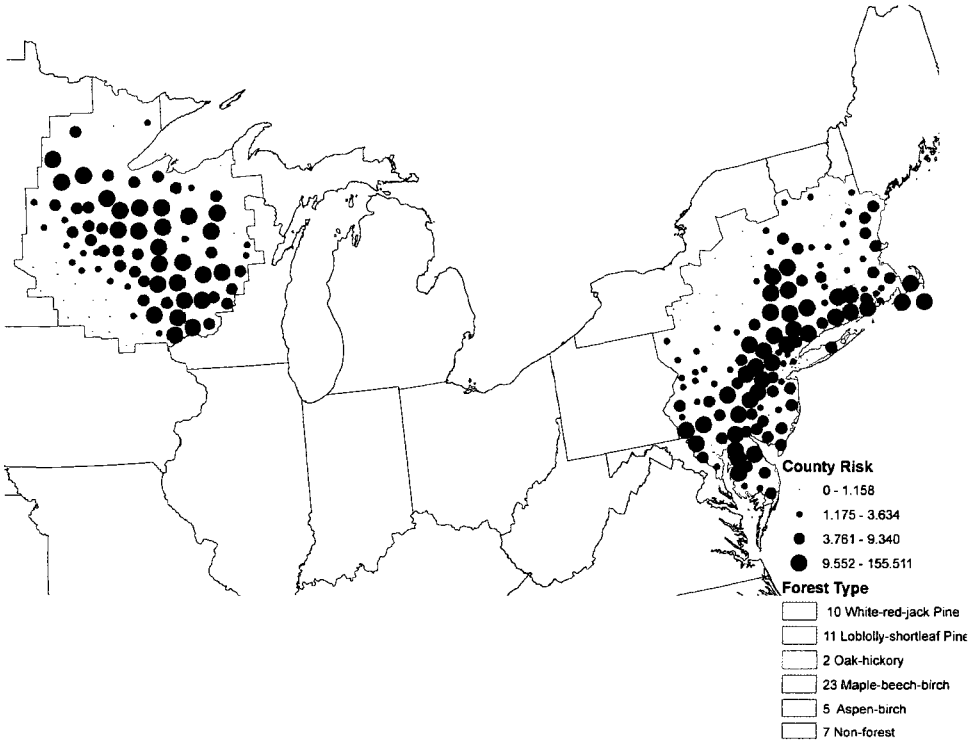


*additional information on this outcome variable was provided in Table 5.6.1

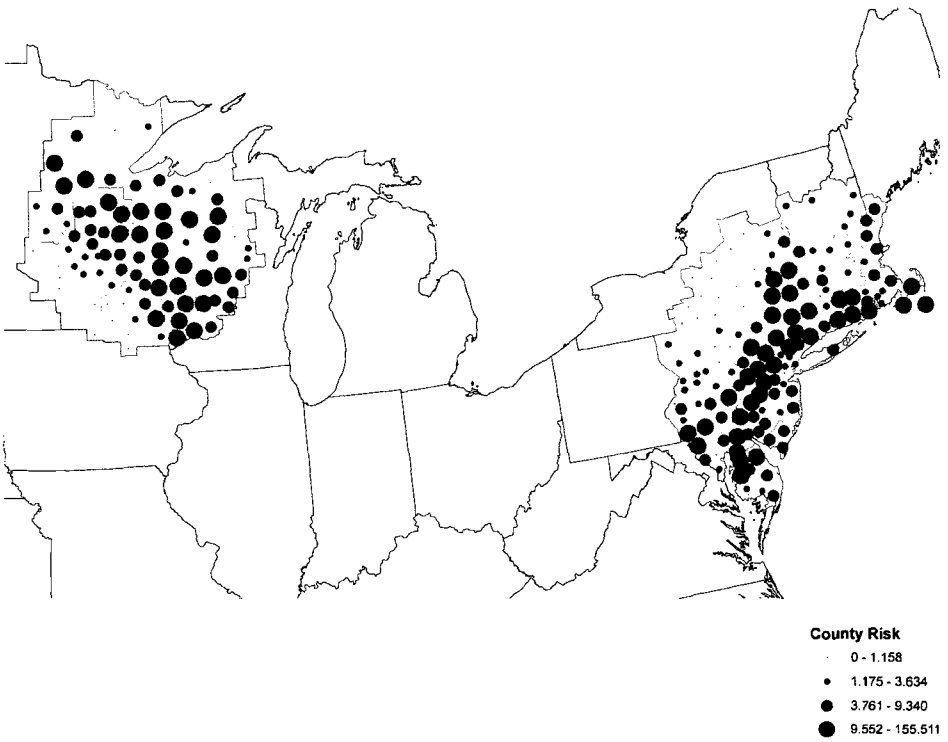
5.7.17. Bailey's ecoregion division and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006



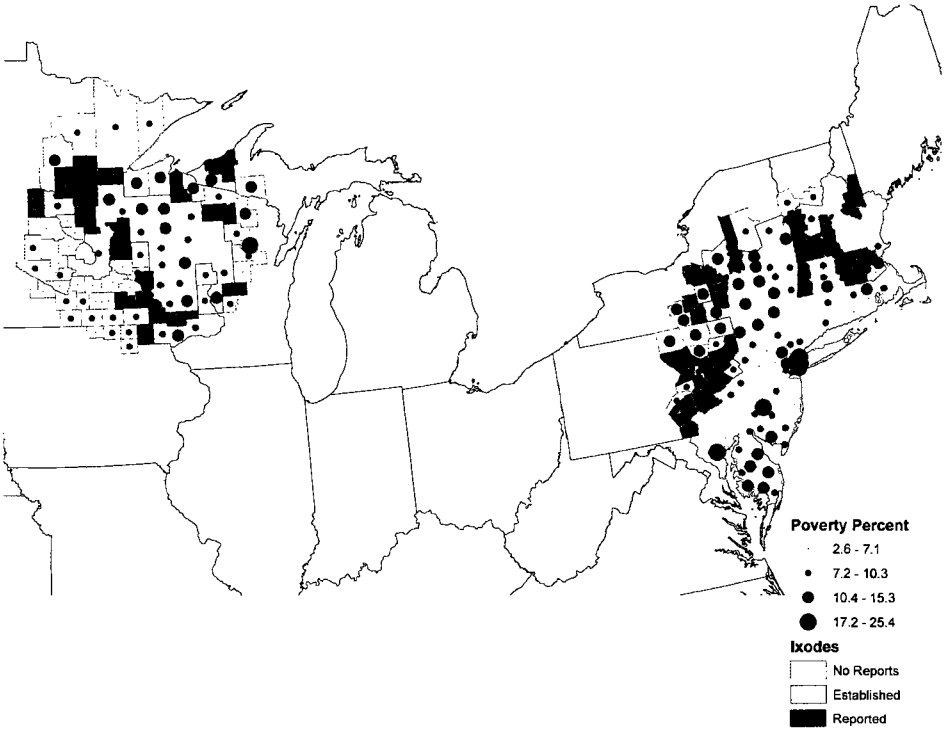
5.7.18. Forest type and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006



5.7.19. Urban influence code and county risk by quartile within two active clusters of reported Lyme disease cases during 1992 – 2006

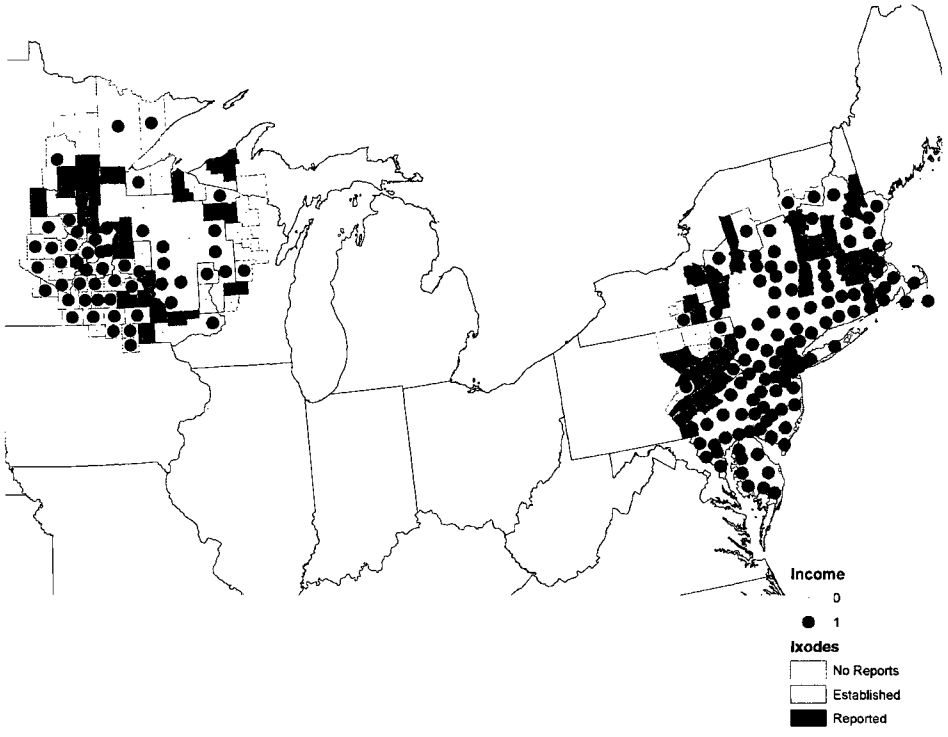


5.7.20. Poverty* and *Ixodes** distribution within two active clusters of reported Lyme disease cases during 1992 – 2006



*additional information on this outcome variable was provided in Table 5.6.1

5.7.21. Income* and *Ixodes** distribution within two active clusters of reported Lyme disease cases during 1992 – 2006



*additional information on this outcome variable was provided in Table 5.6.1

CHAPTER 6

6. CONCLUSIONS

The studies presented were designed to increase knowledge about the epidemiology of hard tick-associated illness in the United States and we present our final conclusions here. From the prospective health assessment of Fort Campbell, Kentucky patrons bitten by ticks described in Chapter 2, we learned that the proportion of participants who removed their embedded tick by hand was 42% higher among those who reported at least one symptom compared to those who reported none. No participant reported fever following the bite of *A. americanum* suggesting the lack of infection with a tick-transmitted viral or bacterial agent. No participants were bitten by *Ixodes* and all *A. americanum* tested negative for *B. burgdorferi* providing additional support that true Lyme disease is rare in this region.

In Chapters 3 and 4 we presented trends in Lyme disease cases reported 1992 – 2006 that underscored the need for prevention strategies targeted to populations with increasing risk. During the 15-year study period, incidence increased disproportionately among children, particularly males. Arthritis was reported more frequently (and conversely erythema migrans less frequently) among children age 10 – 14 years among women aged 30 – 39 years. The percentage of cases for which signs of disseminated infection were reported did not decrease during the reporting period, demonstrating the need for

continued education about early disease recognition and treatment. Geographic expansion was apparent in Minnesota, Pennsylvania and Wisconsin. The age, sex, seasonality of illness onset and frequency of symptoms among cases reported from highly endemic states differed from those reported by all other states suggesting aberrant reporting or fundamental differences in the epidemiology.

We showed the usefulness of national Lyme disease surveillance data in Chapter 5, an ecologic study of social and economic risk factors for Lyme disease. We contributed to the body of literature suggesting that Lyme disease continued to emerge in northeastern and north central United States. We provided data to support the hypothesis that high income was a possible risk factor for increased risk in the northeast and, for the first time, showed that high socioeconomic status was less likely and low socioeconomic status was more likely in counties with higher Lyme disease risk in north central states. These differences demonstrated that results of studies conducted in the northeast may not apply to north central states. However, further studies conducted on smaller geographic scale (e.g., census block, neighborhood, or individual level) are required to determine if these ecologic factors are associated with risk below the county level.

These findings will benefit researchers looking for the etiologic agent of *Amblyomma americanum* associated illness, inform physicians evaluating humans at risk of exposure to hard ticks, and help public health practitioners focus prevention campaigns and resources on the populations at greatest risk for the most common vector-borne disease in the United States, Lyme disease.

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APPENDIX A

Become a TickPro!

The U.S. Army and the Centers for Disease Control and Prevention are studying sickness caused by tick bites.

Most people who are bitten by ticks do not get sick, but some ticks in this area can carry germs.

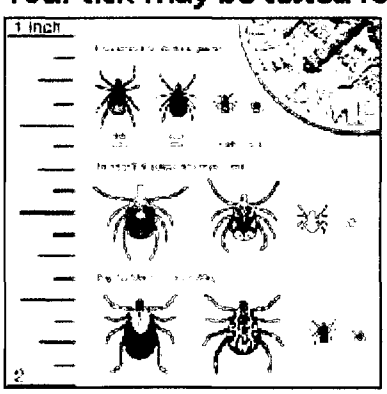
We need to learn which ticks at Fort Campbell make people sick.

If you are bitten by a tick, don't throw it away!

Bring it to: Fort Campbell Environmental Health, Bldg 6903, Desert Storm Avenue, 270-798-8695

It will be tested for germs.
It's a for military personnel, their dependents, military retirees, civilian employees, contractors, and reserve or National Guard components.

Your tick may be tested for these diseases that can occur after tick bite:



- **anaplasmosis**
- **babesiosis**
- **ehrlichiosis**
- **Lyme disease**
- **rickettsiosis**
- **Rocky Mountain spotted fever**
- **STARI** - southern tick-associated rash illness

MILITARY MEDICINE, 174, 3:1, 2009

Prospective Health Assessment of Fort Campbell, Kentucky Patrons Bitten by Ticks

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ABSTRACT *Amblyomma americanum* is an aggressive human-biting tick that transmits several known human pathogens and is associated with a Lyme disease-like illness of unknown etiology. To determine the frequency, distinguishing clinical characteristics, and etiology of *A. americanum*-associated illness and identify associated risk factors, a prospective study of adult tick-bite victims was conducted at Fort Campbell from 2004 to 2006. Forty-two participants submitted ticks, none of which contained *Borrelia lonestari* or *B. burgdorferi* DNA. Thirty-three participants completed a follow-up health survey; 14 reported at least one symptom; two had erythema migrans-like rash; eight sought medical evaluation for their symptoms. Findings suggest that a variety of symptoms are temporally associated with tick bite but data provide no clear evidence that reported symptoms were caused by an infectious process. Removing a tick by hand or being bitten on a limb may be a risk factor for illness.

INTRODUCTION

Each year, several hundred cases of Lyme disease are reported in areas that do not support the known zoonotic cycle for *Borrelia burgdorferi*, the agent of Lyme disease.[1] Retrospective studies have shown that these cases are associated with the bite of *Amblyomma americanum* ticks,[2–4] which do not transmit *B. burgdorferi*.[5–9] We sought to learn more about illness associated with *A. americanum* ticks in an area where the risk of Lyme disease is rare (<1 case per 100,000 population reported annually).

A. americanum (the lone star tick) is an aggressive human-biting tick distributed throughout the southeastern United States (U.S.) and along the eastern coast as far north as Maine.[10] The agents of tularemia and human monocytic ehrlichiosis are the most frequently reported bacteria transmitted to humans during the bite of *A. americanum*, but rare transmission of *Ehrlichia ewingii* and potential transmission of *Rickettsia* spp. and *Coxiella burnetii* to humans has also been reported.[10–13] Although *A. americanum* do not transmit the agent of Lyme disease, *B. burgdorferi*,[5–9] these ticks have been associated with a Lyme disease-like rash illness of unknown etiology.[2–4,14,15] The classic Lyme disease rash is erythema migrans (EM); a red expanding rash, with or without central clearing.[2–4,15] EM is often accompanied by symptoms of fever, malaise, fatigue, headache, myalgia, or arthralgia.

In 1996, a bacterium named *B. lonestari* was suggested as a possible cause of EM-like rash illness following *A. americanum* tick bite.[16] Using polymerase chain reaction (PCR), the presence of *B. lonestari* DNA has been reported in 1–12% of *A. americanum* from Alabama, Arkansas, Delaware, Georgia, Kansas, Kentucky, Maryland, Missouri, New Jersey, New York, North Carolina, South Carolina, Tennessee, Texas, and Virginia,[9,17–24] blood samples from white-tailed deer, *O. virginianus*, in Arkansas, Georgia, North Carolina, and South Carolina,[25] and the skin biopsy sample and attached *A. americanum* from one human patient with possible exposure in Maryland or North Carolina.[14] However, subsequent investigations by Wormser et al.[26] and Johnson et al. (Centers for Disease Control and Prevention [CDC], unpublished data) failed to find evidence of *B. lonestari* infection in patients with *A. americanum*-associated EM-like rash illness.

Epidemiologic study or surveillance for *A. americanum*-associated illness is difficult because there is no uniform case definition or diagnostic criteria for this illness and the etiology is unknown. There are two reports of analytic epidemiologic studies of persons with EM-like rash illness in areas where *A. americanum* is the predominant human biting tick and true Lyme disease is rare.[2,27] In a retrospective study of persons with EM-like rash, Campbell et al. found that when compared with controls, case patients were statistically more likely to live near a pond or lake, recall recent chigger bite, hunt, and be male.[2] Armstrong et al. conducted a prospective study of residents living in a coastal Maryland *A. americanum*-infested community[27] and found that the perceived risk of Lyme disease was high although 95% of ticks submitted were *A. americanum*. Patients with a history of Lyme disease diagnosis were significantly more likely to garden, have more than one tick bite per week, and use personal protection measures when compared to residents lacking a history of Lyme disease diagnosis.

Here we describe TickPro, a prospective study of Fort Campbell, Kentucky patrons bitten by ticks. The aims of this study were to: (1) further define the frequency and

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distinguishing clinical characteristics of *A. americanum*-associated illness; (2) develop alternative hypotheses for *Amblyomma*-associated illness, such as hypersensitivity to tick bite, misdiagnosis of tick-borne illness, or another etiology; and (3) determine if illness is related to tick characteristics, host traits, or other risk factors.

METHODS

Participants were recruited and enrolled by the Fort Campbell Preventive Medicine, Environmental Health Section. The study population included any person aged 18 or older of any gender or race who was eligible for health services at Fort Campbell. Participants must have recently removed an embedded tick from their skin and the tick must have been submitted concurrent with enrollment. Fort Campbell is well within the geographic range of *A. americanum* and the risk of locally acquired Lyme disease is rare.

Consenting adults completed an enrollment survey providing information about demographics, tick exposure, and general health. The self-administered surveys were designed to collect data related to the index tick bite (e.g., crawling or embedded, date of removal, method of removal, length of attachment, and location of tick bite), host traits (e.g., age, sex, race, military status, previous diagnosis of tick-borne illness or chronic disease, antibiotic or immunosuppressive drug therapy, and tick-bite history), or place and time (e.g., exposure location and month or year of tick bite).

Index ticks were sent to the U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM) Entomological Sciences Program laboratory where they were identified and tested for known pathogens under the Human Tick Test Kit Program. CHPPM determined the species, sex, life stage, engorgement level, and viability of all ticks. Tick DNA was extracted using standard research methods.[28] *A. americanum* were tested for the presence of *B. lonestari* DNA using PCR with melting curve analysis of a portion of the glycerophosphodiester phosphodiesterase (glpQ) gene,[29] *B. burgdorferi* DNA using PCR amplification of the OspA gene,[30] and *E. chaffeensis* DNA by PCR using melting curve analysis of amplification of the groESL gene.[31] *D. variabilis* were tested for the presence of spotted fever group *Rickettsia* spp. using primers for the OmpB gene[32] and confirmed using amplification of the OmpA gene (Rr190.70p and Rr190.602n).[33]

Approximately 30–45 days after enrollment, CDC asked each participant to complete a 30-day follow-up survey by mail, e-mail, or telephone. The survey collected information on selected symptoms of acute illness including rash, joint pain, joint swelling, swollen lymph nodes/glands, headache, stiff neck, paralysis, generalized weakness, fever, chills, fatigue, impaired memory, confusion, abdominal pain, nausea, vomiting, diarrhea, cough, difficulty breathing, jaundice, and photosensitivity. Participants were also asked about physician visits, diagnoses, prescribed therapy, clinical outcome, and additional tick exposures.

Because of the small sample size, a dichotomized outcome variable was created by classifying illness on the basis of the report of no symptom after tick bite (no disease) or at least one symptom reported after tick bite (disease). Categorical variables were compared in cross-reference tables with Fisher's exact test. For continuous variables, the difference between sample means was tested using Student's *t*-test.

The study was reviewed and approved by the CDC Human Research Protection Office Institutional Review Board and Colorado State University Research Integrity and Compliance Review Office Human Research Committee to ensure compliance with the Health and Human Services Policy for Protection of Human Research Subjects codified in the Code of Federal Regulations at 45 CFR part 46.

RESULTS

Forty-two adults participated. Geographic exposure location was provided by 36 participants (Fig. 1); 6 (14%) reported an unknown exposure location. The average age of participants was 41 years (range 20–79); 23 (55%) were male, 25 (60%) were white, and 9 (21%) black. Thirteen participants (30%) were active duty military, 10 (24%) retired, 10 (24%) military dependents, 5 (12%) civilian employees, and 3 (6%) were another status (National Guard, Reserves, or other). F1

Participants were bitten by *A. americanum* ($n = 36$, 86%) or *D. variabilis* ($n = 6$, 14%). Among those bitten by a single *A. americanum* ($n = 29$), 19 (65%) were bitten by adult ticks (11 female; 8 male) and 10 (34%) by a nymphal tick. Twenty-four (83%) participants removed and submitted an unengorged *A. americanum*, four (14%) submitted a partially engorged *A. americanum*, and one (3%) submitted a fully engorged *A. americanum*. Month of tick bite correlated well with known tick activity patterns (except for July, which may be an artifact of troop movement in 2005–2006 and a long hot and dry spell in 2007) (Fig. 2). Laboratory tests for the presence of *B. lonestari* and *B. burgdorferi* in *A. americanum* were negative. *E. chaffeensis* DNA was detected in the *A. americanum* removed from one participant. The tick fragment from one participant was too small for DNA extraction and, therefore, was not tested by PCR. Laboratory tests for the presence of *R. rickettsii* in *D. variabilis* were negative. F2

Participants reported removing embedded ticks with tweezers ($n = 18$, 43%) or by hand ($n = 24$, 57%). Anatomical location of tick bite was available for 36 participants (90%) reporting a single tick bite. Among these, 19 (53%) were bitten on the torso, 12 (33%) on a limb, 5 (14%) on the head or neck. On the day of enrollment (generally the same day the embedded tick was noticed), participants were asked if they had a skin rash or lesion at the tick bite site. Most did not ($n = 24$, 57%). Among 16 participants that did, 7 indicated that the lesion was less than 1 inch wide and 9 reported a 1- to 3-inch lesion; 12 reported pain or itch at the bite site, 9 reported the rash was raised or bumpy, and six reported redness. Two participants were unsure if a skin rash or lesion developed at the tick bite site.

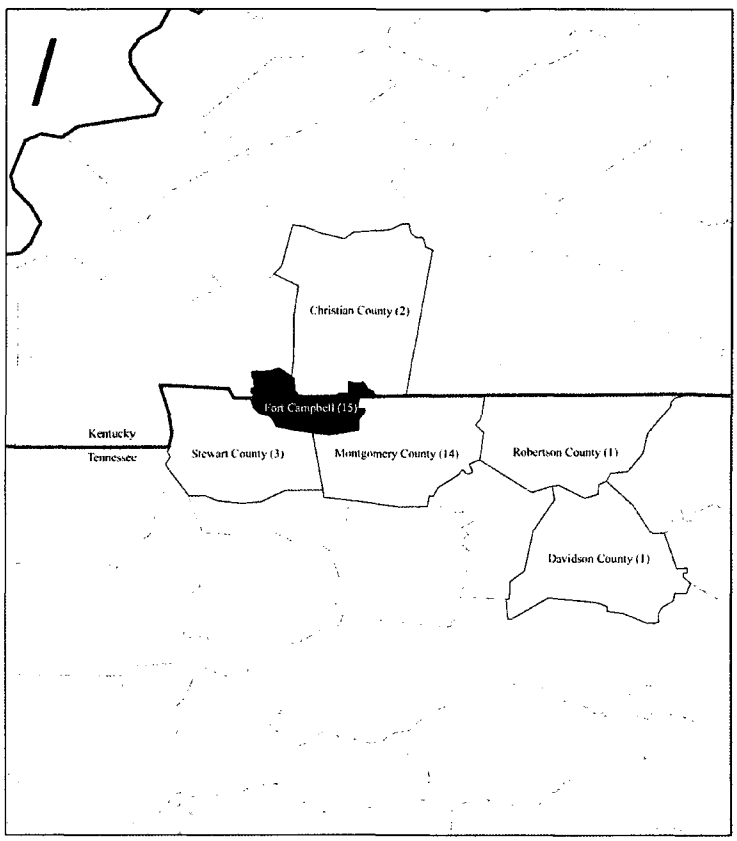

FIGURE 1. County of exposure among TickPro participants.

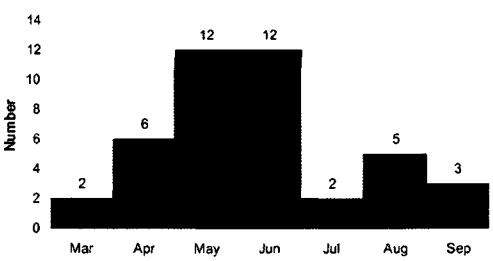

FIGURE 2. Month of tick bite among TickPro participants.

Fourteen of 33 participants for whom follow-up data were available reported at least one symptom (range 1–7). Among those with symptoms, fatigue was reported most often (43%), followed by headache/stiff neck (36%), cough (29%), sore throat (21%), joint swelling (14%), EM-like rash (14%), and other rash (14%). Diarrhea, joint pain, numbness/paralysis, chills, confusion, vomiting, difficulty breathing, and light sensitivity were each reported once. No participant reported fever, skin ulcer, jaundice, impaired memory, or difficulty concentrating.

Two participants reported EM-like rash after tick bite. One 53-year-old black female military dependent reported expanding circular red skin rash 3 or more inches in diameter, unexplained chills, unexplained fatigue, confusion, vomiting, diarrhea, cough, and sore throat for which she sought medical evaluation. She reportedly received no diagnosis or therapy. She reported no prior history of health conditions, antibiotic use, or immunosuppressive therapy. Her index tick was a partially engorged *A. americanum* nymph that was PCR negative for specific pathogens tested. One 23-year-old black female military dependent reported expanding circular red skin rash 3 or more inches in diameter, severe headache or stiff neck, and

numbness or paralysis of the face, arms, or legs for which she sought medical evaluation. She was diagnosed with Lyme disease and was prescribed doxycycline. She reported no prior history of health conditions, antibiotic use, or immunosuppressive therapy. Her index tick was a partially engorged *A. americanum* nymph that was PCR negative for specific pathogens tested.

Of 14 participants reporting at least one symptom within 30 days of tick bite, 8 sought medical attention for their symptoms; 3 were told their symptoms were related to tick bite (one was diagnosed with Lyme disease); therapy was administered or prescribed for 5 (antibiotics, steroid cream, or injectable headache relief). Six participants were prescribed antibiotics for their symptoms or reported taking them during the study period. On the date of follow-up survey, 4 reported persistent symptoms.

There were no significant differences (p value ≤ 0.05) in demographic features or seasonality of tick bite between participants reporting no and at least one symptom after tick bite (Table I). The data suggest, however, that persons bitten during late spring or early summer may have increased risk of having at least one symptoms compared with those bitten in late summer (Fig. 3).

There were no significant differences in the occurrence of tick-borne disease symptoms in the 12 months before study enrollment or reported lifetime health history between participants reporting no and at least one symptom after tick bite (Table II). The proportion of immunosuppressive or antibacterial therapy in the month before enrollment or during the study period did not differ significantly between these two groups.

The proportion of participants who removed the index tick by hand (as opposed to tweezers or some other method) and the proportion who were bitten on a limb was significantly higher among those with at least one symptoms as compared to those with no symptom ($p = 0.03$ and $p = 0.02$, respectively) (Table III). However, there is strong association between these

TABLE I. Demographics of TickPro Participants, Seasonality of Tick Bite and Presence or Absence of Reported Symptoms

	No Acute Symptoms $n = 19$ (%)	=>1 Acute Symptom $n = 14$ (%)	P Value
Age			
Mean	41	47	0.2018[a]
Range	20–59	23–79	
Male	9 (47)	7 (50)	1.0000[b]
Race			
White	12 (63)	10 (71)	0.0910[b]
Black	2 (11)	4 (29)	
Other	5 (26)	0 (0)	
Active duty	4 (21)	3 (21)	1.0000[b]
Year of enrollment			
2005	4 (21)	2 (14)	1.0000[b]
2006	10 (53)	8 (57)	
2007	5 (12)	4 (29)	
Season of enrollment			
Mar–May	6 (32)	9 (64)	0.0853[b]
Jun–Sep	13 (68)	5 (36)	

[a] p value from two sample t-test.

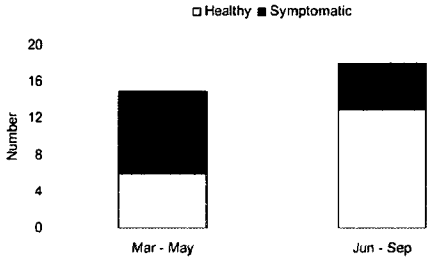

FIGURE 3. Season of tick bite and presence or absence of symptoms among TickPro participants.

TABLE II. TickPro Participant Health and Presence or Absence of Reported Symptoms

	No Acute Symptoms $n = 19$ (%)	=>1 Acute Symptom $n = 14$ (%)	Fisher's Exact Test P-Value
Symptom of tick-borne disease in past 12 months	1 (5)	3 (21)	0.2882
Lifetime health history			
Any condition	11 (58)	9 (64)	1.0000
Allergy	4 (21)	6 (43)	0.2569
Skin condition	9 (47)	3 (21)	0.1604
Joint/muscle disorder	1 (5)	3 (21)	0.2882
Tick-borne disease	1 (5)	0 (0)	1.0000
Therapy in month before tick bite			
Immunosuppressive	0 (0)	1 (7)	0.4062
Antibacterial	1 (5)	4 (29)	0.1420
Therapy during study period			
Immunosuppressive	0 (0)	1 (7)	0.4242
Antibacterial	2 (11)	4 (29)	0.3649
Tick bite in month before study enrollment	6 (32)	4 (29)	1.0000

TABLE III. Tick Bite Characteristics and Presence or Absence of Reported Symptoms among TickPro Participants

	No Acute Symptoms $n = 19$ (%)	=>1 Acute Symptom $n = 14$ (%)	Fisher's Exact Test P-Value
Species			
A. americanum	15 (79)	13 (93)	0.3662
D. variabilis	4 (21)	1 (7)	
More than one tick bite at enrollment	4 (21)	2 (14)	1.0000
Removed tick(s) by hand	7 (37)	11 (79)	0.0329
Skin reaction at bite site	5 (26)	7 (54)	0.1502
Anatomical location of bite			
Torso	10 (55)	5 (36)	0.4824
Limb	2 (11)	7 (50)	0.0191
Head or neck	4 (22)	1 (7)	0.3662
Other unspecified	2 (11)	1 (7)	1.0000
Exposure location on base	4 (25)	5 (39)	0.6822
Occupational exposure	2 (11)	2 (14)	1.0000
Additional tick bites during study period	4 (21)	2 (14)	1.0000

two risk factors (Fisher's exact p value = 0.02). That is, most participants who removed the index tick by hand were bitten on a limb.

DISCUSSION

The proportion of participants who removed their embedded tick by hand was significantly higher among those reporting disease than those reporting no disease. This observation supports recommendations by CDC and others who suggest tick removal by grasping the tick with tweezers very close to human skin.[34–37] This presumably reduces the chance that tick midgut contents (where pathogens may reside) are forced into the host because of grasping, squeezing, or crushing a tick with your fingers.[34,38]

The proportion of participants who reported anatomical site of tick bite as the limb (arm, hand, upper leg, lower leg, or foot) was significantly higher among those with disease compared to those with no disease. This finding is consistent with the prospective study conducted by Kirkland[4] but differs from findings published by Campbell, Felz, and Wormser[2,3,39] who reported that most patients were bitten on the torso. This discrepancy may result from a difference in the definition of disease. Whereas previous studies recruited patients with EM-like rash, many of our participants reported symptoms other than rash.

A few other findings are worth noting even though they lack statistical significance. Sixty percent of participants were white. This contrasts sharply with known demographics for persons with Lyme disease where >95% are white. This may simply reflect diversity in the Fort Campbell sample population rather than suggest a biological difference in zoonotic risk between persons exposed to *A. americanum* and those exposed to *I. scapularis*. At enrollment (presumably within a day or 2 of tick removal), almost half of participants (43%) reported the development of a skin lesion at the tick bite site. This swift reaction was likely caused by a local inflammatory response to the tick bite rather than infection by a bacterial or viral etiologic agent.[40]

All PCR tests were negative except for *E. chaffeensis* in one tick removed from an asymptomatic participant. This finding is not surprising considering the small number of participants and expected low infection rates for the agents tested. It is important to note that no participants were bitten by *Ixodes* ticks and all *A. americanum* tested negative for *B. burgdorferi*.

It appears that participants accurately recalled and reported length of tick attachment; 88% self-reported length of tick attachment as less than 1–3 days and 86% of ticks were identified as unengorged by CHPPM. This is consistent with feeding habits of *A. americanum* as they take in very little blood during the first 3–4 days of feeding and then take a "big sip" during the last 12 hours of attachment.[41]

In previous studies of *A. americanum*-associated illness and in this study, reports of fatigue, headache and/or stiff neck, and malaise or musculoskeletal pains were most frequent. Unfortunately these influenza-like symptoms are common to many infectious diseases including most known tick-borne diseases and do not provide much unique data on which to draw new hypotheses about the etiology of *A. americanum*-associated illness. Our participants did not report fever, which differs from previous investigations of *A. americanum*-associated rash illness where 5–29% of patients reported fever.[2–4,39]

Several study limitations are worth mentioning. Characteristics of the Fort Campbell sample population likely differed from the larger population of persons who experience tick bite and, therefore, reduce the extent to which findings can be generalized. Tick density, activity, and infection rates are often spatially and temporally focused because of variance in reservoir host availability and microclimate conditions. This may have reduced the number of persons subject to tick bite (e.g., when climate conditions are unsuitable for ticks) or reduced the potential for exposure to agents in ticks (e.g., tick infection rates are low when few reservoirs hosts are available for tick feeding). Advertising the study to recruit tick bite victims may have caused people to check for ticks more frequently and therefore reduce the amount of time that a tick has fed on participants. Participants may have had difficulty accurately recalling prior illnesses or tick encounters resulting in misclassification of exposure. Signs of symptoms of disease were self-reported by study participants and were not corroborated by a health care provider or with standardized diagnostic testing. Although physician-diagnosed illness with laboratory support of infection is a gold standard in epidemiologic studies, achieving this was not possible in our study because there is no standard case definition for *A. americanum*-associated illness, the illness is mild and may not require a physician visit, there are no standard serologic assays for *B. lonestari*, and PCR testing for *B. lonestari* in human specimens is complex. Finally, data on the frequency of symptoms reported among individuals without known tick bite was not available for comparison.

One of the novel components of this study, compared with other epidemiologic studies of *A. americanum*-associated illness, was recruitment of participants with known tick bite coupled with laboratory identification and PCR testing of the index tick(s) and prospective evaluation of a variety of acute symptoms. Previous retrospective studies used a strict definition of rash to recruit patients and may have limited observation of a spectrum of clinical outcomes following *A. americanum* tick bite.[2–4,27,39] A strength of the current study is long-term storage of DNA extracted from ticks removed from study participants, allowing an opportunity to test for future suspect etiologies of *A. americanum*-associated illness.

In summary, we found diversity in the type and frequency of symptoms reported by persons bitten by *A. americanum*. We were unable to identify any sequelae that distinguished *A. americanum*-associated illness from Lyme disease.

We also did not identify a specific etiology; however, the lack of fever suggests a noninfectious process. Removing a tick by hand or finding it embedded on a limb was reported more frequently among persons with at least one reported symptom, but small sample size limited examination of the relationships between many other risk factors and specific symptoms of illness.

ACKNOWLEDGMENTS

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Tick Illness Study

Consent for Participation in a Research Study

Introduction

The Centers for Disease Control and Prevention (or CDC), Colorado State University (CSU), and the United States Army are studying sickness caused by tick bites. Most people who are bitten by ticks do not get sick, but some ticks can carry germs that make people sick.

We are asking you to join this research study since you have been bitten by a tick that might have a germ in it. This form gives you details about the research study. After you have read this form, please ask the study staff to explain any words or parts that you do not clearly understand. Once you understand the study, you can decide if you want to join the study or not.

If you do not join the study, it will not change the health care you get. You can have your tick tested for germs whether you join the study or not.

What is the purpose of this study?

We are doing this study to answer three questions:

1. How often do people get sick after being bitten by a tick?
2. What kind of symptoms do they have?
3. Is their sickness caused by a tick germ? Particularly a germ called *Borrelia lonestari*?

What is the process?

We are asking you to join this research study since you have been bitten by a tick. We want you to give us your tick and answer some questions about your health and your contact with ticks. This year, we want to invite 1,000 people to be in this study.

TODAY

Tick – Your tick will be sent to a US Army lab and tested for germs. If any test result seems to have meaning for your health, we will tell this clinic.

Enrollment Survey – You will complete a form asking questions about your health and your contact with ticks. It will take about 10 minutes to finish this form.

IN 30 DAYS

Follow Up Survey – In about one month, CDC will contact you and ask you to answer more questions about your health, visits to your doctor, and contact with ticks. This survey will take less than 20 minutes to finish.

Are there any risks to me?

1. Tick removal – If your tick is still attached to your skin, a clinic worker will remove it using sterile tweezers. This should not hurt you, but it will take a minute or two of your time.
2. Survey questions - None of the questions we ask should make you feel uneasy. But it is ok to skip any question you don't want to answer for any reason.

It is not possible to identify all potential risks in research procedures, but the researchers have taken reasonable safeguards to minimize any known and potential, but unknown, risks.

Are there any benefits?

You will not benefit directly from being a part of this study. But helping to carry out this research has a chance to tell us more about diseases from ticks. This could be of future benefit to you, someone you know, or other people living in the United States.

How will CDC protect my privacy?

CDC will keep your personal information in a locked file and only the study staff will be allowed to look at it. After we receive both of your surveys, we will delete your name, street address, and other personal information from all of our records. Your name or other facts that might point to you will not appear when we present this study or publish its results.

Will I have to pay for anything?

You will not pay for the research tests that the US Army will do with your tick. The only cost to you for being in this study is the time you will spend here today and the time you will spend filling out the second survey.

Will I receive any payment?

We can not pay you to join this study. And neither CDC, CSU, nor the US Army has money to pay for costs that might result from your visits to this clinic. You (or your health provider) are responsible for health care or treatment that might result from your visits to this clinic.

Can I refuse to join the study?

You are free to join this study or not. If you join the study, you can drop out later for any reason. If you don't join the study or decide later to drop out, it will not in any way affect your relationship with your own doctors, this clinic, CDC, CSU, or the US Army.

What if I have questions?

If you have any concerns about your rights as a volunteer in this study, or if you think that you have been hurt by being in this study, call the CDC Deputy Associate Director of Science at 1-800-584-8814 for information about your rights and advice on what to do. If the Deputy Director does not answer directly, leave a message including your name, your phone number, and protocol number 4492, so that you may be called back as soon as possible. The Deputy Director is not affiliated with this study in any way.

Or, you can call Celia Walker, CSU Director of Regulatory Compliance at 970-491-1533. The Colorado Governmental Immunity Act determines and may limit Colorado State University's legal responsibility if an injury happens because of this study. Claims again the University must be filed within 180 days of the injury.

If you have any questions about how the study works or have complaints or comments about the study, please write or call Rendi Bacon, CDC, P.O. Box 2087, Fort Collins, CO 80525, (970) 266-3528.

Consent

I have read this consent form and my questions have been answered. I have been told the risks and benefits to me from being in this study. I have been given a copy of the consent form for me to keep. After joining this study, I may leave it at any time by calling Rendi Bacon with CDC at 970-266-3528.

Sign your name: _____

Print your name: _____

Today's date is: _____

ID#

VOLUNTEER CONTACT INFO

CDC will use the following information to contact you in about 30 days to complete your Follow-Up survey. This form will then be destroyed to protect your privacy.

Please indicate your preference:

☐ call me on the telephone

☐ send me an email

☐ send it to me in the mail

Today's Date (mm/dd/yy) _____

Your Name:		
last	*first*	*middle*
Mailing Address:		
City:	State:	Zip Code:
Email:		Phone:

ID#

Today's Date		POC Name	
Clinic		POC COM	
Installation			

TICK ILLNESS ENROLLMENT SURVEY

The following sections should be completed by the volunteer.

Your age:_____

☐ Male ☐ Female

☐ American Indian or Alaskan Native
☐ Asian
☐ Black or African-American
☐ Hispanic
☐ Native Hawaiian or other Pacific Islander
☐ White, Non-Hispanic
☐ Other or Decline to Answer

☐Active Duty ☐Nat'l Guard ☐Retired ☐Reserves ☐Military Dependent ☐Civilian ☐Other

The following questions refer to the tick you are submitting today.

1. Was the tick crawling on your skin or clothing or was it embedded in your skin?
   ☐crawling (skip to question 5) ☐embedded ☐unknown

2. How did you remove this tick?
   ☐by hand ☐tweezers ☐other _____

3. How many days do you think this tick was attached to your skin?
   ☐less than 1 day ☐1-3 days ☐more than 3 days ☐unknown

4. Do you have/Did you develop a skin reaction (rash or lesion) at the tick bite site?
   ☐yes ☐no ☐unknown

   If yes, how wide is/was the rash?
   ☐less than 1 inch ☐1-3 inches ☐more than 3 inches ☐unknown

   If yes, is/was the rash?
   ☐itchy or painful ☐raised or bumpy ☐just red ☐other_____

5. Where did you acquire this tick?
   ☐on this base ☐another location ☐unknown
   If another location, give City _____County _____State _____

6. On what date did you remove this tick from your skin?
   (mm/dd/yy) _____/_____/_____

7. Where on your body did you find this tick?
   ☐head ☐neck ☐arm ☐hand ☐chest ☐abdomen ☐shoulder
   ☐upper leg ☐lower leg ☐groin ☐foot ☐back ☐buttocks ☐other

8. How many ticks have you removed from your body in the past 30 days?
   ☐just this one ☐more than one, but less than 5 ☐5 to 10 ☐more than 10 ☐unknown

Please turn to back page...

In the past 12 months, have you had any of the following symptoms or conditions?

9. Expanding circular red skin rash, 3 or more inches in diameter ☐ Yes ☐ No ☐ Unknown
10. Severe joint pain or swelling of your knees unrelated to injury ☐ Yes ☐ No ☐ Unknown
11. Severe headache or stiff neck lasting more than 2 days ☐ Yes ☐ No ☐ Unknown
12. Numbness or paralysis of the face, arms, or legs ☐ Yes ☐ No ☐ Unknown

Have you ever had any of the following?

13. Severe allergic reaction to bee sting ☐ Yes ☐ No ☐ Unknown
14. Allergies for which you took/take medicine ☐ Yes ☐ No ☐ Unknown
15. Hives ☐ Yes ☐ No ☐ Unknown
16. Skin ulcer ☐ Yes ☐ No ☐ Unknown
17. Eczema/Atopic Dermatitis or Psoriasis ☐ Yes ☐ No ☐ Unknown
18. Contact Dermatitis ☐ Yes ☐ No ☐ Unknown
19. Vitiligo ☐ Yes ☐ No ☐ Unknown
20. Recurrent rash on wrists, ankles, or elbows ☐ Yes ☐ No ☐ Unknown
21. Any other type of skin rash or rash illness ☐ Yes ☐ No ☐ Unknown
    If yes, please describe _____
22. Scabies, Head Lice, or Body Lice ☐ Yes ☐ No ☐ Unknown
23. Impetigo ☐ Yes ☐ No ☐ Unknown
24. Lupus ☐ Yes ☐ No ☐ Unknown
25. Rheumatoid Arthritis ☐ Yes ☐ No ☐ Unknown
26. Fibromyalgia ☐ Yes ☐ No ☐ Unknown
27. Mixed Connective Tissue Disorder ☐ Yes ☐ No ☐ Unknown
28. Chronic Fatigue Syndrome ☐ Yes ☐ No ☐ Unknown
29. Lyme disease ☐ Yes ☐ No ☐ Unknown
30. Babesiosis ☐ Yes ☐ No ☐ Unknown
31. Ehrlichiosis ☐ Yes ☐ No ☐ Unknown
32. Rocky Mountain Spotted Fever ☐ Yes ☐ No ☐ Unknown
33. Colorado Tick Fever ☐ Yes ☐ No ☐ Unknown
34. Powassan Virus ☐ Yes ☐ No ☐ Unknown
35. Southern Tick Associated Rash Illness (STARI) ☐ Yes ☐ No ☐ Unknown

36. Are you currently taking steroid or other immunosuppressive drugs? ☐ Yes ☐ No ☐ Unknown
    If yes, please list each drug on a separate line?

_____ _____

_____ _____

36. Have you taken antibiotics for any reason in the last 4 weeks? ☐ Yes ☐ No ☐ Unknown
    If yes, please list each antibiotic on a separate line?

_____ _____

_____ _____

Thank you for your time!

INSTRUCTIONS FOR TICK TEST KIT

Express Shipping Address (e.g. FedEx):
Commander
U.S. Army Center for Health Promotion and Preventive Medicine
ATTN: Entomological Sciences Program
40th St. & Atkisson Rd., Bldg. E5800
Aberdeen Proving Ground, MD 21010-5403
Phone (410) 436-3613

Fastest method

First Class Mail Address:
Commander
U.S. Army Center for Health Promotion and Preventive Medicine
ATTN: Entomological Sciences Program
5158 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5403

MEMORANDUM FOR HEALTH CARE PROVIDER:

This kit provides you with a quick, easy means of obtaining an identification of a tick removed from a patient, and an assay of that tick for infection with a variety of tick-borne pathogens, including those that cause Lyme disease, southern tick-associated rash illness (STARI), Rocky Mountain spotted fever, human monocytic ehrlichiosis, human granulocytic ehrlichiosis, and babesiosis. Since different species of ticks transmit different diseases, and since most tick-borne diseases have very similar early symptoms, knowing the species and infection status of the tick greatly enhances the physician's ability to accurately diagnose and treat the patient.

The tick should be removed from the patient with as little trauma to the tick as possible (i.e., attempt to keep mouthparts intact; do not puncture or crush body). These precautions will decrease the likelihood of pathogens being injected into the wound site during removal and facilitate identification.

Tests performed on live ticks are the most accurate. Negative test results for dead ticks can be unreliable (i.e., they may be False Negative), because the DNA of pathogenic organisms begins to degrade after the tick dies. Therefore, in the case of negative results for dead ticks, the patient should remain alert for symptoms of tick-borne diseases that appear 1 to 4 weeks following the tick bite.

> We will test dead ticks, but the condition of the tick specimen affects the quality of the test. A complete specimen, kept cool and dry and **mailed to us promptly**, will yield more reliable results than a specimen that is kept in warm, moist conditions (mold develops, and the tick degrades rapidly). A tick that is in pieces, or even just a piece of a tick, can sometimes be identified and tested, but intact, whole ticks will yield more reliable results.

Be sure to fill out the accompanying data sheet carefully and return it to us with the tick. We will contact you telephonically with the results of identification and analysis, followed by written confirmation, so be sure to include a POC name, address, and phone number. Provide a sample identification number of your choice so that we can link our results to your specimen, but DO NOT USE PATIENT NAME OR SOCIAL SECURITY NUMBER as this information is protected by the Privacy Act and is not desired for our records.

Follow these instructions:

1. Place the tick into the vial. Do NOT add water.
2. Screw the cap onto the vial securely and seal it in the ziplock plastic bag by "zipping" it closed.
3. Fill out the front of the data form SUBMISSION OF TICK SPECIMENS FROM HUMAN SUBJECTS (CHPPM FORM 321-R) completely and fold it in half crosswise.
4. Place the data form and the ziplock bag inside the preaddressed mailing envelope. **Be sure to stamp the mailing envelope with your return address.**
5. Mail immediately by **FIRST CLASS MAIL** (envelope provided) **or EXPRESS SHIPPING SERVICE (e.g. FedEx)**.
6. Results of tick identification and/or analyses will be provided to you within 3-4 days of receipt of your tick specimens by this laboratory. Replacement kits are available upon request.

If you have any questions, or need more TICK TEST KITS, contact:

DSN 584-3613; CM (410) 436-3613; FAX -2037
Ellen.Stromdahl@apg.amedd.army.mil

U.S. Army Center for Health Promotion and Preventive Medicine
DOD HUMAN TICK TEST KIT PROGRAM

SUBMISSION OF TICK SPECIMENS FROM HUMAN TICK-BITE PATIENTS

CLINIC MAILING ADDRESS (Please print clearly and accurately):

_____

_____

_____

_____

CLINIC POC NAME:_____

CLINIC POC PHONE: DSN_____ **COM**_____

CDC ID#_____

PATIENT INFORMATION

SERVICE ASSOCIATION: ARMY (Circle one)
NAVY
AIR FORCE
MARINES

STATUS (Circle): ACTIVE DUTY
NAT'L GUARD
RETIRED
RESERVES
MILITARY DEPENDENT
CIVILIAN
OTHER_____

AGE_____ **SEX:** M F

TICK-BITE INFORMATION

WHERE WAS TICK-BITE ACQUIRED?

ON-POST? (Circle if the tick-bite was acquired on-post, and give the name of the installation):
_____

OFF-POST? (Circle if the tick was acquired off-post, and enter the following information, if known):

CITY_____

COUNTY_____

STATE_____

UNKNOWN (Circle if you do not know where the tick-bite was acquired)

DATE OF TICK REMOVAL_____ **UNKNOWN**
mo/da/yr

WAS THIS AN OCCUPATIONAL EXPOSURE? YES NO

REMARKS_____

_____

_____

_____

_____

U.S. Army Center for Health Promotion and Preventive Medicine
DOD HUMAN TICK TEST KIT PROGRAM

TICK ANALYSIS DATA SHEET

Page __ of ___ pages	Installation sample #	CHPPM sample #	Date rec'd:

Identified by:	Call-in date (I.D.) to: by:	Tested by:	Call-in date (tests) to: by:

TICK IDENTIFICATION	Sex & Stage			Engorgement			Condition	
Species	Adult M/F	Nymph	Larva	flat	part	full	alive	dead *
Amblyomma americanum lone star tick								
Dermacentor variabilis American dog tick								
Ixodes scapularis blacklegged tick (a.k.a. deer tick)								
Other:								

THIS TICK WAS TESTED FOR:	Pos	Neg *	REMARKS:
Anaplasma phagocytophilum (human granulocytic ehrlichiosis, HGE)			
Babesia microti (babesiosis)			
Borrelia burgdorferi (Lyme disease, LD)			
Borrelia lonestari (southern tick-associated rash illness, STARI)			
Ehrlichia chaffeensis (human monocytic ehrlichiosis, HME)			
Rickettsia rickettsii (Rocky Mountain spotted fever)			

* Tests performed on live ticks are the most accurate. Negative test results for dead ticks can be unreliable (i.e., they may be False Negative), because the DNA of pathogenic organisms begins to degrade once the tick dies. Therefore, the patient should be alert for symptoms of tick-borne diseases appearing 1 to 4 weeks following the tick bite.

ID#

30-DAY FOLLOW-UP SURVEY

Health

Since the day you submitted your tick to DOD, have you had any of the following symptoms (#1 through #19) lasting for at least 2 days?

1. Expanding circular red skin rash, 3 or more inches in diameter ☐ Yes ☐ No ☐ Unknown
   If yes, where on your body was this rash (check all that apply)?
   ☐head ☐neck ☐arm ☐hand ☐chest ☐abdomen ☐shoulder
   ☐upper leg ☐lower leg ☐groin ☐foot ☐back ☐buttocks ☐other
2. Severe joint pain or swelling unrelated to injury ☐ Yes ☐ No ☐ Unknown
   If yes, where was this pain (check all that apply)?
   ☐wrist ☐elbow ☐shoulder ☐hip ☐knee ☐ankle ☐other
3. Severe headache or stiff neck ☐ Yes ☐ No ☐ Unknown
4. Numbness or paralysis of the face, arms, or legs ☐ Yes ☐ No ☐ Unknown
5. Skin ulcer ☐ Yes ☐ No ☐ Unknown
6. Recurrent rash on wrists, ankles, or elbows ☐ Yes ☐ No ☐ Unknown
7. Any other type of skin rash or rash illness ☐ Yes ☐ No ☐ Unknown
   If yes, please describe in your own words _____
8. Unexplained fever ☐ Yes ☐ No ☐ Unknown
9. Unexplained chills ☐ Yes ☐ No ☐ Unknown
10. Unexplained fatigue ☐ Yes ☐ No ☐ Unknown
11. Confusion ☐ Yes ☐ No ☐ Unknown
12. Vomiting ☐ Yes ☐ No ☐ Unknown
13. Diarrhea ☐ Yes ☐ No ☐ Unknown
14. Cough ☐ Yes ☐ No ☐ Unknown
15. Sore throat ☐ Yes ☐ No ☐ Unknown
16. Difficulty breathing ☐ Yes ☐ No ☐ Unknown
17. Jaundice (yellow coloring of the skin and eyes) ☐ Yes ☐ No ☐ Unknown
18. Sensitivity to bright light ☐ Yes ☐ No ☐ Unknown
19. Impaired memory or difficulty concentrating ☐ Yes ☐ No ☐ Unknown

If you checked "yes" to any of the questions above (#1 through #19), please answer the following questions, Otherwise, skip to # 25 on the back page .

20. Did you visit a health clinic or doctor's office for any of these symptoms? ☐ Yes ☐ No ☐ Unknown
    If yes, which symptoms (#1 through #19) prompted a doctor's visit?

    _____ _____ _____ _____ _____ _____ _____ _____ _____

21. Were you given an explanation for these symptoms? ☐ Yes ☐ No ☐ Unknown
    If yes, what were you told? _____
22. Did you receive any treatment or medication for these symptoms? ☐ Yes ☐ No ☐ Unknown
    If yes, what treatment or medication? _____
23. Are you well now? ☐ Yes ☐ No ☐ Unknown
24. If you answered "no", what symptoms (#1 through #19) do you still have?

    _____ _____ _____ _____ _____ _____ _____ _____ _____

Please turn to back page...

Since the day you submitted your tick to DOD, have you been diagnosed with?

25. Lyme disease ☐ Yes ☐ No ☐ Unknown
26. Babesiosis ☐ Yes ☐ No ☐ Unknown
27. Ehrlichiosis ☐ Yes ☐ No ☐ Unknown
28. Rocky Mountain Spotted Fever ☐ Yes ☐ No ☐ Unknown
29. Colorado Tick Fever ☐ Yes ☐ No ☐ Unknown
30. Powassan Virus ☐ Yes ☐ No ☐ Unknown
31. Southern Tick Associated Rash Illness (STARI) ☐ Yes ☐ No ☐ Unknown

32. Are you currently taking steroid or other immunosuppressive drugs? ☐ Yes ☐ No ☐ Unknown
    If yes, please list each drug on a separate line?

_____ _____

_____ _____

33. Have you taken antibiotics for any reason in the last 4 weeks? ☐ Yes ☐ No ☐ Unknown
    If yes, please list each antibiotic on a separate line?

_____ _____

_____ _____

Recent Tick Bites

The following questions are about new tick bites.

34. Have you been bitten by a tick(s) in the last 30 days?
    ☐yes ☐no ☐unknown
    If "yes", please answer questions 35 through 37 below. If "no" or "unknown" you are done with this survey.

35. How many ticks have you removed from your body in the past 30 days?
    ☐one ☐more than one, but less than 5 ☐ 6 to 10 ☐more than 10 ☐unknown

36. Where on your body were you bitten (check all that apply)?
    ☐head ☐neck ☐arm ☐hand ☐chest ☐abdomen ☐shoulder
    ☐upper leg ☐lower leg ☐groin ☐foot ☐back ☐buttocks ☐other

37. Where did you acquire this/these ticks?
    ☐on base ☐another location ☐unknown
    If another location, give City _____ County _____ State _____

Your age:_____

☐ Male ☐ Female

☐ American Indian or Alaskan Native
☐ Asian
☐ Black or African-American
☐ Hispanic
☐ Native Hawaiian or other Pacific Islander
☐ White, Non-Hispanic
☐ Other or Decline to Answer

☐Active Duty ☐Nat'l Guard ☐Retired ☐Reserves ☐Military Dependent ☐Civilian ☐Other

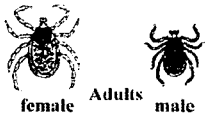
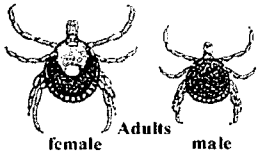
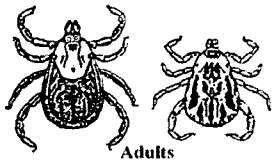
Thank you for your time.

Protect Yourself from Tick-Borne Diseases

- Ticks can carry and transmit (vector) a wide variety of disease-causing organisms (pathogens). Different kinds (species) of ticks generally transmit different pathogens, that is, they are considered vectors for specific disease organisms. Some ticks can be vectors for more than one kind of pathogen.

- Not all ticks are infected, so a tick bite does not necessarily mean you will get a disease. In addition, even if a tick is infected, it must be attached to your skin for at least several hours before it can successfully transmit the pathogens to you. Therefore, the sooner you remove attached ticks, the safer you will be.

Tick Species and Life Stages Most Likely to Bite Humans in the U.S. and the Diseases They May Cause

Tick Species	Disease	Pathogen
 ***Ixodes scapularis*** **(blacklegged tick, also known as the deer tick)**	**Lyme disease** **Human granulocytic ehrlichiosis** **Babesiosis**	***Borrelia burgdorferi*** ***Anaplasma phagocytophilum*** ***Babesia microti***
 ***Amblyomma americanum*** **(lone star tick)**	**Human monocytic ehrlichiosis** **Southern tick-associated rash illness (STARI)**	***Ehrlichia chaffeensis*** ***Borrelia lonestari***
 ***Dermacentor variabilis*** **(American dog tick)**	**Rocky Mountain spotted fever**	***Rickettsia rickettsii***

- There are additional tick species that bite humans in limited areas of the United States. They include: *Ixodes pacificus* (western blacklegged tick) which looks identical to *Ixodes scapularis* and transmits the same or closely related pathogens as that tick species, but is present only in the Pacific Coast states; and *Dermacentor andersoni* (Rocky Mountain wood tick), which looks very similar to *Dermacentor variabilis*, and transmits RMSF, but only in the Rocky Mountain states.

- Ticks go through several stages in their life cycle: egg, larva, nymph, and adult (male and female at this stage). For all tick species, the larva is very tiny (a mere speck), the nymph is a little larger (but still very small, about the size of a pin head), and the adults are larger and easy to see. Although larval ticks will bite man, they rarely transmit pathogens, but both nymphs and adults may do so. Nymphs are of greatest concern, owing to their small size which makes them easy to overlook.

- A tick needs a blood meal from a host in order to molt (progress to the next stage of its life cycle), and to reproduce (mate and lay eggs) as adults. This feeding process continues for several days to a week until the tick is fully engorged with blood. It then releases its hold from the host, drops off, and subsequently molts or lays eggs. If the tick is infected with pathogens, it can transmit the infection to the host (this could be you!) during the feeding process.

DO THIS:

- Wear the proper clothing:
  - Long pants tucked into boots or socks;
  - Long sleeves;
  - Shirt tucked into pants;
  - Light-colored clothing makes it easier to spot ticks.
- Use these safe and effective insect repellents:
  - Treat clothing with permethrin repellent. When ticks crawl onto the fabric, they absorb a tiny amount of permethrin, making them too sick to bite you. Follow application directions on the repellent label. For military uniforms, order aerosol spray can (NSN 6840-01-278-1336), or impregnation (IDA) kit (NSN 6840-01-345-0237).
  - Apply DEET repellent to skin that is not covered by clothing. Follow application directions on the label. Order NSN 6840-01-284-3982.
- Check yourself for ticks routinely:
  - Use the buddy system;
  - When you go indoors, remove your clothes and shower, checking your skin carefully;
  - You can place your clothes in a hot dryer for 20 minutes to ensure that any ticks you failed to notice will be killed;
  - Check children and pets carefully.
- Remove attached ticks immediately:
  - Grasp the tick's mouthparts as close to the skin as possible with fine-tipped tweezers; pull back slowly and steadily with firm force until the barbed mouthparts can be eased out of the skin. Be patient.
  - DO NOT squeeze the body of the tick as this may force infective fluid into you.
  - DO NOT apply any substance, including petroleum jelly, finger nail polish, finger nail polish remover, repellents, pesticides, or a lighted match to the tick *while it is attached.* These materials are either ineffective, or worse, might agitate the tick, causing it to regurgitate infective fluid into the bite site.
  - Wash the bite site and apply an antiseptic.
  - Save the tick for future identification should you develop disease symptoms. Preserve it by placing it in a clean, dry jar or other container and keeping it in the freezer. Discard after one month as all known tick-borne diseases will generally display symptoms within this time period.
  - If you develop flu-like illness or otherwise feel sick following a tick bite, seek medical attention immediately.

USACHPPM
Readiness thru Health
U.S. Army Center for Health Promotion and Preventive Medicine
ATTN: Entomological Sciences Program
Aberdeen Proving Ground, Maryland 21010-5403

Please take this page with you.
It contains valuable information that you should read and remember.

It is important to follow these recommendations

to reduce your risk of tick-borne disease!

- Avoid ticks if possible.
- Check your body for ticks every day.
- Remove any attached ticks promptly.
- Use insect repellant on skin and clothing.
- Use these tips around your home to reduce ticks:
  - Use pesticides once or twice a year.
  - Remove leaf litter.
  - Create wood chip barriers between lawn and forest or dense vegetation.
  - Keep deer and rodents away if possible.
- Seek prompt medical attention for signs and symptoms of tick-borne disease. These include:
  - Rash
  - Fever
  - Headache or stiff neck
  - Joint pain or swelling
  - Fever or chills
  - Severe fatigue
  - Paralysis

Some Guidelines for Use of the Tick Test Kit and Evaluation of Results

Most people cannot identify the species of tick that has bitten them. You CANNOT determine the species of a tick by its size because ALL ticks are extremely tiny in their immature stages, and then get progressively larger as they progress through their life cycle. In addition, you CANNOT tell if a tick is infected by looking at it.

Our Tick Test Kit program provides information about ticks that have been removed *from tick-bite patients, to include identification of tick species, relative engorgement* level, and infection status. This information is useful for the following reasons:

1. Tick species: Different tick species only transmit certain pathogens, or groups of pathogens. Therefore, knowing the species involved in the tick bite incident alerts the patient/physician to watch for specific disease(s), and may aid in differential diagnosis if clinical symptoms are present.

2. Relative engorgement level: If a tick is infected, it may transmit that infection when it bites an individual. However, transmission does NOT happen immediately. The tick must be attached for at least several hours in order to effectively transmit pathogens. In the case of Rocky Mountain spotted fever, infection may take place in as little as 4 - 6 hours, and in the case of Lyme disease, 24 - 48 hours is usually required; however, there is no EXACT time frame for any pathogen. Engorgement level (flat or unengorged, partly engorged, fully engorged) is simply a relative indication of attachment duration. The longer a tick is attached, the more engorged it becomes, and the longer an **infected** tick is attached, the greater the risk that transmission will take place. So, risk increases with engorgement level.

3. Infection status. (We currently analyze ticks for the agents of six diseases: Lyme disease, Rocky Mountain spotted fever, human monocytic ehrlichiosis, human granulocytic ehrlichiosis, babesiosis, and STARI, i.e. Southern Tick-Associated Rash Illness, a Lyme-like illness seen in the southeastern/south central U.S.):

a. Ticks can ONLY transmit infection if they bite (attach to) a person. Ticks found just crawling on a person's skin or clothing cannot have transmitted infection, unless the tick appears to be fully engorged. A fully engorged tick indicates that the tick has just fed to repletion (completed its blood meal). In this rare or unlikely scenario, the tick would have been feeding on the person for several days, becoming fully engorged in the process, then detached and been immediately located by the individual before falling off. The Tick Test Kit program is designed to identify and test only those ticks that were actually attached to a person, because they are the only ticks that present a health risk. Submitting unattached ticks, therefore, is generally not justified unless there are extenuating circumstances, such as in the example just stated above.

b. If a tick is analyzed and found to be negative for a particular pathogen, the person cannot have acquired infection from that tick. If the tick is positive, the **potential** for infection to have taken place is increased, **but not confirmed**. We analyze both live and dead ticks. Analytical results are most reliable for live tick specimens. Once the tick dies, its cells begin to break down and pathogen DNA, if it is present, may begin to degrade. The longer a tick is dead, and the poorer the condition of the tick (e.g. moldy,

burned, etc.), the greater the chance for a false negative analytical result. However, we have a high degree of confidence in analytical results for a dead tick that we receive within a reasonable period of time following removal and that appears to be in fairly good condition.

Additional facts to keep in mind when evaluating the results of the Tick Test Kit:

1. Tick Test Kit results do NOT represent human diagnosis; they merely provide additional information that may facilitate evaluation of the patient and may assist the physician in making diagnostic/treatment decisions.

2. Identification and analysis of the submitted tick do not rule out the possibility that the individual may have had other undetected tick bites. **Actual clinical symptoms in an individual should never be discounted based on Tick Test Kit results.**

3. Regardless of the species, engorgement level, and infection status of a tick, **prevailing philosophy in the reputable medical literature is that antibiotic therapy is generally not indicated unless there are supporting clinical symptoms.** The decision to administer antibiotics for a tick bite victim should be made by the physician on a case-by-case basis, after full evaluation of, and discussion with, the patient. Certain circumstances might justify prophylactic treatment, such as removal of a fully engorged, infected tick from a pregnant female. High infection rates in local tick populations, a high reported incidence of tick-borne disease in the area, underlying medical conditions of the patient, and even level of patient anxiety are also factors that might contribute to decisions to administer prophylactic therapy in some cases.

4. In the absence of symptoms, blood tests immediately following a tick bite are unproductive, as antibody titers or pathogen populations (IF infection did indeed take place) have not yet had enough time to develop to levels sufficient for measurement or detection.

5. It is important that telephonic results of identification and analysis be provided immediately to the patient's health care professional (e.g. physician) for his/her evaluation. Once CHPPM Form 321-R is mailed back to the clinic from CHPPM with the official written results of identification and analysis, it is very important that the form, along with the accompanying transmittal letter discussing the results, be provided to the physician, and that the **form and transmittal letter be placed in the individual's medical file for future reference, even if the results of analysis are negative.** We do not test ticks for every possible known pathogen, and specific tick species may eventually be found to harbor as yet unidentified or 'emerging' pathogens. CHPPM Form 321-R serves as the record of a tick bite, contains the results of identification and analysis, and is an important aspect of the patient's medical record.

US Army Center for Health Promotion and Preventive Medicine
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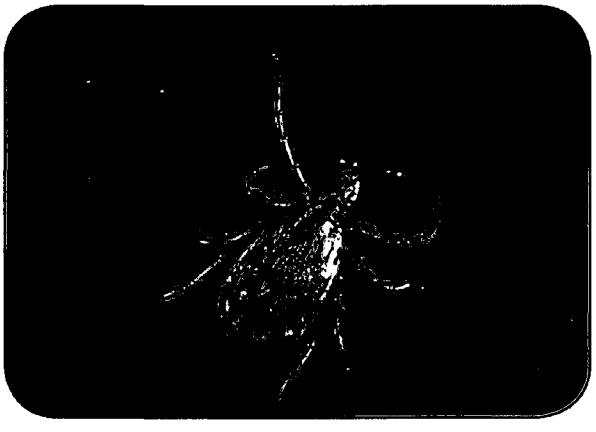
APPENDIX B

MMWR™
Morbidity and Mortality Weekly Report
www.cdc.gov/mmwr

Surveillance Summaries | October 3, 2008 / Vol. 57 / No. SS-10

Surveillance for Lyme Disease — United States, 1992–2006



DEPARTMENT OF HEALTH AND HUMAN SERVICES
CENTERS FOR DISEASE CONTROL AND PREVENTION

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CONTENTS

On the Cover: *Ixodes scapularis* (also known as the blacklegged or deer tick), which transmits the agent of Lyme disease, *Borrelia burgdorferi*, to humans.

Surveillance for Lyme Disease — United States, 1992–2006

Rendi Murphree Bacon, MS, Kiersten J. Kugeler, MPH, Paul S. Mead, MD
Division of Vector-Borne Infectious Diseases, National Center for Zoonotic Vector-Borne and Enteric Diseases

Abstract

Problem/Condition: Lyme disease is a multisystem disease that occurs in North America, Europe, and Asia. In the United States, the etiologic agent is *Borrelia burgdorferi sensu stricto*, a spirochete transmitted to humans by infected *Ixodes scapularis* and *I. pacificus* ticks. The majority of patients with Lyme disease develop a characteristic rash, erythema migrans (EM), accompanied by symptoms of fever, malaise, fatigue, headache, myalgia, or arthralgia. Other manifestations of infection can include arthritis, carditis, and neurologic deficits. Lyme disease can be treated successfully with standard antibiotic regimens.

Reporting Period: 1992–2006.

Description of System: U.S. health departments report cases of Lyme disease voluntarily to CDC as part of the National Notifiable Disease Surveillance System. Variables collected include patient age, sex, race, county and state of residence, date of illness onset, and reported signs and symptoms.

Results: During 1992–2006, a total of 248,074 cases of Lyme disease were reported to CDC by health departments in the 50 states, the District of Columbia, and U.S. territories; the annual count increased 101%, from 9,908 cases in 1992 to 19,931 cases in 2006. During this 15-year period, 93% of cases were reported from 10 states (Connecticut, Delaware, Massachusetts, Maryland, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island, and Wisconsin). Incidence was highest among children aged 5–14 years, and 53% of all reported cases occurred among males. More than 65% of patients with EM had illness onset in June and July, compared with 37% of patients with arthritis.

Interpretation: Lyme disease is the most commonly reported vectorborne illness in the United States. The geographic distribution of cases is highly focused, with the majority of reported cases occurring in the northeastern and north-central states. During 1992–2006, the number of reported cases more than doubled. A disproportionate increasing trend was observed in children and in young males compared with other demographic groups.

Public Health Action: The results presented in this report underscore the continued emergence of Lyme disease and the need for tick avoidance and early treatment interventions. Public health practitioners can use the data presented in this report to target prevention campaigns to populations with increasing incidence (i.e., children and young males).

Introduction

Lyme disease was first described in 1977 following investigation of a cluster of arthritis cases among children living near Lyme, Connecticut (*1*). Further study indicated that arthritis was a late manifestation of a multisystem, tick-transmitted disease. In 1981, a bacterial spirochete, *Borrelia burgdorferi*, was identified in *Ixodes scapularis* (*2*) and later demonstrated to be the etiologic agent of Lyme disease (*3,4*).

B. burgdorferi occurs naturally in reservoir hosts, including mice, squirrels, shrews, and other small vertebrates (*5*). *Ixodes scapularis* and *I. pacificus* (also referred to as blacklegged or deer ticks) become infected with *B. burgdorferi* while feeding on the blood of natural reservoir hosts. During subsequent blood meals, the ticks can transmit infection among reservoir hosts or to incidental hosts, including humans. Although deer are not infected with *B. burgdorferi*, they play a role in transporting ticks and maintaining tick populations.

In humans, infection with *B. burgdorferi* can result in dermatologic, musculoskeletal, neurologic, or cardiac abnormalities (*6–8*). In approximately 70%–80% of cases, patients develop a characteristic rash, erythema migrans (EM), within 30 days of infection with *B. burgdorferi*. EM is a red expanding rash, with or without central clearing, which often is accompanied by symptoms of fatigue, fever, headache, mild stiff neck, arthralgia, or myalgia. Within days or weeks, untreated infection can spread to other parts of the body, causing more serious neurologic conditions (e.g., meningitis, radiculopathy, and facial palsy) or cardiac abnormalities (e.g., carditis with

Corresponding author: Paul S. Mead, MD, Division of Vector-Borne Infectious Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, CDC, 3150 Rampart Rd., MS P-02, Fort Collins, CO 80521. Telephone: 970-221-6474; Fax: 970-221-4257; E-mail: pfm0@cdc.gov.

atrioventricular heart block). Over a period of months or years, untreated infection can lead to mono- or oligoarticular arthritis, peripheral neuropathy, or encephalopathy.

Lyme disease is diagnosed on the basis of physician-observed clinical manifestations and a history of probable exposure to infected ticks (*8*). Laboratory tests are neither suggested nor required to confirm diagnosis for patients with recent onset (2–3 weeks) of a characteristic EM rash (*9*). However, positive results of recommended two-tiered serologic testing (*10*) can provide confirmation of infection in patients with musculoskeletal, neurologic, or cardiac symptoms. Testing methods that have not been adequately validated can be misleading (*11*) and are not recommended (*12*).

The majority of infections can be cured with use of recommended antimicrobials. Patients with physician-diagnosed EM can be treated with oral doxycycline, amoxicillin, or cefuroxime axetil (*7,8*). Patients with other manifestations of Lyme disease are treated with either oral or intravenous antimicrobials (e.g., ceftriaxone), depending on the specific clinical condition.

Measures to prevent Lyme disease and other tickborne infections include avoiding tick-infested areas when possible, using insect repellents containing 20%–30% DEET (*N,N*-diethyl-*m*-toluamide) on exposed skin and clothing, and performing daily self-examination for ticks (*13*). Tick abundance can be reduced around private homes and in recreational areas by removing brush and leaf litter, creating a buffer zone of wood chips or gravel between forests and lawn, applying acaricides, and excluding deer (*13,14*). Tickborne illness can be mitigated by prompt and proper tick removal and by recognizing and seeking treatment for early signs of illness (*8,15,16*). A single dose of doxycycline should be considered for prophylaxis of Lyme disease in persons aged ≥8 years who have been bitten by a nymph or adult *I. scapularis* or *I. pacificus* tick in an area in which at least 20% of ticks are thought to be infected with *B. burgdorferi* (*8*). The tick must have been attached for ≥36 hours and prophylactic antibiotic administered within 72 hours of tick removal (*8*).

With the cooperation of state and local health departments, CDC initiated surveillance for Lyme disease in 1980; the first summary of 226 cases was published in 1981 (*17*). Before 1991, Lyme disease surveillance case definitions and reporting practices varied among states and between states and CDC. Standardized surveillance and reporting for Lyme disease began in 1991 after the Council of State and Territorial Epidemiologists (CSTE) designated Lyme disease as a nationally notifiable disease and published a standardized surveillance case definition* (*18*). This report describes the characteristics and distribution of Lyme disease cases reported in the United States during 1992–2006, providing 15-year trends and the frequency of reported symptoms. In addition, it details differences between cases reported from within and outside of the 10 states (Connecticut, Delaware, Maryland, Massachusetts, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island, and Wisconsin) in which Lyme disease is highly endemic† (*19*). These results underscore the continued emergence of Lyme disease and provide a basis for targeting prevention campaigns to populations with increasing incidence.

Methods

Surveillance Case Definitions

During 1991–1996, a case of Lyme disease was defined for national surveillance purposes as 1) physician-diagnosed EM of ≥5 cm in diameter or 2) at least one objective late manifestation (i.e. musculoskeletal, cardiovascular, or neurologic) with laboratory confirmation of infection with *B. burgdorferi* (*18*). Laboratory confirmation required 1) isolation of *B. burgdorferi* from clinical specimens, 2) demonstration of diagnostic levels of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies to *B. burgdorferi* in serum or cerebrospinal fluid (CSF), or 3) significant change in IgM or IgG antibody response in paired serum samples. In 1997, CSTE and CDC implemented a revised surveillance case definition on the basis of the availability of improved serologic testing (*20*). Clinical criteria were not changed; however, laboratory confirmation was modified to require 1) isolation of *B. burgdorferi* from a clinical specimen or 2) demonstration of diagnostic levels of IgM or IgG antibodies to *B. burgdorferi* in serum or CSF. A two-test approach (a sensitive enzyme immunoassay or immunofluorescence antibody assay followed by Western blot) was recommended but not required (*10*).

Data Sources

U.S. state and territorial health departments report cases of Lyme disease voluntarily to CDC as part of the National Notifiable Disease Surveillance System (NNDSS). Provisional data are transmitted to CDC weekly using the National Electronic Telecommunications System for Surveillance,

* The Lyme disease surveillance case definition was developed to standardize national public health surveillance and reporting of Lyme disease cases; it is not meant to be used as absolute criteria for clinical diagnosis.

† In 2000, these 10 states were defined as Healthy People 2010 (HP2010) Lyme disease reference states. A Healthy People 2010 goal (objective no. 14-8) is to reduce Lyme disease to 9.7 new cases per 100,000 population in the 10 HP2010 reference states (*19*) through the implementation of community-based prevention programs, host-targeted acaricides to reduce the numbers of vector ticks, and appropriate use of Lyme disease vaccine. However, the only vaccine approved by the Food and Drug Administration for use against Lyme disease in humans was removed from sale by the manufacturer in February 2002 citing low demand, greatly reducing the possibility of achieving this objective.

and final data are published annually in CDC's *Summary of Notifiable Diseases* (available at http://www.cdc.gov/mmwr). State or local health departments are responsible for ensuring that cases reported to CDC meet the case definition.

This report is based on data for all Lyme disease cases reported to CDC for 1992–2006.[§] During this 15-year period, state health officials used various methods to ascertain cases, including provider-initiated passive surveillance, laboratory-based surveillance, and enhanced or active surveillance. Basic demographic data (e.g., age, sex, race, and county of residence) were available for >90% of reported cases; however, information specific to Lyme disease (e.g., county of exposure, symptoms and signs, antibiotic treatment, and laboratory results) was incomplete. For example, only 61% of case reports contained data for reported signs and symptoms.

Analyses

Annual U.S.-, state-, county-, sex-, and age group–specific incidence rates per 100,000 population were calculated using U.S. Census Bureau population estimates for July 1 for each year of the reporting period (1992–2006). Analyses of symptom data were restricted to case reports for which at least one symptom was coded as "yes" (n = 150,829 records). Characteristics of cases reported from the 10 HP2010 reference states were compared with cases reported from all other (non-HP2010) states and territories.

Results

U.S. Case Counts and Rates

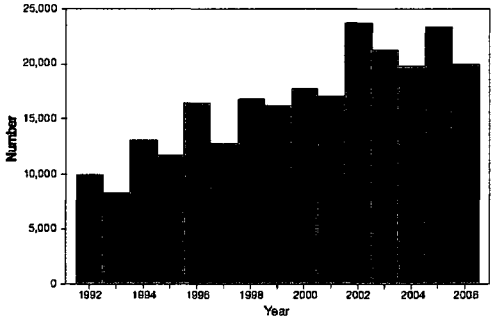
During 1992–2006, a total of 248,074 Lyme disease cases were reported to CDC. Although annual counts fluctuated by as much as 57% from year to year, the overall trend indicates a steady increase in the number of reported cases (Figure 1). During the 15-year study period, the number of cases reported increased 101%, from 9,908 cases in 1992 to 19,931 cases in 2006.

State Rates

The 15-year mean annual rate for all states ranged from <0.01 cases per 100,000 population in Montana and Colorado to 73.6 cases per 100,000 population in Connecticut (median: 0.5 cases) (Table 1). The 10 HP2010 reference states accounted for 229,782 cases, representing 92.6% of overall cases and at least 88% of cases reported in any single year. Reported annual rates

[§] Although data for 1991 were available, these data were excluded from the analysis because certain states reported aggregate case counts rather than information for individual case reports.

FIGURE 1. Number* of reported Lyme disease cases, by year — United States, 1992–2006



*N = 248,074.

for seven HP2010 reference states (Maryland, Massachusetts, Minnesota, New Jersey, New York, Pennsylvania, and Wisconsin) were relatively stable during 1992–2006. Annual rates were more variable in three states (Connecticut, Delaware, and Rhode Island), in part because of changes in surveillance practices. In Connecticut, annual rates per 100,000 population increased from 53.7 cases in 1992 to 133.9 cases in 2002; in 2003, the rate decreased to 40.3 cases. In Delaware, the number of cases increased from 339 in 2004 to 646 in 2005, boosting the annual rate per 100,000 population from 40.9 to 76.7 cases. The annual rate per 100,000 population reported in Rhode Island increased from 27.5 cases in 1992 to 68.5 cases in 2003, then declined to 23.1 cases in 2004 and 3.6 cases in 2005; 28.9 cases were reported in 2006.

County Rates

County of residence was provided for 243,430 (98.1%) cases. The mean number of counties reporting at least one case of Lyme disease was 714 (range: 625–796). In all years, the percentage of counties reporting at least one case was >75 in six states (Connecticut, Delaware, Massachusetts, Maryland, New Jersey, and Rhode Island). In contrast, during 1992–2006, the percentage of counties reporting at least one case increased from 33% to 74% in Minnesota, from 79% to 97% in Pennsylvania, and from 76% to 97% in Wisconsin. In New York, the percentage of counties reporting at least one case ranged from 61% to 85%, with no obvious increasing or decreasing temporal trend.

The 15-year average county-specific rate for counties reporting at least one case during 1992–2006 ranged from <0.01 cases per 100,000 population in Honolulu County, Hawaii,

TABLE 1. Annual rate* of Lyme disease, by state/area and year — United States, 1992–2006

State/Area	Year															
	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	Average
Alabama	0.2	0.1	0.1	0.3	0.2	0.3	0.6	0.5	0.1	0.2	0.3	0.2	0.1	0.1	0.2	**0.2**
Alaska	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.0	0.3	0.3	0.5	0.5	0.5	0.6	0.5	**0.2**
Arizona	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.2	0.2	0.2	**0.1**
Arkansas	0.8	0.3	0.6	0.4	1.1	1.1	0.3	0.3	0.3	0.2	0.1	0.0	0.0	0.0	0.0	**0.4**
California	0.8	0.4	0.2	0.3	0.2	0.5	0.4	0.4	0.3	0.3	0.3	0.2	0.1	0.3	0.2	**0.3**
Colorado	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.0	0.0	**0.0**
Connecticut†	53.7	41.3	62.1	47.4	95.0	70.3	104.9	98.0	110.6	104.8	133.9	40.3	38.6	51.7	51.0	**73.6**
Delaware†	31.7	20.4	15.0	7.8	23.8	14.8	10.4	22.2	21.2	19.1	24.1	26.0	40.9	76.8	56.5	**27.4**
District of Columbia	0.5	0.4	1.6	0.5	0.6	1.9	1.5	1.2	1.9	2.9	4.3	2.4	2.8	1.7	10.7	**2.3**
Florida	0.2	0.2	0.2	0.1	0.4	0.4	0.5	0.4	0.3	0.3	0.5	0.3	0.3	0.3	0.2	**0.3**
Georgia	0.7	0.6	1.8	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	**0.3**
Hawaii	0.2	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Idaho	0.2	0.2	0.3	0.0	0.2	0.3	0.6	0.2	0.3	0.4	0.3	0.2	0.4	0.1	0.5	**0.3**
Illinois	0.4	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.3	0.3	0.4	0.6	0.7	1.0	0.9	**0.4**
Indiana	0.4	0.6	0.3	0.3	0.6	0.6	0.7	0.4	0.4	0.4	0.3	0.4	0.5	0.5	0.4	**0.5**
Iowa	1.2	0.3	0.6	0.6	0.7	0.3	0.9	0.8	1.2	1.2	1.4	2.0	1.7	3.0	3.3	**1.3**
Kansas	0.7	2.1	0.7	0.9	1.4	0.2	0.5	0.6	0.6	0.1	0.3	0.2	0.1	0.1	0.1	**0.6**
Kentucky	0.8	0.4	0.6	0.4	0.7	0.5	0.7	0.5	0.3	0.6	0.6	0.4	0.4	0.1	0.2	**0.5**
Louisiana	0.2	0.1	0.1	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.0	**0.2**
Maine	1.3	1.5	2.7	3.6	5.1	2.7	6.3	3.3	5.6	8.4	16.9	13.4	17.1	18.7	25.6	**8.8**
Maryland†	3.7	3.6	6.8	9.0	8.8	9.7	12.9	17.4	13.0	11.3	13.6	12.6	16.1	22.1	22.2	**12.2**
Massachusetts†	3.7	2.5	4.1	3.1	5.3	4.8	11.4	12.8	18.2	18.2	28.1	23.8	23.8	36.3	22.3	**14.5**
Michigan	0.4	0.2	0.3	0.1	0.3	0.3	0.2	0.1	0.2	0.2	0.3	0.1	0.3	0.6	0.6	**0.3**
Minnesota†	4.4	3.1	4.6	4.5	5.4	5.5	5.5	5.9	9.4	9.3	17.3	9.4	20.1	17.9	17.7	**9.3**
Mississippi	0.0	0.0	0.0	0.6	0.9	1.0	0.6	0.1	0.1	0.3	0.4	0.8	0.0	0.0	0.1	**0.3**
Missouri	2.9	2.1	1.9	1.0	1.0	0.5	0.2	1.3	0.9	0.7	0.7	1.2	0.4	0.3	0.1	**1.0**
Montana	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	**0.0**
Nebraska	1.4	0.4	0.2	0.4	0.3	0.1	0.2	0.7	0.3	0.2	0.4	0.1	0.1	0.1	0.6	**0.4**
Nevada	0.1	0.4	0.1	0.4	0.1	0.1	0.3	0.1	0.2	0.2	0.1	0.1	0.0	0.1	0.2	**0.2**
New Hampshire	4.0	1.3	2.7	2.4	4.1	3.3	3.8	2.3	6.8	10.3	20.5	14.8	17.4	20.3	46.9	**10.7**
New Jersey†	8.8	10.0	19.4	21.4	27.3	25.3	23.6	21.1	29.2	23.8	27.4	33.4	31.1	38.6	27.9	**24.6**
New Mexico	0.1	0.1	0.3	0.1	0.1	0.1	0.2	0.1	0.0	0.1	0.1	0.1	0.1	0.2	0.2	**0.1**
New York†	19.1	15.5	28.6	24.5	29.2	18.3	25.6	24.2	22.8	21.4	28.9	28.1	26.4	28.8	23.1	**24.3**
North Carolina	1.0	1.2	1.1	1.2	0.9	0.5	0.8	1.0	0.6	0.5	1.7	1.9	1.4	0.6	0.4	**1.0**
North Dakota	0.2	0.3	0.0	0.0	0.3	0.0	0.0	0.2	0.3	0.0	0.2	0.0	0.0	0.5	1.1	**0.2**
Ohio	0.3	0.3	0.4	0.3	0.3	0.4	0.4	0.4	0.5	0.4	0.7	0.6	0.4	0.5	0.4	**0.4**
Oklahoma	0.8	0.6	3.1	1.9	1.3	1.4	0.4	0.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	**0.7**
Oregon	0.4	0.3	0.2	0.6	0.6	0.6	0.6	0.5	0.4	0.4	0.3	0.5	0.3	0.1	0.2	**0.4**
Pennsylvania†	9.8	9.0	11.9	13.0	23.4	18.2	23.0	23.2	19.1	22.8	32.4	46.4§	32.2	34.6	26.1	**23.0**
Rhode Island†	27.5	27.3	47.4	34.9	54.1	44.8	79.9	55.1	64.2	48.2	79.7	68.5	23.1	3.6	28.9	**45.8**
South Carolina	0.1	0.3	0.2	0.5	0.2	0.1	0.2	0.2	0.6	0.2	0.6	0.4	0.5	0.4	0.5	**0.3**
South Dakota	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.3	0.1	0.1	0.3	0.1	**0.1**
Tennessee	0.6	0.4	0.3	0.5	0.5	0.8	0.9	1.1	0.5	0.5	0.5	0.3	0.3	0.1	0.3	**0.5**
Texas	0.6	0.3	0.3	0.4	0.5	0.3	0.2	0.4	0.4	0.4	0.6	0.4	0.4	0.3	0.1	**0.4**
Utah	0.3	0.1	0.2	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.2	0.1	0.0	0.1	0.2	**0.1**
Vermont	1.6	2.1	2.8	1.5	4.4	1.4	1.9	4.4	6.6	2.9	6.0	7.0	8.1	8.7	16.8	**5.1**
Virginia	1.9	1.5	2.0	0.8	0.9	1.0	1.1	1.8	2.1	2.2	3.6	2.6	2.9	3.6	4.7	**2.2**
Washington	0.3	0.2	0.1	0.2	0.3	0.2	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1	**0.2**
West Virginia	0.8	2.8	1.6	1.4	0.7	0.6	0.7	1.1	1.9	0.9	1.4	1.7	2.1	3.4	1.5	**1.5**
Wisconsin†	10.5	7.9	8.0	7.2	7.7	9.2	12.6	9.3	11.7	11.1	20.0	13.5	20.8	26.4	26.4	**13.5**
Wyoming	1.1	1.9	1.1	0.8	0.6	0.6	0.2	0.6	0.6	0.2	0.4	0.4	0.8	0.6	0.2	**0.7**

* Per 100,000 population using U.S. Census Bureau population estimates for July 1 for each year of the reporting period.
† *Healthy People 2010* reference states in which Lyme disease is endemic.
§ Includes 4,722 confirmed and 1,008 suspected cases.

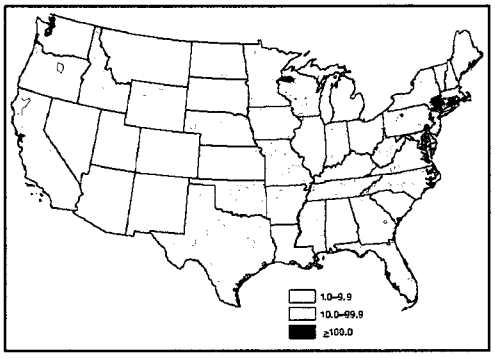
to 595.1 cases per 100,000 population in Nantucket County, Massachusetts (median: 0.7 cases per 100,000 population) (Figure 2). Counties with the highest average county-specific rate for three 5-year periods during the 15-year reporting period (1992–1996, 1997–2001, and 2002–2006) are presented (Table 2). Five counties ranked among the top 10 incidence counties for each 5-year period: Windham County, Connecticut; Nantucket County, Massachusetts; Hunterdon County, New Jersey; Dutchess County, New York; and Putnam County, New York. The only counties outside the northeast

TABLE 2. Average rate* and number of cases of Lyme disease, by county and 5-year period — United States, 1992–2006

	1992–1996			1997–2001			2002–2006		
Rank	County	Rate	(No. cases)	County	Rate	(No. cases)	County	Rate	(No. cases)
1	Nantucket County, MA	755	(55)	Nantucket County, MA	669	(60)	Columbia County, NY	962	(609)
2	Hunterdon County, NJ	337	(385)	Columbia County, NY	639	(403)	Dutchess County, NY	439	(1281)
3	Dutchess County, NY	337	(899)	Dutchess County, NY	445	(1234)	Nantucket County, MA	361	(36)
4	Putnam County, NY	278	(248)	Hunterdon County, NJ	443	(535)	Dukes County, MA	337	(52)
5	Washington County, RI	227	(262)	Windham County, CT	304	(330)	Hunterdon County, NJ	276	(356)
6	Middlesex County, CT	197	(290)	Washington County, RI	296	(361)	Greene County, NY	271	(133)
7	Washburn County, WI	182	(27)	Putnam County, NY	222	(211)	Cameron County, PA	239	(14)
8	Burnett County, WI	161	(23)	Dukes County, MA	201	(30)	Washburn County, WI	238	(39)
9	New London County, CT	156	(400)	Litchfield County, CT	195	(355)	Windham County, CT	220	(249)
10	Windham County, CT	130	(137)	New London County, CT	183	(472)	Putnam County, NY	219	(219)

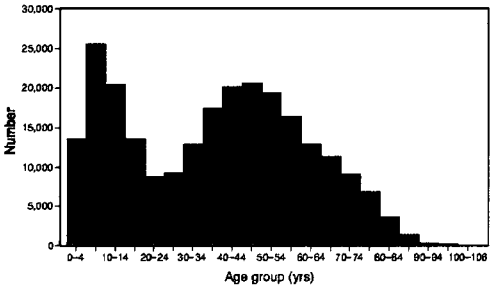
*Per 100,000 population.

FIGURE 2. Average rate* of Lyme disease, by county of residence† — United States, 1992–2006§



*Per 100,000 population.
†County of residence was available for 98.1% of cases reported during 1992–2006.
§During 2003, Pennsylvania reported 4,722 confirmed cases and 1,008 suspected cases.

FIGURE 3. Number* of reported Lyme disease cases, by age group — United States, 1992–2006



*N = 241,931.

to rank among the top 10 counties for any 5-year period were Washburn County and Burnett County, Wisconsin. Because of marked differences in population size across counties, a high rate does not necessarily indicate a substantial number of reported cases.

Selected Demographics

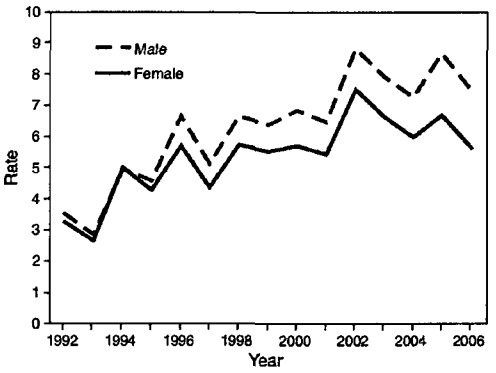
Information regarding age was available for 241,931 (97.5%) reported cases. Reported ages ranged from <1–106 years and were bimodal in distribution (Figure 3). Average annual rates peaked among children aged 5–9 years (8.6 cases per 100,000 population) and adults aged 55–59 years (7.8 cases per 100,000 population). The lowest rate was reported among adults aged 20–24 years (3.0 cases per 100,000 population).

Information about sex was available for 243,564 (99.1%) reported cases. Of these, 129,349 (53.1%) occurred among males, yielding an average annual rate per 100,000 population of 6.3 cases for males and 5.4 cases for females. During 1992–2006, rates increased disproportionately among males compared with females (Figure 4). This trend was most pronounced among persons aged 5–19 years; rates per 100,000 population in this age group increased 194% in males, from 3.5 cases in 1992 to 10.3 cases in 2006, and 114% in females, from 2.9 cases in 1992 to 6.2 cases in 2006.

Information regarding race was available for 166,194 (70.0%) reported cases. Of these, 156,346 (94.1%) patients were identified as white, 2,765 (1.7%) as black, 1,299 (0.8 %) as Asian/Pacific Islander, and 452 (0.3%) as American Indian/Alaska Native.

Age and sex of persons with Lyme disease differed among the 10 HP2010 reference states compared with other states. In the reference states, the modal age was 7 years, and males accounted for 120,369 (53.4%) reported cases. In the remaining states, the modal age was 44 years, and males accounted for 8,890 (49.4%) cases.

FIGURE 4. Rate* of Lyme disease,† by sex and year — United States, 1992–2006



* Per 100,000 population
† N = 243,564.

Seasonality

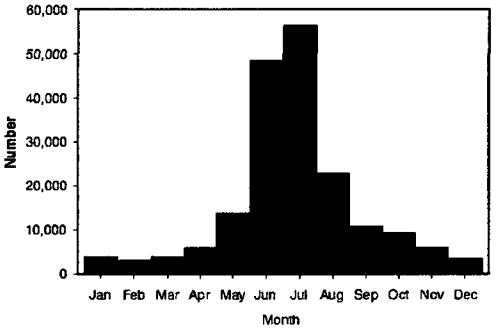
Month of disease onset was available for 188,340 (75.9%) reported cases (Figure 5). Although cases occurred in all months of the year; the majority (48,413 [25.7%]) of patients had onset in June, July (56,507 [30.0%]), or August (22,867 [12.1%]), the 3 months in which ticks actively seek mammalian hosts and human outdoor activity is greatest. In the HP2010 reference states, 99,762 (56.5%) cases had onset during June or July, compared with 5,518 (44.2%) among non-HP2010 reference states. Among 150,829 cases with reported clinical features, seasonal variation was most pronounced for cases with EM (Figure 6). Approximately 67% of patients with EM had onset in June and July, compared with 37% of those with arthritis.

Clinical Features

Information on clinical features of illness was available for 150,829 (60.8%) cases. Among these, EM was reported for 104,387 (69.2%) cases, arthritis characterized by brief attacks of joint swelling for 48,272 (32.0%) cases, neurologic symptoms (facial palsy or cranial neuritis, radiculoneuropathy, lymphocytic meningitis, encephalitis, or encephalomyelitis) for 18,157 (12.0%) cases, and second- or third-degree atrioventricular block for 1,222 (0.8%) cases. More than one clinical manifestation was reported for 19,321 (12.8%) cases. Data on clinical features of cases from all states was representative of data on clinical features of cases from the HP2010 reference states. By comparison, among 7,745 cases reported from non-HP2010 states, EM was reported less frequently (4,887 cases [63.0%]), and musculoskeletal, neurologic, and cardiac manifestations were reported more frequently (3,285 cases [42.4%], 1,442 cases [18.6%], and 100 cases [1.3%], respectively).

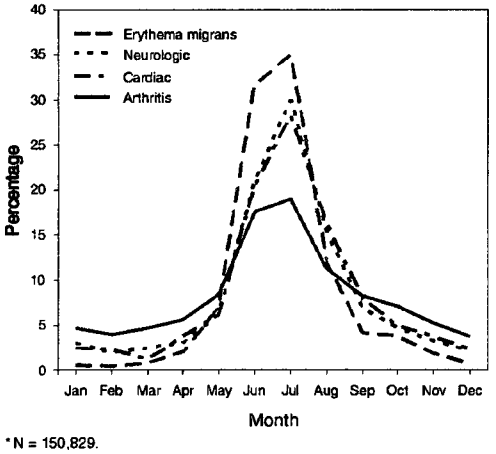
Temporal trends in national data indicate that the overall frequency of reported clinical features were generally stable over time (Figure 7). However, the frequency of reported symptoms was highly variable across the youngest age categories (Figure 8) and among HP2010 reference states (Table 3).

FIGURE 5. Number* of reported Lyme disease cases, by month of illness onset — United States, 1992–2006



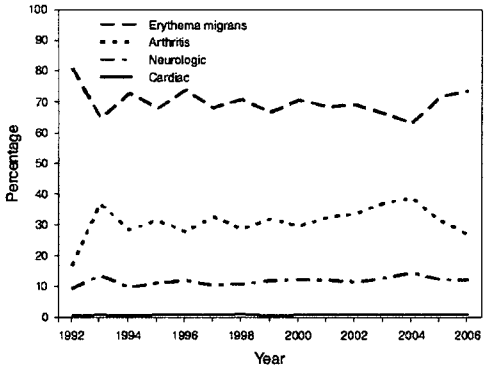
* N = 188,340.

FIGURE 6. Percentage of symptoms reported among Lyme disease patients,* by month of illness onset — United States, 1992–2006



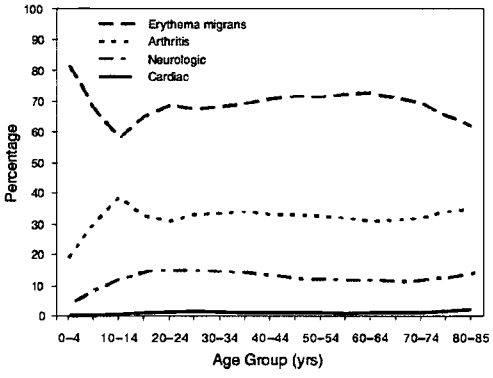
* N = 150,829.

FIGURE 7. Percentage of symptoms reported among Lyme disease patients,* by year — United States, 1992–2006



*N = 150,829.

FIGURE 8. Percentage of symptoms reported among Lyme disease patients,* by age group — United States, 1992–2006



*N = 148,899.

TABLE 3. Number and percentage* of reported symptoms among Lyme disease patients, by state† — *Healthy People 2010* (HP2010) reference states, 1992–2006

State	EM§		Arthritis		Neurologic		Cardiac	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Connecticut	25,538	(74)	7,845	(23)	3,305	(10)	171	(0.5)
Delaware	846	(51)	828	(50)	263	(16)	14	(0.9)
Massachusetts	8,196	(68)	3,948	(33)	1,849	(15)	179	(1.5)
Maryland	4,908	(60)	2,919	(36)	1,738	(21)	80	(1.0)
Minnesota¶	218	(87)	48	(19)	15	(6)	1	(0.4)
New York	33,024	(74)	10,953	(25)	4,047	(9)	362	(0.8)
Pennsylvania	17,014	(61)	13,093	(47)	4,040	(15)	215	(0.8)
Rhode Island	4,189	(65)	2,375	(37)	599	(9)	45	(0.7)
Wisconsin	5,567	(70)	2,978	(37)	859	(11)	55	(0.7)

* Total percentages exceed 100% because certain patients had multiple symptoms.
† Data represent approximately 60% of reported cases from HP2010 reference states. States did not report data on symptoms for all years during the 15-year study period, and one state (New Jersey) did not report any data on symptoms.
§ Erythema migrans.
¶ Data regarding symptoms reported only for 1996.

Discussion

During 1992–2006, the annual number of Lyme disease cases reported to CDC increased considerably, while remaining highly focused in northeastern and north-central states. Multiple reasons might explain this increase, including a true increase in the number of infections, enhanced surveillance, increased awareness among health-care professionals and the public, misdiagnosis, and reporting errors (*21–23*). In six HP2010 reference states (Connecticut, Delaware, Massachusetts, Maryland, New Jersey, and Rhode Island) in which the majority of counties regularly reported cases, a true increase in transmission might have resulted from greater tick densities and encroachment of human development into rural and suburban areas. In other HP2010 reference states, particularly Minnesota, Pennsylvania, and Wisconsin, the number of counties reporting cases increased appreciably, suggesting an additional role for geographic expansion of reservoir mammals and vector ticks into new areas. In certain states, especially those in the southeastern United States, Lyme disease surveillance is complicated by the occurrence of southern tick-associated rash illness, a condition that can resemble early Lyme disease but is not caused by *B. burgdorferi* (*24–26*).

Overall, features of reported cases changed little over time. Peak rates were reported among children, males, and whites in each year throughout the 15-year period. However, rates increased disproportionately among young males compared with young females; the reasons for this difference are not

known. The proportion of cases with EM and arthritis, the most commonly reported symptoms, has been relatively stable since 1993. However, across age categories, the frequency of reported symptoms varied widely among persons aged <20 years, with the lowest percentage of EM (58.2%) and the highest percentage of arthritis (38.7%) reported for children aged 10–14 years. These findings provide a basis for targeting prevention campaigns to populations with increasing incidence.

The findings in this report highlight both the benefits of infectious disease surveillance and the opportunity for improvement. Detailed analysis of reported cases enables public health authorities to define the demographics and distribution of disease and to survey trends. However, growing case counts and the implementation of electronic laboratory reporting have created a substantial reporting burden on certain state and local health departments as they attempt to verify compliance with the surveillance case definition (*27,28*). This burden has caused certain states to curtail or modify portions of their surveillance system, resulting in fluctuations in case tallies. In 2007, CSTE revised the national surveillance case definition for Lyme disease with the twin goals of reducing the burden of reporting while potentially enhancing the system's ability to capture a broader range of clinical manifestations. The revised case definition, which was implemented in January 2008, specifies required laboratory evidence in more detail than previous iterations and allows reporting of confirmed and, for the first time, probable cases of Lyme disease to CDC (*29*).

Limitations

The findings in this report are subject to at least three limitations. First, an unknown portion of all Lyme disease cases are reported; cases probably are underreported in areas in which the disease is endemic and overreported in areas in which the disease is not endemic. Misdiagnosis and overreporting from areas in which the disease is not endemic might explain the demographic differences noted between cases reported from HP2010 and non-HP2010 reference states. Second, variation in reporting practices and adherence to the surveillance case definition occurs among states, in part because states invest unequally in infrastructure for Lyme disease surveillance. As a result, Lyme disease–specific variables for cases reported by certain states are incomplete, unavailable, or not transmitted to CDC. Finally, cases are reported on the basis of the patient's state of residence rather than on the state in which the exposure occurred. Therefore, Lyme disease in a traveler returning from an area in which the disease is highly endemic cannot be construed as evidence of local transmission.

Conclusion

The number of reported cases of Lyme disease continues to increase, underscoring the need for targeted prevention strategies, early disease recognition and treatment, and a sustainable surveillance system. During the 15-year study period, incidence increased disproportionately among children, particularly males. Geographic expansion was apparent in Minnesota, Pennsylvania, and Wisconsin. Differences in the features of cases reported from HP2010 reference states and all other states suggest either aberrant reporting or fundamental differences in the epidemiology of Lyme disease in areas in which the disease is not endemic. The percentage of cases for which signs of disseminated infection were reported did not decrease during the reporting period, underscoring the need for continued education about early disease recognition and treatment. Despite the limitations of national surveillance data, these findings are useful in defining demographics, distribution, and trends in Lyme disease cases. Intensive surveillance methodologies, such as active population-based surveillance and the use of nonhuman data (e.g., serologic testing of dogs and surveillance for vectors), could be used to augment these data and provide a better understanding of this emerging infectious disease.

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