Published in final edited form as:
J Wildl Dis. 2013 April ; 49(2): 294–302. doi:10.7589/2012-06-172.

SERUM ANTIBODIES TO *BORRELIA BURGDORFERI, ANAPLASMA PHAGOCYTOPHILUM*, AND *BABESIA MICROTI* IN RECAPTURED WHITE-FOOTED MICE

Louis A. Magnarelli[1,4], **Scott C. Williams**[1], **Steven J. Norris**[2], and **Erol Fikrig**[3]

[1]Center for Vector Biology and Zoonotic Diseases, The Connecticut Agricultural Experiment Station, 123 Huntington Street, P. O. Box 1106, New Haven, Connecticut 06504, USA

[2]Department of Pathology and Laboratory Medicine and Microbiology and Molecular Genetics, University of Texas Medical School, 6431 Fannin Street, Houston, Texas 77030, USA

[3]Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine, 300 Cedar Street, P. O. Box 208031, New Haven, Connecticut 06520, USA

Abstract

A mark-release-recapture study was conducted during 2007 through 2010 in six, tick-infested sites in Connecticut, United States to measure changes in antibody titers for *Borrelia burgdorferi* sensu stricto, *Anaplasma phagocytophilum, and Babesia microti* in *Peromyscus leucopus* (white-footed mice). There was an overall recapture rate of 40%, but only four tagged mice were caught in ≥2 yr. Sera from 561 mice were analyzed for total antibodies to B. *burgdorferi* and A. *phagocytophilum* by using whole-cell or recombinant (VlsE or protein 44) antigens in a solid-phase enzyme-linked immunosorbent assay or to whole-cell B. *microti* by indirect fluorescent antibody staining. Antibody prevalences were highly variable for *B. burgdorferi* (from 56% to 98%), A. *phagocytophilum* (from 11% to 85%), and *B. microti* (from 11% to 84%) depending on the site and time of sampling. Of 463 mice with antibodies, 206 (45%) had antibodies to all three pathogens. Changes in antibody status for some mice from negative to positive (117 seroconversions) or from positive to negative (55 reversions) were observed. Seroconversions were observed in 10.1% of 417 mice for B. *burgdorferi*, 18.0% of 306 mice for *A. phagocytophilum*, and 6.6% of 304 mice for *B. microti*; reversion rates were 5.3, 5.9, and 4.9%, respectively. Antibodies to all pathogens persisted in some mice over several weeks while, in others, there were marked declines in titration end points to negative status. The latter may indicate elimination of a certain pathogen, such as *A. phagocytophilum*, or that mouse immune systems ceased to produce antibodies despite an existing patent infection.

Keywords

Anaplasma phagocytophilum; antibodies; *Babesia microti*; *Borrelia burgdorferi*; *Peromyscus leucopus*

INTRODUCTION

Larval and nymphal *Ixodes scapularis* ticks parasitize white-footed mice (*Pero-myscus leucopus*) in or near forested habitats of the eastern and upper midwestern United States.

[4]Corresponding author (Louis.Magnarelli@ct.gov).

These ticks and rodents can simultaneously harbor multiple human pathogens: *Borrelia burgdorferi* sensu stricto, *Anaplasma phagocytophilum*, and *Babesia microti* (Anderson et al., 1986, 1987; Tokarz et al., 2010; Johnson et al., 2011). Geographic ranges of these pathogens are expanding in North America and Eurasia, mainly due to the dispersal of *I. scapularis* or related *Ixodes* species such as *Ixodes pacificus, Ixodes ricinus*, and *Ixodes persulcatus* via the movement of vertebrate hosts frequently parasitized by these ectoparasites. Seabirds, fed upon by numerous tick species, play an important role in distributing ticks over long distances (Dietrich et al., 2011).

Serologic and molecular methods can determine if one or more human pathogens infect ticks or vertebrate hosts in selected sites. Automated antibody tests, such as enzyme-linked immunosorbent assays (ELISA), can be particularly helpful in quickly assessing if vertebrate hosts have been exposed to pathogens at specific sites; assays have been developed that contain highly specific recombinant antigens for *B. burgdorferi* sensu stricto or *A. phagocytophilum* (Magnarelli et al., 2006). If white-footed mice have antibodies to one or more disease organisms, DNA detection methods can then be used to confirm specific identities of the pathogens infecting ticks or rodents (Tokarz et al., 2010; Johnson et al., 2011).

Surveillance programs, which monitor *P. leucopus* in small private or public properties, have several advantages. These rodents are relatively easy to capture and recapture; their home ranges are limited (normally >2 ha) compared to deer, most other mammals, and birds; and robust concentrations of serum antibodies are produced by these mice to multiple pathogens (Magnarelli, et al., 2006). However, it is unclear if antibody concentrations change over several weeks in *P. leucopus*, particularly when *I. scapularis* nymphs are not actively feeding on these rodents or depending on how long tagged mice live in tick-infested areas. We measured antibody titers for different pathogens in marked white-footed mice recaptured over a 4-yr period in sites not chemically treated for ticks.

MATERIALS AND METHODS

Sherman box traps (H. B. Sherman Traps, Inc., Tallahassee, Florida, USA), baited with peanut butter, were used during 2007 through 2010 to capture white-footed mice in forested areas of Connecticut, United States, where *I. scapularis* ticks are abundant. Six sampling sites were located in the following towns: two in Redding (41°17′N, 73°22′W), two in North Branford (41°22′N, 72°46′W), one in Mansfield (41°46′N, 72°13′W), and one in Storrs (41°49′N, 72°15′W). The two sites in Redding (Fairfield County) were about 1 km apart whereas the two sites in North Branford (New Haven County) were about 0.5 km apart. The remaining two sites in Tolland County were about 5 km apart. Each site had mixed hardwoods and understory vegetation, typical habitats for white-tailed deer (*Odocoileus virginianus*), rodents, and tick populations. White-footed mice were captured from late June to mid-October; frequency of trapping was highest (at least weekly) during July and August. In Connecticut, populations of questing *I. scapularis* nymphs, the most important stage in the transmission of the aforementioned pathogens, normally peak in June and decline to low densities by late July (Stafford et al., 1998). This finding was based on dragging flannel over vegetation at 10 residential properties in eastern Connecticut once or twice per month over 9 yr.

Captured mice were sedated by using the inhalant anesthetic isofluorane (Piramal Critical Care, Inc., Bethlehem, Pennsylvania, USA), which has a rapid induction time (2–3 min) with a relatively short, complete recovery time of about 10 min. Blood samples were collected usually within 5 min of the time an animal was completely sedated. A 0.1-cc whole-blood sample was obtained by cardiac puncture by using a 1 cc syringe tipped with a

27-gauge, 15.9-mm needle. No diluent was added to blood samples at the field sites. Whole blood samples were transported to the laboratory with cold packs in an insulated container. Following centrifugation of undiluted whole bloods, sera were stored at −60 C until analysis. The amount of serum recovered per blood sample varied depending on the amount of whole blood acquired; at best, about 25 μL of serum per sample were available for testing. At the time of first capture, each mouse received a numbered ear tag (National Band and Tag Co., Newport, Kentucky, USA) and was released into the habitat where captured. Mouse trapping and handling protocols were approved by the Wildlife Division of the Connecticut Department of Energy and Environmental Protection and the Connecticut Agricultural Experiment Station's Institutional Animal Care and Use Committee. In general, guidelines of the American Society of Mammalogists for the use of wild animals in research (Sikes and Gannon, 2011) were followed.

To measure total antibodies, separate polyvalent, solid-phase ELISA or indirect fluorescent antibody (IFA) staining methods were used (Magnarelli et al., 1997, 2006). Briefly, in analyses for *B. burgdorferi* antibodies, whole-cell lysate (strain 2591) or recombinant (fusion protein) VlsE antigen was coated at respective concentrations of 5 μg/mL and 1 μg/mL to flat-bottom polystyrene plates (NUNC A/S Roskilde, Denmark). This recombinant antigen, a His_6-tagged version of the full-length, surface-exposed VlsE, was produced at the University of Texas (Houston, Texas, USA) and found to be highly specific in tests of human and dog sera (Lawrenz et al., 1999; Liang et al., 2000; Magnarelli et al., 2002). The VlsE antigen was incorporated into an ELISA and tested with a subset of mouse sera to confirm *B. burgdorferi* infections in all sampling areas. A previously developed ELISA (Magnarelli et al., 2006) was also used to measure total antibodies to recombinant protein (p) 44 of *A. phagocytophilum*. This fusion protein was produced at Yale University (New Haven, Connecticut, USA) from a human isolate (NCH-1 strain) and coated to plates at a concentration of 2.5 μg/mL. In IFA analyses, *B. microti* antigen, which consisted of infected erythrocytes obtained from golden Syrian hamsters (*Mesocricetus auratus*), was fixed to glass microscope slides. The hamsters were inoculated with whole blood from an infected person diagnosed with human babesiosis (Anderson et al., 1991). Materials and methods used to conduct all antibody tests, including positive and negative controls, washing solutions, conjugates, production of whole-cell and recombinant antigens, and critical regions for positive results, have been reported (Magnarelli et al., 1997, 2006). Greater than fourfold change in titration endpoints for paired sera was considered significant in recording seroconversions and reversions. Groups of the same sera were retested weeks after initial analyses to assess variability of results. There was no commercial affinity-purified horseradish peroxidase-labeled goat anti-*P. leucopus* reagent available to measure immunoglobulin M antibodies. Requests for reagents should be directed to those authors who produced the antigens or who have reference mouse sera.

Statistical analyses to determine significant differences in percentages of antibody-positive white-footed mice were performed by using a z-test. The software program (SigmaStat, SPSS, Inc., Chicago, Illinois, USA) used included the Yate's correction in analyses.

RESULTS

White-footed mice were recaptured during each year. The overall recapture rate (40%) for 561 subjects paralleled those of 40–45% recorded in 2007, 2008, and 2010 (Table 1). Mouse populations during 2009 were low at all study sites. Although the maximum number of recaptures for a given mouse was five, 117 (53%) of 223 mice were recaptured once. The mean number of days between first and last capture for all mice in a given year varied between 26 and 50. The latter, recorded in 2008, reflected the maximum number of days (n=111) and the highest number of mice recaptured (n=78).

Peromyscus leucopus had antibodies to one or more pathogens (Table 2). Of the 463 antibody-positive mice, 206 (45%) had antibodies to all three agents. The remaining mice had serologic evidence of exposure to *B. burgdorferi* and *A. phagocytophilum* (n=69), *B. burgdorferi* and *B. microti* (n=76), *A. phagocytophilum* and *B. microti* (n=12), or only to *B. burgdorferi* (n=66), *A. phagocytophilum* (n=18), or *B. microti* (n=13). When the number of sera tested was ≥19 for a given month, results for a subset of recaptured mice show that antibody prevalences were generally high (Table 2). Prevalence for *B. burgdorferi* antibodies exceeded 77% for most sites in at least 1 mo during 2007 and 2008, except for Storrs in 2007 (73%) and in 2008 (69%). Antibody prevalences of ≥80% for *A. phagocytophilum* and *B. microti* were far less frequent compared to those recorded for *B. burgdorferi*. Percentages of antibody-positive sera for *A. phagocytophilum* and *B. microti* were lowest (range=15–21%) in Storrs during 2008.

White-footed mice captured and tagged in a given location did not enter another study site. There were notable differences in antibody prevalences among study sites, even those ≤1 km apart. During 2008, analyses for 86 and 58 sera (including samples from recaptured mice) revealed no significant difference (z=0.848, P=0.396) in percentages (79% and 85%) of antibody-positive samples for *B. burgdorferi* at two sites in New Haven County. Similar results were recorded for *A. phagocytophilum*; 70% and 74% were not statistically significant (z=0.334, P=0.739). However, prevalences of *B. microti* antibodies (74% vs. 53%) were different statistically (z=2.422, P =0.015). Statistical analyses for results obtained for 71 and 68 sera, representing mice captured in these sites in 2010, revealed no significant differences in percentages of antibody-positive samples for *B. burgdorferi* (90% vs. 91%, z=−0.088, P=0.930), *A. phagocytophilum* (58% vs. 56%, z=0.067, P=095), or *B. microti* (83% vs. 88%, z=0.595, P=0.552).

When results were compared by county and year (Table 3), antibody prevalences for 561 *P. leucopus* were highly variable. An overall antibody prevalence of 75% for *B. burgdorferi* in all sites exceeded the figures recorded for both *A. phagocytophilum* (55%) and *B. microti* (54%). During 2008, a time when relatively large numbers of *P. leucopus* were captured in all three counties, the overall percentage of mice with antibodies to *B. burgdorferi* in New Haven County (84%) was not significantly greater than the value calculated for Fairfield County (74%, z=1.294, P=0.196). The rate for New Haven County, however, was significantly greater than the value for Tolland County (61%, z=2.662, P=0.008). The prevalence recorded that year for *A. phagocytophilum* antibodies in mice captured in New Haven County (74%) was not significantly greater than that recorded for mice captured in Fairfield County (61%, z=1.511, P=0.131) but was significantly different when compared to results for Tolland County (23%, z=5.312, P<0.001). Prevalence of antibody to *B. microti* antibodies in New Haven County (71%) was not significantly different than the prevalence for Fairfield County (62%, z=0.981, P=0.326). Similar to the results obtained for the other two pathogens, the prevalence for New Haven County was statistically different from that for Tolland County (18%, z=5.492, P<0.001).

Groups of the same positive and negative sera were retested weeks after initial analyses to assess variability of results. In analyses of 11 sera containing *B. burgdorferi* antibodies to whole-cell antigens, ELISA results were identical (n=3), or varied by twofold (n=6) or fourfold (n=2), compared to results obtained 23 wk earlier. Five negative sera remained nonre-active in the second trial. Similar results were recorded by an ELISA for *A. phagocytophilum* antibodies. In duplicate tests of seven sera, conducted over 3 wk, there was no change in titers for two sera while findings for the remaining samples varied by twofold (n=3) or fourfold (n=2). The status of five negative sera was unchanged. Application of IFA staining methods for *B. microti* antibodies revealed no change in results for four sera, twofold variation for three sera, and a fourfold difference for two sera when

samples were reanalyzed 4 wk after initial testing. Results for the five negative sera remained nonreactive.

Sera were analyzed with the VlsE antigen of *B. burgdorferi* in an ELISA to compare results recorded for whole-cell antigens. Of the 107 sera representing all sampling sites and screened separately for antibodies, 66 of the positives for whole-cell antigen also reacted with the VlsE antigen while an additional 17 sera were negative in both tests (concordance=78%). These results confirmed *B. burgdorferi* presence at all sampling sites. The remaining sera contained antibodies to whole-cell antigens (titer range=1:320 to 1:40,960) but were negative in tests with VlsE antigen (n=22) or vice versa (n=2). In the latter, there was a low concentration of antibodies (titer=1:160) to the VlsE antigen in both sera.

There were changes beyond fourfold normal assay variation in antibody titers recorded for all antigens tested during a given year. In analyses with whole-cell *B. burgdorferi* antigens, seroconversions (negative to positive) and reversions (positive to negative) were documented for 42 and 22 mice, respectively. For *A. phagocytophilum*, there were 55 seroconversions and 18 reversions in antibody status, while 20 seroconversions and 15 reversions were recorded when testing for *B. microti* antibodies.

Selected examples for nine mice (Table 4) demonstrate extensive variation in results and, in some cases, show persistence of antibodies. There were instances when antibody titers for multiple samples were equivalent for *B. burgdorferi* (mouse no. 8) and *A. phagocytophilum* (mouse nos. 2, 4, 7, and 9). Seroconversions for *B. burgdorferi* (mouse nos. 2–4, 6, and 9), *A. phagocytophilum* (mouse nos. 3, 6, and 8), and *B. microti* antibodies (mouse nos. 2–4) were recorded for other blood samples collected during the summer. Reversions in antibody titers were also documented for *B. burgdorferi* (mouse no. 5), *A. phagocytophilum* (mouse no. 5), and *B. microti* (mouse no. 9). The reversion for mouse no. 5 (captured in Redding) occurred during a 3-yr period.

Recapturing tagged mice over multiple years was rare (4 of 561 mice). Aside from mouse no. 5 (Table 4), there were three other individuals recaptured during a 2-yr period. One mouse, caught in North Branford on 3 August 2007 and recaptured on 29 July 2008, seroconverted for *B. burgdorferi* antibodies with a maximum titer of 1:1,280. Antibody titers for *A. phagocytophilum* and *B. microti* were unchanged (titers=1:20,480 and 1:80, respectively). Another mouse caught in Redding on 11 July 2007 and recaptured on 26 June 2008 had the same antibody concentration (titer=1:20,480) for *B. burgdorferi*, a fourfold rise in titer to 1:5, 120 for *A. phagocytophilum*, and a seroconversion for *B. microti* (maximum titer=1:160). Analyses for the third mouse, captured in Redding on 19 August 2009 and 1 July 2010, revealed seroconversions for *B. burgdorferi* (titer=1:40,960) and *A. phagocytophilum* (titer=1:2,560) but showed no reactivity to *B. microti* antigen.

DISCUSSION

Longevity for tagged *P. leucopus* beyond 1 yr was very limited. Nonetheless, mouse numbers were sufficient to perform statistical analyses for data collected in all years except 2009, when increased predation, lack of food, winter conditions, or other factors may have suppressed populations. The relatively low recapture rate for *P. leucopus* from one year to the next may reflect correspondingly low long-term survival time. It is unlikely that these rodents leave their home range and relocate to other sites. Relatively low survival time beyond 1 yr for *P. leucopus* underscores the importance of overwintering infected *I. scapularis* nymphs in maintaining pathogens in foci.

Antibodies to all three etiologic agents were detected in white-footed mice captured in each study site and at relatively high prevalences. Numerous seroconversions and reversions in antibody status were also documented. These rodents are heavily parasitized by nymphal *I. scapularis* in Connecticut during mid- to late May with peak tick numbers normally reached in June and early July (Stafford et al., 1998). In a long-term study, these authors reported an overall nymphal infection rate of 14.3% (n=3,866 ticks tested) for *B. burgdorferi* but, in 1996, an infection rate of 24.4% of 513 ticks tested was recorded. Co-infections of unrelated human pathogens have been documented for *Ixodes* species (Tokarz et. al., 2010; Burri et al., 2011; Gigandet et al., 2011) and *P. leucopus* (Anderson et al., 1986; Johnson et al., 2011). Therefore, frequent feedings by infected ticks on white-footed mice would tend to elevate antibody titers and antibody prevalences during summer. These antibodies persisted in some mice well beyond the nymphal tick-feeding periods. Previous work verified that *B. burgdorferi*, or the DNA of this agent, persists in *P. leucopus* for several days (Anderson et al., 1987; Bunikis et al., 2004). This pathogen was cultured from mouse tissues during winter (Anderson et al., 1987). Different results were reported by Lindsay et al. (1997); *P. leucopus* was infective to feeding *I. scapularis* larvae for about 49 days postinoculation or postinfection. The reversions in antibody status documented in our study may indicate waning infections in certain mice. Some mice may be able to eliminate certain pathogens, as is reported for *A. phagocytophilum* (Johnson et al., 2011). It is also possible that mouse immune systems ceased to produce antibodies despite an existing patent infection. It is unclear how increased age or other factors affect mouse immune systems or if decreased antibody concentrations during times of existing patent infections enhance reservoir competency during late summer and early fall when *I. scapularis* larvae parasitize white-footed mice.

Based on our serologic results, there appears to be a greater prevalence of mouse infections in New Haven County (southern Connecticut) as compared to the northern sites in Tolland County. These differences may be due, in part, to higher populations of white-tailed deer, chief hosts for *I. scapularis* adults, and correspondingly higher tick populations in New Haven County. Another important factor might be higher populations of other infected reservoir hosts for different pathogens such as eastern gray squirrels (*Sciurus carolinensis*), meadow voles (*Microtus pennsylvanicus*), and eastern chipmunks (*Tamias striatus*). These animals, along with *P. leucopus*, may serve to infect more ticks and enhance amplification of *B. burgdorferi* in southern Connecticut. Raccoons (*Procyon lotor*) and eastern gray squirrels are competent reservoirs for *A. phagocytophilum* (Levin et al., 2002). Serologic evidence of *B. microti* infections in northern Connecticut reaffirms that this pathogen is more widely distributed away from the coastal areas of northeastern United States.

Human babesiosis continues to emerge as an important public health problem. A review of medical records for elderly persons revealed relatively high babesiosis rates for Connecticut, Rhode Island, New York, and Massachusetts and possible presence of this disease in other eastern states (Menis et al., 2012). Moreover, *B. microti* coexists with *Babesia divergens* or *Babesia venatorum* (also known as *Babesia EU1*) in Europe (Gigandet et al., 2011; Lempereur et al., 2011). Therefore, in the Holarctic where *Ixodes* species exist and different strains or genospecies of *B. burgdorferi* sensu lato and *A. phagocytophilum* likewise occur, *B. microti* should be considered in ecologic and epidemiologic studies as well as in the differential diagnosis of unknown febrile human illnesses.

The use of whole-cell *B. burgdorferi* antigens in an ELISA facilitates rapid, initial screening of white-footed mice for antibodies to *Borrelia* species. Subsequent testing with the highly specific recombinant VlsE antigen can help confirm *B. burgdorferi* exposure in these rodents. There are other bacterial pathogens present, however, such as *Borrelia miyamotoi* (Scoles et al., 2001; Ullmann et al., 2005; Tokarz et al., 2010), *Borrelia andersonii* (Marconi

et al., 1995), and *Borrelia bissettii* (Schneider et al., 2008) in the northeastern United States. Although these organisms do not appear to be as prevalent as *B. burgdorferi*, positive reactions to whole-cell *B. burgdorferi* antigens, coupled with negative results with the VlsE antigen, might indicate the presence of antibodies to one or more of these other *Borrelia* species. Therefore, DNA analyses would ultimately be required for more-specific pathogen identifications in foci. Two sera were positive in an ELISA with the VlsE antigen and negative in tests with whole-cell *B. burgdorferi*. The presence of several nonspecific antigens in plate wells can sometimes block specific antibody-antigen complex formation and cause false negatives, particularly when there is a low concentration of specific antibodies.

Ear-tagged white-footed mice did not appear to be long-lived. Maximal life expectancy for these animals in the wild is about 2–3 yr (Burt and Grossenheider, 1964). Nonetheless, these rodents are ecologically important as reservoir hosts for at least three important human pathogens. In a Minnesota study of white-footed mice (Johnson et al., 2011), infection rates for *A. phagocytophilum* and *B. burgdorferi* were 20% and 42%, respectively, whereas antibody prevalences were 29% and 48%. Coinfection was documented in 14% of the mice tested. Culturing the pathogens and detecting their DNA are the best methods to monitor infections in *P. leucopus*, and serology is useful for assessing the host's responses to pathogens. There have been extensive efforts aimed at reducing tick populations and infection rates at selected sites (Eisen et al., 2012). Depending on the habitat, sampling questing ticks can be more difficult than capturing mice. The apparent low year-to-year survival rate for *P. leucopus* is an advantage in field studies designed to assess effectiveness of chemical or biologic tick management programs in selected sites. If intervention strategies are effective in significantly reducing target tick populations, there should be a corresponding decline in infection and antibody prevalence for *P. leucopus* over a 2–3-yr period.

Acknowledgments

We are grateful to the following persons for technical assistance: Tia Blevins, Michael Short, Joseph Barsky, Geoffrey Picard, Emily Picard, Rhyan Wilcox, Maribeth Chassey, Lara Ariori, and Elizabeth White. We also acknowledge financial support (Hatch funds) administered by the US Department of Agriculture.

LITERATURE CITED

Anderson JF, Johnson RC, Magnarelli LA, Hyde FW, Myers JE. *Peromyscus leucopus* and *Microtus pennsylvanicus* simultaneously infected with *Borrelia burgdorferi* and *Babesia microti* . J Clin Microbiol. 1986; 23:135–137. [PubMed: 3517038]

Anderson JF, Johnson RC, Magnarelli LA. Seasonal prevalence of *Borrelia burgdorferi* in natural populations of white-footed mice, *Peromyscus leucopus* . J Clin Microbiol. 1987; 25:1564–1566. [PubMed: 3624451]

Anderson JF, Mintz ED, Gadbaw JJ, Magnarelli LA. *Babesia microti*, human babesiosis, and *Borrelia burgdorferi* in Connecticut. J Clin Microbiol. 1991; 29:2779–2783. [PubMed: 1757548]

Bunikis J, Tsao J, Luke CJ, Luna MG, Fish D, Barbour AG. *Borrelia burgdorferi* infection in a natural population of *Peromyscus leucopus* mice: A longitudinal study in an area where Lyme borreliosis is highly endemic. J Infect Dis. 2004; 189:1515–1523. [PubMed: 15073690]

Burri C, Dupasquier C, Bastic V, Gern L. Pathogens of emerging tick-borne diseases, *Anaplasma phagocytophilum, Rickettsia* spp., and *Babesia* spp., in *Ixodes* ticks collected from rodents at four sites in Switzerland (Canton of Bern). Vector-Borne and Zoonotic Dis. 2011; 11:939–944.

Burt, WH.; Grossenheider, RP. A field guide to the mammals. Boston, Massachusetts: Houghton Mifflin Company; 1964. p. 284

Dietrich M, Gomez-Diaz E, McCoy KD. Worldwide distribution and diversity of seabird ticks: Implications for the ecology and epidemiology of tick-borne pathogens. Vector-Borne and Zoonotic Dis. 2011; 11:453–470.

Eisen RJ, Piesman J, Zielinski-Gutierrez, Eisen L. What do we need to know about disease ecology to prevent Lyme disease in northeastern United States? J Med Entomol. 2012; 49:11–22. [PubMed: 22308766]

Gigandet L, Stauffer E, Douet V, Rais O, Moret J, Gern L. Prevalence of three zoonotic *Babesia* species in *Ixodes ricinus* (Linne, 1758) nymphs in a suburban forest in Switzerland. Vector-Borne and Zoonotic Dis. 2011; 11:363–366.

Johnson RC, Kodner C, Jarnefeld J, Eck DK, Xu Y. Agents of human anaplasmosis and Lyme disease at Camp Ripley, Minnesota. Vector-Borne and Zoonotic Dis. 2011; 11:1529–1534.

Lawrenz MB, Hardham JM, Owens RT, Nowakowski J, Steere AC, Wormser GP, Norris SJ. Human antibody responses to VlsE antigenic variation protein of *Borrelia burgdorferi* . J Clin Microbiol. 1999; 37:3997–4004. [PubMed: 10565921]

Lempereur L, Decat A, Caron Y, Madder M, Claerebout E, Saegerman C, Losson B. First molecular evidence of potentially zoonotic *Babesia microti* and *Babesia* sp. EU1 in *Ixodes ricinus* ticks in Belgium. Vector-Borne and Zoonotic Dis. 2011; 11:125–130.

Levin ML, Nicholson WL, Massung RF, Sumner JW, Fish D. Comparison of the reservoir competence of medium-sized mammals and *Peromyscus leucopus for Anaplasma phagocytophilum* in Connecticut. Vector-Borne and Zoonotic Dis. 2002; 2:125–136.

Liang FT, Jacobson RH, Straubinger RK, Grooters A, Phillipp MT. Characterization of a *Borrelia burgdorferi* VlsE invariable region useful in canine Lyme disease serodiagnosis by enzyme-linked immunosorbent assay. J Clin Microbiol. 2000; 38:4160–4166. [PubMed: 11060084]

Lindsay LR, Barker IK, Surgeoner GA, McEwen SA, Campbell GD. Duration of *Borrelia burgdorferi* infectivity in white-footed mice for the tick vector *Ixodes scapularis* under laboratory and field conditions in Ontario. J Wildl Dis. 1997; 33:766–775. [PubMed: 9391960]

Magnarelli LA, Anderson JF, Stafford KC III, Dumler JS. Antibodies to multiple tick-borne pathogens of babesiosis, ehrlichiosis, and Lyme borreliosis in white-footed mice. J Wildl Dis. 1997; 33:466–473. [PubMed: 9249691]

Magnarelli LA, Lawrenz M, Norris SJ, Fikrig E. Comparative reactivity of human sera to recombinant VlsE and other *Borrelia burgdorferi* antigens in class-specific enzyme-linked immunosorbent assays for Lyme borreliosis. J Med Microbiol. 2002; 51:649–655. [PubMed: 12171295]

Magnarelli LA, Stafford KC III, IJdo JW, Fikrig E. Antibodies to whole-cell or recombinant antigens of *Borrelia burgdorferi, Anaplasma phagocytophilum*, and *Babesia microti* in white-footed mice. J Wildl Dis. 2006; 42:732–738. [PubMed: 17255439]

Marconi RT, Liveris D, Schwartz I. Identification of novel insertion elements, restriction fragment length polymorphism patterns, and discontinuous 23S rRNA in Lyme disease spirochetes: Phylogenetic analyses of rRNA genes and their intergenic spacers in *Borrelia japonica* sp. nov. and genomic group 21038 (*Borrelia andersonii* sp. nov.) isolates. J Clin Microbiol. 1995; 33:2427–2434. [PubMed: 7494041]

Menis M, Anderson SA, Izurieta HS, Kumar S, Burwen DR, Gibbs J, Kroop G, Erten T, MaCurdy TE, Worrall CM, et al. Babesiosis among elderly Medicare beneficiaries, United States, 2006–2008. Emerg Infect Dis. 2012; 18:128–131. [PubMed: 22257500]

Schneider BS, Schriefer ME, Dietrich G, Dolan MC, Morshed MG, Zeidner NS. *Borrelia bissettii* isolates induce pathology in a murine model of disease. Vector-Borne and Zoonotic Dis. 2008; 8:623–634.

Scoles GA, Papero M, Beati L, Fish D. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. Vector-Borne and Zoonotic Dis. 2001; 1:21–34.

Sikes RS, Gannon WL. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J Mammalogy. 2011; 92:235–253.

Stafford KC III, Cartter ML, Magnarelli LA, Ertel SH, Mshar PA. Temporal correlations between tick abundance and prevalence of ticks infected with *Borrelia burgdorferi* and increasing incidence of Lyme disease. J Clin Microbiol. 1998; 36:1240–1244. [PubMed: 9574684]

Tokarz R, Komal J, Bennett A, Briese T, Lipkin WI. Assessment of polymicrobial infections in ticks in New York State. Vector-Borne and Zoonotic Dis. 2010; 10:217–221.

Ullmann AJ, Gabitzsch ES, Schulze TL, Zeidner NS, Piesman J. Three multiplex assays for detection of *Borrelia burgdorferi* sensu lato and *Borrelia miyamotoi* sensu lato in field-collected *Ixodes* nymphs in North America. J Med Entomol. 2005; 42:1057–1062. [PubMed: 16465748]

Table 1

Total number of white-footed mice (*Peromyscus leucopus*) recaptured at six sites in Connecticut, United States during 2007–2010.

Years	Total mice captured	No. (%) of mice recaptured	No. of recaptures[a]				No. of days between first and last capture[a]		
			1	2	3	4	Mean	SD	Range
2007	140	63 (45)	38	18	7	0	29	15.6	12–71
2008	192	78 (41)	42	24	7	5	50	29.0	9–111
2009	40	7 (18)	3	3	1	0	26	11.7	11–44
2010	189	75 (40)	34	27	14	0	35	16.2	8–71
Totals	561	223 (40)	117	72	29	5	38	23.1	9–111

[a]Number of mice recaptured once, twice, etc. during a given year; excludes subjects captured in different years. Mean 5 total number of days between first and last recapture divided by the number of recaptured mice.

Table 2

Prevalence of serum antibodies to whole-cell *Borrelia burgdorferi* or recombinant protein 44 *Anaplasma phagocytophilum* (as determined by enzyme-linked immunosorbent assay) or to whole-cell *Babesia microti* (as determined by indirect fluorescent antibody staining) in white-footed mice (*Peromyscus leucopus*) captured in Connecticut, United States.

Sampling			Total sera tested[a]	No. (%) sera with antibodies to:		
Towns (counties)	Years	Months		*B. burgdorferi*	*A. phagocytophilum*	*B. microti*
Redding	2007	July	43	36 (84)	18 (42)	27 (63)
(Fairfield)		August	28	25 (89)	14 (50)	10 (36)
North Branford	2007	July	37	35 (95)	31 (84)	31 (84)
(New Haven)		August	48	47 (98)	41 (85)	39 (81)
Storrs (Tolland)	2007	August	33	24 (73)	12 (36)	11 (33)
Redding	2008	June	32	18 (56)	16 (50)	9 (28)
		July	19	13 (68)	8 (42)	11 (58)
		August	22	17 (77)	14 (64)	11 (50)
North Branford	2008	July	81	64 (79)	60 (74)	44 (54)
		August	33	29 (88)	25 (76)	27 (82)
Storrs	2008	August	48	33 (69)	10 (21)	7 (15)

[a]Includes multiple sera per mouse from a subset of recaptured mice. A minimum of 19 sera were required for a table listing.

Table 3

Prevalence of white-footed mice (*Peromyscus leucopus*) with serum antibodies to whole-cell *Borrelia burgdorferi* or recombinant protein 44 of *Anaplasma phagocytophilum* (as determined by an enzyme-linked immunosorbent assay) or to whole-cell *Babesia microti* (as determined by indirect fluorescent antibody staining) at six sites in Connecticut, United States, 2007–2010.

Sampling			No. (%)[a] of mice with antibodies to:		
Counties	**Years**	**Total mice captured 57**	***B. burgdorferi***	***A. phagocytophilum***	***B. microti***
Fairfield	2007	57	45 (79)	26 (46)	29 (51)
	2008	66	49 (74)	40 (61)	41 (62)
	2009	13	8 (62)	7 (54)	5 (39)
	2010	91	62 (68)	33 (36)	35 (39)
New Haven	2007	52	50 (96)	49 (94)	46 (89)
	2008	82	69 (84)	61 (74)	58 (71)
	2009	9	9 (100)	9 (100)	7 (78)
	2010	69	57 (83)	42 (61)	53 (77)
Tolland	2007	31	20 (65)	12 (39)	11 (36)
	2008	44	27 (61)	10 (23)	8 (18)
	2009	18	13 (72)	15 (83)	2 (11)
	2010	29	13 (45)	7 (24)	8 (28)
Totals		561	422 (75)	311 (55)	303 (54)

[a]Percent positive = the number of antibody-positive mice divided by total number of mice tested in the respective categories by year and county. Any one positive serum sample equals a positive mouse.

Table 4

Reciprocal antibody titers to whole-cell *Borrelia burgdorferi* or recombinant protein 44 of *Anaplasma phagocytophilum* (as determined by enzyme-linked immunosorbent assay) or to whole-cell *Babesia microti* (as determined by indirect fluorescent antibody staining) for white-footed mice (*Peromyscus leucopus*) recaptured in Connecticut, USA, 2007–2010.

	Capture date	Reciprocal antibody titers[b] to:		
Mouse[a]	mo/day/yr	*B. burgdorferi*	*A. phagocytophilum*	*B. microti*
1A	7/11/07	5,120	20,480	640
1B	9/07/07	1,280	10,240	320
2A	7/02/08	N	N	N
2B	8/14/08	2,560	N	640
2C	10/10/08	5,120	N	2,560
3A	7/03/08	N	N	N
3B	8/13/08	1,280	20,480	160
4A	6/29/10	N	N	N
4B	8/13/10	10,240	N	160
5A	8/09/07	5,120	640	N
5B	7/23/10	N	N	N
6A	8/19/09	N	N	N
6B	7/11/10	40,960	2,560	N
7A	6/29/10	20,480	N	N
7B	8/13/10	5,120	N	N
8A	6/25/10	40,960	N	320
8B	7/28/10	40,960	10,240	80
9A	7/09/10	N	N	1,280
9B	8/24/10	2,560	N	N

[a] Letters refer to different captures of same mouse.

[b] Each positive mouse serum had antibodies to the VlsE recombinant antigen in a separate ELISA; N = negative.