

# *Borrelia burgdorferi* Infection in a Natural Population of *Peromyscus Leucopus* Mice: A Longitudinal Study in an Area Where Lyme Borreliosis Is Highly Endemic

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Blood samples from *Peromyscus leucopus* mice captured at an enzootic site in Connecticut were examined for antibodies to and DNA of *Borrelia burgdorferi*, to characterize the dynamics of infection in this reservoir population. From trappings conducted over the course of 2 transmission seasons, 598 (75%) of 801 serum samples from 514 mice were found to be positive by enzyme immunoassay. Seropositivity correlated with date of capture and mouse age, was similar among locations within the site, increased from 57% to 93% over the course of the transmission season, and was associated with antibodies to outer surface protein (Osp) C, but not to OspA. Longitudinal samples from 184 mice revealed an incidence of 0.2 cases/mouse/week. Nineteen (10%) of 187 samples were found by polymerase chain reaction to be positive for *B. burgdorferi*, and, of those, 14 (74%) were found to be seropositive. Nearly the entire population of *P. leucopus* mice became infected with *B. burgdorferi* by late August, coinciding with the peak activity period of host-seeking larvae uninfected with the spirochete *Ixodes scapularis*, thereby perpetuating the agent through succeeding generations of ticks.

Vectorborne zoonotic infections are important causes of morbidity and mortality in humans and agricultural animals throughout the world. For the most part, understanding the pathogenesis and dynamics of immune responses during these infections comes from studies of model laboratory animals. Less is known about in-

fections in the natural populations of vertebrates that serve as reservoirs of pathogens. Since the risk of infection for humans is correlated with the prevalence of infection in reservoirs and vectors [1], disease control programs, such as those targeting interventions to reservoirs, would likely benefit from a better understanding of the transmission dynamics of these infectious agents among their hosts in their natural setting. With this goal in mind, we began a field-based study, in a reservoir host population, of infection with *Borrelia burgdorferi*, which is an agent of Lyme borreliosis (LB), the most common vectorborne zoonosis in the United States and in much of Europe [2, 3].

In the Midwestern and northeastern United States, the white-footed mouse, *Peromyscus leucopus*, is a major reservoir host for *B. burgdorferi* [4–6]. The role of this rodent as a source of *B. burgdorferi* infection for immature *Ixodes scapularis* ticks has been documented [7, 8]. Limited serologic surveys of natural *P. leucopus* populations have reported that, in areas in the northeastern United States where LB is highly endemic, 30%–80%

Received 20 June 2003; accepted 6 October 2003; electronically published 30 March 2004.

Presented in part: 9th International Conference on Lyme Borreliosis and Other Tick-Borne Diseases, New York, 18–22 August 2002 (abstract O-82).

Financial support: National Institutes of Health (grant AI37248 to A.G.B.); National Science Foundation (grant support to J.T.); The Harold G. and Leila Y. Mathers Charitable Foundation (grant support to D.F.); US Department of Agriculture (grant 58-1265-5023 to D.F.); Centers for Disease Control and Prevention (cooperative agreement 919558-01 to J.B.).

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The Journal of Infectious Diseases 2004;189:1515–23

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0022-1899/2004/18907-0023\$15.00

of captured mice have antibodies to *B. burgdorferi*, and seroprevalence peaks during summer months [9–11]. Seasonal variation of infection of *P. leucopus* mice with *B. burgdorferi* has been demonstrated by cultivation of the pathogen from the mice [6, 12]. To date, there have been few studies of antibody responses to and infection with *B. burgdorferi* in a natural population of *P. leucopus* mice during the spirochete's transmission season [6, 13]. Experimental infection of *P. leucopus* mice with *B. burgdorferi* has demonstrated that immunity in the mice is strain specific [14], but the relationship between infection and immune responses among reservoir mice in nature has not been well characterized.

Moreover, the characteristics of the antibody response of a natural population of any *B. burgdorferi* reservoir are incompletely understood. There have been few studies to determine which *B. burgdorferi* antigens are immunologically recognized by *P. leucopus* (or any other reservoir species) in the field. The results of those studies that have examined this aspect are conflicting. For example, in one study [15] but not in others [6, 13], the presence of what appear to be antibodies to outer surface protein (Osp) A in the serum samples from field *P. leucopus* mice has been reported to be common. This is not a trivial point. If *P. leucopus* mice commonly do have antibodies to OspA, these may affect the transmission of *B. burgdorferi* to the host mouse by killing the spirochetes in the midgut of the engorging tick [16]. On the other hand, if *P. leucopus* mice commonly do not have antibodies to OspA, then this reservoir species could be an appropriate target for a field-administered vaccine designed to prevent the transmission of *B. burgdorferi* between vectors and reservoirs [17].

For the present study, we used both serologic assays and a polymerase chain reaction (PCR) assay to characterize *B. burgdorferi* infection in *P. leucopus* mice both captured and released at a field site in Connecticut during the summer months of 2 consecutive years. By the end of the transmission season by *I. scapularis* nymphs, almost all the field mice from which samples had been obtained were found to be seropositive for the spirochete, and *B. burgdorferi* DNA was detectable in ~10% of blood samples, even in those from mice with antibodies to *B. burgdorferi*. OspC was the most commonly recognized antigen, but the occurrence of antibodies to OspA was rare.

## MATERIALS AND METHODS

**Field study.** Mice were trapped on nine 1.2-hectare plots, in a mixed hardwood forest, on property owned by a private water company near New Haven, Connecticut. The plots were distributed among 3 sites (1, 2, and 3) that were separated by 100–1500 m. Each plot consisted of a 9 × 9 array of live traps that were baited with peanut butter for 2–4 consecutive nights/trapping period. We rotated sequentially among the 3 sites, with

~21 days between visits to the same plot, resulting in 4 trapping periods/plot and a total of 12 trapping periods during late May to August, for both 1998 and 1999. Captured mice were marked with uniquely numbered ear tags. Mice were classified as juveniles (if they were <15 g and if their pelage [fur] was entirely gray), as subadults (if they were 15–17 g and/or if their pelage was a mix of gray and brown), and as adults (if they were >17 g and if their pelage was entirely brown) [18]. Blood samples were obtained from the retro-orbital sinus while mice were under ketamine anesthesia; after recovery, mice were released at the point of capture. For mice recaptured within a trapping period, samples were not obtained a second time. The vertebrate animal protocol #07596 was approved for the present study by Yale University.

In 1998, but not in 1999, mice were subjected to a pilot study of field immunizations by injection with either recombinant OspA of *B. burgdorferi* or a negative control immunogen, glutathione S-transferase [19]. The mice captured at the negative control plots formed the basis for the longitudinal analyses. To increase the analytical power of the prevalence-over-time survey, we also used serum samples from first-time-captured mice, samples obtained before vaccination with OspA.

**Laboratory infections and immunizations.** Twenty adult, female, laboratory-reared *P. leucopus* mice (Genetic Stock Center, University of South Carolina, Columbia) were infected with the Valhalla strain of *B. burgdorferi* via feeding by laboratory-reared, infected nymphs of *I. scapularis*, as described elsewhere [20]. Infection of mice was confirmed by use of xenodiagnostic larval *I. scapularis* [7]. Blood was obtained from the mice before infestation with the ticks and again 4 weeks later. Two additional mice were infected by intradermal injection of 10<sup>4</sup> cells of the Sh-2-82 strain of *B. burgdorferi* [21]. Blood samples were obtained 60 weeks later from these mice and from 6 uninfected *P. leucopus* mice of the same age. For immunizations, 5 *P. leucopus* mice were injected intramuscularly with 1 µg of purified, lipidated recombinant OspA (rOspA) that was derived from *B. burgdorferi* strain B31 [22, 23] and diluted in normal saline, and 5 mice received saline alone. Mice were injected on days 0 and 14, and blood samples were obtained on day 42.

**EIA and immunoblot analysis.** For all assays, serum samples were diluted 1:100 in PBS. For whole-cell EIA of serum samples obtained from field mice in 1998 and of serum samples obtained from laboratory-infected mice, a commercial kit (MarDx) based on strain B31 of *B. burgdorferi* was used (B31 EIA). For whole-cell EIA of serum samples obtained in 1999, strain B314, a B31 derivative that expresses OspC in great abundance but does not express OspA or OspB, was used (B314 EIA) [24]. The procedure for this and for the EIA using purified rOspA has been described elsewhere [25]. Goat anti-*P. leucopus* IgG heavy and light chains conjugated to alkaline phosphatase (Kirkegaard Perry Laboratories) were used as secondary anti-

body. The cutoff value for a positive value by EIA was 3 SD above the mean optical density value of serum samples from noninfected, laboratory-reared *P. leucopus* mice.

Immunoblot assays using rOspA or the lysate of strain B312, which produces both OspA and OspC abundantly [24], were performed as described elsewhere [25]. Different proteins were identified by use of monoclonal antibodies to OspA [26], FlaB [27], P66 [28], BmpA (P39) [29], the 80/93/100-kDa protein [30], and OspC (provided by Denee Thomas, University of Texas Health Science Center, San Antonio). The 18-kDa protein was identified by use of commercial blot strips based on strain B31 (MarDx).

**DNA extraction and PCR.** Total DNA was extracted from 100- $\mu$ L blood clots of field mice by use of the FastDNA kit (BIO 101 Systems) under a biological hood in an isolated room. A 684-bp fragment of the chromosomal *p66* gene of *B. burgdorferi* [31] was amplified by use of nested PCR using the following primers (Genbank number X87725 coordinates): external forward, 5'-GATTTTCTATATTTGGACACAT-3' (positions 985–1007); external reverse, 5'-TGTAATCTTATTAGTTTTTCAAG-3' (positions 1717–1740); internal forward, 5'-CAAAAAA-GAAACACCCTCAGATCC-3' (positions 1026–1049); and internal reverse, 5'-CCTGTTTTTAAATAAATTTTTGTAGCATC-3' (positions 1681–1709). PCR conditions were the following: 94°C for 3 min followed by 40 cycles of 94°C for 1 min; 50°C for 2 min; and 74°C for 2 min. Negative controls were included throughout the DNA extraction and PCR preparation steps, for each run of the assay. Amplicons were directly sequenced in both directions, by use of the internal primers, on a CEQ8000 capillary DNA sequencer (Beckman Coulter).

**Statistical analyses.** The means were determined for numerical data and, unless indicated otherwise, are followed throughout the text by 95% confidence intervals in parentheses. Unpaired or paired *t* tests were used for comparing mean optical density values for independent or dependent samples, respectively. Multiple comparisons of means were analyzed by analysis of variance (ANOVA) or covariance (ANCOVA). Proportions were compared by use of  $\chi^2$  test or Fisher's exact test.

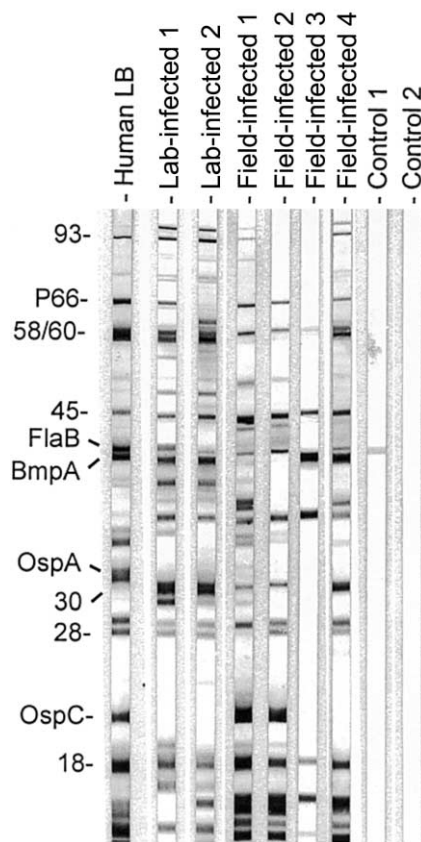
**Supplementary material.** Some results are provided in supplementary figures accompanying the online version of this paper, at <http://www.journals.uchicago.edu/JID/journal/issues/v189n8/31322/31322.html>.

## RESULTS

**Laboratory infections and OspA immunization.** To determine baseline antibody levels and cutoff values for EIAs using serum samples from *P. leucopus* mice, immune responses to whole-cell *B. burgdorferi* antigens were first evaluated in serum samples from 26 uninfected and 20 experimentally infected *P. leucopus* mice. By the whole-cell B31 EIA, the uninfected *P.*

*leucopus* mice had a mean OD value of 0.09 (0.08–0.10), with a cutoff value of 0.15, and the infected *P. leucopus* mice had a mean OD value of 0.27 (0.23–0.31) 4 weeks after infestation with the ticks. For 6 uninfected, laboratory-reared *P. leucopus* mice, by the whole-cell B314 EIA, the mean OD value was 0.10 (0.09–0.11), and the corresponding cutoff value was 0.25.

Although, in serum samples from uninfected *P. leucopus* mice, antibodies to *B. burgdorferi* were not detectable by immunoblot, in the serum samples from laboratory-infected *P. leucopus* mice, antibodies reacted with several *B. burgdorferi* antigens, including the 83/93/100-kDa protein, P66, FlaB, BmpA, and the 18-kDa protein (figure 1). Neither the serum samples from laboratory-infected *P. leucopus* mice nor those from the control mice had antibodies to OspA detectable by either immunoblot or EIA with rOspA, but serum samples from the 5 *P. leucopus* mice vaccinated with OspA had antibodies to



**Figure 1.** Immunoblot analysis of antibodies to *Borrelia burgdorferi* antigens in serum samples from laboratory-infected, field-infected, and uninfected (control) *Peromyscus leucopus* mice. The serum samples were incubated with commercial strips. The binding of antibodies was detected by use of alkaline phosphatase-conjugated goat anti-*P. leucopus* immunoglobulin antiserum. Serum from a human patient with Lyme borreliosis (LB) was the positive control. The location of selected *B. burgdorferi* antigens (83/93/100-kDa protein, P66, FlaB, BmpA, outer surface protein [Osp] A, and OspC) were identified by use of monoclonal antibodies and are shown on the left.

**Table 1. Summary of serum samples from *Peromyscus leucopus* mice captured at a field site in Connecticut in 1998 and 1999 and descriptive statistics of the results of whole-cell *Borrelia burgdorferi* EIAs.**

Year	No. of mice	No. of serum samples	Antigen <sup>a</sup>	EIA OD <sub>405</sub>		
				Mean (range)	Percentile	
					10th	90th
1998	267	355	B31	0.21 (0.05–0.57)	0.07	0.35
1999	247	446	B314	0.70 (0.06–2.02)	0.12	1.28

<sup>a</sup> B31, outer surface protein (Osp) A<sup>+</sup>OspC<sup>+</sup> phenotype; B314, OspA<sup>-</sup>OspC<sup>+</sup> phenotype.

OspA detectable by immunoblot and had reciprocal titers of  $\geq 320$  detected by the EIA using rOspA, 6 weeks after the first immunization.

**Field studies.** The mark-recapture data indicated that 79% (72%–86%) and 78% (74%–82%) of the resident mouse populations in 1998 and 1999, respectively, were captured [32]. Among mice captured at the 3 sites, 62% (50%–73%) in 1998 and 77% (65%–89%) in 1999 were recaptured by the last trapping period. The mean (range) estimated abundance at negative control plots was 17 (4–30) mice/ha in 1998 and 42 (23–62) mice/ha in 1999 ( $P < .001$ , ANOVA).

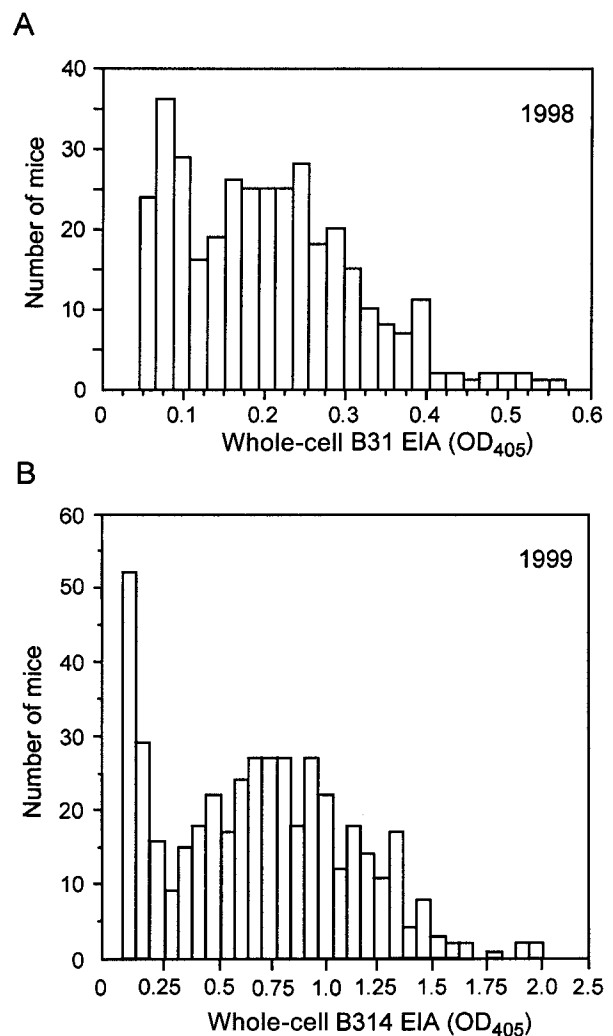
**Antibodies of field *P. leucopus* mice to whole cells of *B. burgdorferi*.** To assess the prevalence and yearly dynamics of *B. burgdorferi* infection of *P. leucopus* mice captured at the enzootic area, we analyzed a total of 801 serum samples obtained from 514 mice during both years, by use of whole-cell EIA. There was a greater range of values and a higher maximum optical density by the B314 EIA in 1999 than by the B31 EIA in 1998, but the minimum of the range and the 10th percentile optical density values for low-reactivity samples were similar for both assays, an indication of comparable specificities for both assays (table 1). Within different treatment plots or sites in 1998 and 1999, there were no significant differences in mean optical density values for the samples ( $P > .1$ , ANCOVA), and the findings were pooled for each year.

Serum samples from the field mice showed a bimodal distribution of optical density values by whole-cell EIA, for each of the years (figure 2). The first peaks ( $\sim 0.08$  by the B31 EIA [figure 2A] and  $\sim 0.13$  by the B314 EIA [figure 2B]) corresponded to the range of values observed with uninfected *P. leucopus* mice in the laboratory and suggests that field mice with optical density values distributed around these first peaks were uninfected. The distribution of samples around the second peak ( $\sim 0.25$  by the B31 EIA and  $\sim 0.80$  by the B314 EIA) corresponded to the range of values for serum samples from laboratory-infected *P. leucopus* mice.

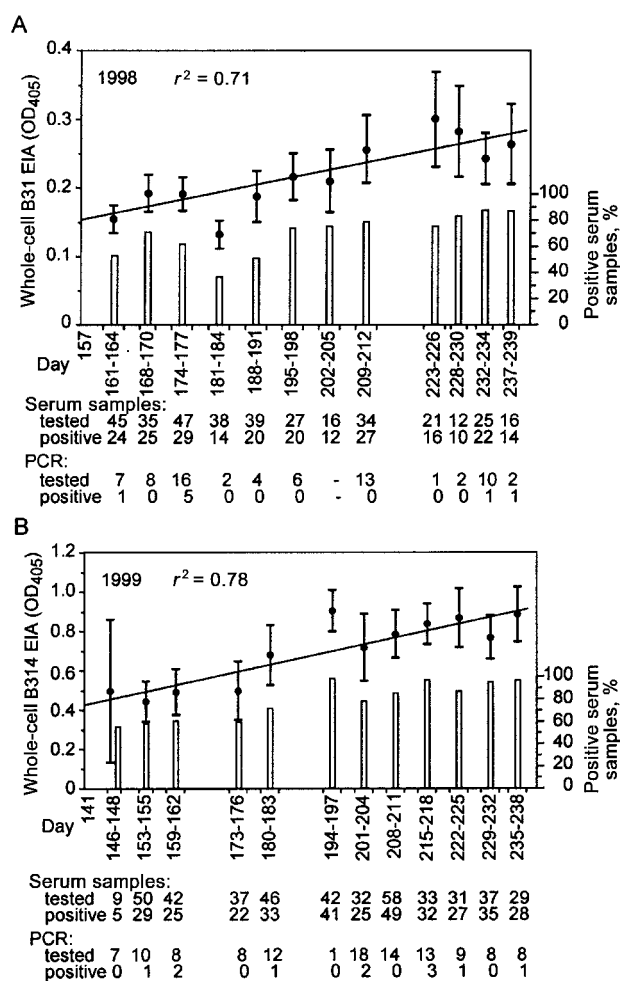
Figure 3 shows the mean values and percentages of seropositivity detectable by whole-cell EIA, by sampling intervals over the course of the studies during 1998 and 1999. For com-

bined collection periods, the prevalence of seropositivity increased from 83 (57%) of 146 samples at the start of the sampling (late May to mid-June [days 146–164]) to 42 (93%) of 45 samples by the end of August (days 235–239) ( $\chi^2 = 18.7$ ;  $P < .001$ ). There was a high coefficient of determination ( $r^2$ ) between calendar day and mean values by EIA in both years, and the regression curves for the plot of day against values by EIA had similar slopes for 1998 and 1999. Extrapolating from the regression curves, we estimated that the populations of *P. leucopus* mice first had mean values by EIA above the cutoff point by May 11 (day 130) in 1998 and by April 16 (day 106) in 1999.

**Antibodies of *P. leucopus* mice by age.** We next determined the relationship between the estimated age of individual mice and their respective antibody levels by use of whole-cell EIA for serum samples obtained in 1999 ( $n = 429$ ); also in-



**Figure 2.** Frequency distribution of whole-cell *Borrelia burgdorferi* EIA OD<sub>405</sub> values for serum samples obtained in 1998 (A) and 1999 (B) from a natural population of *Peromyscus leucopus* mice.



**Figure 3.** Infection of *Peromyscus leucopus* mice with *Borrelia burgdorferi* over a transmission season, as determined by whole-cell *B. burgdorferi* EIA of serum samples and by *p66* gene polymerase chain reaction (PCR) assay of blood samples in 1998 (A) and 1999 (B). B31 cells were used for EIA in 1998, and B314 cells were used in 1999. Sampling-day intervals are indicated on the X-axis. EIA OD<sub>405</sub> value intervals and the percentage of positive serum samples for a sampling period are shown on the left and the right Y-axis, respectively. Below the graph are the total number of blood samples subjected to EIA and PCR analysis, from different collection periods, as well as the number of samples that were found to be positive by these assays. Coefficient of determination ( $r^2$ ) values between the collection dates and the values by EIA are shown.

cluded in this analysis were samples obtained from recaptured mice. The average body mass and OD value, respectively, were 11.5 g (10.0–13.1 g) and 0.20 (0.11–0.29) for juveniles, 15.0 g (14.8–15.4 g) and 0.49 (0.40–0.58) for subadults, and 21.5 g (21.1–22.0 g) and 0.83 (0.77–0.90) for adults. Although the juvenile and subadult groups did not differ significantly in optical density values, both had significantly lower optical density values than did the adult group ( $P < .05$ , Bonferonni-corrected  $t$  test).

If younger mice have a lower prevalence of anti-*B. burg-*

*dorferi* antibodies than do older mice, then one would expect that mice captured for the first time during a trapping period would have lower antibody levels than mice that were recaptured on the same day. Accordingly, for both years, we matched mice captured for the first time with recaptured mice, by use of the date at which mice were sampled. The mean body mass for mice captured for the first time and for recaptured mice in 1998 was 15.9 g (14.8–16.9 g) and 17.8 g (16.9–18.7 g), respectively, ( $P < .004$ , paired  $t$  test), and that in 1999 was 17.5 g (16.6–18.4 g) and 19.4 g (18.7–20.4 g), respectively ( $P < .003$ ). The mean OD values for 32 pairs of mice captured for the first time and for recaptured mice in 1998 were 0.16 (0.12–0.20) and 0.28 (0.24–0.32), respectively, ( $P < .0001$ , paired  $t$  test). The corresponding OD values for 61 pairs of mice, in 1999, were 0.58 (0.47–0.68) and 0.89 (0.78–0.99), respectively, ( $P < .001$ ).

**Antibodies to specific antigens of *B. burgdorferi*.** A random sample of 206 serum samples from mice captured in 1998 was analyzed by immunoblot, for antibodies to OspC, BmpA, FlaB, P66, the 18-kDa and 80/93/100-kDa proteins, which are included in a standard set of antigens for diagnostic testing [33], and OspA. Figure 1 shows the patterns of reactivities of 4 representative serum samples from the mice. Of 206 serum samples from *P. leucopus* mice captured at the field site, 138 (67%) had antibodies to OspC detectable by immunoblot. The proportions with which other antigens were recognized were the following: 37% for BmpA, 36% for the 18-kDa protein, 24% for the 83/93/100-kDa protein, 19% for P66, and 12% for FlaB. Values by whole-cell B31 EIA were generally higher among OspC-positive serum samples (mean OD, 0.26) than among OspC-negative serum samples (mean OD, 0.13) ( $P < .0001$ ,  $t$  test). The mean OD value for 44 samples producing only 1 of the 6 bands (0.21 [0.18–0.23]) was lower than that for 73 samples producing  $\geq 3$  of the 6 bands (0.28 [0.26–0.29]), by immunoblot ( $P < .001$ ,  $t$  test). None of the 206 serum samples had antibodies to OspA detectable by immunoblot.

Of 355 serum samples from 1998 that were examined by EIA for antibodies to OspA, 353 (99.4%) had values less than the cutoff value established for uninfected laboratory mice (0.12). The only 2 serum samples (0.6%) that had optical density values above the cutoff value were confirmed by immunoblot to have antibodies to OspA.

**Longitudinal studies of anti-*B. burgdorferi* antibodies in *P. leucopus* mice.** Table 2 summarizes the sampling frequencies and median collection days for the longitudinal studies of the total 184 mice captured at least twice during 1998 and 1999. In 1998, 34 (59%) of 58 mice were found to be seropositive at the first sampling, and, in 1999, 86 (68%) of 126 mice were found to be seropositive at the first sampling ( $P > .2$ ). For both years, only 10 mice (5.4%) were found to be seronegative at each sampling, and only 3 (1.6%) mice were

**Table 2. Summary of serum samples and results of whole-cell *Borrelia burgdorferi* EIA, for a longitudinal study of *Peromyscus leucopus* mice captured at a field site in Connecticut.**

Year	No. of mice	No. of captures (median day of capture)				Capture, mean EIA OD <sub>450</sub> ± 95% CI			
		1	2	3	4	1st	2nd	3rd	4th
1998	57	57 (175)	57 (195)	19 (223)	7 <sup>a</sup> (232)	0.19 ± 0.03	0.24 ± 0.03	0.33 ± 0.04	0.36 ± 0.06
1999	126	126 (174)	126 (202)	65 (222)	8 (229)	0.58 ± 0.07	0.83 ± 0.07	0.90 ± 0.08	0.80 ± 0.29

**NOTE.** CI, confidence interval.

<sup>a</sup> One mouse was sampled 6 times in 1998.

found to be seropositive at the first sampling and seronegative subsequently. For both years, most mice either continued to be seropositive (64%) or seroconverted (29%) during subsequent captures. Table 2 also shows the mean values by EIA, for each capture for both years, which increased between the first and second captures (1998 and 1999) and then again between the second and third captures (1998) ( $P < .01$ , Bonferroni-corrected ANOVA).

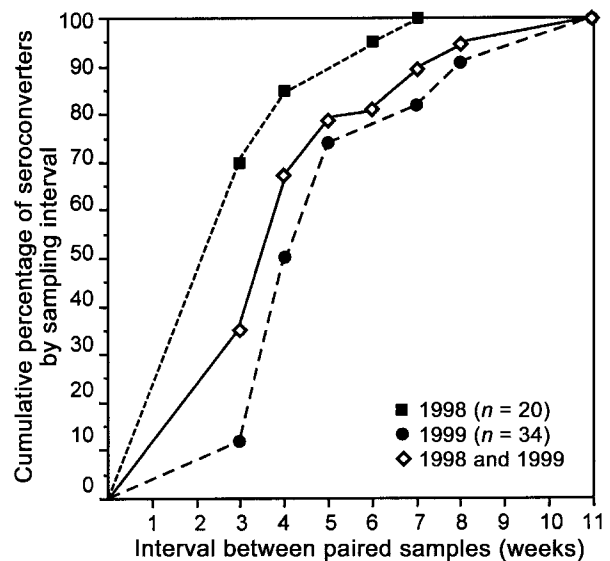
To estimate the incidence rate of *B. burgdorferi* infection among the longitudinally sampled *P. leucopus* mice, we used seroconversion from negative to positive, in the EIA, to define a new case of infection. Figure 4 shows the cumulative seropositive prevalence over time for each year and for the combined data. Infections accumulated in mice earlier in 1998 than in 1999: 14 (70%) of 20 mice in 1998 and 4 (12%) of 34 mice in 1999 ( $\chi^2 = 16.7$ ;  $P < .001$ ) seroconverted within the first 3-week sampling period.

For both years, the frequency of seroconversion was the same among mice from which samples were first obtained after day 194 (July 13) (9 of 10), a time representing peak host-seeking activity by *I. scapularis* nymphs [34], as among those from which samples were first obtained on or before that date (25 of 32) ( $P > .1$ ). Accordingly, we estimated a weighted average of the incidence rate of seroconversion in *P. leucopus* mice for the entire trapping season. In total, 18 of 26 mice seroconverted within 3 weeks, and 16 of 21 mice seroconverted within 4 weeks. The weighted average incidence rate over the 3- and 4-week periods was 0.2 cases/mouse/week, or 29 infections/1000 mouse-days. From the combined data in figure 4, we estimated that 50% of uninfected *P. leucopus* mice became infected with *B. burgdorferi* within 23 days. For samples obtained longitudinally in 1998, seroconversion in whole-cell EIA was associated with a higher prevalence of antibodies to OspC detectable by immunoblot: 14 (82%) of 17 mice that seroconverted had antibodies to OspC in the second sample.

**Detection of *B. burgdorferi* DNA in mice blood.** Infection of *P. leucopus* was further investigated by a PCR assay for the *p66* gene in a random selection of 187 blood samples from 152 mice obtained during different collection periods during both years. Eight (11.3%) of 71 blood samples from 1998 and 11 (9.5%) of 116 samples from 1999 ( $P > .1$ ) were found to be

positive for *B. burgdorferi* DNA (figure 3). The frequencies of PCR-positive samples were similar during different trapping periods: 10 (13.2%) of 76 samples from days 146–181, 2 (3.4%) of 58 samples from days 182–212, and 7 (13.2%) of 53 samples from days 215–239 ( $P > .1$ ). Among paired samples from 32 individual mice, 3 (10%) were found to be positive on 1 occasion but not on the other: samples from 2 mice at the first testing and from 1 mouse at the second testing.

The mean OD value, by whole-cell EIA, for the PCR-positive and -negative samples was 0.16 (0.10–0.22) and 0.23 (0.20–0.25), respectively, in 1998 ( $P > .05$ ), and 0.82 (0.64–1.00) and 0.77 (0.68–0.86), respectively, in 1999 ( $P > .1$ ). In contrast, when immunoblot analysis was performed on the same blood sample, antibody to OspC was detected in 42 (70%) of 60 PCR-negative samples but in only 2 (29%) of 7 PCR-positive samples ( $P < .05$ , Fisher's exact test).



**Figure 4.** Dynamics of seroconversion in field *Peromyscus leucopus* mice. Cumulative plots for 20 and 34 mice that seroconverted in whole-cell *B. burgdorferi* EIA in 1998 (black squares) and 1999 (black circles), as well as an aggregate for both years (white squares), are shown. Intervals, in weeks, between dates that paired serum samples were obtained from mice that seroconverted are indicated on the X-axis. The Y-axis shows the cumulative percentage of mice that seroconverted, by sampling interval.

## DISCUSSION

Our study has elaborated on the findings of previous investigations of *B. burgdorferi* infection of *P. leucopus* mice in the field and in the laboratory [6–15] and is the most comprehensive to date, in terms of the following: (1) the inclusion of both laboratory and field *P. leucopus* mice; (2) the extensiveness of the trapping of *P. leucopus* mice in a single, well-characterized locale; (3) the continuation of the study over the course of 2 transmission seasons; and (4) the use of PCR for direct pathogen detection, as well as serologic testing to estimate incidence and prevalence of infection. On the basis of our review of the literature, the present study of enzootic infection in a reservoir population appears to be the most comprehensive to date, for any vectorborne zoonosis.

Our estimates of the population sizes of *P. leucopus* mice and their fluctuations at the experimental sites were within the range found by investigators in other studies of the northeastern United States [35–38]. Although population sizes varied between the 2 years, the number of the studied field mice was highly representative and included nearly 80% of the populations from both years.

For the samples obtained in 1998, the whole-cell EIA was based on *B. burgdorferi* B31 cells producing comparatively high amounts of OspA and low amounts of OspC [39], a protein profile similar to those of most commercial and reference EIA kits [33]. For the samples obtained in 1999, we used B314 cells of the same genetic background, but they produced no OspA and abundant OspC [24], which presumably improved the assay's performance by yielding wider range of values above the cutoff, without a loss of specificity. Among the several antigens that were examined, OspC was most frequently bound by antibodies in the samples from the mice, as found elsewhere [6].

Longitudinal sampling allowed us to relate the dynamics of the *B. burgdorferi* infection to the established pattern of activity of *I. scapularis* ticks during transmission season. Because nymphs become active in May [34, 40], the samples found to be seropositive at the outset of the study period are attributable, in part, to early infections, presumably beginning in mid-April or early May. Maternal transfer of antibodies, as suggested by Mather et al. [41], and overwintering by adult mice are 2 possible contributors to seropositivity in the spring. However, our finding that juvenile and subadult mice had significantly lower antibody levels than did adult mice indicates that transplacental antibodies account for only a minority of the seropositive mice in the spring. Overwintering is also not likely a major factor in early seropositivity; only ~2% of mice overwinter (J.T., unpublished data).

Both the prevalence of seropositive mice and the mean levels of anti-*B. burgdorferi* antibody in the population increased over the course of trapping in both years. This increase could partly be explained by a collective increase in antibody titers in in-

dividual mice as their immune responses to the organism develop. In addition, the steady incidence rate of 0.2 cases/mouse/week and the PCR-positivity rate of ~10% over both years' trapping periods indicate that the risk of infection remains the same during the spring and summer, as suggested elsewhere [12]. One possible explanation for a constant risk of infection of mice while numbers of host-seeking *I. scapularis* nymphs fluctuate is the combination of abundant infected nymphs and the high efficiency of transmission of the spirochete by nymphs to mice [7].

The incidence rate from the seroconversion data was 3 times higher than the rate estimated by Hofmeister et al. for a natural population of *P. leucopus* mice in Maryland [6]. Maryland generally has lower endemicity of LB than does Connecticut [2], but, even so, these investigators may have underestimated the incidence, because they used in vitro cultivation of the spirochete. Our PCR assay of the blood samples indicated a prevalence of bacteremia of ~10%, but this estimate also probably underestimated the prevalence of infected mice that could transmit *B. burgdorferi* to feeding larvae. Given the transient presence and low numbers of spirochetes in the blood [12, 42], seroconversion may be preferable to culture as an indicator of first-time infections with *B. burgdorferi*.

One drawback, however, of using seroconversion figures to estimate incidence is that reinfections would be difficult to detect without a strain-specific *B. burgdorferi* immunoassay. The whole-cell and immunoblot assays used in the present study were not strain specific [43]. The finding that a majority of mice were seropositive at or soon after the outset of obtaining samples, with the prevalence of PCR positivity more-or-less constant throughout the study, indicates that second, third, and even fourth infections occur in individual mice during the transmission period. Anti-*B. burgdorferi* antibodies may take 2–3 weeks to appear in the blood after infection, but, thereafter, antibodies are present in the blood of mice for several months [14]. Bacteremia with *B. burgdorferi*, on the other hand, lasts no longer than a few weeks [44, 45]. The great majority of PCR-positive mice in the present study were also found to be seropositive, an indication that the antibodies to the spirochete predated the current infection. If the antibodies were already present, then they had either decreased below a level sufficient for prevention or were ineffective against the newly infecting strain [46].

One of the determinants of strain-specific immunity to *B. burgdorferi* appears to be antibody to OspC [47]. The frequent occurrence of antibodies to OspC among the mice, detectable by immunoblot, indicates that this protein is expressed by *B. burgdorferi* during infection of *P. leucopus* mice. The finding that PCR-positive mice were less likely to have anti-OspC antibodies detectable by immunoblot than were PCR-negative

mice supports the proposal that immunity to OspC may provide positive selection of *ospC* genes in this area [48, 49].

There was no apparent illness among the mice, whether seropositive or not, which is an observation that is consistent with the report of Hofmeister et al. [6]. Mild or no disease is a characteristic of infection in enzootic reservoirs [50], but the high incidence of infection with *B. burgdorferi* is unusual in comparison with other human zoonotic diseases with enzootic transmission cycles [51, 52]. Nearly all the *P. leucopus* mice in the study area became infected during the transmission season, a finding more typical of an epizootic outbreak (e.g., of plague or Sin Nombre virus) [53–55].

High prevalence of antibodies to *B. burgdorferi* appears to have no effect on the reservoir competence of *P. leucopus* mice for passing the spirochete to naive *I. scapularis* larvae later in the same transmission season. Monitoring the prevalence of *B. burgdorferi* infection of *I. scapularis* nymphs in the study area for several consecutive years revealed no major fluctuations (J.T., unpublished data). This is in contrast to the reduction of the reservoir competence of *P. leucopus* mice for *Anaplasma phagocytophilum* after experimentally induced immunity to this other pathogen, which is transmitted by *I. scapularis* ticks in the same area [56].

Although the sizes of the mouse populations differed during the 2 seasons, the levels and monthly patterns of seroprevalence and PCR positivity in mice were similar for both years. These findings, together with the short duration of bacteremia and the infrequent survival of *P. leucopus* mice through the winter at the field site are further evidence that overwintering ticks are critical for maintenance of *B. burgdorferi* in the area. This is in contrast to hantavirus enzootic infection, which has a prevalence that is inversely related to the size of the population of the reservoir *Peromyscus* mice, and long-lived, persistently infected rodents are thought to maintain the virus over winter [57].

In conclusion, almost all *P. leucopus* mice became infected with *B. burgdorferi* during a single transmission season at a study site in Connecticut that is representative of the landscape of much of the northeastern United States. The findings indicate that reinfection of mice is common and that the presence of antibodies to the organism does not prevent bacteremia, which probably occurs with a different strain of *B. burgdorferi*. The finding of a high prevalence of antibodies to OspC among the *P. leucopus* mice in the population studied is consistent with the proposal that OspC is an important determinant of strain-specific immunity [47]. On the other hand, although *P. leucopus* mice in the laboratory could produce an immune response to injected OspA in this or another study [19], naturally infected mice rarely had antibodies to this antigen, a finding that confirms the reports of Hofmeister et al. [6] and Brunet et al. [13]. This supports the application of OspA (or another appropriate

antigen) as a transmission-blocking vaccine directed at *P. leucopus* mice and other reservoirs of *B. burgdorferi* [17, 19].

## Acknowledgments

We thank Sven Bergström for providing recombinant outer surface protein A, Erol Fikrig for providing glutathione S-transferase, Hossein Mirian and Kristin Mayeda for technical assistance, Tim Wootton for his advice and support as thesis advisor to J.T., and Rick Ostfeld for advice on field methods. We are grateful to the South Central Connecticut Regional Water Company for permission to work on their property.

## References

1. Daniels TJ, Boccia TM, Varde S, et al. Geographic risk for Lyme disease and human granulocytic ehrlichiosis in southern New York state. *Appl Environ Microbiol* **1998**; 64:4663–9.
2. Centers for Disease Control and Prevention. Lyme disease—United States, 1996. *MMWR Morb Mortal Wkly Rep* **1997**; 46:531–5.
3. World Health Organization (WHO). Report of WHO workshop on Lyme borreliosis diagnosis and surveillance, Warsaw, Poland [WHO/CDS/VP/95]. Geneva: WHO, **1996**:141.
4. Levine JF, Wilson ML, Spielman A. Mice as reservoirs of the Lyme disease spirochete. *Am J Trop Med Hyg* **1985**; 34:355–60.
5. Spielman A, Wilson ML, Levine JF, Piesman J. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annu Rev Entomol* **1985**; 30:439–60.
6. Hofmeister EK, Ellis BA, Glass GE, Childs JE. Longitudinal study of infection with *Borrelia burgdorferi* in a population of *Peromyscus leucopus* at a Lyme disease-enzootic site in Maryland. *Am J Trop Med Hyg* **1999**; 60:598–609.
7. Donahue JG, Piesman J, Spielman A. Reservoir competence of white-footed mice for Lyme disease spirochetes. *Am J Trop Med Hyg* **1987**; 36: 92–6.
8. Mather TN, Wilson ML, Moore SI, Ribeiro JM, Spielman A. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). *Am J Epidemiol* **1989**; 130:143–50.
9. Magnarelli LA, Anderson JF, Hyland KE, Fish D, McAninch JB. Serologic analyses of *Peromyscus leucopus*, a rodent reservoir for *Borrelia burgdorferi*, in northeastern United States. *J Clin Microbiol* **1988**; 26: 1138–41.
10. Magnarelli LA, Oliver JH Jr, Hutcheson HJ, Boone JL, Anderson JF. Antibodies to *Borrelia burgdorferi* in rodents in the eastern and southern United States. *J Clin Microbiol* **1992**; 30:1449–52.
11. Stafford KC 3rd, Massung RF, Magnarelli LA, Ijdo JW, Anderson JF. Infection with agents of human granulocytic ehrlichiosis, Lyme disease, and babesiosis in wild white-footed mice (*Peromyscus leucopus*) in Connecticut. *J Clin Microbiol* **1999**; 37:2887–292.
12. Anderson JF, Johnson RC, Magnarelli LA. Seasonal prevalence of *Borrelia burgdorferi* in natural populations of white-footed mice, *Peromyscus leucopus*. *J Clin Microbiol* **1987**; 25:1564–6.
13. Brunet LR, Sellitto C, Spielman A, Telford SR 3rd. Antibody response of the mouse reservoir of *Borrelia burgdorferi* in nature. *Infect Immun* **1995**; 63:3030–6.
14. Schwan TG, Kime KK, Schrupf ME, Coe JE, Simpson WJ. Antibody response in white-footed mice (*Peromyscus leucopus*) experimentally infected with the Lyme disease spirochete (*Borrelia burgdorferi*). *Infect Immun* **1989**; 57:3445–51.
15. Magnarelli LA, Anderson JF, Flavell RA, Fikrig E. Reactivity of white-footed mouse and raccoon sera to whole cell and recombinant antigens of *Borrelia burgdorferi*. *J Wildl Dis* **1995**; 31:339–44.



16. de Silva AM, Telford SR 3rd, Brunet LR, Barthold SW, Fikrig E. *Borrelia burgdorferi* OspA is an arthropod-specific transmission-blocking Lyme disease vaccine. *J Exp Med* **1996**; 183:271–5.
17. Kurtenbach K, Dizij A, Voet P, Hauser P, Simon MM. Vaccination of natural reservoir hosts with recombinant lipidated OspA induces a transmission-blocking immunity against Lyme disease spirochaetes associated with high levels of LA-2 equivalent antibodies. *Vaccine* **1997**; 15:1670–4.
18. Schug MD, Vessey SH, Korytko AI. Longevity and survival in a population of white-footed mice (*Peromyscus leucopus*). *J Mammal* **1991**; 72:360–6.
19. Tsao J, Barbour AG, Luke CJ, Fikrig E, Fish D. OspA immunization decreases transmission of *Borrelia burgdorferi* spirochetes from infected *Peromyscus leucopus* mice to larval *Ixodes scapularis* ticks. *Vector Borne Zoonotic Dis* **2001**; 1:65–74.
20. Levin M, Papero M, Fish D. Feeding density influences acquisition of *Borrelia burgdorferi* in larval *Ixodes scapularis* (Acari: Ixodidae). *J Med Entomol* **1997**; 34:569–72.
21. Schwan TG, Burgdorfer W, Garon CF. Changes in infectivity and plasmid profile of the Lyme disease spirochete, *Borrelia burgdorferi*, as a result of in vitro cultivation. *Infect Immun* **1988**; 56:1831–6.
22. Erdile LF, Brandt MA, Warakomski DJ, et al. Role of attached lipid in immunogenicity of *Borrelia burgdorferi* OspA. *Infect Immun* **1993**; 61: 81–90.
23. Fikrig E, Barthold SW, Kantor FS, Flavell RA. Protection of mice against the Lyme disease agent by immunizing with recombinant OspA. *Science* **1990**; 250:553–6.
24. Sadziene A, Wilske B, Ferdows MS, Barbour AG. The cryptic OspC gene of *Borrelia burgdorferi* B31 is located on a circular plasmid. *Infect Immun* **1993**; 61:2192–5.
25. Luke CJ, Huebner RC, Kasmiersky V, Barbour AG. Oral delivery of purified lipoprotein OspA protects mice from systemic infection with *Borrelia burgdorferi*. *Vaccine* **1997**; 15:739–46.
26. Barbour AG, Tessier SL, Todd WJ. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. *Infect Immun* **1983**; 41:795–804.
27. Barbour AG, Hayes SF, Heiland RA, Schrumph ME, Tessier SL. A *Borrelia*-specific monoclonal antibody binds to a flagellar epitope. *Infect Immun* **1986**; 52:549–54.
28. Bunikis J, Luke CJ, Bunikiene E, Bergstrom S, Barbour AG. A surface-exposed region of a novel outer membrane protein (P66) of *Borrelia* spp. is variable in size and sequence. *J Bacteriol* **1998**; 180:1618–23.
29. Simpson WJ, Cieplak W, Schrumph ME, Barbour AG, Schwan TG. Nucleotide sequence and analysis of the gene in *Borrelia burgdorferi* encoding the immunogenic P39 antigen. *FEMS Microbiol Lett* **1994**; 119:381–7.
30. Volkman DJ, Luft BJ, Gorevic PD, Schultz J, Padovano L. Characterization of an immunoreactive 93-kDa core protein of *Borrelia burgdorferi* with a human IgG monoclonal antibody. *J Immunol* **1991**; 146: 3177–82.
31. Bunikis J, Barbour AG. Access of antibody or trypsin to an integral outer membrane protein (P66) of *Borrelia burgdorferi* is hindered by Osp lipoproteins. *Infect Immun* **1999**; 67:2874–83.
32. Otis DL, Burnham KP, White GC, Anderson DR. Statistical inference from capture data on closed animal populations. *Wildlife Monogr* **1978**: 1–135.
33. Dressler F, Whalen JA, Reinhardt BN, Steere AC. Western blotting in the serodiagnosis of Lyme disease. *J Infect Dis* **1993**; 167:392–400.
34. Fish D. Environmental risk and prevention of Lyme disease. *Am J Med* **1995**; 98:2S–8S.
35. Elkinton JS, Healy WM, Buonaccorsi JP, et al. Interactions among gypsy moths, white-footed mice, and acorns. *Ecology* **1996**; 77:2332–42.
36. Brooks RT, Smith HR, Healy WM. Small-mammal abundance at three elevations on a mountain in central Vermont, USA: a sixteen-year record. *For Ecol Manage* **1998**; 110:181–93.
37. LoGiudice K, Ostfeld RS, Schmidt KA, Keesing F. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. *Proc Natl Acad Sci USA* **2003**; 100:567–71.
38. Hansen L, Batzli GO. Influence of supplemental food on local populations of *Peromyscus leucopus*. *J Mammal* **1979**; 60:335–42.
39. Sadziene A, Rosa PA, Thompson PA, Hogan DM, Barbour AG. Antibody-resistant mutants of *Borrelia burgdorferi*: in vitro selection and characterization. *J Exp Med* **1992**; 176:799–809.
40. Piesman J, Mather TN, Dammin GJ, Telford SR 3rd, Lastavica CC, Spielman A. Seasonal variation of transmission risk of Lyme disease and human babesiosis. *Am J Epidemiol* **1987**; 126:1187–9.
41. Mather TN, Telford SR 3rd, Adler GH. Absence of transplacental transmission of Lyme disease spirochetes from reservoir mice (*Peromyscus leucopus*) to their offspring. *J Infect Dis* **1991**; 164:564–7.
42. Schwartz I, Bittker S, Bowen SL, Cooper D, Pavia C, Wormser GP. Polymerase chain reaction amplification of culture supernatants for rapid detection of *Borrelia burgdorferi*. *Eur J Clin Microbiol Infect Dis* **1993**; 12:879–82.
43. Bunikis J, Barbour AG. Laboratory testing for suspected Lyme disease. *Med Clin North Am* **2002**; 86:311–40.
44. McLean R, Ubico S, Cooksey L. Experimental infection of the eastern chipmunk (*Tamias striatus*) with the Lyme disease spirochete (*Borrelia burgdorferi*). *J Wildl Dis* **1993**; 29:527–32.
45. Wang G, Ojaimi C, Iyer R, et al. Impact of genotypic variation of *Borrelia burgdorferi* sensu stricto on kinetics of dissemination and severity of disease in C3H/HeJ mice. *Infect Immun* **2001**; 69:4303–12.
46. Piesman J, Dolan MC, Happ CM, et al. Duration of immunity to reinfection with tick-transmitted *Borrelia burgdorferi* in naturally infected mice. *Infect Immun* **1997**; 65:4043–7.
47. Probert WS, Crawford M, Cadiz RB, LeFebvre RB. Immunization with outer surface protein (Osp) A, but not OspC, provides cross-protection of mice challenged with North American isolates of *Borrelia burgdorferi*. *J Infect Dis* **1997**; 175:400–5.
48. Qiu WG, Bosler EM, Campbell JR, et al. A population genetic study of *Borrelia burgdorferi* sensu stricto from eastern Long Island, New York, suggested frequency-dependent selection, gene flow and host adaptation. *Hereditas* **1997**; 127:203–16.
49. Wang IN, Dykhuizen DE, Qiu W, Dunn JJ, Bosler EM, Luft BJ. Genetic diversity of *ospC* in a local population of *Borrelia burgdorferi* sensu stricto. *Genetics* **1999**; 151:15–30.
50. Poland J, Barnes A. Plague. In: Steele J, ed. *CRC handbook series in zoonoses. Section A: Bacterial, rickettsial, and mycotic diseases. Vol. 1. Boca Raton: CRC Press, 1979*:515–97.
51. Calisher CH, Sweeney W, Mills JN, Beaty BJ. Natural history of Sin Nombre virus in western Colorado. *Emerg Infect Dis* **1999**; 5:126–34.
52. Gruwell JA, Fogarty CL, Bennett SG, et al. Role of peridomestic birds in the transmission of St. Louis encephalitis virus in southern California. *J Wildl Dis* **2000**; 36:13–34.
53. Kartman L, Hudson BW. The effect of flea control on *Yersinia (Pasteurella) pestis* antibody rates in the California vole, *Microtus californicus*, and its epizootiological implications. *Bull World Health Organ* **1971**; 45:295–301.
54. Hudson BW, Goldenberg MI, Quan TJ. Serologic and bacteriologic studies on the distribution of plague infection in a wild rodent plague pocket in the San Francisco Bay area of California. *J Wildl Dis* **1972**; 8:278–86.
55. Childs JE, Ksiazek TG, Rollin PE, et al. Hantaviruses and their rodent reservoirs in the United States. In: Halverson WS, Crabb AC, eds. *Proceedings of the 16th Vertebrate Pest Conference*. Davis, CA: University of California Press, **1994**:188–91.
56. Levin ML, Fish D. Immunity reduces reservoir host competence of *Peromyscus leucopus* for *Ehrlichia phagocytophila*. *Infect Immun* **2000**; 68:1514–8.
57. Calisher CH, Mills JN, Sweeney WP, et al. Do unusual site-specific population dynamics of rodent reservoirs provide clues to the natural history of hantaviruses? *J Wildl Dis* **2001**; 37:280–8.