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Borrelia burgdorferi Has Minimal Impact on the Lyme Disease Reservoir Host *Peromyscus leucopus*

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Abstract

The epidemiology of vector-borne zoonotic diseases is determined by encounter rates between vectors and hosts. Alterations to the behavior of reservoir hosts caused by the infectious agent have the potential to dramatically alter disease transmission and human risk. We examined the effect of *Borrelia burgdorferi*, the etiological agent of Lyme disease, on one of its most important reservoir hosts, the white-footed mouse, *Peromyscus leucopus*. We mimic natural infections in mice using the vector (Black-legged ticks, *Ixodes scapularis*) and examine the immunological and behavioral responses of mouse hosts. Despite producing antibodies against *B. burgdorferi*, infected mice did not have elevated white blood cells compared with uninfected mice. In addition, infected and uninfected mice did not differ in their wheel-running activity. Our results suggest that infection with the spirochete *B. burgdorferi* has little impact on the field activity of white-footed mice. Lyme disease transmission appears to be uncomplicated by pathogen-altered behavior of this reservoir host.

Key Words: Black-legged ticks—Host–vector encounter rates—Lyme disease—Spirochete—White-footed mice.

Introduction

Infection with a pathogen may alter host physiology or behavior in ways that positively or negatively influence disease transmission (Dobson 1988, Holmes and Zohar 1990, Poulin et al. 1994). Pathogens may manipulate hosts to enhance their encounter rates with uninfected hosts, thereby increasing the basic reproductive rate of the pathogen yet often decreasing the fitness of the host (Poulin et al. 1994, Koella et al. 1998). Host behavior may also change because of disease pathology or to compensate for the impacts of infection on host fitness in ways that positively or negatively impact pathogen transmission (e.g., sickness lethargy or terminal investment behaviors) (Minchella and LoVerde 1981, Holmes and Zohar 1990). As an example, mice infected with rodent malaria have reduced defensive behaviors against mosquito vectors, which benefits both the mosquito and the *Plasmodium* parasite, but has unknown consequences for the mouse (Day and Edman 1983). For zoonotic diseases, the behavior of reservoir hosts (those hosts that maintain the pathogen and serve as a source of infection) influences encounter rates with humans or vectors and, thus, the risk of human exposure to the pathogen. Effects of zoonotic pathogens on behavior of reservoir hosts have rarely been examined.

The etiological agent of Lyme disease (LD) in northeastern United States, *Borrelia burgdorferi*, is transmitted among vertebrate hosts via blood meal of the vector, the black-legged tick, *Ixodes scapularis* (Burgdorfer et al. 1982, Lane et al. 1991). Humans acquire the *B. burgdorferi* spirochete bacterium primarily from nymphal ticks, and therefore, the LD risk depends strongly on the density of infected nymphs (DIN) (Mather et al. 1996, Stafford et al. 1998). The DIN, in turn, is determined by encounter rates between uninfected larvae and infected hosts that are efficient at transmitting *B. burgdorferi* to the larvae (hosts with high reservoir competence) (LoGiudice et al. 2003). The white-footed mouse, *Peromyscus leucopus*, has been identified as one of the main reservoir hosts of *B. burgdorferi* (Levine et al. 1985, Donahue et al. 1987, LoGiudice et al. 2003, Brisson et al. 2008). The majority of white-footed mice in populations in northeastern United States become infected with *B. burgdorferi* by late summer (LoGiudice et al. 2003, Bunikis et al. 2004, Brunner et al. 2008). White-footed mice successfully feed a large proportion of the larval ticks that encounter them (Keesing et al. 2009) and transmit *B. burgdorferi* to 70–90% of the ticks (Levine et al. 1985, Donahue et al. 1987, LoGiudice et al. 2003, Brunner et al. 2008).

In addition to having high reservoir competence, white-footed mice influence the density of ticks. High mouse

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population densities in the year following oak masts (*Quercus* spp.) lead to high DIN in the subsequent year (Jones et al. 1998, Goodwin et al. 2001, Ostfeld et al. 2001, 2006). High relative abundance of mice in the small-mammal community increases the likelihood that larval ticks will feed on a mouse and acquire *B. burgdoreri*, thus influencing nymphal infection prevalence (Ostfeld and Keesing 2000, Schmidt and Ostfeld 2001, LoGiudice et al. 2003, 2008).

Identifying the factors that influence encounter rates between mice and immature ticks provides valuable ecological insight into the epidemiology of LD. Mice that are more active behaviorally may have larger home ranges and may investigate a greater proportion of the space within their home range, thereby increasing the chances of encountering host-seeking immature ticks, which are patchily distributed in the environment (Ostfeld et al. 1996a, 1996b, Schmidt and Ostfeld 2003). If infection with *B. burgdorferi* influences the activity levels of infected mice, the tick encounter rate should be affected. Humans and laboratory mice are impacted strongly by infection with *B. burgdorferi*, showing joint inflammation, lethargy, and neurologic damage (Burgess et al. 1990, Barthold et al. 1991, Moro et al. 2002, Stanek and Strie 2003). Impacts of *B. burgdorferi* on native reservoir hosts, such as mice and chipmunks, are less well known. White-footed mice are susceptible to infection and respond immunologically to the spirochete (Anderson et al. 1987, Schwan et al. 1988, Burgess et al. 1990, Bunikis et al. 2004). In animals, mounting an immune response is often costly energetically and behaviorally and can lead to lethargy or altered reproductive or foraging behavior in hosts (Zuk and Stoehr 2002, Demas 2004). In addition, given the growing evidence that immunological measures (e.g., blood cell counts) provide an indication of overall animal condition and susceptibility to infection (Beldomenico et al. 2008a, 2008b, Beldomenico and Begon 2010), mounting an immune response against *B. burgdorferi* could make white-footed mice more susceptible to other parasites (including ticks) and lead to a cycle of decreasing overall health and activity (Martin et al. 2006). However, the few studies examining the influence of *B. burgdorferi* infection on the physiology and behavior of host white-footed mice have provided conflicting evidence (Burgess et al. 1990, Moody et al. 1994, Hofmeister et al. 1999). Based on two observational studies, *B. burgdorferi* may be correlated with behaviors consistent with neurologic damage (Burgess et al. 1990), but appears to have no effect on the field survival of naturally infected white-footed mice (Hofmeister et al. 1999). In this study, we infected *P. leucopus* with *B. burgdorferi* via nymphal tick bite to experimentally examine the effect of infection on hosts. We compared the immunological and behavioral response of infected and uninfected mice to determine whether *B. burgdorferi* affects this reservoir host in ways that may alter disease transmission dynamics.

Materials and Methods

Mice and infection using naturally infected nymphs

Twenty adult male white-footed mice (*P. leucopus*) from the *Peromyscus* Genetic Stock Center were maintained at the Cary Institute of Ecosystem Studies. Mice were held individually in wire mesh cages suspended over plastic tubs and maintained on a 14:10 light: dark cycle. Food (standard rodent blocks) and water were provided *ad libitum*.

The mice were randomly assigned to two experimental groups: infected with *B. burgdorferi* and uninfected. To establish these groups, mice were infested with nymphal blacklegged ticks, *I. scapularis*. Larval *I. scapularis* were collected from either *P. leucopus* or raccoons (*Procyon lotor*) trapped from wild populations near Millbrook, NY. After feeding to repletion, larvae were kept in moist glass tubes, where they molted into nymphs. Because $\sim$90% of tick larvae that feed on mice at our study site are infected, whereas $<$10% of larvae that fed on raccoons are infected (LoGiudice et al. 2003), we are able to establish infected and control treatments that simulate the natural circumstances of infection. We therefore expected mice inoculated with *P. leucopus*-fed nymphs to be exposed to and become infected with *B. burgdorferi* (exposed; E), and mice inoculated with *P. lotor*-fed nymphs would remain unexposed and uninfected (control; C). Five nymphs from one species of host were applied to the back of each mouse. Each mouse was maintained in a polyvinyl chloride (PVC) tube (1.25 inches in diameter) for 4 h to inhibit the initial grooming response against ticks. Tick infection status was not confirmed because we were interested primarily in the infection status of mice. Following confirmation of infection status with enzyme-linked immunosorbent assay (ELISA) (see ELISA analyses below), mice were compared across treatment groups of infected (I) and uninfected (U) to allow for direct tests of the influence of *B. burgdorferi* infection.

We collected 20–120 μL of blood from each mouse via submandibular puncture the day before tick infestation and 34–37 days postinfestation (p.i.). A portion of blood was collected into heparinized microcapillary tubes to perform a complete blood cell count as an indicator of general health (see below) (Beldomenico et al. 2008a). We performed an ELISA on the remainder of the p.i. blood sample to determine whether mice were infected with *B. burgdorferi*.

Outer surface protein C ELISA to confirm infection status of mice

To confirm that our infection method worked, we used ELISA to compare the immune response of exposed and control mice to *B. burgdorferi* outer surface protein C (OspC). The OspC protein is expressed by *B. burgdorferi* during tick feeding and induces a strong immune response in *P. leucopus* (Schwan et al. 1995, Hofmeister et al. 1999, Bunikis et al. 2004). The OspC protein is highly variable (Wang et al. 1999), so we used seven OspC groups (A, B, E, G, H, K, and N) commonly found in the eastern United States (Qiu et al. 2002).

We performed ELISAs as in the work by Ivanova et al. (2009). We coated 96-well NUNC MaxiSorb plates overnight with each of seven different OspC proteins (A, B, E, G, H, K, N) and bovine serum albumin (BSA) (as a control). After blocking with 2% BSA, we added 100 μL of 1:100 sera from each mouse to each of the seven OspC types and BSA. We used a secondary antibody (anti-*P. leucopus* immunoglobulin G) conjugated to horseradish peroxidase and added 1-step Ultra TMB to initiate the color reaction. We used a plate reader to read the absorbance at 652 nm, which reached equilibrium after 30 min. We repeated the ELISA to determine its consistency.

Hematology

To determine counts of red and white blood cells, as in the work by Beldomenico et al. (2008a), 2 μL of whole blood was immediately mixed with 18 μL of 0.01 M phosphate-buffered saline (1:10 dilution). For red blood cell counts, 2 μL of the 1:10 blood dilution was mixed with 1 mL of phosphate-buffered saline (1:5000 dilution). For white blood cell counts, the remaining 1:10 blood dilution was used to prepare a 1:20 dilution in 4% acetic acid with 1% crystal blue. Within 3 h of blood collection, these diluted blood samples were loaded into Kova Glasstic® slides with grids (Hycor Biomedical, Garden Grove, CA). We counted the blood cells in predetermined grids and calculated the number of cells per microliter of whole blood. To determine counts of each type of white blood cell (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), we completed a blood cell differential on blood smears that were air-dried and stained with Wright stain (Sigma 45253). One-hundred cells were counted per slide and identified by white blood cell type (Feldman et al. 2000).

Wheel-running behavior of mice

Wheel-running behavior was recorded for all mice at 8 days prior to infestation, as well as 1, 2, 3, and 6 weeks p.i. For each week of wheel running, mice were placed in automated wheel running chambers (Lafayette Instruments) between 16:00 and 17:30 and removed 2 days later between 08:00 and 09:00 (i.e., an ~40-h period). Wheel revolutions were counted automatically every minute between these times using Activity Wheel Monitor Software (Lafayette Instruments). The chambers contained shaved aspen bedding and *ad libitum* food and water.

Statistical analysis

For the ELISA results from each mouse, we standardized the equilibrium absorbance of each OspC type by dividing it by the equilibrium background absorbance of the BSA control (hereafter referred to as the standardized absorbance). We then calculated the geometric mean standardized absorbance of the seven OspC types for each mouse, which represents the binding affinity of mouse antibodies to the seven OspC types. Across the two replicate ELISAs, antibody binding affinity was highly correlated among the 19 mice ($r = 0.99$, $p < 0.001$). For each tick treatment (E, C), we used a one-sample *t*-test to test whether the standardized absorbance was significantly different from 1.0 (i.e., the BSA control). We used a two-sample *t*-test to compare the standardized absorbance between the two tick treatments. For each analysis, we log-transformed the data to ensure normality but presented the parameter estimates as the back-transformed standardized absorbance.

We analyzed each of the blood cell components using a mixed effects model with treatment (infected versus uninfected mice), time (pre- and postinfestation), and the treatment×time interaction as fixed effects and mouse identity as a random effect. Mass was initially included in the model but then removed because it did not improve the fit.

Virtually no wheel activity occurred during daylight hours. We used the wheel running behavior data from the second night of every two-night sampling period between 20:00 (when the lights turn off) and 07:00 for analyses. We calculated four different running behaviors including (1) total distance run (m), (2) average speed (m/min), (3) total time spent running (min), and (4) average speed while running (the average speed during the minutes when wheel revolutions were recorded, m/min). We analyzed each of these four running behaviors using a mixed effects model with treatment (I, C) as a fixed factor, mouse body mass and week of sample (pre-infestation and 1, 2, 3, and 6 weeks post-infection) as covariates, the treatment * week interaction and mouse identity as a random effect. We also examined whether nocturnal activity patterns differed between infected and uninfected mice by analyzing the running speed for each minute in the second night of running in weeks 3 and 6 using a similar mixed effects model as earlier.

Results

Infection status

One mouse in the exposed treatment group died during the course of the experiment (28 day p.i.), reducing the final sample size of exposed and control mice to 9 and 10, respectively. The geometric mean standardized absorbance of exposed mice was 4.8 times the BSA control ($t = 6.66$, df $= 8$, $p < 0.001$). In contrast, the geometric mean standardized absorbance of control mice was only 1.1 times the BSA control ($t = 0.64$, df $= 9$, $p = 0.540$). The geometric mean standardized absorbance of the exposed mice was 4.6 times higher than that of the control mice, a statistically significant difference ($t = 6.01$, df $= 17$, $p < 0.001$). Seven of 9 exposed mice were clearly infected with *B. burgdorferi*, whereas only 1 of 10 control mice was infected (Fig. 1).

Blood cells

Infection status did not influence blood cell counts as seen by nonsignificant treatment×time terms. Over the course of the experiment, infected and uninfected mice showed an increase in total white blood cell volume, because of increases in neutrophils, lymphocytes, and basophils (Table 1; Fig. 2).

Wheel running behavior

Over the course of the experiment, infection status had no effect on any of the running variables (Table 2; Fig. 3). Running speed each minute declined over the course of the second night but was not influenced by infection 3 weeks p.i. (p[treatment] $= 0.93$, p[time] < 0.0001, p[treatment×time] $= 0.23$) or 6 weeks p.i. (p[treatment] $= 0.55$, p[time] < 0.0001, p[treatment×time] $= 0.26$).

Discussion

For vector-borne diseases, transmission dynamics depend on interactions among hosts, pathogens, and vectors. If a pathogen alters the physiology or behavior of a host in a manner that affects encounter rates between infected hosts and vectors, transmission dynamics will be altered. This study suggests that the etiological agent of LD in the northeastern United States has little effect on the behavior of its main reservoir host, the white-footed mouse. We found no evidence for a transitory or persistent effect of experimental infection by *B. burgdorferi* on activity levels of white-footed mice, as measured by wheel running behavior.

The finding that *B. burgdorferi* infection did not affect the running behavior of white-footed mice is somewhat surprising because pathological effects of the spirochete have been well established. In non-*P. leucopus* hosts, *B. burgdorferi*

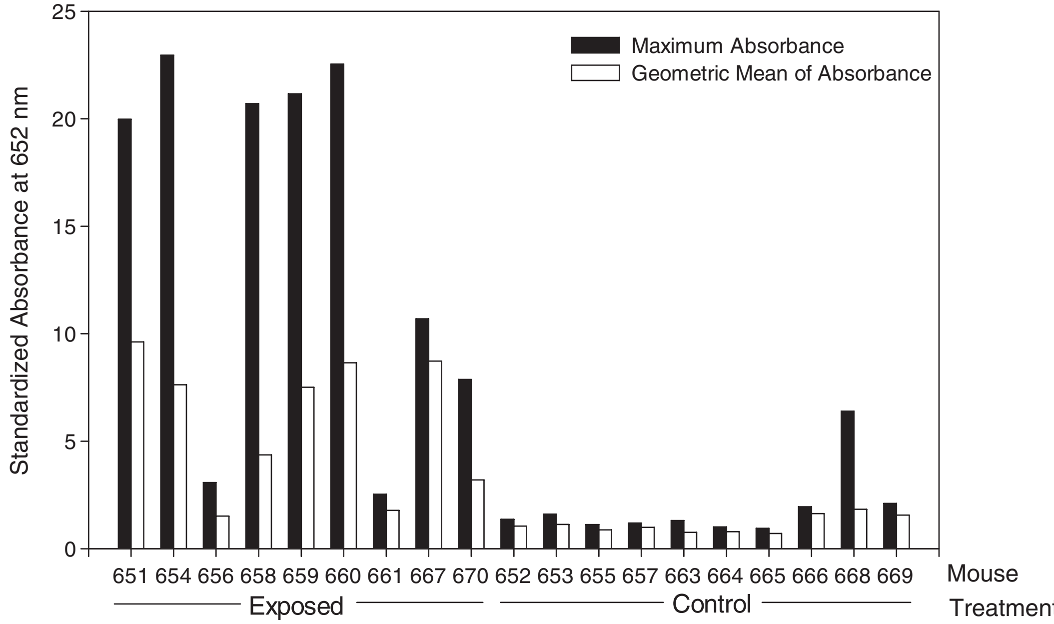

FIG. 1. The strength of the mouse immune response to seven common *Borrelia burgdorferi* outer surface protein C types (A, B, E, G, H, K, N) was measured as the maximum and geometric mean absorbance (at 652 nm) in 19 *Peromyscus leucopus* mice. Mice in the exposed group ($n = 9$) had been inoculated with mouse-fed ticks, whereas mice in the control group ($n = 10$) had been inoculated with raccoon-fed ticks. Mice 656 and 661 in the exposed group were determined to be uninfected, whereas mouse 668 in the control group was determined to be infected with *B. burgdorferi*.

damages skeletomuscular and neurological tissues, which often leads to lethargy (Burgess et al. 1990, Barthold et al. 1991, Moro et al. 2002, Stranek and Strie 2003). *B. burgdorferi* can disseminate throughout the body tissues of white-footed mice (Anderson et al. 1987, Schwan et al. 1988). Moody et al. (1994) showed that experimentally infected infant white-footed mice suffer from carditis and arthritis, although experimentally infected adult mice did not. These results suggest that juvenile mice are more susceptible to *B. burgdorferi*-induced joint damage and may display greater behavioral changes. More broadly, we expect that white-footed mice that are in poor condition, experiencing food restriction, or otherwise compromised immunologically will be more likely to suffer disease symptoms (Beldomenico et al. 2008a, 2008b, Pederson and Grieves 2008, Beldomenico and Begon 2010). Individual mouse variation may help explain variation seen among field studies on the impacts of *B. burgdorferi* infection. Burgess et al. (1990) found motor dysfunction in field-caught white-footed mice, and thorough investigation uncovered *B. burgdorferi* as the potential causative agent. In contrast, a 2-year field study of white-footed mice by Hofmeister et al. (1999) found no measur-

TABLE 1. POSTTREATMENT BLOOD CELL COUNTS[a]

			Mixed effects model			
	Infected	*Uninfected*	*Week*	*Treatment*	*Treatment × week*	*Mass*
RBCs	12.39 ± 2.00	14.00 ± 1.44	1.38 (0.25)	2.12 (0.16)	0.52 (0.48)	0.42 (0.52)
WBCs	7.01 ± 0.96	7.76 ± 1.25	10.14 (0.003) Post > Pre	0.11 (0.74)	0.24 (0.63)	0.20 (0.66)
Neutrophils[b]	0.87 ± 0.23	0.81 ± 0.14	9.44 (0.007) Post > Pre	0.72 (0.41)	0.77 (0.39)	0.32 (0.57)
Lymphocytes	5.67 ± 0.85	6.32 ± 0.98	12.15 (0.001) Post > Pre	0.03 (0.87)	0.42 (0.52)	0.01 (0.90)
Monocytes[b]	0.09 ± 0.03	0.11 ± 0.05	0.76 (0.39)	0.11 (0.75)	0.58 (0.46)	0.16 (0.70)
Eosinophils[b]	0.23 ± 0.04	0.40 ± 0.14	0.00 (1.00)	0.27 (0.61)	0.11 (0.74)	0.71 (0.41)
Basophils[b]	0.15 ± 0.04	0.12 ± 0.04	11.30 (0.004) Post > Pre	0.03 (0.86)	1.19 (0.29)	1.26 (0.27)

F-statistics are presented (*p*-values) from mixed effects model with individual identity as a random effect. Treatment refers to infected ($n = 8$) or uninfected ($n = 11$) group with *Borrelia burgdorferi*. Mass refers to mouse body mass.

[a]Values are mean ± standard error, where RBC values are $\times 10^6/\mu L$ and all types of WBC values are $\times 10^3/\mu L$.

[b]Data were log-transformed for statistical analyses to normalize data.

RBCs, red blood cells; WBCs, white blood cells.

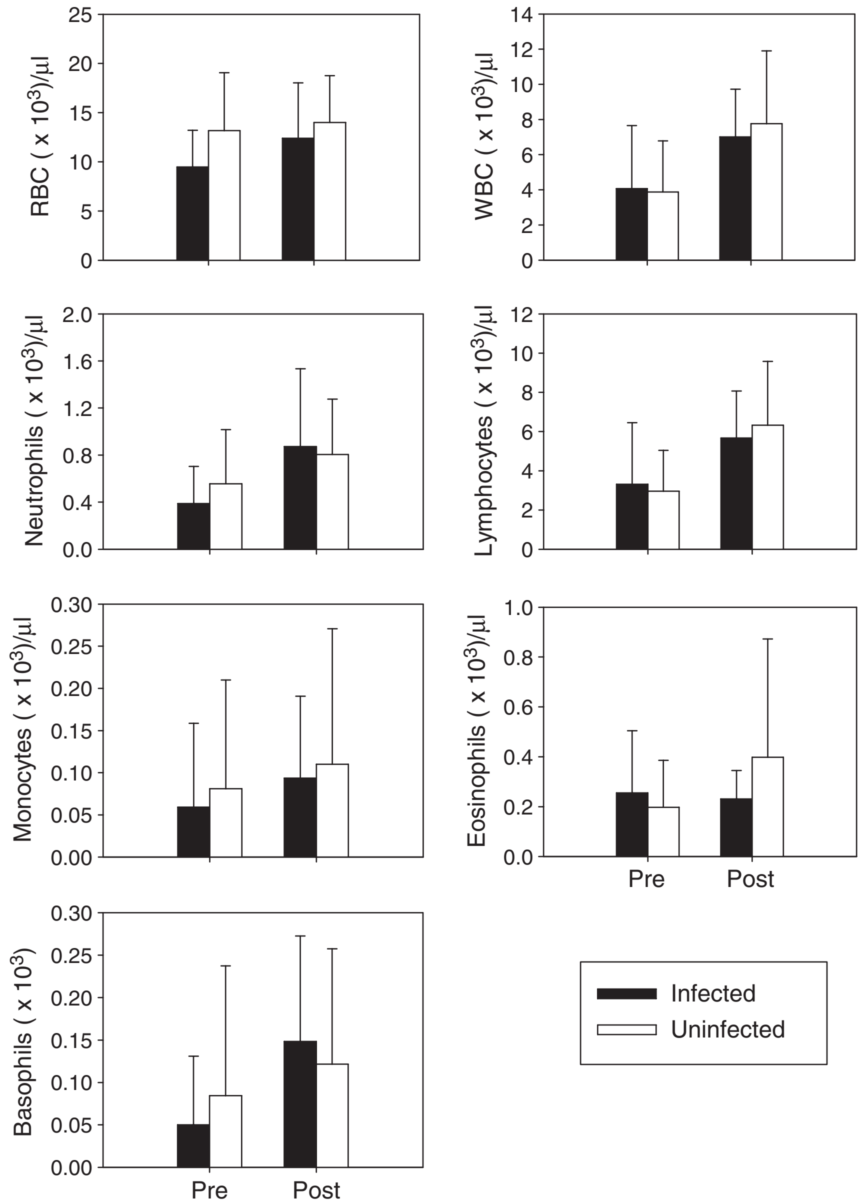

FIG. 2. Blood cell counts (per microliter whole blood) for mice infected ($n = 8$) and uninfected ($n = 11$) with *B. burgdorferi.* Panels show mean ± 1 standard deviation for each treatment group preinfestation and 5 weeks postinfestation.

able effect of *B. burgdorferi* infection on the survival of mice. The findings from our laboratory experiment are more consistent with those of Hofmeister et al., although we did not investigate whether there were any signs of pathology associated with tissue damage or different measures of motor function.

In our study, a humoral immune response against *B. burgdorferi* was observed in the majority of mice inoculated with mouse-fed ticks. Despite the robust recruitment of antibodies, blood cell counts (including lymphocytes) were not higher in infected compared with uninfected mice. However, white blood cell counts increased in both treatments over the course of the experiment, suggesting that experimental procedures or exposure to additional pathogens affected immune function. In addition, it is possible that infection caused a transitory change in white blood cell counts that diminished in our 5-week p.i. blood sample. Our results suggest that high anti-*B. burgdorferi* antibody titers in infected mice were independent of the density of circulating lymphocytes at 1 month p.i. Moreover, it is surprising that the blood cell counts, which should provide an indicator of general immunological health (Beldomenico et al. 2008b, Beldomenico and Begon 2010), revealed no indication of infection with *B. burgdorferi.* The finding that the infection did not cause persistent changes in this measure of immunological health suggests that mounting an immune response to *B. burgdorferi* may be relatively cheap and thus have low impact on behaviors such as activity level. In addition, this result does not support an increased probability of coinfection with additional pathogens that may otherwise lead to a cycle of decreasing condition (Beldomenico and Begon 2010).

TABLE 2. WHEEL RUNNING BEHAVIOR (MEAN ± STANDARD ERROR) COMBINED FOR 1, 2, 3, AND 6 WEEKS POSTINFECTION

			Mixed effects model		
	Infected	*Uninfected*	*Week*	*Treatment*	*Treatment×week*
Total distance (m)	5435 ± 668	5860 ± 504	0.57 (0.68)	0.03 (0.87)	0.54 (0.71)
Average speed (m/min)	8.23 ± 1.01	8.75 ± 0.70	0.44 (0.78)	0.00 (0.95)	0.64 (0.64)
Running time (min)	331 ± 33.4	351 ± 20.7	0.87 (0.49)	0.06 (0.82)	0.54 (0.71)
Running speed (m/min)	15.17 ± 1.75	16.03 ± 1.03	0.03 (0.88)	1.15 (0.34)	0.76 (0.55)

F-statistics (*p*-values) are presented from mixed effects model with individual identity as a random effect. Treatment refers to infected ($n = 8$) and/or uninfected ($n = 11$) group with *B. burgdorferi.*

Our study took advantage of natural variation among ticks in infection with *B. burgdorferi* to successfully create naturalistic infected and uninfected groups of hosts. White-footed mice carry persistent body burdens of black-legged ticks during summer (Ostfeld et al. 1996b, Brunner and Ostfeld 2008), and escaping infection of hosts requires escaping infestation with *B. burdorferi*-infected ticks. In natural settings, this is determined by whether ticks have previously fed on hosts of high reservoir competence (LoGiudice et al. 2003). Nymphs that completed their larval blood meal on white-footed mice have a high likelihood (~90%) of carrying the spirochete, whereas those that completed their larval meal on raccoons or opossums have a very low likelihood of being infected (<10%). Performing experimental infections via naturalistic means is important because acquiring *B. burgdorferi* in the presence of tick saliva increases infection success and spirochete dissemination (Gern et al. 1993, Zeidner et al. 2002, Nuttall and Labuda 2004, Ramamoorthi et al. 2005, Horká et al. 2009). We argue that an understanding of the natural host response requires imitating natural infection routes (e.g., Donahue et al. 1987, Bunikis et al. 2004).

Our results suggest that infection by the etiological agent of LD does not affect the activity levels of a main reservoir host, the white-footed mouse, and therefore does not affect encounter rates between ticks and mice in natural settings. Enzootic transmission of LD, therefore, appears to be uncomplicated by pathology of *B. burgdorferi* in the white-footed mouse. This conclusion would best be confirmed with further experiments on the field behavior and space use of infected wild mice. In addition, it is possible that variation among wild mice in condition and immunocompetence might influence this generalization. Previous research on wild *P. leucopus* has demonstrated considerable intraspecific variation in susceptibility to tick infestation (Brunner and Ostfeld 2008), which suggests that individuals may also vary in their immunological susceptibility to LD pathology. Further investigation of the effects of *B. burgdorferi* infection on female, juvenile, and poor-condition mice, as well as in years of varying food abundance, are warranted.

The finding that *B. burgdorferi* does not appear to affect healthy white-footed mouse hosts (here and Hofmeister et al. 1999) potentially explains why *P. leucopus* is such a competent host for this pathogen. Infected *P. leucopus* persist in the habitat with a sustained infection of *B. burgdorferi* (Schwan et al. 1989, Hofmeister et al. 1999), which can be transmitted to uninfected ticks for the rest of the summer (Donahue et al. 1987, Ostfeld [unpublished data], but see Lindsay et al. 1997). When a pathogen is transmitted via a vector with asynchronous life stages, such as *I. scapularis* in the northeastern United

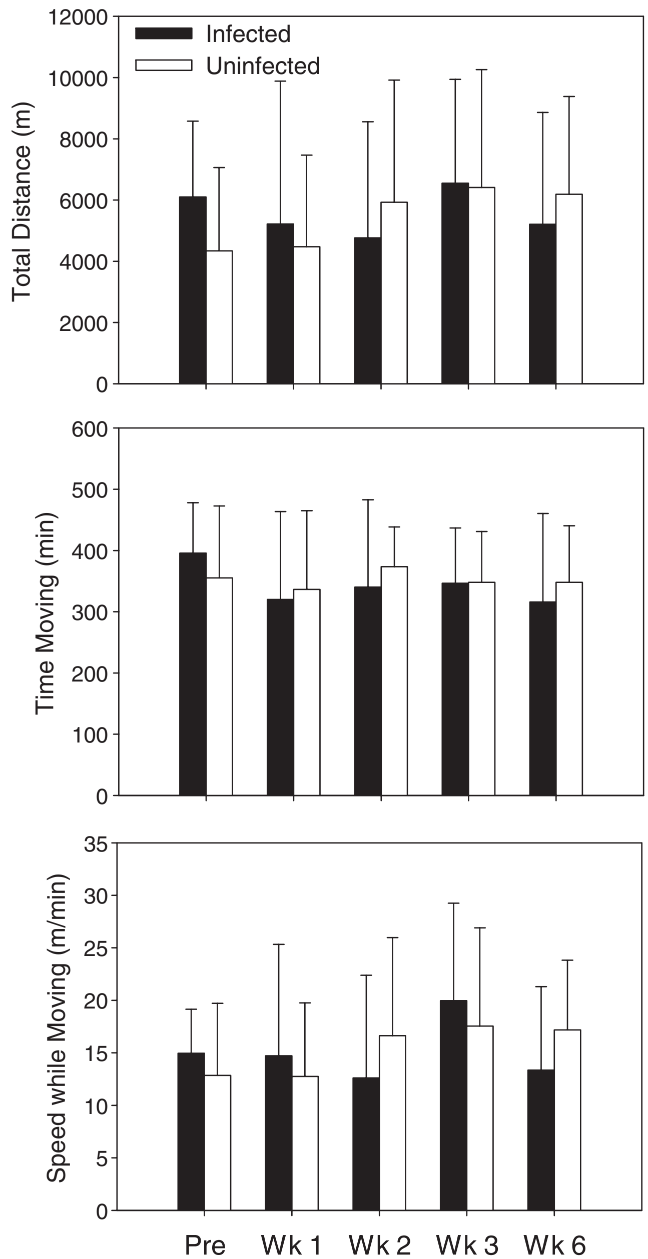

FIG. 3. Wheel running behavior across the duration of the experiment. Values are mean ± 1 standard deviation.

States (larval densities peak 1–2 months after nymphal densities peak) (Ostfeld et al. 1996a, Goodwin et al. 2001), persistence of infective reservoir hosts is necessary for pathogen persistence (Ogden et al. 1997). Persistence in a host at low levels of parasitemia appears to also be an important component of transmission and population persistence for other vector-borne pathogens, such as *Bartonella* and *Babesia* (Chauvin et al. 2009, Chomel et al. 2009). For *B. burgodorferi*, the low level of pathogenicity in white-footed mice may be the result of the relatively benign nature of the specific immunological response of white-footed mice (Martin et al. 2007) or evolution of reduced virulence of the spirochete (Alizon 2008).

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Disclosure Statement

All procedures were approved by the Institutional Animal Care and Use Committee at the Cary Institute. No competing financial interests exist.

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