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Mammal Diversity and Infection Prevalence in the Maintenance of Enzootic *Borrelia burgdorferi* along the Western Coastal Plains of Maryland

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Abstract

The primary vector of *Borrelia burgdorferi* in North America, *Ixodes scapularis*, feeds on various mammalian, avian, and reptilian hosts. Several small mammal hosts; *Peromyscus leucopus*, *Tamias striatus*, *Microtus pennsylvanicus*, and *Blarina* spp. can serve as reservoirs in an enzootic cycle of Lyme disease. The primary reservoir in the northeast United States is the white-footed mouse, *P. leucopus*. The infection prevalence of this reservoir as well as the roles of potential secondary reservoirs has not been established in southern Maryland, a region of low to moderate *Borrelia* infection in humans. Intensive trapping at 96 locations throughout the western Coastal Plains of Maryland was conducted and we found that 31.6% of *P. leucopus* were infected with *B. burgdorferi*. Sequence and phylogenetic analysis revealed that only *B. burgdorferi* sensu stricto circulated in southern Maryland. Feral house mice and voles also were infected and may serve as secondary hosts. *Peromyscus* gender, age and month of capture were significantly associated with infection status. Larval *I. scapularis* were the dominant ectoparasite collected from captured rodents even though host seeking *A. americanum* and *D. variabilis* were collected in greater numbers across the sampling region. Our findings illustrate that the enzootic cycle of LD is maintained in the western Coastal Plains region of southern Maryland between *I. scapularis* and *P. leucopus* as the dominant reservoir.

Keywords

Lyme disease; *Ixodes scapularis*; *Peromyscus leucopus*; *Borrelia burgdorferi*

INTRODUCTION

Peromyscus leucopus Rafinesque, the white-footed mouse, is the primary reservoir for *Borrelia burgdorferi* sensu stricto, the etiologic agent of Lyme disease (LD), in the northeastern United States (Burgdorfer et al. 1982, Johnson et al. 1984, Lane et al. 1991). This mouse is also one of the primary blood meal sources for immature black-legged ticks,

Ixodes scapularis, the most important vector of *B. burgdorferi* s.s. in this region (Magnarelli et al. 1988). *Borrelia burgdorferi* is not maintained transovarially within the tick population nor is it transferred horizontally (from mouse to mouse) or vertically (from mother to offspring) within the *Peromyscus* population (Piesman et al. 1986). Thus, maintenance of a zoonotic cycle between ticks and vertebrates is critical for pathogen transmission (Burgdorfer et al. 1988, Lane et al. 1987, Magnarelli et al. 1987).

In addition to *P. leucopus*, immature *I. scapularis* parasitize many other small mammalian, reptilian and avian hosts (Keirans et al. 1996). Among small mammal hosts, eastern chipmunks (*Tamias striatus*), red and gray squirrels (*Sciurus carolinensis* and *Tamiasciurus hudsonicus*), meadow voles (*Microtus pennsylvanicus*), and shrews (*Blarina* sp.) are commonly parasitized by *Ixodes* (Anderson et al. 1984, Battaly et al. 1993, Carey et al. 1980, Magnarelli et al. 1992). Several of these hosts play an important role in maintaining tick populations but serve only as minor or transient reservoirs for *B. burgdorferi* (Markowski et al. 1997, Markowski et al. 1998). In LD endemic areas of the northeastern United States, larval ticks that feed to repletion on an infected *P. leucopus* have a 40–90% chance of becoming infected with *B. burgdorferi* (Mather et al. 1989, Schmidt et al. 1999) whereas only 10–50% of ticks that feed on other rodents such as the eastern chipmunk, tree squirrels and voles become infected (LoGiudice et al. 2003).

Lyme disease is primarily found in the northeast and northern mid-west United States, with 95% of all cases occurring in only 12 states. Maryland (MD) is found at the southern edge of this region (CDC-MMWR 2004b). In Maryland case prevalence varies greatly, with counties to the northeast reporting the majority of the cases while counties to the west and south report far fewer. Reduced populations of *I. scapularis* or *P. leucopus* or a reduction in the prevalence of infection in either population could contribute to the decline in LD cases among humans in these areas. One of the largest studies of *Borrelia* infection in a natural population of *P. leucopus* was conducted in an endemic region of northern Baltimore County, MD, where it was established that 25–42% of mice were infected with *B. burgdorferi* s.s. (Hofmeister et al. 1995, 1999a,b). Other studies conducted along the northeastern U.S. have revealed much higher rates of infection in *P. leucopus* (Anderson et al. 1986, Anderson et al. 1985, Mather et al. 1989). More recently, Bunikis et al. (2004) conducted a longitudinal study at a single location in New Haven, CT and found that 57% of *P. leucopus* were infected with *B. burgdorferi*. Larger regional studies of *B. burgdorferi* infection in populations of white-footed mice have not been conducted thus far. In this present study we conducted a cross-sectional study of 96 sites in a five county region located along the western Coastal Plains of southern MD to determine the infection prevalence of *B. burgdorferi* in the primary reservoir, *P. leucopus*, as well as to identify potential secondary small rodent reservoirs. This is the first large-scale study of the infection prevalence of *B. burgdorferi* in a natural *Peromyscus* population within a low-endemic region of LD transmission.

METHODS

Field study

From June through October 2001, mice and ticks were collected from 96 sites located in a five county region of the western Coastal Plains of MD covering approximately 1,960 square miles (3,154 sq km; Fig. 1). One 20 × 200 meter grid (0.40 hectare) was selected at each site within which a total of 60 baited live traps (H. B. Sherman, Tallahassee, FL) were placed in three parallel lines of 20 traps for two consecutive days and nights. Captures were identified to species, weighed and sexed. Age was determined by body mass and pelage coloration (Jackson 1961, Schug et al. 1991, Wolfe 1985). Animals were lightly anesthetized with halothane, then one ear was swabbed with ethanol before a 2-mm ear tissue biopsy was harvested (Falco et al. 1992, Hofmeister et al. 1992). The tissue was immediately frozen until further processing. If present, ticks were removed from the anesthetized animals and frozen. Rodents were released at the location of capture. No additional tissue was collected from recaptured animals. Ticks were collected within each trapping grid by flagging (Falco et al. 1992). All ticks were identified to species and life stage and held at −80°C until further processing.

DNA extraction from rodent and tick samples

Whole genomic DNA was extracted from surface sterilized ear tissue biopsies using the QIAamp DNA Mini Kit (Qiagen Corp., Madison, WI) following the manufacturer's recommendations with slight modification including the removal of undigested rodent hair. Purified DNA was eluted in 50 µL of HPLC grade water and stored at −80°C. Whole ticks were extracted using hexadecyltrimethylammonium bromide (CTAB) DNA extraction method (Black IV et al. 1997, Johnson et al. 1992) with slight modifications (Ammerman et al. 2004). Extractions were rehydrated in 50 µL (adult) or 30 ×L (nymph) 0.5× TE buffer and held at −20°C for short term storage and archived at −80°C.

Determination of *Borrelia* infection

To verify DNA extraction fidelity, rodent DNA preparations were subjected to PCR amplification of cytochrome oxidase b (*cytb*) and tick DNA preparations were subjected to PCR amplification of mitochondrial rRNA. Degenerate PCR primers corresponding to the 5' (*cytb*F: CCA CTC CTT CAT TGA TCT CCC) and 3' (*cytb*R ACC CTA TGA ATG CTG TTG CT) ends of the gene encoding cytochrome oxidase b from an alignment of several rodent species were designed using Primer3 (Rozen et al. 2000). Amplification of the mitochondrial 16S gene was achieved using 16S +1 and −1 primers as previously described (Norris et al. 1999). All successful rodent and tick extractions were screened for *Borrelia* by nested-PCR amplification of the flagellin B gene (*flaB*) as described by Johnson et al. (1992). All rodent and tick samples that tested positive for *Borrelia* sp. based on the *flaB* gene were then amplified with a secondary marker for *B. burgdorferi* s.s. infection using PCR primers designed to amplify the plasmid encoded *Borrelia ospA* gene (Guttman et al. 1996). The *ospA* nested-PCR reaction produced a 345-bp fragment.

Sequence and phylogenetic analysis

PCR products from *flaB* and *ospA* were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA) and eluted with 30 μ L of deionized water. Purified products were sequenced directly in both directions and sequence fragments were assembled into contigs using SeqManII (DNASTar Corp., Madison WI). Resulting *flaB* sequences were compared to sequences in GenBank using the BLASTn algorithm (Altschul et al. 1990). Derived sequences were aligned with ClustalX (Thompson et al. 1997) then manually realigned with BioEdit software (Hall 1999). Based on analysis of the *flaB* alignment using Modeltest 3.5 (Posada et al. 1998) the GTR+ Γ model of substitution and calculated base frequencies, gamma distribution, invariant sites, transition/transversion ratios, and rate matrix values were employed as distance parameters for maximum likelihood optimality criteria using PAUP* (Felsenstein 1989, Swofford 2003). Tree structure was verified by bootstrap analysis (1000 replicates) using maximum likelihood distance estimates (50% majority rule) (Felsenstein 1985).

Statistical analysis

Prevalence estimates were calculated using STATA (Version 7.0; Stata Corp., College Station, TX) and were reported as percentages with exact 95% confidence intervals (CI) based on a binomial distribution. Significance testing comparing *Borrelia* infection with small animal characteristics (animal age, sex and month of collection) was conducted using Pearson's Chi-Square and Fisher's exact statistics. The associations between *Borrelia* infection and rodent sex, weight, age, and month of collection were quantified as odds ratios (OR), calculated using univariate logistic regression, to measure the degree of the association between risk factors and infection prevalence. Significant associations from the univariate model ($p \leq 0.05$) were included in a multivariate model to identify confounding and interaction effects. Those that ceased to be significant in the multivariate model were eliminated in a step-wise manner, yielding a final model in which all variables were independently associated with *Borrelia* infection.

RESULTS

Small animal collections

Eight hundred two rodents were captured during 11,520 trap nights. Six hundred forty-two (80%) were primary captures (Fate 1) and 139 (17%) were recaptures (Fate 2); the remainder escaped before processing. Five hundred fifty-three (86%) of all primary captures were *P. leucopus*. Eighty-nine (13.7%) of the captures were species other than *P. leucopus*: feral house mice (*Mus musculus*) (42), voles (*Microtus pennsylvanicus*) (31), shrews (*Blarina brevicauda*) (7), birds (6), chipmunks (*Tamias striatus*) (2), a flying squirrel (*Glaucomys volans*) (1), a meadow jumping mouse (*Zapus hudsonius*) (1), and an eastern box turtle (*Terrapene carolina*) (1). Of the *P. leucopus* collected 236 (43%) were female and the majority (66%) were considered to be sexually mature adults based on pelage and body mass (Table 1). Two hundred six ticks were removed from 80 animals (Table 1). Of the ticks removed 182 were *I. scapularis* (180 larvae and 2 nymphs) and 24 ticks were classified as metastriata (10 larvae and 14 nymphs). Identification of metastriata ticks revealed that all nymphal and larval ticks were *D. variabilis*. The majority of ticks ($n = 177$) were found on

Peromyscus captures ($n = 68$) with an average of 0.320 ticks per primary capture ($n = 553$). Considering only animals with ticks attached, the mean number of *I. scapularis* larvae per mouse was 2.6 (range, 1–26). Two animals were found with >25 ticks each.

Flagging collections

Five hundred ninety-three ticks were collected by flagging from 43 of 96 sites during the 2001 collection season (June through October; Table 2). Of the ticks collected, 484 were *Amblyomma americanum* (150 larvae, 292 nymphs and 42 adults), 89 *Dermacentor variabilis* (73 larvae and 16 adults) and 26 were *I. scapularis* (7 larvae, 13 nymphs and 6 adults). *Amblyomma* ticks were found at 30 sites (11.26 ticks/site), *Dermacentor* ticks were collected from 12 sites (2.97 ticks/site) and *Ixodes* ticks were only found at 11 sites (2.36 ticks/site).

Detection of *Borrelia* infection in rodent captures

Six hundred twenty-eight of 642 rodent extractions were screened by PCR amplification of *flaB* and *ospA*, of which 29% tested positive (Table 3). By species, 31.6% of *P. leucopus*, 11.9% of *M. musculus*, and 6.45% of *M. pennsylvanicus* were positive for *B. burgdorferi* based on the PCR assays. No *B. brevicauda* were infected with *Borrelia*. More male small animal captures (31.8%) were infected with *Borrelia* than female animals (25.2%; Table 3). Among the *P. leucopus* captures, the highest infection prevalence (37%) was observed in Charles and Prince George's counties. Infection prevalence was lowest in captures from Calvert County (21.3%). Of the 5 infected *M. musculus*, two were collected in Anne Arundel County and both infected voles (*M. pennsylvanicus*) were collected in St. Mary's County. Temporal heterogeneity was apparent during the study period as the prevalence of *Borrelia*-positive *P. leucopus* varied with month of capture ($p < 0.027$). Prevalence of infection among *P. leucopus* was 58% lower in July compared to June (OR = 0.417, 95% CI 0.184–0.945, p value = 0.036).

Several small mammal characteristics were associated with *B. burgdorferi* infection: species of rodent, age, weight and sex of animal (Table 4). *Peromyscus leucopus* was more likely to be infected than other mammal species ($p < 0.001$). Among *P. leucopus*, female mice were 34% less likely to be infected with *B. burgdorferi* (OR = 0.652, 95% CI 0.449–0.945, $p = 0.02$). The odds of collecting *Borrelia*-positive adult *P. leucopus* was 2.8 times that of juvenile mice (95% CI 1.14–6.92, $p = 0.02$). Infestation with ectoparasites (ticks) was not significantly associated with *Borrelia* infection among *P. leucopus* ($p = 0.441$) nor *M. musculus*. Multivariate analysis revealed that the risk of infection with *B. burgdorferi* among *P. leucopus* was significantly associated with the age and month of capture (Table 4).

Detection of *Borrelia* infection in tick collections

DNA was successfully extracted from 5 of the 6 adults and all nymphal ($n = 13$) *Ixodes* ticks collected by flagging based on PCR amplification of the mtRNA 16S gene fragment. Of the 18 ticks examined, 66.6 % ($n = 12$) were positive for *B. burgdorferi* s.s. (3 adult and 9 nymphs) based on *flaB* amplification.

Phylogenetic analysis of gene fragments

Phylogenetic analysis of the *flaB* gene fragment (335 bp) indicated that the *Borrelia* amplicons recovered from rodents and ticks collected in MD belonged to the clade containing other, known *B. burgdorferi* s.s. strains and were 99% identical to *B. burgdorferi* strains 297 and B31 (GenBank accession nos. AB035616 and AF416433). Specimen MDPI550 was identical in sequence to *B. burgdorferi* strain 297 within the amplified region of the *flaB* gene. Additionally, the specimens collected in MD were phylogenetically distinct from *B. lonestari*, *B. bissettii* and *B. andersonii* (Lin et al. 2001, Oliver et al. 2000) (Fig. 2). The placement of the MD *Borrelia* samples in the clade containing other *B. burgdorferi* s.s. sequences was strongly supported (83%) by bootstrap replication (Fig. 2). Additionally, BLAST analysis of *ospA* fragments revealed that the majority of MD samples were identical to the previously established *ospA* mobility class variants (Guttman et al. 1996) with the exception of 2 amplicons that were 98% similar to known *B. burgdorferi* s.s. isolates published in GenBank.

DISCUSSION

The objective of this study was to determine the infection prevalence of *B. burgdorferi* in *P. leucopus* and other potential small animal reservoirs in southern MD where relatively few LD cases are reported annually (I: 6.5 per 100,000 population) (CDC-MMWR 2002) and prevalence of infection among the tick population is moderate (8.2%) (Amerasinghe et al. 1992). Our observations indicate that *B. burgdorferi* s.s. appears to be the only member of this pathogen group circulating in southern MD and that the prevalence of infection in rodents, primarily *P. leucopus*, is much higher than previously considered.

The majority (87.9%) of rodents collected at the 96 sites were identified as *P. leucopus* ($n = 705$). Other rodent reservoirs for *B. burgdorferi* were rarely captured (Humair et al. 1999, Mannelli et al. 1993, McLean et al. 1993, Schmidt et al. 1999). *Ixodes scapularis* larvae were the most prominent ectoparasite collected from rodents. In contrast with reports from highly enzootic sites where mice are often parasitized with >1 nymphal *Ixodes* tick (Carey et al. 1980, Lane et al. 1991, Spielman et al. 1985), we found only 2 nymphs on all of our *Peromyscus* captures. Recently, LoGiudice et al. (2003) reported an average of 28 *I. scapularis* per *P. leucopus* capture, a much higher infestation level than we observed from rodents collected in MD. The paucity of *Ixodes* ticks may be due to the timing of the collection period; the majority of the trapping period was conducted during the summer months (June through September) when nymphal and larval *I. scapularis* ticks are presumably less active in this region (Sonenshine 1993) or an overall lower abundance of ticks present, compared to the northeastern United States (Brownstein et al. 2003).

The infection status of *P. leucopus* has not been previously established along the western Coastal Plains of MD. Previous reports by Hofmeister et al. (1999a, 1992) and Markowski et al. (1998) established that 29–66% of *P. leucopus* mice collected in Baltimore County, MD, north of our study region, were infected. Our findings, using the same gene target (*ospA*) as well as an additional gene target, *flaB*, closely agree with the 1992 study by Hofmeister et al. While it is known that *Microtus* are competent reservoirs for *B. burgdorferi*, especially in areas where *P. leucopus* are not abundant (Markowski et al. 1998), this is the first report of

infection in this host collected in the mid-Atlantic region. The finding of infected feral house mice was somewhat surprising, although one previous report by (Oliver et al. 1999) found 13.6% of mice collected in 1987 on Assateague Island, MD had antibodies to *B. burgdorferi*, which is consistent with the prevalence of infection we observed in our study region. These data illustrate that the aforementioned minor reservoirs are exposed to ticks and become infected with *Borrelia* even in areas of moderate to low LD endemicity.

Species and age appeared to be significant predictors of infection among the rodent captures. Both feral house mice and voles were less likely to be infected with *B. burgdorferi* than *P. leucopus* (OR 0.307 and 0.156, respectively; Table 4). *P. leucopus* is likely the primary reservoir of *B. burgdorferi* as well as the primary host for *I. scapularis* larvae. Although presence of ticks on *P. leucopus* was not significantly associated with infection ($p = 0.442$) it is important to note that the burden of *I. scapularis* larvae on white-footed mice was much higher than on other small animal captures (Table 1), which most likely contributed to the disparity in infection prevalence in each host. Although suggested by LoGiudice et al. (2003), the presence of alternate tick hosts such as voles, shrews and feral house mice appears to have little appreciable effect on the enzootic cycle of borreliosis in this region.

Infection prevalence in *I. scapularis* adult populations collected by Qiu et al. (2002) of 42.1% ($n = 38$) and 81.1% ($n = 11$) in Cecil and Somerset counties of the eastern Coastal Plains of MD, respectively, suggests that the infection prevalence in ticks reported in this study (63%) may be within reason. Additionally, an unpublished report from our laboratory found ~50% of adult ticks collected in 2000 in Baltimore County, located just north of our study region, were infected with *B. burgdorferi*.

Our data illustrate that *B. burgdorferi* s.s. circulates throughout southern MD in an enzootic cycle involving *I. scapularis* and *P. leucopus* as the primary reservoir. There is evidence that *M. musculus* and *M. pennsylvanicus* act as secondary reservoirs in this region. Three species of Ixodid ticks were collected by flagging; *I. scapularis*, *D. variabilis*, and *A. americanum*, yet *I. scapularis* were the predominate ectoparasites on rodent captures. Previous work by Amerasinghe et al. (1992, 1993) suggested that lower numbers of *I. scapularis* ticks collected from flagging and deer sampling studies could explain the reduction of human LD cases along the western Coastal Plains of MD, yet this study suggests that both ticks and mice are infected with *B. burgdorferi* throughout the study area. Since the risk of human infection is related to exposure to infected ticks (Barbour et al. 1993), low incidence of human infection in this region may not be explained by the observed tick and mouse infection levels or the low, overall density of questing ticks, particularly questing nymphal ticks present in this region, although based on the current study the final assertion is more difficult to assess. Clearly *B. burgdorferi* s.s. circulates within the tick and rodent population in southern Maryland, therefore, further analysis considering other variables such as ecological conditions or social behaviors that may address the focality of enzootic cycles should be conducted. *Peromyscus* species mice are ubiquitous throughout the eastern United States and can be easily collected in regions where ticks are not abundant and during periods of tick inactivity thus, *Peromyscus* spp. are useful as sentinels to establish whether *B. burgdorferi* is endemic in a particular region.

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FIG. 1.

Trapping locations in the western Coastal Plains of southern Maryland. Circles represent each of the 96 trapping sites visited during the summer of 2001. Inset represents the location of the rodent collections relative to the entire state.

FIG. 2. Maximum likelihood cladogram based on multiple alignments of *flaB* gene fragments (334 bp) derived using PAUP. Maximum likelihood bootstrap values (1000 replicates) are listed for each supported node. Maryland (MD) samples collected from *P. leucopus* (Pl) are indicated by specimen capture number. Accession number follows sample name.

Table 1

Total Number of Primary Captures, Including Sex, Weight, and Age of Captured Rodents

Species	Sex (%)			Body mass (g)				Age (%)			Ectoparasitites ^b				
	Total (%)	Male	Female	Average	SD	Min	Max	Juvenile	Sub-adult	Adult	Total ticks	Ixodes larvae	Ixodes nymph	Metastriata larvae	Metastriata nymph
<i>Peromyscus</i>	553 (86)	317 (57)	236 (42)	19.296	3.52	8	33.5	36 (6.5)	135 (24.4)	382 (69)	200	176	1	9	14
<i>Mus</i>	42 (6.5)	24 (57)	18 (42)	15.357	3.57	7	22	15 (35.7)	9 (21.4)	18 (43)	1	—	—	1	—
<i>Microtus</i>	31 (4.8)	18 (58)	13 (41)	36.129	12.02	12	58	10 (32.2)	—	21 (67.7)	3	2	1	—	—
<i>Blarina</i>	7 (1.09)	1 (14)	6 (85)	11.928	1.86	9	14	7 (100)	—	—	—	—	—	—	—
Other	11 (1.7)	—	—	—	—	—	—	—	—	—	2	2	—	—	—
Total	642	360	273								206	180	2	10	14

^aFor *Peromyscus* captures, age was determined by body mass and pelage coloration (Schug et al. 1991); age of other captures was estimated based on weight compared to referenced average adult weight (Jackson 1961, Wolfe, 1985). *Peromyscus* juvenile < 15 g, sub-adult = 15–17 g, and adult > 17 g. *Mus* average adult weight = 21 g (range 17–25 g). *Microtus* average adult weight = 43 g (range 33–65 g). *Blarina* adult weight = 21.6 g (range 18–30 g).

^bMetastriata are non-Ixodes, such as *Dermacentor variabilis* or *Amblyomma americanum*.

Table 2
Total Number of Metastriata and Prostriaata Ticks Collected by Flagging Techniques by County

County	Total sites with ticks	Total ticks collected	Total Amblyomma collected	Total sites with Amblyomma	Total Dermacentor collected	Total site with Dermacentor	Total Ixodes collected	Total sites with Ixodes
Anne Arundel	11	147	128	6	12	2	15	6
Charles	10	88	82	10	1	1	5	2
Calvert	5	85	40	1	44	2	1	1
Prince George's	5	50	25	2	20	1	5	2
St. Mary's	12	223	209	11	12	6	0	0
Total	43	593	484	30	89	12	26	11
Average tick per site ^a			11.256		2.967		2.364	
Average tick per 96 sites ^b			5.047		0.927		0.271	

^a Average number of ticks found per site where ticks were collected.

^b Average number of ticks found per site for all 96 sites.

Table 3

Infection Prevalence of Small Animal Captures Collected in Maryland, 2001

Characteristic	N	All species	% Infected with <i>B. burgdorferi</i> ss ^a (95% CI)					
			N	P. leucopus	N	M. musculus	N	M. pennsylvanicus
Overall		$p = 0.001$						
	628	29.0 (25.4–32.5)	548	31.6 (27.6–35.1)	42	11.9 (1.6–22)	31	GAS (–2.7–15)
Sex		$p = 0.069$		$p = 0.024$		$p = 0.636$		$p = 0.168$
Male	358	31.8 (26.9–36.3)	313	35.4 (30.1–40.8)	24	8.3 (–3.6–20.2)	13	15.3 (–7–33)
Female	270	25.2 (20.0–30.3)	235	26.4 (20.7–32.1)	18	16.6 (–2.4–35.7)	18	0
Age ^c		$p = 0.185$		$p = 0.003$		$p = 1.0$		$p = 1.0$
Juvenile	56	10.7 (4–21.8)	36	16.6 (6.3–32)	15	0	1	0
Sub-adult	148	22.2 (15.8–29.8)	134	23 (16.3–31.2)	12	16.6 (2.1–48.4)	1	0
Adult	424	33.7 (29.2–38.4)	378	35.9 (31.1–41)	15	20 (4–48)	29	6.8 (0.8–22.7)
County								
Anne arundel	78	26.9 (17.5–33.2)	58	32.8 (20–46.3)	7	28.6 (–16–73.3)	13	0
Calvert	81	19.8 (11.7–30.1)	75	21.3 (11.8–30.8)	6	0	0	0
Charles	158	36.1 (28.6–44.1)	146	37.7 (29–45.6)	4	25 (–54.5–104)	3	0
Prince George's	109	34.8 (25.9–44.6)	99	37.4 (27.6–47)	5	20 (–35.3–75.5)	4	0
St. Mary's	202	24.7 (18.9–31.3)	170	27 (20.3–33.8)	20	5 (–5.4–15.4)	11	18.2 (–8.9–45.3)
Month		$p = 0.09$		$p = 0.027$		$p = 0.312$		$p = 0.637$
June	51	29.4 (17–43.8)	31	41.9 (24.5–60.9)	7	28.5 (3.6–70.9)	13	0
July	142	22.5 (15.9–30.2)	134	23.1 (16.2–31.2)	7	14.2 (0.3–57.8)	1	0
August	317	33.4 (28.2–38.9)	274	36.4 (30.7–42.5)	24	8 (1–26.9)	13	15.3 (1.9–45.4)
September	89	26.9 (18.1–37.4)	84	28.5 (19.2–39.4)	4	0	0	0
October	29	17.2 (58.4–35.7)	25	20 (6–40)	0	0	4	0

^a Infection prevalence based on *flab* and *ospA* PCR amplification.^b Fisher's ($n < 10$) or Pearson's chi-square p -value; significant values are in bold.^c Age based on weight.

Table 4Univariate and Multivariate Odds Ratio (OR) Associated with *B. burgdorferi* s.s. Infection

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	p-value	OR	95% Ci	p-value
Species						
<i>Peromyscus</i>	1.0	Reference				
<i>Mus</i>	0.292	0.113–0.578	0.011			
<i>Microtus</i>	0.110	0.035–0.6333	0.010			
<i>P. leucopus</i>						
Sex						
Male	1.0	Reference				
Female	0.652	0.449–0.945	0.024			
Weight (g)	1.07	1.01–1.12	0.007			
Age						
Juvenile	1.0	Reference				
Sub-adult	1.5	0.573–3.946	0.406			
Adult	2.8	1.140–6.920	0.025	2.85	1.11–7.28	0.029
Ecto-parasite	1.182	0.772–1.812	0.442			
Month						
June	1.0	Reference				
July	0.417	0.184–0.945	0.036	0.313	0.133–0.735	0.009
August	0.796	0.374–1.692	0.553			
September	0.553	–0.235–1.303	0.176	0.343	0.138–0.849	0.021
October	0.346	0.103–1.163	0.086	0.225	0.64–0.787	0.02
<i>M. musculus</i>						
Sex						
Male	1.0	Reference				
Female	2.2	0.327–14.8	0.417			
Weight (g)	1.5	0.968–2.32	0.069			
Age						
Juvenile	1.0	Reference				
Adult	2.01		<0.001			
Ecto-parasite	NA					
Month						
June	1.0	Reference				
July	0.417	0.0286–6.063	0.522			
August	0.227	0.184–2.025	0.0255039			
September	NA					
October	NA					