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Peromyscus maniculatus (Rodentia: Cricetidae): An overlooked reservoir of tick-borne pathogens in the Midwest, USA?

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Abstract. Mice belonging to the genus *Peromyscus* are one of the most important reservoirs of tick-borne pathogens in the United States. However, the composition and abundance of *Peromyscus* species may vary geographically. The woodland deer mouse, *Peromyscus maniculatus gracilis,* is often abundant in the northern counties of Minnesota, Wisconsin, and Michigan. In these states, multiple pathogens transmitted by the blacklegged tick, *Ixodes scapularis*, are endemic. In comparison to the well-studied white-footed mouse, *Peromyscus leucopus*, little is known about the importance of *P. maniculatus* in maintaining natural cycles of tick-borne pathogens. We conducted small mammal trapping in north-central Wisconsin and compared the modified reservoir potential of *P. maniculatus* to *P. leucopus*. Based on mixed-model regression analysis, individual *P. maniculatus* were 2.07 (1.07–4.01) times more likely to be infected with *Borrelia burgdorferi* compared with *P. leucopus*. We report the first detection of three emerging tick-borne pathogens (*Babesia microti*, *Borrelia mayonii,* and *Ehrlichia muris eauclairensis*) in *P. maniculatus*. Additionally, *P. maniculatus* infected with *Ba. microti* were 4.8 (2.74–8.50) times more likely to be concurrently infected with *Bo. burgdorferi* compared with *P. leucopus*. While we found individual *P. leucopus* to be more infested with both larval and nymphal *I. scapularis*, *P. maniculatus* was the more abundant species. As a result, *P. maniculatus* had a higher modified reservoir potential in our study area and was likely responsible for feeding and infecting more ticks with pathogens than *P. leucopus*. Overall, our results illustrate that *P. maniculatus* is an important reservoir in areas of the Midwest, where it occurs in high abundance.

Key words: *Babesia microti; Borrelia burgdorferi;* coinfection; Lyme disease; *Peromyscus leucopus.*

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Introduction

The Midwest, USA, is a hotspot for emerging and endemic tick-borne pathogens transmitted through the bite of the blacklegged tick, *Ixodes scapularis* Say (Eisen et al. 2017). The white-footed mouse, *Peromyscus leucopus* Rafinesque, occurs throughout much of the Midwest (Long 1996, Jannett et al. 2007, Myers et al. 2009) and is considered an important host for immature *I. scapularis* as well as a reservoir for multiple tick-borne pathogens including *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia burgdorferi*, *Bo. mayonii*, *Bo. miyamotoi*, *Ehrlichia muris eauclairensis,* and Powassan virus (Ruebush et al. 1981, Levine et al. 1985, Telford et al. 1996, Scoles et al. 2001, Johnson et al. 2011, 2017, Castillo et al. 2015, Barbour 2017). However, the abundance of *P. leucopus* varies geographically and the woodland deer mouse, *Peromyscus maniculatus gracilis* Wagner, is also common in the northernmost counties of Minnesota, Wisconsin, and Michigan where the two mice occupy similar habitats (Long 1996, Myers et al. 2009, Stephens and Anderson 2014). Here, we assessed the importance of each of these *Peromyscus* species as tick-borne pathogen reservoirs at a field site in the Midwest.

While *P. maniculatus* is known to serve as a host to immature *I. scapularis* and play a role in maintaining natural cycles of *Bo. burgdorferi* (Rand et al. 1993), their importance as a reservoir of tick-borne pathogens is not well understood. *Peromyscus leucopus* and *P. maniculatus* were found to have similar *Bo. burgdorferi* infection rates when compared directly in field studies (Friedrich 2003, Simon et al. 2014, Larson et al. 2018). The two species also showed comparable susceptibility to *Bo. burgdorferi* when intravenously inoculated with spirochetes in a laboratory experiment (Burgess et al. 1986). By contrast, *P. maniculatus* was found to have a higher prevalence of *A. phagocytophilum* (Larson et al. 2018), which suggests that the two species might not be equally susceptible to all pathogens. Of the tick-borne pathogens that are known to occur in the Midwest and cause human disease, only *A. phagocytophilum*, *Bo. burgdorferi, Bo. miyamotoi,* and Powassan virus have been reported from field-caught *P. maniculatus* (Rand et al. 1993, Deardorff et al. 2013, Larson et al. 2018, Sambado et al. 2020).

In addition to the host pathogen prevalence, host densities and tick infestations rates are also important factors in estimating the number of ticks infected by a given host species (Mather et al. 1989, Hamer et al. 2012). Immature *I. scapularis* infestations on *P. leucopus* and *P. maniculatus* were compared in allopatric populations in Maine (Rand et al. 1993) and sympatric populations in Michigan (Friedrich 2003), Wisconsin (Larson et al. 2018), and Quebec (Bouchard et al. 2011). With the exception of the study in Quebec, *P. leucopus* was more frequently infested with larval *I. scapularis* and at higher intensity than *P. maniculatus*. In contrast, differences in nymphal infestation between *P. maniculatus* and *P. leucopus* were less consistent across previous studies (Rand et al. 1993, Friedrich 2003, Bouchard et al. 2011, Larson et al. 2018). While *P. leucopus* appears to have a higher propensity for being infested with larval *I. scapularis*, the relative abundance of each host species could vary. Therefore, despite being associated with lower tick infestations, *P. maniculatus* could potentially feed and infect more ticks in areas where it occurs in higher abundance.

It has been suggested that the distribution and relative abundances of these *Peromyscus* spp. are linked to climate and that warming temperatures in the Midwest are favoring *P. leucopus* (Long 1996, Myers et al. 2005, 2009). The relative abundances of these mouse species are known to fluctuate in response to temperature and resource availability with *P. maniculatus* being more adapted to cold weather and *P. leucopus* a more prolific breeder (Drickamer and Vestal 1973, Long 1996, Wolff 1996, Myers et al. 2005). Roy-Dufresne et al. (2013) found the current distribution of *P. leucopus* in Quebec to be highly correlated with winter length and winter temperatures in that the probability of occurrence increased with milder winters. Moreover, long-term observations suggest that warming temperatures are responsible for *P. leucopus* replacing *P. maniculatus* on Michigan's Lower Peninsula (Myers et al. 2005, 2009). Therefore, understanding differences in pathogen prevalence and tick infestations between these species is a timely topic that may provide some insights on how warming temperature may affect tick-borne disease risk in the Midwest. Based on its propensity to have higher infestations with larval *I. scapularis*

(Larson et al. 2018), we expect *P. leucopus* to be feeding and infecting more larval ticks with tick-borne pathogens.

To further elucidate the importance of *P. maniculatus* as a reservoir of tick-borne pathogens in the Midwest, we compared the modified reservoir potential by assessing tick infestations, abundance, and pathogen prevalence of *P. maniculatus* to the more extensively studied *P. leucopus*. We tested individual mice for *A. phagocytophilum*, *Bo. burgdorferi*, and *Bo. miyamotoi* as well as *Ba. microti*, *Bo. mayonii*, and *E. muris eauclairensis* which have not been previously reported as infecting *P. maniculatus* (Barbour 2017). Previous studies examining *P. leucopus* have found that concurrent infection with *Bo. burgdorferi* and *Ba. microti* were higher than expected and that *Ba. microti* infection preceded *Bo. burgdorferi* (Hersh et al. 2014, Tufts and Diuk-Wasser 2018). Therefore, we examined the coinfection status of all mice.

Materials and Methods

Small mammal trapping and sample collection

Small mammals were live-trapped at 10 hardwood forest sites within the Chequamegon-Nicolet National Forest located in Taylor County, WI, where both *P. leucopus* and *P. maniculatus* are known to occur (Long 1996). This is the same county in which the first known case of erythema migrans, the hallmark symptom of Lyme disease, was reported (Scrimenti 1970). Fig. 1 displays the approximate range of *P. maniculatus* based on occurrence records obtained from the Global Biodiversity Information Facility (GBIF) database as well as the study site location (*Peromyscus maniculatus* subsp. *gracilis* GBIF Secretariat 2017). Trapping was conducted from June to August in 2018 and 2019. All trapping was conducted in accordance with Institutional Animal Care and Use Committee protocol (AA005400) approved by University of Wisconsin–Madison. Trapping grids of at least 0.5 ha were established with 12.5 m or 15 m trap spacing and 60 to 81 traps at each site. Sherman traps, 28 × 9 × 23 cm (H.B. Sherman Inc., Tallahassee, Florida, USA) and Heslinga live traps (Heslinga traps, Groningen, The Netherlands), were baited before sunset and checked in the morning of the following day. Grids were pre-baited for one night and trapped for two to four nights per month. Captured animals were sexed, ear-tagged, weighed, examined for ticks, and ear biopsies and blood samples were collected. Mice were classified as immature or adult based on their mass; mice which had a mass of 16 g or more were classified as adults and mice <16 g as juveniles (Larson et al. 2018). Ear punch biopsies were collected using a two-mm (Integra Miltex, York, Pennsylvania, USA) disposable biopsy punch and placed in 70% ethanol. Blood was collected via tail vein sampling and stored on filter paper (Whatman 903 Multipart Neonatal Protein Saver Cards, Pittsburgh, Pennsylvania, USA) at 4°C. Ticks were placed in vials containing 70% ethanol and stored at room temperature until identification. All ticks were identified to life stage and species according to established taxonomic keys (Clifford et al. 1961, Durden and Keirans 1996).

DNA extraction tissue and blood

Ear punch biopsy samples.—A sterile 18-gauge needle (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) was used to bisect each ear biopsy. We used the ISOLATE II Genomic DNA Kit (Meridian Life Sciences, Memphis, Tennessee, USA) in accordance with the manufacturer's protocol to isolate DNA from bisected biopsies. Extracted DNA was eluted to a total volume of 75 μL.

Blood samples.—The adjusted methods of Bereczky et al. (2005) previously reported in Larson et al. (2018) were used to extract DNA from blood cards. Briefly, a six mL sample of each blood card was removed using a disposable biopsy punch (Integra Miltex). Erythrocytes were lysed by incubating blood punches in 1 mL of a 0.5% saponin solution at 4°C overnight. Following incubation, the saponin solution was removed and replaced with one mL of a 1X PBS solution for 30 min at 4°C. Filter paper punches were transferred to new preheated tubes containing 50 μL of 20% Chelex-100 (20 mg/80 mL) in 1X PBS solution and 150 μL of distilled water and incubated for 10 min at 100°C. Tubes were then centrifuged at 10,000 g for two minutes before the supernatant was transferred to a new tube and centrifuged for an additional two minutes at 10,000 g. Finally, the resulting supernatant was transferred to a new tube and stored at −20°C until *Peromyscus* spp. identification and pathogen testing was performed.

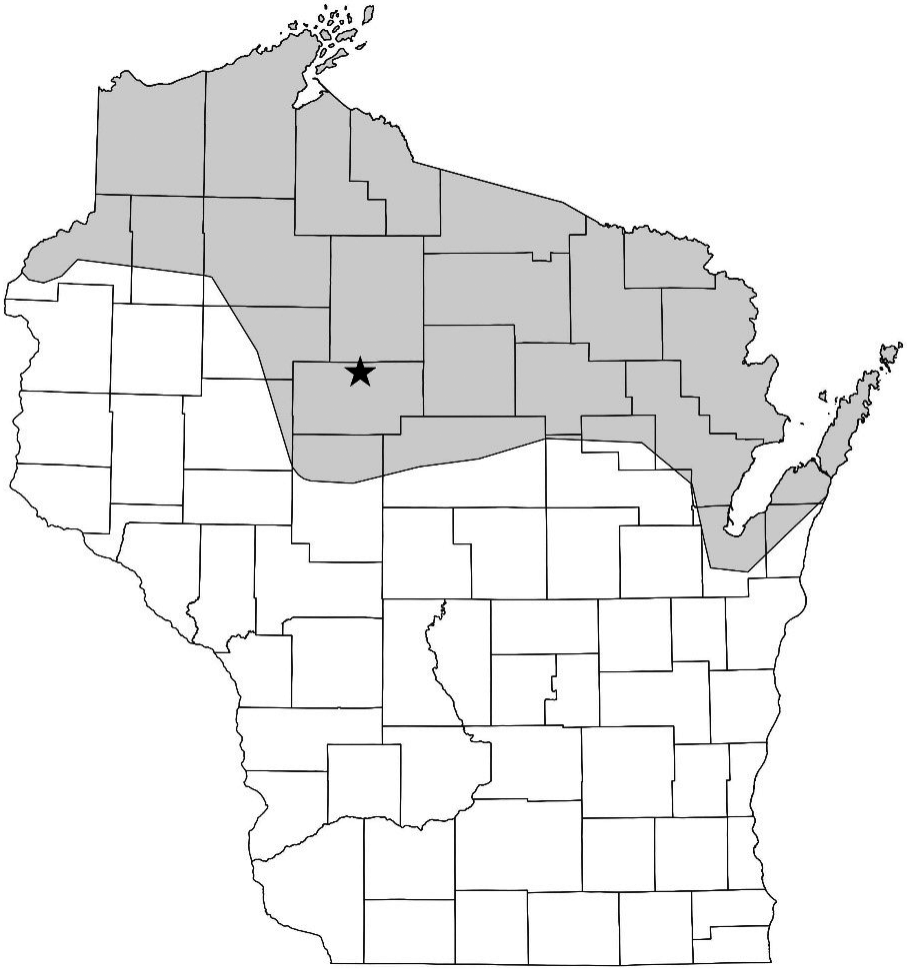

Fig. 1. Approximate range of *Peromyscus maniculatus gracilis*, indicated by the shaded gray area, in Wisconsin (*P. maniculatus* subsp. *gracilis* in GBIF Secretariat 2017). *Peromyscus leucopus* occurs throughout the state of Wisconsin. The star indicates our study site at the Chequamegon Nicolet National Forest located in Taylor County, WI.

PCR identification of Peromyscus *spp.*

Peromyscus maniculatus and *P. leucopus* were differentiated using multiplex primers targeting COIII in polymerase chain reactions (PCR) as described by Tessier et al. (2004). We separated PCR products on a 2% agarose gel stained with Gel Red (Biotium, Inc., Fremont, California, USA) and visualized under UV light.

Pathogen detection

For pathogen detection, we modified the methods of Babady et al. (2008) and Stauffer et al. (2020), which use quantitative PCR based on fluorescent resonance energy transfer probes. We used DNA extracted from ear tissue biopsies for detection of *Bo. burgdorferi* and *Bo. mayonii*, while DNA extracted from blood cards was used for detecting *A. phagocytophilum*, *Bo. miyamotoi*, *E. m. eauclairensis*, and *Ba. microti*. Primer, probes, melting temperatures, and limits of detection for each pathogen can be found in Appendix S1: Table S1. Briefly, reactions of 20 µL were prepared by adding five µL of DNA eluate to 15 µL of 1× LightCycler FastStart DNA Master HyProbe (Roche, Indianapolis, Indiana, USA), magnesium chloride, primers, and probes (Appendix S1: Table S1). All reactions were run using a LightCycler 2.0 instrument (Roche)

following the cycling conditions as described in Babady et al. (2008). Constructed plasmids (TIB Molbiol, LLC, Adelphia, New Jersey, USA) diluted in PCR grade water served as the positive controls, and five μL of PCR grade water served as negative control. Limits of detection for each pathogen were established by running standard curves in triplicate. For each standard curve, plasmids were diluted in PCR grade water ranging from 10^7 to 10^{-2} copies/μL.

Statistical analysis

All data analyses and modeling were conducted in R 3.4.4 (R Development Core Team 2018). For each pathogen tested, prevalence was calculated by dividing the number of positive animals by the total number of individuals tested. We determined the mean intensity of tick infestations by calculating the average tick infestations of mice that were infested with at least one tick.

Modeling tick abundance.—Captures with missing variables were removed from the data set. We fit generalized linear mixed models (GLMM) from package *lme4* (Bates et al. 2015) to assess tick abundance. We hypothesized that tick abundance on a mouse could be due to characteristics of the mouse (species, sex, and age) as well as the sample month, year, and site. We estimated the abundance of larval *I. scapularis* on individual mice by fitting a GLMM with a negative binomial distribution using the function "glmer.nb." For negative binomial models, we used a bound optimization by quadratic approximation optimizer and 100,000 iterations to improve model convergence. Tick abundance was based on the first time an individual animal was captured each month. Fixed effects in our full model included species (*P. leucopus* or *P. maniculatus*), age (adult or juvenile), sex (male or female), month (June, July, or August), and year (2018 or 2019). Site was used as a random effect in our models to account for potential effects of spatial autocorrelation on tick abundance (Cayol et al. 2018). We fit a full model including species, sex, age, month, and year as a starting point for model comparison. As an alternative to manually fitting models, we used the "dredge" function from the *MuMIn* package (Bartoń 2019) to assess all possible model combinations ranked by small-sample corrected Akaike information criterion (AIC_c). In lieu of using model selection to identify a singular best model, we employed multimodel inference to consider information from all competing models (Burnham and Anderson 2002, Kaizer et al. 2015). Following VanAcker et al. (2019), all models within two AIC_c of the best fit model were considered competitive and were selected for model averaging. We used conditional model averaging, which only uses estimates of parameters if they appeared in models and does not substitute parameter estimates with zero in cases where they did not appear in models (full model averaging). Model averaging was conducted using the "model.avg" function, which is also available through the *MuMIn* package (Bartoń 2019). Finally, we displayed the average prediction of all models with $\Delta AIC_c < 2$.

Modeling infection prevalence.—We repeated the same approach for modeling *Bo. burgdorferi* infection and added concurrent infection with *Ba. microti* as a variable, because this has been shown to be associated with *Bo. burgdorferi* in mice (Dunn et al. 2014, Hersh et al. 2014). We modeled the probability of individual mice being infected with *Bo. burgdorferi*, the most commonly detected pathogen, upon first capture by fitting a logistic regression with random effects using the function "glmer." Fixed effects of the pathogen prevalence model included host characteristics (age, sex, year, and concurrent infection with *Ba. microti)* and sampling time (month and year). Site was included as a random effect. We exponentiated model estimates to obtain adjusted odds ratios (AOR), which displays how a one unit increase in explanatory variable changes the ratio of individual mice being infected.

Assessing temporal patterns of coinfection.—We assessed temporal patterns of pathogen coinfections for mice that were captured in at least two separate months and concurrently tested positive for *Ba. microti* and *Bo. burgdorferi* within at least one of the months captured. These patterns were displayed using the "geom_tile" function in the *ggplot2* package (Wickham 2016).

Host density estimation.—Detailed methods describing our host density estimation can be found in Appendix S2. Briefly, to estimate host density, we modeled mouse abundance and estimated the effective area trapped. *Peromyscus leucopus* and *P. maniculatus* abundance were

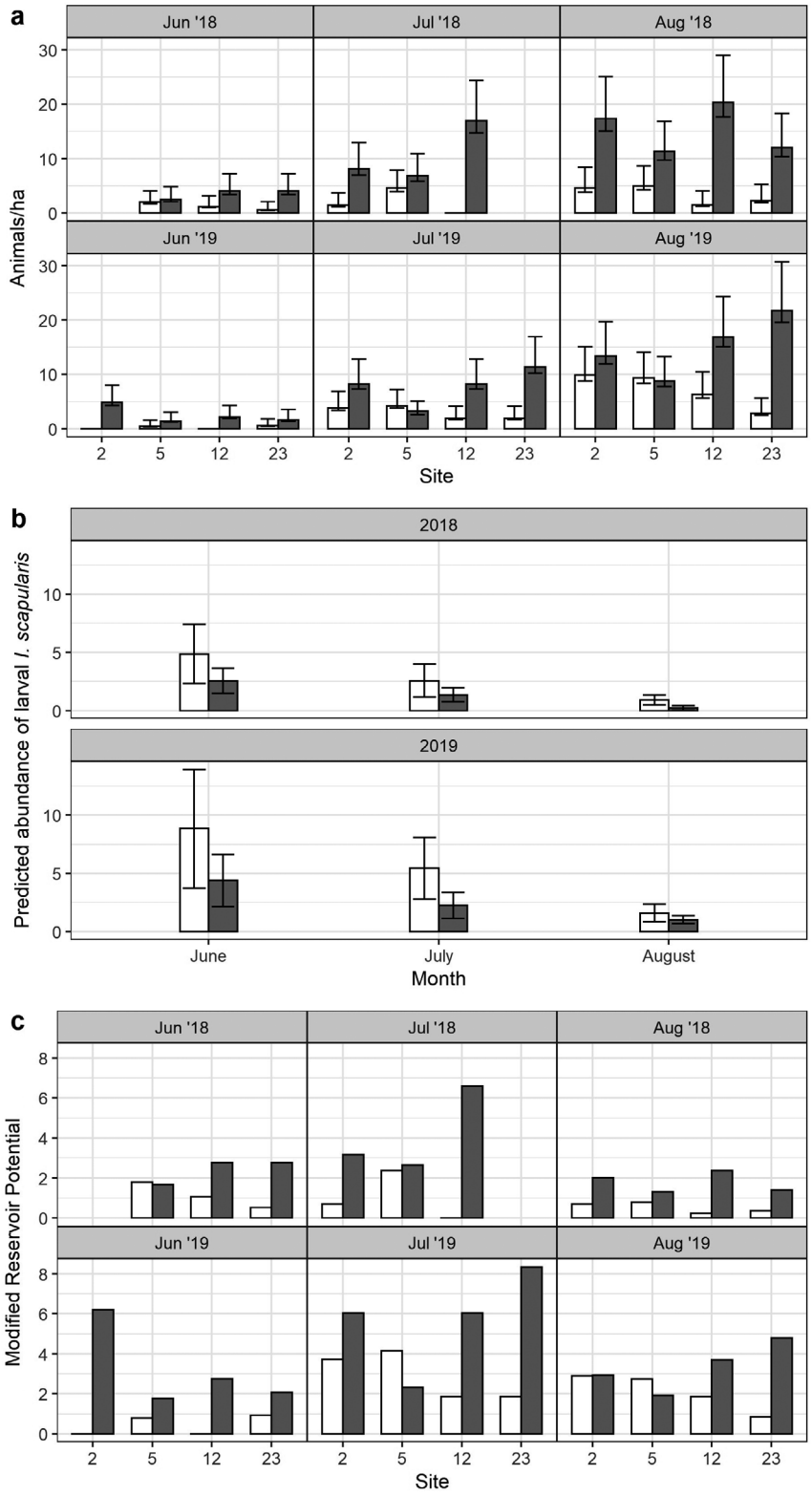

Fig. 2. (a) Estimated host density (error bars represent 95% confidence intervals), (b) predicted abundance of

(Fig. 2. *Continued*)
larval *Ixodes scapularis* infestations (error bars represent 95% confidence intervals), and (c) monthly modified reservoir potential of *Peromyscus leucopus* (white bars) and *Peromyscus maniculatus* (grey bars). Modified reservoir potential is an estimate of the number of larval *I. scapularis* that each host species is responsible for infecting with *Borrelia burgdorferi*.

estimated for four sites that were sampled in both years with Huggins robust design models using RMark (Huggins 1989, Laake 2013). Abundances (*N)* were grouped by species (n = 2), trap session (n = 6), and site (n = 4). To convert N to densities, we estimated the effective trapping area for each species, trap session, and site, by adding a buffer strip equal to one half the mean maximum distance moved (MMDM) of mice to the trapping grid area (Parmenter et al. 2003, Schwemm et al. 2018). Maximum distance moved was defined as the distance between the two farthest traps used by an individual mouse within monthly trapping sessions. Densities were obtained by dividing N by the effective trapping area.

Comparing modified reservoir potential.—For each species, we calculated the modified reservoir potential as described by Hamer et al. (2012), which was an estimate of the number of ticks that feed on Bo. burgdorferi-infected hosts of a certain species. Modified reservoir potential was obtained by the product of density of ticks expected to be fed by a given host species (feeding density) and the proportion of a given host species that was infected with *Bo. burgdorferi*. We compared monthly modified reservoir potential at four sites in which we were able to estimate densities across years and months. Monthly feeding density was obtained for each species by multiplying model-averaged predictions of larval infestations (see *Modeling tick abundance*) and the estimated host density for each year, month, and site. The proportion of a given host species that was infected with *Bo. burgdorferi* was estimated from the species-specific *Bo. burgdorferi* prevalence across all months, years, and sites. For each month and site in which both species were present, we compared the difference in modified reservoir potential between species using a paired t-test.

Results

Throughout 7,877 trap nights, we captured 438 unique *Peromyscus* spp. individuals 1041 times in total, and 549 times when considering the first time an animal was captured each month. Twenty-four of the 549 monthly captures (representing 15 individuals) were removed from analysis due to missing information. After removing incomplete observations, the data used for our tick abundance and pathogen prevalence models included 423 individual *Peromyscus* (97 *P. leucopus* and 326 *P. maniculatus*), which were captured 525 times (120 *P. leucopus* and 405 *P. maniculatus*).

Host density estimation

Modeling results for host abundance estimates and effective area trapped are contained in Appendix S2. *Peromyscus maniculatus* densities were higher than *P. leucopus* across all sites, years, and months (Fig. 2a). Site 2 was not trapped in June 2018, and site 23 was removed from density estimation analysis in July 2018 due to a heavy rainfall event, which prevented the processing of animals captured during the third trapping night. Mouse densities increased throughout the summer and were highest in August (Fig. 2a).

Tick infestations

We collected 986 larval and 56 nymphal *I. scapularis* from 296 mice. The overall prevalence of larval *I. scapularis* infestation for *P. leucopus* and *P. maniculatus* was 71% (85/120) and 50% (204/405), respectively (Table 1). Overall mean intensities of infestation with larval *I. scapularis* for *P. leucopus* and *P. maniculatus* were 3.94 (median = 3; range = 1, 18) and 3.19 (2; 1, 26), respectively (Table 2). During each trapping session, *P. leucopus* had a higher prevalence of both larval and nymphal ticks (Table 1). With the exception of the August trapping session in 2019, *P. leucopus* was also found to have higher mean intensities of infestation with larval *I. scapularis* (Table 2). We also collected a total of 247 immature *Dermacentor variabilis* Say (229 larvae and 18 nymphs) from 94 individual mice. The prevalence of infestation of immature *D. variabilis* on *P. leucopus* and *P. maniculatus* was 23% (27/120) and

Table 1. Prevalence of infestation with *Ixodes scapularis* by trapping session.

Year	Life stage	Species	June	July	August
2018	Larvae	*Peromyscus leucopus*	88% (7/8)	75% (12/16)	42% (8/19)
		Peromyscus maniculatus	80% (33/41)	58% (40/69)	19% (15/80)
	Nymphs	*Peromyscus leucopus*	25% (2/8)	0% (0/16)	5% (1/19)
		Peromyscus maniculatus	17% (7/41)	0% (0/69)	1% (1/79)
2019	Larvae	*Peromyscus leucopus*	100% (2/2)	95% (19/20)	67% (37/55)
		Peromyscus maniculatus	72% (13/18)	79% (52/66)	39% (51/131)
	Nymphs	*Peromyscus leucopus*	100% (2/2)	35% (7/20)	5% (3/55)
		Peromyscus maniculatus	28% (5/18)	12% (8/66)	5% (6/131)

Table 2. Mean intensity (median, range) of infestation with *Ixodes scapularis* by trapping session.

Year	Life stage	Species	June	July	August
2018	Larvae	*Peromyscus leucopus*	10.1 (10, 5–18)	3.83 (3.5, 1–8)	1.75 (1, 1–4)
		Peromyscus maniculatus	5 (4, 1–14)	2.33 (1, 1–15)	1.53 (1, 1–4)
	Nymphs	*Peromyscus leucopus*	1.5 (1.5, 1–2)	N/A	1 (1, 1–1)
		Peromyscus maniculatus	1 (1, 1–3)	N/A	1 (1, 1–1)
2019	Larvae	*Peromyscus leucopus*	5.5 (5.5, 3–8)	5.68 (5, 1–15)	2.30 (1, 1–6)
		Peromyscus maniculatus	2.31 (2, 1–7)	3.56 (2, 1–26)	3.04 (2, 1–16)
	Nymphs	*Peromyscus leucopus*	1 (1, 1–1)	1.29 (1, 1–2)	1 (1, 1–1)
		Peromyscus maniculatus	1.4 (1, 1–3)	1.75 (1, 1–4)	1.17 (1, 1–2)

17% (67/405), respectively. The overall mean intensities of infestation with immature *D. variabilis* on *P. leucopus* and *P. maniculatus* were 2.6 (median = 2; range = 1, 36) and 2.6 (2; 1, 11), respectively.

Modeling tick abundance

The two selected models describing larval *I. scapularis* abundance on mice both included month, species, and year as covariates (Appendix S3: Table S1). Larval *I. scapularis* infesting mice was highest in June and decreased throughout the summer (Fig. 2b. and Table 3). Additionally, larval *I. scapularis* abundance in 2019 was 1.88 times higher than in 2018 (Fig. 2b and Table 3). Larval abundance on *P. maniculatus* was 0.47 times lower than on *P. leucopus* (IRR = 0.47, 95% C.I. 0.36–0.63, $P < 0.001$). We found no evidence of an association between either mouse age or sex on larval *I. scapularis* abundance.

Pathogen prevalence

In *P. maniculatus*, we detected all six pathogens: *Ba. microti*, *Bo. burgdorferi*, *Bo. mayonii*, *Bo.*

Table 3. Conditional average for two models describing the abundance of infestation of larval *Ixodes scapularis*.

		Larval *Ixodes scapularis* abundance			
Fixed Effect	Level	Coefficient (SE)	*Z*	*P*	IRR (95% CI)
	(Intercept)	1.59 (0.27)	6.0	<2e−16***	4.87 (2.89–8.20)
Age	Immature	−0.07 (0.14)	0.49	0.63	0.93 (0.71–1.23)
Month	July	−0.56 (0.21)	2.73	0.0064**	0.57 (0.38–0.85)
	August	−1.77 (0.21)	8.44	<2e−16***	0.17 (0.11–0.26)
Species	*P. maniculatus*	−0.75 (0.14)	5.26	1.0e−07***	0.47 (0.36–0.63)
Year	2019	0.63 (0.14)	4.50	6.9e−06***	1.88 (1.43–2.48)

Notes: Fixed effects included: Month = month trapped (two levels, June is the reference). Species = *Peromyscus* species (two levels, *P. leucopus* is the reference). Year = year sampled (two levels, 2018 is the reference). Age = age of mouse (two levels, adult is the reference). Random effect included site. Number of models refers to the subset of models with $AIC_c < 2$. Number of observations = 525. IRR, incidence rate ratio. ** means parameter significantly affected the abundance of larvae on mice at $P < 0.01$ level. *** means the parameter significantly affected the abundance of larvae on mice at $P < 0.001$ level.

miyamotoi, *A. phagocytophilum*, and *E. m. eauclairensis*. *Borrelia mayonii* and *E. m. eauclairensis* were not detected in samples collected from *P. leucopus* (Table 4). At first capture, *Bo. burgdorferi* prevalence in *P. leucopus* and *P. maniculatus* was 19% (18/94) and 30% (94/318), respectively. The prevalence of *Ba. microti* in *P. leucopus* and *P. maniculatus* was 15% (14/94) and 25% (80/318), respectively. Prevalence of *Bo. burgdorferi* and *Ba. microti* coinfection was 9% (8/94) in *P. leucopus* and 16% (51/318) in *P. maniculatus* (Table 4). While we detected coinfections involving other pathogens, we had too few to analyze.

Peromyscus maniculatus were 2.06 times more likely to be infected with *Bo. burgdorferi* in comparison to *P. leucopus* (95%CI: 1.07–3.98, $P = 0.03$) (Table 5). Mice infected with *Ba. microti* were 4.78 times more likely to be concurrently infected with *Bo. burgdorferi* (95%CI: 2.70–8.34, $P < 0.001$). Juvenile mice were less likely to be infected with *Bo. burgdorferi* compared to adult mice (AOR = 0.06, 95% CI: 0.02–0.18, $P < 0.001$). No associations between *Bo. burgdorferi* infection status and sex, month, or year were found (Appendix S3: Table S2).

Table 4. Pathogen prevalence at first capture.

Pathogen (s)	*Peromyscus leucopus*		*Peromyscus maniculatus*	
Anaplasma phagocytophilum	1%	(1/94)	0.6%	(2/318)
Babesia microti	15%	(14/94)	25%	(80/318)
Borrelia burgdorferi	19%	(18/94)	30%	(94/318)
Borrelia mayonii	0%	(0/94)	1.6%	(5/318)
Borrelia miyamotoi	1%	(1/94)	0.6%	(2/318)
Ehrlichia muris eauclairensis	0%	(0/94)	0.9%	(3/318)
Bo. burgdorferi + *Ba. microti*	8.5%	(8/94)	16%	(51/318)

Temporal detection of coinfection

Twenty-four of the *Bo. burgdorferi* and *Ba. microti* coinfected mice ($n = 59$) were captured in more than one month. At the first capture of individual animals, 42% (10/24) were positive for both, 25% (6/24) were positive for *Ba. microti* only, 4% (1/24) were positive for *Bo. burgdorferi* only, and neither pathogen was detected in 29% (7/24) of mice (Fig. 3). In 67% (16/24) of mice, the first detection of either *Bo. burgdorferi* or *Ba. microti* occurred simultaneously (mice were coinfected with both pathogens). *Babesia microti* also appeared to be more persistent in mice compared to *Bo. burgdorferi*; after first detection in an individual mouse, *Ba. microti* DNA was detected in all subsequent captures. However, detection of *Bo. burgdorferi* failed in four mice that were previously identified as coinfected with both *Ba. microti* and *Bo. burgdorferi*. Within this subset of coinfected mice, we also detected *A. phagocytophilum* (1/24), *Bo. mayonii* (1/24), *Bo. miyamotoi* (1/24), and *E. m. eauclairensis* (1/24) (Fig. 3).

Modified reservoir potential

Modified reservoir potential ranged from zero to an estimated 8.3 larval ticks fed by *Bo. burgdorferi*-infected *P. maniculatus* or *P. leucopus* per ha (Fig. 2c). While *P. leucopus* had higher larval infestations (Fig. 2b; Tables 1, 2), *P. maniculatus* had a higher infection prevalence (Table 4) and was the more abundant species (Fig. 2a) compared to *P. leucopus*. Overall, *P. maniculatus* had a higher modified reservoir potential than *P. leucopus* ($t = -3.6$, df = 18, $P < 0.001$). Additionally, *P. maniculatus* had a higher estimated modified

Table 5. Probability of infection of *Borrelia burgdorferi* in two *Peromyscus* species. Conditional average of four models predicting the likelihood of infection of *Bo. burgdorferi* in two *Peromyscus* species.

Fixed effect	Level	Coefficient (SE)	*Z*	*P*	AOR (95% CI)
	(Intercept)	−1.70 (0.40)	4.19	2.8e−05***	0.18 (0.08–0.40)
Age	Immature	−2.81 (0.54)	5.15	2e−07***	0.06 (0.02–0.18)
Ba. microti	Positive	1.56 (0.29)	5.42	1e−07***	4.75 (2.70–8.34)
Species	*P. maniculatus*	0.72 (0.33)	2.16	0.03*	2.06 (1.07–3.98)
Sex	Male	0.05 (0.31)	0.54	0.77	1.18 (0.69–2.05)
Year	2019	0.24 (0.31)	0.77	0.44	1.56 (0.89–2.76)

Notes: Fixed effects included: Species = *Peromyscus* species (two levels, *P. leucopus* is the reference). Year = year sampled (two levels, 2018 is the reference). Sex: sex of mouse (female is the reference). Age = age of mouse (two levels, adult is the reference). Concurrent infection with *Babeisa microti* (two levels, positive is reference). Random effect included site. AOR, adjusted odds ratio; CI, confidence interval. Number of observations = 412. We removed 11 individuals in which blood samples were not collected from the model analysis. * indicates parameter significantly affected the probability of infection at $P < 0.05$. *** indicates parameter significantly affected the probability of infection at $P < 0.001$.

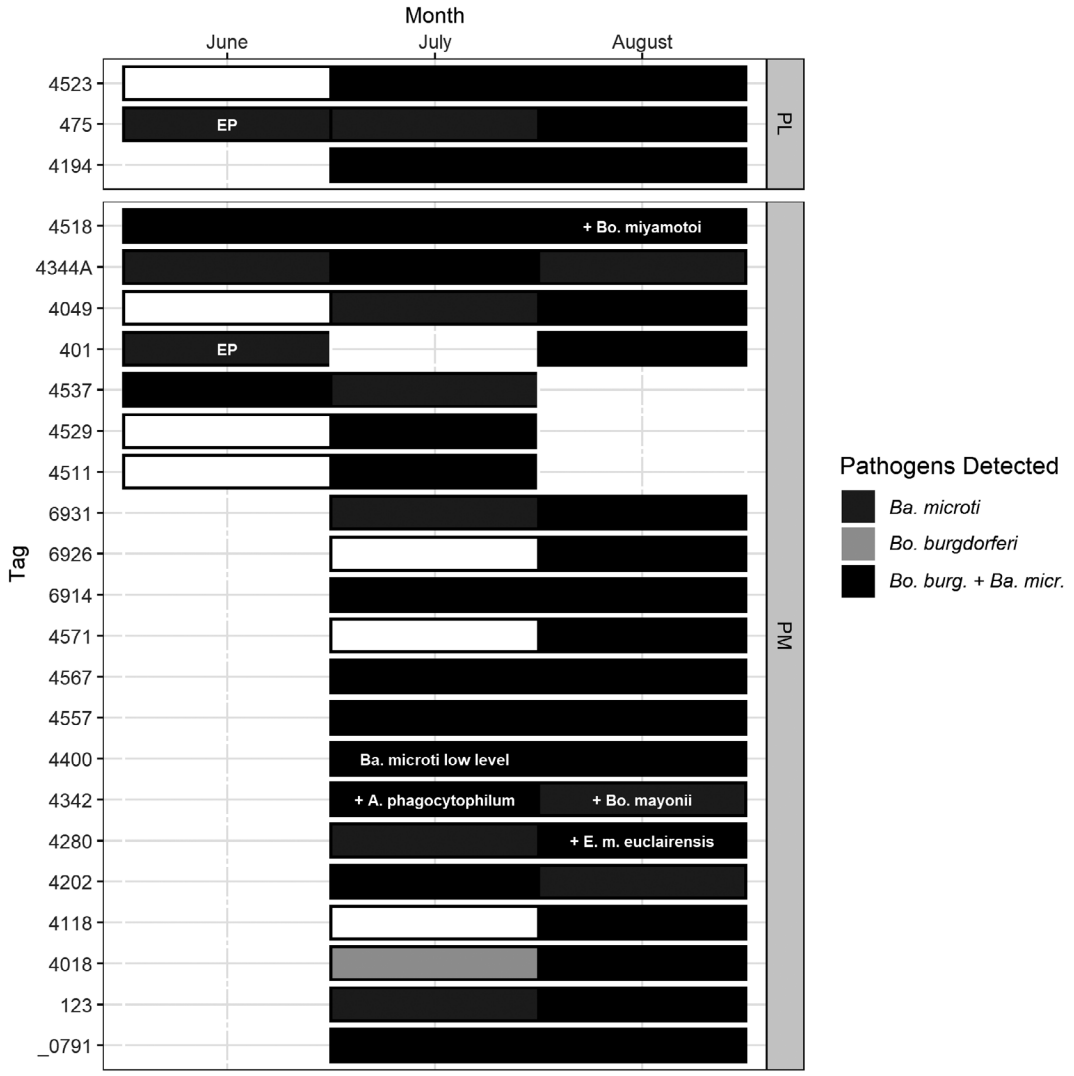

Fig. 3. Temporal detection of coinfection in *Peromyscus leucopus* and *Peromyscus maniculatus* (Taylor County WI, 2018–2019). Mice included in this subset were captured in at least two separate months and coinfected with *Babesia microti* and *Borrelia burgdorferi* in at least one of those months. Tag = the unique ear tag identifying each animal. PL and PM refer to *P. leucopus* and *P. maniculatus*, respectively. Black borders with white fill indicate that the animal was captured but no pathogens were detected. *Ba. microti* low level: pathogen concentration was below the limits of reliable detection based on RT-PCR crossing point values. Pathogens preceded by a plus sign (in example + *Anaplasma phagocytophilium*, + *Borrelia mayonii*, + *Borrelia miyamotoi*, and + *Ehrlichia muris eauclairensis*) annotates that these pathogens were concurrently detected. EP, indicates ear punch biopsy was used for testing *Ba. microti* because blood sample a was not collected from this mouse. Ear punch samples were not included in pathogen analysis or modeling.

reservoir potential in 17 out the 19 monthly site visits, in which both species were captured (Fig. 2c). The two occasions when *P. leucopus* had a higher modified reservoir potential occurred at site 5 in July and August of 2019, when animal densities of the two species were very similar (Fig. 2b). The highest modified reservoir potentials for both species were recorded in July 2019.

Discussion

Our results show that *P. maniculatus* was responsible for infecting more larval ticks with *Bo. burgdorferi* at our field site (Taylor County, WI, 2018–2019) in comparison to *P. leucopus*. *Peromyscus leucopus* has sometimes been described as the "primary" or "most important" host of both larval *I. scapularis* and *Bo. burgdorferi* (Levine et al. 1985, Donahue et al. 1987, Ostfeld et al. 2006). However, Brisson et al. (2008) showed that shrews (*Blarina* and *Sorex* species) may be responsible for infecting more larval ticks than *P. leucopus*. Here, we demonstrate that *P. leucopus* may not always be the most important mouse host in terms of infecting larval ticks. While individual *P. leucopus* were more frequently infested with larval *I. scapularis*, *P. maniculatus* was the more abundant of the two species at our field site and, consequently, was feeding more larval ticks in total. Ultimately, the proportion of ticks that these two species are responsible for feeding and infecting is not only dependent on larval infestations but is also driven by the densities of each species.

In contrast to previous studies (Friedrich 2003, Bouchard et al. 2011, Larson et al. 2018), we found moderate evidence that *P. maniculatus* was more likely to be infected with *Bo. burgdorferi*. While this finding may be an artifact of the small number of *P. leucopus* represented in the study, both mice appear to be highly susceptible to *Bo. burgdorferi* as well as numerous other tick-borne pathogens. In *P. maniculatus,* we detected not only *Bo. burgdorferi* and *A. phagocytophilum* but also *Ba. microti, Bo. mayonii,* and *E. muris eauclairensis*. According to Barbour (2017), these three pathogens had not been previously detected in *P. maniculatus*. With the exception of *Ba. microti*, in which transplacental transmission is possible (Tufts and Diuk-Wasser 2018), mice are thought to first acquire these infections through an infectious tick bite and *P. leucopus* is known to be a competent reservoir host for these pathogens. Assuming that *P. maniculatus* is a competent reservoir and can efficiently transmit these pathogens to ticks, this species should not be overlooked as a potential reservoir host for tick-borne pathogens in the Midwest.

In our study, we used modified reservoir potential to compare reservoir competency (Hamer et al. 2012). This approach assumes that once infected, both mice species are equally efficient in transmitting pathogens to feeding larval ticks. This assumption could be tested by determining the proportion of ticks that acquire pathogens while feeding on an infected host and successfully molt into infected nymphs (Mather et al. 1989). Pathogen transmission efficiencies could be combined with our field-collected data to obtain more precise reservoir competence estimates (Mather et al. 1989). Further assessments of the reservoir competence of *P. maniculatus* are warranted to better understand their overall importance in tick-borne disease ecology.

Our results suggest that there is a positive association between *Bo. burgdorferi* and concurrent infection with *Ba. microti* in both *P. leucopus* and *P. maniculatus*. This relationship was previously described in *P. leucopus* in the Northeast (Dunn et al. 2014, Hersh et al. 2014), and we show that *P. maniculatus* may also be an important source of coinfections in the Upper Midwest. Concurrent infection with *Bo. burgdorferi* and *Ba. microti* is thought to increase severity and persistence of disease symptoms in humans (Krause et al. 1996). To better understand temporal patterns of pathogen coinfection, we examined infection statuses of coinfected mice over time. Within this subset of mice, we found that both pathogens were often first detected within the same month. In cases where pathogens were first detected singularly (only *Bo. burgdorferi* or *Ba. microti* was detected), we found that infection with *Ba. microti* preceded *Bo. burgdorferi* more frequently than the reverse. Although transplacental transmission has been shown to promote earlier infection with *Ba. microti* in mice, the first *Ba. microti* detection for several mice in our study did not occur until subsequent captures, which suggests that at least some of the mice are first acquiring *Ba. microti* from ticks in the environment rather than through transplacental infection (Tufts and Diuk-Wasser 2018). Further studies are needed to elucidate relationships between *Ba. microti* and the acquisition and persistence of other tick-borne pathogens in *Peromyscus* hosts.

While *P. maniculatus* was the most abundant mouse at our field site, warming temperatures may favor *P. leucopus* in the future. Long-term observational studies have documented the

northward range expansion of *P. leucopus* in the Midwest (Long 1996, Myers et al. 2005, 2009). Additionally, increases in the relative abundance of *P. leucopus* and even replacement of *P. maniculatus* by *P. leucopus* have been described in this region (Long 1996, Myers et al. 2005, 2009). It has been posited that shorter, milder winters may be a primary driver of range expansions and increases in relative abundance of *P. leucopus* (Long 1996, Myers et al. 2005, 2009, Roy-Dufresne et al. 2013). Based on our findings, tick-borne disease risk may increase in areas where *P. leucopus* becomes the more dominant species. Compared to its congener, we found *P. leucopus* to be infested with more than twice as many larval *I. scapularis* at our study area. Similarly, Larson et al. (2018) found approximately three times more larval *I. scapularis* infesting *P. leucopus* compared to *P. maniculatus*. Therefore, shifts in favor of *P. leucopus* may result in more larval ticks being fed overall. To further illustrate this point, we considered a hypothetical scenario in which a population comprised of 10 *P. maniculatus* is completely replaced by 10 *P. leucopus*, assuming all other factors are held constant. Based on the modified reservoir potential values for July 2019 (the month of highest modified reservoir potential during our study), complete replacement of *P. maniculatus* with *P. leucopus* would result in 113% increase in the number of ticks fed by mice and a 35% increase in the number of larval ticks infected with *Bo. burgdorferi* by mice.

Here, we established the first detection of three emerging tick-borne pathogens in *P. maniculatus*. Our results suggest that this mouse species is likely an important reservoir for tick-borne pathogens in the northernmost areas of the Midwest—in the areas where *I. scapularis* and the hallmark sign of Lyme disease were first reported and numerous tick-borne pathogens are endemic (Eisen et al. 2017). There is an urgent need to better understand differences in reservoir competency of these two species for the different tick-borne pathogens as the distributions and relative abundance of these mice species has been linked to climate (Myers et al. 2005, 2009, Roy-Dufresne et al. 2013). While future climate projections suggest the range of *P. leucopus* will expand poleward (Roy-Dufresne et al. 2013), it is not known if this species will also become the more dominant of the two forest-dwelling *Peromyscus* species throughout the Midwest. This region contains the trailing edge of *P. maniculatus* (Fig. 1); thus, future studies should examine how the distribution and relative abundances of these species will respond to warming temperatures as well as the potential impacts on human tick-borne disease risk.

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Data Availability

Data are available from the Center for Open Science: https://osf.io/xmtpe/?view_only=242ac04e5db44feb90361500f15046b8

Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3831/full