Ticks and Tick-borne Diseases 10 (2019) 1162–1167

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Rodent species as possible reservoirs of *Borrelia burgdorferi* in a prairie ecosystem

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ARTICLE INFO

Keywords:
Borrelia burgdorferi
Ictidomys
Microtus
Prairie
Reservoir
Zapus

ABSTRACT

Lyme borreliosis is the most commonly reported vector-borne disease in the United States and Europe. It is caused by a group of spirochete bacteria belonging to the *Borrelia burgdorferi* sensu lato complex. These pathogens are transmitted among vertebrate reservoir hosts through the bite of hard-bodied ticks. While the enzootic cycle of *Borrelia* transmission is well understood in its primary reservoir, the white-footed mouse, *Peromyscus leucopus*, far less is known about other reservoir hosts, particularly in grassland ecosystems. This study assessed the prevalence of *B. burgdorferi* s. l. among four non-*Peromyscus* rodents in a prairie ecosystem in the Midwestern United States over a four-year period. We found high prevalences of the bacteria in all four species studied. Our results help to support the roles of *Microtus* species as reservoirs of *B. burgdorferi* and add to the literature that suggests *Zapus hudsonius* may also be a reservoir. Additionally, we identified a previously unknown possible reservoir, *Ictidomys tridecemlineatus*. Our study also identifies the need to study the dynamics of Lyme borreliosis in habitats and areas outside of the typical range of *P. leucopus*.

1. Introduction

Lyme borreliosis is the most commonly diagnosed tick-borne disease in both the United States and Europe (Bratton et al., 2008; Stanek et al., 2011). In the United States, there has been a 60% increase in confirmed cases between 1996 and 2016 (CDC, 2017), with an additional 10,000 probable diagnoses in 2016. Additionally, diagnoses have occurred in each of the 48 contiguous states and Alaska, suggesting a widespread disease distribution (CDC, 2017). Extensive research has focused on the causative agent of Lyme borreliosis, *Borrelia (B.) burgdorferi* sensu lato (s. l.) (Rauter and Hartung, 2005; Rudenko et al., 2011), as well as the primary vectors of the disease in the US, the blacklegged tick, *Ixodes (I.) scapularis*, and the western black-legged tick, *I. pacificus* (Eisen et al., 2015; Halsey et al., 2018; Xu et al., 2018). Far fewer studies have focused on the reservoirs of the pathogen (Piesman and Gern, 2004; Tsao, 2009). The primary reservoir of *B. burgdorferi* is known to be the white-footed mouse, *Peromyscus leucopus* (Schwanz et al., 2011). However, Lyme diagnoses often occur outside of the range of *P. leucopus* (Oliver, 1996; Rudenko et al., 2016) and *B. burgdorferi* s. l. has been identified in ticks in habitats that are not utilized by *P. leucopus* (Burkot et al., 2000). As such, it is necessary to identify and assess other reservoirs of the bacteria.

Transmission of *B. burgdorferi* s. l. occurs through an enzootic cycle (Obiegala et al., 2017). Ticks typically acquire the spirochete in their larval or nymphal stage when consuming a blood meal from an infected reservoir host. While these stages commonly parasitize small mammals, nymphs are also known to feed on hosts of a wide variety of sizes (Keirans et al., 1996). Once infected, ticks retain the bacteria transstadially and are capable of transmitting it in subsequent feedings (Oliver et al., 2015). As such, infected nymphs are capable of transmitting the bacteria to new reservoir hosts or larger dead-end hosts (*e.g.* deer, dogs, humans) (Little et al., 2010). By comparison, adult ticks primarily feed on large hosts such as deer, which are incompetent for the bacterium (Randolph, 1998). Given that there is no evidence of transovarial transmission of *B. burgdorferi* s. l. in ticks (Kwan et al., 2017; Rollend et al., 2013), reservoir species play an essential role in the transmission cycle.

While the role of *P. leucopus* as the primary reservoir of *B. burgdorferi* sensu stricto (s. s.) is well established, other studies have identified several non-Peromyscus species as possible disease reservoirs (Hanincová et al., 2006; Tsao, 2009; Vuong et al., 2014). Of those, the majority are woodland species: *Sciurus* spp. (Roy et al., 2017) and *Tamias striatus* (Slajchert et al., 1997), with the exception of *Blarina brevicauda* which is a fossorial species (Telford et al., 1990). Among non-Peromyscine reservoirs, the role of the meadow vole, *Microtus pennsylvanicus*, is of particular interest. Several studies indicate that *M.*

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https://doi.org/10.1016/j.ttbdis.2019.06.011
Received 7 December 2018; Received in revised form 11 June 2019; Accepted 12 June 2019

pennsylvanicus is a reservoir of *B. burgdorferi* s. l. (Callister et al., 1991. However, in areas of co-habitation with *P. leucopus* and *T. striatus*, infection rates are markedly lower in *M. pennsylvanicus* (Mather et al., 1989). This is consistent with other studies that have demonstrated low tick loads in non-Peromyscine species when *P. leucopus* is present (Oliver et al., 1999). Combined, these findings suggest that *Microtus* spp. may be an important reservoir host in ecosystems where *P. leucopus* is absent or in low abundance.

While *P. leucopus* is a habitat generalist with the ability to utilize a variety of habitats, it has a preference for woodland and brush habitats (Berl et al., 2018). Compared to woodland systems, grassland systems have high *Microtus* abundance and low *P. leucopus* abundance (Payne, 1999; Snyder and Best, 1988). Yet little attention has been dedicated to the role of these ecosystems in maintaining Lyme borreliosis. While previous research suggested that prairie systems are less suitable for *I. scapularis* than woodland areas (Mather et al., 1989), the few studies that have focused on prairie systems have found high levels of *B. burgdorferi* infection (Rydzewski et al., 2011; Zeidner et al., 2000). In one such study, *B. burgdorferi* s. l. was identified in 86.6% of prairie voles, *Microtus ochrogaster*, in northern Colorado (Zeidner et al., 2000). Subsequent experiments also demonstrated that ticks isolated from *M. ochrogaster* at the same site were able to transmit *Borrelia bissettiae* to other *M. ochrogaster* in a laboratory setting (Burkot et al., 2000), suggesting a possible reservoir role for this species.

Studies in prairie systems should be of particular interest given that the upper Midwestern US is one of two major foci of Lyme infections (Walter et al., 2017). Additionally, this area is home to a large percentage of the remaining tallgrass prairies in the US. While more than 90% of tallgrass prairie habitats have been converted for residential and commercial use (Samson et al., 2004), recent years have seen extensive efforts to preserve remnant prairie habitats and restore historic habitats (Ruiz-Jaen and Aide, 2005; Wortley et al., 2013). One consequence of this restoration effort, is that it brings humans into close proximity to these ecosystems, as many restoration efforts occur in and near suburban areas (Fischer et al., 2013; Klaus, 2013). While this has marked benefits from conservation, education, and recreation perspectives, it also increases the likelihood of pathogen spillover due to contact with tick vectors and reservoir hosts (Alexander et al., 2018; McDaniel et al., 2018). As such, it is imperative to assess the prevalence of tick-borne diseases and the reservoir status of infected animals in prairie systems.

In this study, we sought to identify the presence and prevalence of *B. burgdorferi* s. l. among prairie-dependent rodent species in a restored suburban tallgrass prairie system. We hypothesized that overall infection prevalence was high and predicted that several species may represent possible reservoirs for Lyme borreliosis.

2. Material and methods

2.1. Study site

All sampling was conducted at the Distillery Road Conservation Area in Boone County, Illinois (42.2589 °N, 88.9095 °W). This site was previously used for agricultural purposes and was restored to ˜30 ha of tallgrass prairie beginning in 2003. Restoration involved a combination of controlled burns, removal of invasive species, and planting of native grasses and forbs. Primary restoration occurred from 2003 to 2010 in stages, resulting in seven contiguous patches in various stages of ecological succession. The restoration is maintained on a three-year cycle of controlled burns and spot herbicide treatment for invasive species control (BCCD, 2018).

2.2. Sample collection

Two live trapping grids, each with an area of 0.6 ha, were established on the restoration site in 2014. The first and second grids were placed on the patches restored in 2003 and 2008, respectively. The two grids were separated by ˜250 m of contiguous prairie. Each grid consisted of 60 trap stations (four rows of 15 traps) with each trap station separated by 10 m (40 m x 150 m). We placed a modified Fitch trap (Rose, 1973) or Sherman live trap (H. B. Sherman Co., Tallahassee, FL) at each station in an alternating pattern. Animals were trapped twice a week from mid-May to mid-September from 2014 to 2017. During trapping season, each trap was baited with Kaytee Squirrel and Critter Blend (Kaytee Products, Chilton, WI) and set between 1600 and 1800 h. Traps were checked the next morning between 0600 and 0800 h in order to maximize capture of both nocturnal and diurnal species.

We collected demographic data on each captured individual (species, age class, sex, reproductive condition, and mass). The distal phalanx of the second lateral digit of one hind foot was removed from each unique individual for disease identification. Tissue samples were stored in 70% ethanol for later DNA extraction. Toe clips were chosen over other methods for multiple reasons. First, they provided a permanent means of identifying previously captured animals. They were superior to ear punches due to the small pinna size of some of the rodent species. Tail clips were not a viable option due to the tendency of tails to deglove when handled for some rodent species. Additionally, retroorbital bleeding was dismissed as a possibility due to a typical lack of *B. burgdorferi* in circulating blood, the minimal animal handling experience among undergraduate students involved in the study, and the necessity to anesthetize the animals. Following tissue collection, animals were assessed for bleeding and released at the capture site within minutes. In the event of a recapture, tissue samples were not collected again. All protocols for animal handling and tissue sampling were approved by the Illinois Department of Natural Resources and the Boone County Conservation District. Methods complied with the guidelines of the National Institute of Health and the American Society of Mammalogists (National Research Council, 2010, Sikes et al., 2016).

2.3. DNA extraction

Prior to DNA extraction of samples, we used a sterile scalpel blade to cut up toe clips in order to maximize the surface area. Samples were then extracted using a standard ammonium acetate protocol with ethanol precipitation. After extraction was completed, DNA was resuspended in 50 μl of Tris-EDTA buffer and stored at −20 °C. Negative controls were not employed during the extraction process.

2.4. Verification of species identification

It is difficult to distinguish *M. ochrogaster* from *M. pennsylvanicus* in the field, particularly in the case of juveniles. Species identification was achieved by PCR of a microsatellite locus of the *avpr1a* gene following established methods (Henterly et al., 2011). Reactions were performed in 50 μl volumes containing 25 μl GoTaq Master Mix (Promega Corp., Madison, WI), 1 μl $MgCl_2$, 1 μl of each primer in 10- μM concentrations, and 1 μL of sample DNA. Primers were the same as those of Hammock and Young (2005), forward 5′GTATTGCCACAAATAGACCAACG, reverse 5′GTAAGGATGACAGGCGTTACTG. PCR conditions were: 94 °C for 5 min; followed by 10 cycles of (94 °C, 15 s; 58 °C, 30 s; 72 °C, 45 s); then 20 cycles of (94 °C, 15 s; 58 °C, 30 s; 72 °C, 45 s + 5 s each round); then 72 °C for 7 min followed by a 4 °C hold. Products were loaded onto a 2% agarose gel containing 1x TBE buffer and Diamond Nucleic Acid Dye (Promega Corp, Madison, WI). Gels were electrophoresed for 90 min at 100 V before visualization. Samples with products between 600 and 800 bp were identified as *M. ochrogaster* and those with products between 200 and 300 bp were identified as *M. pennsylvanicus*.

2.5. Identification of B. burgdorferi s. l

Nested PCR amplification of *flagellin B* (*FlaB*) and *outer surface protein B* (*ospB*) were conducted separately in order to identify the presence of *Borrelia* DNA in rodent samples. Both loci were amplified

Table 1
Primers and annealing temperatures for *B. burgdorferi* amplification.

Locus	Primer name	Annealing temperature (°C)	Primer sequence
Outer Surface Protein B (*ospB*)	Outer F	52	5'-GGTGCTGAGTCAATTGGTTCT-3'
	Outer R		5'-TTCTAGGCTGGTTCCAGCTGT-3'
	Inner F	52	5'- TTTTCCGACTACAAGACTTCC-3'
	Inner R		5'- TTAGAAGCATTTGATGCCAGC-3'
Flagellin B (*flaB*)	Outer F	52	5'-AARGAATTGGCAGTTCAATC-3'
	Outer R		5'-GCATTTTCWATTTTAGCAAGTGATG-3'
	Inner F	55	5'-ACATATTCAGATGCAGACAGAGGTTCTA-3'
	Inner R		5'-GAAGGTGCTGTAGCAGGTGCTGGCTGT-3'

using primers specific to *B. burgdorferi* s. l. (Table 1) (Clark et al., 2005; Lee et al., 2014). Initial PCR was conducted in a 50 µl reaction containing 25 µl GoTaq Master Mix (Promega Corp., Madison, WI), 1 µl $MgCl_2$, 1 µl of each primer in 10-µM concentrations, and 5 µL of sample DNA. Five microliters of the product of these reactions were then used for nested PCR reactions containing 25 µl GoTaq Master Mix (Promega Corp., Madison, WI), and 1 µl of each inner primer in 10-µM concentrations. The amplification conditions for *OspB* were: 95 °C for 5 min; followed by 30 cycles of (94 °C, 30 s; 52 °C, 60 s; 72 °C, 120 s); then 72 °C for 7 min followed by a 4 °C hold. Conditions were the same for both outer and inner protocols. For *FlaB*, conditions were: 95 °C for 5 min; followed by 40 cycles of (94 °C, 30 s; annealing temp., 30 s; 72 °C, 60 s); then 72 °C for 7 min followed by a 4 °C hold. Outer and inner protocols only differed in their annealing temperature (Table 1). Each set of reactions was run with a single positive control obtained from the Paskewitz lab at the University of Wisconsin-Madison and a single negative control containing sterile water instead of DNA. If a positive control failed to amplify then the set of reactions was re-run.

Following the inner PCR, products were electrophoresed for 120 min at 100 V on a 2% agarose gel containing 1x TBE buffer and Diamond Nucleic Acid Dye (Promega Corp, Madison, WI). The presence of an ~200 bp band indicated a sample that was positive for *B. burgdorferi ospB*. Likewise, the presence of a 389 bp band indicated a sample was positive for *flaB*. In order to be considered positive, a sample must amplify successfully for both genes. In the event of a discrepancy between the two loci, samples were re-run for both loci to verify amplification.

2.6. Nucleotide sequencing

To verify pathogen presence and identity, five positive samples from each of four species (total of 20 samples) were then randomly selected for sequencing at the *flaB* gene to verify that the amplicons represented *B. burgdorferi*. Amplicons were gel-extracted using the method of Sun et al., 2012 and sequenced in both directions (Eurofins MWG). Resulting sequences were aligned using BioEdit version 7.0.5 and sequence identity was determined using BLAST (Altschul et al., 1990). Identity was further confirmed by comparing our sequences to reference sequences obtained from GenBank for *B. burgdorferi* (CP017201), *B. bissettiae* (CP002746), *B. andersonii* (D83763), and *B. afzelii* (CP009058). Sequences were then deposited in GenBank (accession numbers MK716256-MK716275).

2.7. Statistical analyses

Capture rates within species were compared across years using a chi-square test to identify interannual variation. We then calculated the average infection prevalence over the course of the study and used the Clopper-Pearson method to determine the 95% confidence interval of those prevalences. Infection prevalence was compared across species using a chi-square test of independence.

3. Results

Over the four-year study period, 853 unique individuals were captured, sampled, and screened for the presence of *B. burgdorferi*. These captures represented four rodent species: the thirteen-lined ground squirrel (*I. tridecemlineatus*), the meadow jumping mouse (*Zapus hudsonius*), the meadow vole (*M. pennsylvanicus*), and the prairie vole (*M. ochrogaster*). Microsatellite genotyping of the *av1pra* locus confirmed that the majority of voles captured were *M. pennsylvanicus*. *Microtus pennsylvanicus* was the most abundant species overall (349 individuals), followed by *I. tridecemlineatus* (234), *Z. hudsonius* (188), and *M. ochrogaster* (82).

Capture rates of *I. tridecemlineatus* ($\chi^2 = 1.043$, $p = 0.7909$) and *M. ochrogaster* ($\chi^2 = 4.146$, $p = 0.2461$) did not vary significantly over the course of the study. However, both *Z. hudsonius* ($\chi^2 = 31.064$, $p < 0.0001$) and *M. pennsylvanicus* ($\chi^2 = 52.547$, $p < 0.0001$) exhibited significant fluctuations. It is worth noting that when *M. pennsylvanicus* populations were lower, the *Z. hudsonius* populations were higher (Fig. 1).

Of the animals captured, 32.5% (277/853) tested positive for the presence of *B. burgdorferi* s. l. DNA at both loci. No samples were positive at one locus and not the other. All of the negative controls tested negative for *B. burgdorferi* throughout the study and 93% of positive controls amplified during their first run. All positive controls that failed to amplify on their first run, were successfully amplified on a second run. All twenty amplicons of the *flaB* gene that were sequenced were confirmed to be *B. burgdorferi* s. s. with a sequence identity ranging from 99.48 to 100%. Sequences samples exhibited a sequence identity < 97% with reference sequences from other *Borrelia* species.

Among captured species, *M. pennsylvanicus* exhibited the highest prevalence of infection (37.5%, 95% CI: 32.4–42.9%), followed closely by *M. ochrogaster* (35.4%, 95% CI: 25.1–46.7%). The smaller sample

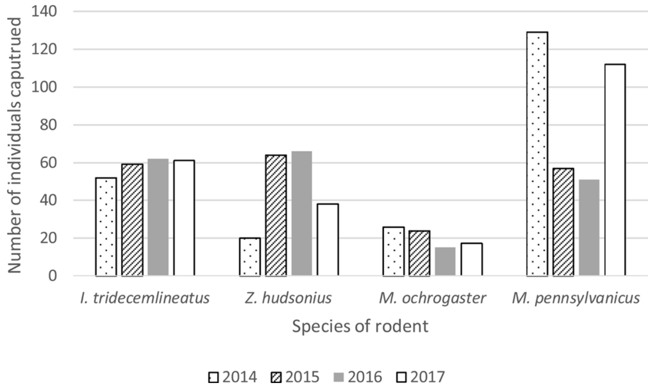

Fig. 1. Captures by species per year. Number of individuals of four rodent species captured over a four year period from 2014 to 2017. Populations of *Ictidomys tridecemlineatus* and *Microtus ochrogaster* did not fluctuate dramatically during the study. Populations of *Zapus hudsonius* ($\chi^2 = 31.064$, $p < 0.0001$) and *Microtus pennsylvanicus* ($\chi^2 = 52.547$, $p < 0.0001$) exhibited significant fluctuations.

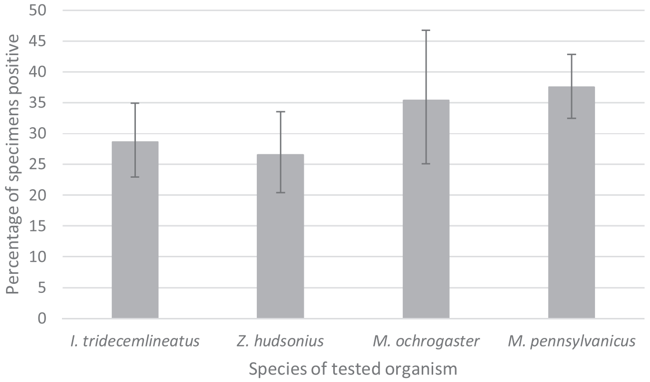

Fig. 2. Prevalence of *Borrelia burgdorferi* by rodent host species. Percentage of captured individuals infected with *Borrelia burgdorferi* sensu lato for each of four species of rodents. Rodents were captured over a four-year period from 2014 to 2017 and infections were identified by PCR amplification of two genes, *ospB* and *flaB*. There are significant differences in infection prevalence between species (χ^2 = 8.928, df = 3, p = 0.0303). Shown are the means and the 95% confidence intervals.

size of *M. ochrogaster* resulted in a much wider confidence interval than *M. pennsylvanicus*. Infection rates in *I. tridecemlineatus* (28.6%, 95% CI: 22.9–34.9%) and *Z. hudsonius* (26.6%, 95% CI: 20.4–33.5%) were lower but still greater than 25%. The results of the chi-square test indicated that there was a significant difference in infection prevalence among the four rodent species (χ^2 = 8.928, df = 3, p = 0.0303) (Fig. 2).

Blacklegged ticks were observed on several animals over the course of the study, but this data was not collected systematically or consistently over the four-year period. When observed, ticks were identified to species and the tick burden was counted for the animal. Only five *Z. hudsonius* were observed with engorged ticks during the study, and in all cases a single tick was found. Ticks were found on 16 voles during the course of the study. In two cases, two ticks were found engorged on the same vole, and in one case, three ticks were found. By comparison, ticks were found on 25 *I. tridecemlineatus*. Numbers ranged from a single tick to eleven on a single animal, with 14 animals (56%) harboring multiple ticks.

4. Discussion

All four rodent species in this study exhibited a high prevalence of *B. burgdorferi* s. l. infections. Consistent with our hypothesis, nearly one-third of the rodents captured over the course of this study were positive for *B. burgdorferi* s. l., indicating a high prevalence of Lyme borreliosis in this particular prairie ecosystem. Additionally, each species exhibited an infection prevalence > 25%, indicating that each of them may play a role in the maintenance and enzootic transmission of *B. burgdorferi* in prairie systems. All four species exhibited a higher infection prevalence than has recently been reported for *B. burgdorferi* s. s. in *P. leucopus* in either the upper Midwest or the Northeast (Larson et al., 2018; Oliver et al., 2006). These data suggest that each of these species may serve as reservoirs of *B. burgdorferi* in the absence of *P. leucopus*. However, given that these are only prevalence data, additional research, including transmission experiments, are needed to validate this.

Of the four rodent species present in this study, the two *Microtus* species are of particular interest. Both *M. ochrogaster* and *M. pennsylvanicus* exhibited the highest prevalence of *B. burgdorferi* among study species. While *M. ochrogaster* was the least abundant species at this site (9.5% of captures), it accounted for 29 positive specimens over the four-year period (35.4% infection prevalence) indicating it likely serves an important role in the enzootic cycle of *B. burgdorferi* in ecosystems where it has higher relative abundance. Previous analyses in the western United States have already indicated that *M. ochrogaster* is a reservoir for *B. burgdorferi* (Burkot et al., 2000; Zeidner et al., 2000). Our study supports these findings and these combined data suggest a reservoir role for this species in Midwestern prairies.

Likewise, the competence of *M. pennsylvanicus* to transmit *B. burgdorferi* has previously been established (Markowski et al., 1998; Mather et al., 1989). However, both Markowski et al. (1998) and Mather et al. (1989) indicated that *P. leucopus* was the preferred host of Lyme-transmitting ticks and that *M. pennsylvanicus* did not play a major role in pathogen transmission when *P. leucopus* was present. In our study *M. pennsylvanicus* had the highest infection prevalence of the four species studied. Additionally, it was the most abundant species in our study (40.9% of captures) and was responsible for nearly half of all samples which tested positive for *B. burgdorferi* (47.3%). These data, combined with the results of experiments by Burkot et al. (2000) and Markowski et al. (1998) suggest that *Microtus* species serve as reservoirs in systems where *P. leucopus* is absent or at low abundance, such as Midwestern prairies. Pathogen transmission experiments between *I. scapularis* and these two *Microtus* species are needed to validate these findings.

Of the other two host species in this study, there is limited information available on their roles as reservoir hosts for *B. burgdorferi*. A previous study identified *B. burgdorferi* s. l. in *Z. hudsonius* in northeastern Illinois (Picken and Picken, 2000), but only five positive individuals were identified over three years, and the study was conducted in an urban setting. Similarly, Hamer et al. (2010) identified *B. burgdorferi* s. s. in *Z. hudsonius* in coastal Michigan, but this study was based on a small sample size (28 samples in five years). In our study, *Z. hudsonius* was highly abundant (22.0% of captures) and exhibited a high prevalence of *B. burgdorferi* (26.6% infection prevalence). However, it should be noted that of the species in this study, it had the lowest relative number of positive samples (18.1% of overall infections). This may be due in part to the small size of *Z. hudsonius*. Members of *Z. hudsonius* typically have a smaller body and weigh less than other small mammals, including *P. leucopus* (Lackey et al., 1985; Whitaker, 1972), which likely limits the tick load of individuals. However, infection rates were still higher than those observed for *B. burgdorferi* s. s. in *P. leucopus* in parts of the Midwest (Hamer et al., 2010; Larson et al., 2018; Wang et al., 2014) and consistent with those observed for *B. burgdorferi* s. l. at other midwestern sites (Hofmeister et al., 1998; Jackson et al., 2002). These data demonstrate a need to further assess the role of this species in the enzootic transmission of *B. burgdorferi* s. l. through transmission experiments and study in other ecosystems.

While no study has previously identified *B. burgdorferi* in *I. tridecemlineatus*, the species has been shown to be infected by other arthropod-borne pathogens (Beckmann et al., unpublished results; Stapp et al., 2008). Additionally, another ground squirrel, *Spermophilus lateralis*, has been shown to harbor *B. bissettiae* (DeNatale et al., 2002), while other Sciurids are reservoirs of *B. burgdorferi* s. l. (Tsao, 2009). The large relative body size of *I. tridecemlineatus* permits for high tick loads and parasitism across all tick life stages. In this study, *I. tridecemlineatus* consistently had the highest tick loads with up to eleven ticks co-feeding on a single animal, as well as engorged ticks of all three life stages. These observations, combined with the infection rate data presented above, indicate that a greater research focus is needed on *I. tridecemlineatus* to determine whether it is a reservoir of *B. burgdorferi* or serves as a dead-end host which may reduce pathogen transmission in prairie systems.

The overall prevalence of *B. burgdorferi* at this site is high (32.5%). This prevalence exceeds that of *B. burgdorferi* s. s. found in mammals, including *Peromyscus*, in Michigan (Hamer et al., 2010) and northern Wisconsin (Larson et al., 2018); and is also higher than the prevalence of *B. burgdorferi* s. l. in Ohio (Wang et al., 2014). It is also comparable to the prevalence of *B. burgdorferi* s. l. identified in *Peromyscus* across sites in Wisconsin and Minnesota (Hofmeister et al., 1998; Jackson et al., 2002). However, it is lower than that identified by Johnson et al (2017) in *P. leucopus* in Minnesota. This is consistent with the high incidence of Lyme disease in people in eastern Minnesota when compared with other parts of the upper Midwest (CDC, 2017). Likewise, the prevalence in the current study exceeds that found for *B. burgdorferi* s. l. in parts of the northeastern United States (Oliver et al., 2006). However, the prevalence in the current study, falls well below that observed in areas such as Connecticut (Bunikis et al., 2004), where prevalence of *B. burgdorferi* s. s. reached 75% in *P. leucopus*, and Maryland (Hofmeister et al., 1999) where prevalence of *b. burgdorferi* s. l. reached 42%. These rates demonstrate the fact that the northeastern United States remains the primary focus of Lyme disease (CDC, 2017).

Overall, our results represent the largest study performed to date to assess the prevalence of *B. burgdorferi* s. l. among prairie rodents in the United States. While Mather et al. (1989) indicated that grasslands were unsuitable for *Borrelia* transmission, our results contribute to a growing literature base that *B. burgdorferi* is prevalent in prairie ecosystems (Gassner et al., 2013; Rydzewski et al., 2011; Zeidner et al., 2000). This is consistent with recent literature that indicates a need to more thoroughly assess non-*Peromyscus* hosts for the prevalence of *B. burgdorferi* (Halsey et al., 2018). Our results identify a clear role of *M. ochrogaster* and *M. pennsylvanicus* in the enzootic cycle of Lyme borreliosis in prairie systems, in addition to the possible role of both *I. tridecemlineatus* and *Z. hudsonius*. In the case of the latter two species, additional research is needed to experimentally assess their reservoir competence and determine if they are involved in pathogen transmission or if they are dead-end hosts. Ultimately, this study demonstrates that additional research is needed to assess the prevalence of *B. burgdorferi*, and tick-borne diseases as a whole, in grassland ecosystems throughout the Midwestern United States.

Declaration of competing interest

The authors affirm they do not have any conflicts of interest related to this manuscript. This research was partially funded by the Community Foundation of Northern Illinois and intramural funding from Rockford University and Stetson University.

Acknowledgements

The authors would like to thank the numerous undergraduates who were involved in the collection and processing of data. In particular we would like to thank Matthew Davis and Michael Hoefer for their contributions to data acquisition and analyses. We would also like to thank Rockford University, Stetson University and the Northern Illinois Community Foundation for funding to complete this study. Dr. Robert Rose provided modified Fitch traps which were essential to the completion of this study. Additionally, the Boone County Conservation District was instrumental in providing support, as well as access to the study site. In particular, the efforts of Dan Kane and Josh Sage were instrumental to the conduct of this study.

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