Assessing *Borrelia burgdorferi* in small mammal communities in Western Pennsylvania

by

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Abstract

Tick-borne disease cases are on the rise in the USA. In Pennsylvania, multiple tick-borne pathogens such as *Borrelia burgdorferi*, *Babesia microti*, and *Anaplasma phagocytophilum* cause disease in humans. These pathogens are transmitted to humans and other animals through tick vectors like *Ixodes scapularis* Say, one of the most common infectious agents in the state and country. Like multiple tick species, *I. scapularis* are hematophagous and feed on birds, amphibians, reptiles, and mammals, including humans. Therefore, they can infect and become infected with pathogens during their blood meals. During a blood meal, pathogens can be transmitted to a competent host that can maintain the pathogens. The competent hosts can be rodents and other small mammals found abundantly in Pennsylvania's wildlife. For example, *Peromyscus leucopus*, the white-footed mouse, is considered a reservoir host for multiple tick-borne pathogens like *B. burgdorferi*, *B. microti*, and *A. phagocytophilum*. The potential infection risk to humans is assessed by looking at the prevalence of the nymphal ticks and the prevalence of the pathogen in the hosts. This study surveys the hosts and the presence of infection in the small mammals of Western Pennsylvania for a period of two years. Small mammals were identified, and samples were collected at six different parks of the region during the summer of 2021 and 2022. A total of 208 and 282 individual small mammals belonging to a wide range of species were collected in 2021 and 2022, respectively. White-footed mice, eastern chipmunks, and shrews were the most abundantly collected species. The density and diversity of these populations

impacted the prevalence of *Borrelia* infection at different sites. The study also showed that the prevalence of *B. burgdorferi* at urban sites became more comparable to the prevalence at rural sites during the two years studied.

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PREFACE

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1.0 INTRODUCTION

1.1 Background

Tick-borne pathogens, including bacteria, viruses, protozoa, and fungi, are a major cause of morbidity and mortality in humans and animals worldwide (Boulanger et al., 2019; Brites-Neto et al., 2015; Lippi et al., 2021; Rochlin & Toledo, 2020). Tick-borne diseases are a financial burden in multiple countries around the world. For example, the cost of diagnosed tick-borne diseases was estimated to cost as much as $969 million to the US economy in 2016 (Hook et al., 2022). Furthermore, treatment of tick-borne disease causes economic hardship in developing countries due to infection in livestock and domesticated animals (Boulanger et al., 2019; Kivaria, 2006; Mac et al., 2019; Rochlin & Toledo, 2020). A recent review on tick-borne diseases concluded that more effort on research, control, and management of tick-borne diseases in the USA and other developed countries is desperately needed (Mac et al., 2019). Since the discovery of the first agent of tick-borne diseases in the 19th century, many more pathogens have been discovered. Multiple studies have been conducted worldwide to understand different tick-borne pathogens and how to prevent or treat them in humans and animals. Current surveillance and control allow us to understand the expansion of tick-borne diseases worldwide, with an emphasis on work occurring in Europe and North America (Lippi et al., 2021).

Tick-borne pathogens and their associative diseases have been emerging and expanding globally. In North America, an increase in the number of cases of tick-borne diseases such as Lyme disease, babesiosis, anaplasmosis, ehrlichiosis, spotted fever rickettsiosis, and tularemia have been recorded. These represent most of the vector-borne diseases in the USA. The total number of all

reported cases of tick-borne diseases has doubled in the past decades and accounts for more than three out of every four cases of vector-borne diseases (Rosenberg et al., 2018). Eastern states of the USA rank among the highest incidences of reported tick-borne diseases (Fig. 1). Pennsylvania is one of the states with the most reported cases and counts, and in 2020 it reported 3334 cases of Lyme disease, 216 cases of anaplasmosis, 33 cases of ehrlichiosis, 29 cases of spotted fever rickettsiosis, and 40 cases of babesiosis in humans (Pennysvania Department of Health, Health, 2022). Although an overall increasing trend has recently been observed in the number of reported cases of all these diseases, the true burden of Babesiosis in PA is unclear because it is still not a reportable disease in the states. Therefore, currently case reporting relies on physicians and laboratories (Pennsylvania Department of Health, 2022).

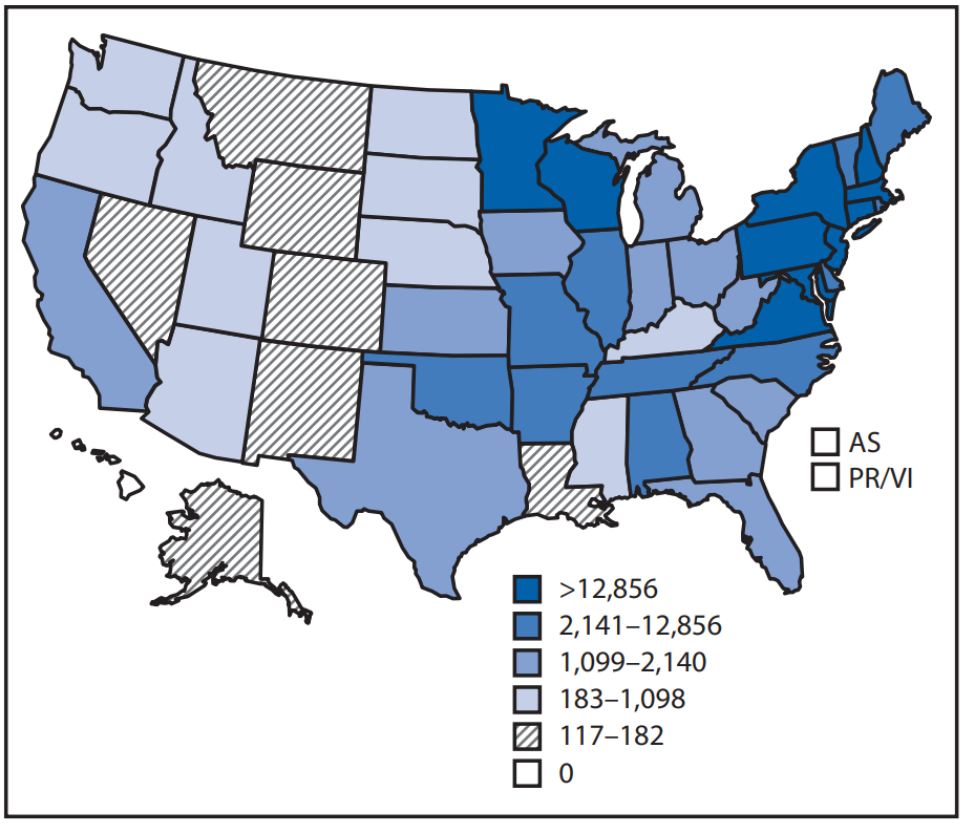


Figure 1. Reported cases of tick-borne diseases in USA and US territories. This map shows the reported cases of tick-borne diseases in the country. They are classified in quintile. Retrieved from Rosenberg et al., 2018.

1.2 Lyme disease and *Borrelia burgdorferi*

Lyme borreliosis is a zoonotic disease caused by the bacterial agent *B. burgdorferi.* It is an acute disease that is treatable if caught early but could lead to chronic conditions, such as Lyme neuroborreliosis (Boulanger et al., 2019; Ford & Tufts, 2021; Steere et al., 2016). The most common clinical manifestation of Lyme borreliosis in the USA includes myalgia, fever, fatigue, headache, and erythema migrans rash (Sanchez, 2015; Steere et al., 2016). Lyme disease is the

most common tick-borne disease and is prevalent in Europe and North America, where it has been expanding and gradually becoming a significant public health issue. Its expansion is associated with the spread of the vector of the pathogen, *Ixodes* spp. ticks (Pepin et al., 2012). In the USA, Lyme disease accounts for most of the vector-borne diseases in the country (Rosenberg et al., 2018). Found in upper midwestern, northeastern, and mid-Atlantic states, it has been increasing in prevalence, incidence, and geographical locations over recent decades (Eisen et al., 2017; Rosenberg et al., 2018) (Fig. 2). Similar to the general trend for tick-borne diseases as a whole, the number of reported cases of Lyme disease in southern states is observably lower than in northeastern states due to factors such as climate, the presence of the vector, and a shift in vector feeding behavior for non-competent hosts (i.e., reptiles) (Ginsberg et al., 2021). In Pennsylvania, Lyme disease was mainly reported in eastern counties in the early 2000s, but by the mid-2000s, the state had an increased number of reported cases in the western counties as well (Eddens et al., 2019). The bacterial agent causing this disease belongs to the *Borrelia* species.

Borrelia species are spirochetal gram-negative bacteria from the Spirochaetaceae family. There are two main groups in the *Borrelia* genus, one that includes species known to cause Lyme disease and the other which includes species that cause relapsing fever (Boulanger et al., 2019; Brites-Neto et al., 2015; Dantas-Torres et al., 2012; Rochlin & Toledo, 2020). The *B. burgdorferi* sensu lato genospecies complex includes multiple genospecies that can cause illness in humans. For instance, *B. garinii* and *B. afzelii* are the main species causing disease in Europe, while *B. burgdorferi* sensu stricto (ss) is the main species that causes disease in North America (Espí et al., 2017; Marques et al., 2021; Sanchez, 2015; Steere et al., 2016). The clinical manifestations exhibited by patients are generally similar in the two regions, however, some differences are observed based on the causing agent of the illness. For instance, erythema migrans (EM or bull's

eye rash) are a common clinical manifestation of the disease in the USA and Europe (Brites-Neto et al., 2015; Marques et al., 2021). However, people with a bull's eye rash caused by with *B. burgdorferi* ss are less likely to remember being bitten than those infected by *B. afzelli* or *B. garini* (Marques et al., 2021). In addition, people infected by *B. burgdorferi* ss are more likely to exhibit arthritic like symptoms and people infected with *B. garini* will more likely exhibit neurological symptoms (Marques et al., 2021).

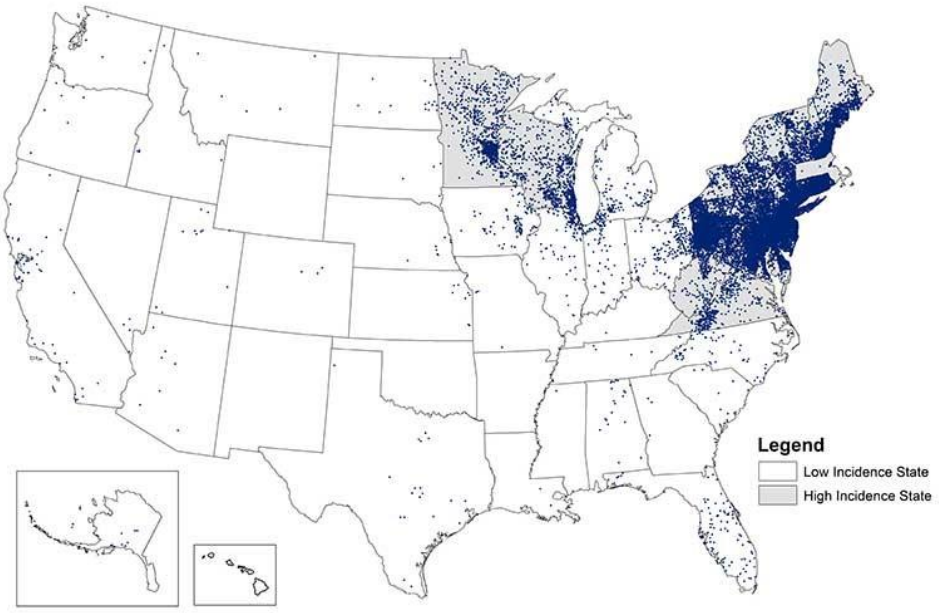


Figure 2. Reported Lyme disease in the USA in 2018. This map shows the reported Lyme disease cases in the USA. Each dot represents one case, and cases are reported from the county of residence of the infected person. Retrieved from CDC 2021.

1.3 Ticks and *Ixodes scapularis* Say (blacklegged tick or deer tick)

Lyme disease is transmitted to humans through the means of tick-vectors. Ticks are hematophagous arthropods that can carry and transmit multiple pathogens to humans and animals. They are subdivided into three families: hard ticks (Ixodidae), soft ticks (Argasidae), and Nuttalliellidae (Boulanger et al., 2019; Brites-Neto et al., 2015; Guglielmone et al., 2010). There are approximately 700 species of Ixodidae, 200 species of Argasidae, and only one species of Nuttalliellidae (Dantas-Torres et al., 2012; Guglielmone et al., 2010; Kolonin, 2007). These arthropods can be very adaptable to surviving in different climates and geographical conditions where they can feed on hosts. Due to their high resiliency, they can be found on all continents (including Antarctica where they survive by feeding on penguins) (Benoit et al., 2007). Although some ticks can be adaptable like *I. ricinus* (found in North Africa and all over Europe, from sea level to 1,500 m elevation in the European Alps), certain tick species like *I. vespertilionis* mainly survive feeding on bats in caves, attics, and other similar sheltered environment in Europe (Estrada-Peña et al., 2018; Gilbert, 2021). While ticks are very adaptable to various areas, they generally thrive the best in humid temperatures near grass and leaf cover where they can easily attach to a host for a blood meal (Sonenshine & Roe, 2013). Ticks are hematophagous meaning they must obtain a blood meal from a host at each life stage in order to molt into the subsequent life stage. Their life cycle includes four development stages, eggs, larvae, nymph, and adult which are normally completed over 2–3 years for Ixodid ticks and up to 20 years for Argasid ticks (Apanaskevich et al., 2013; Boulanger et al., 2019; Parola & Raoult, 2001; Sonenshine & Roe, 2013). Hard and some soft ticks can have one, two, or three host(s) per life cycle (McCoy et al., 2013; Sonenshine & Roe, 2013). In other words, during their life cycle they will either require one, two, or three blood meals taken at a specific life stage. For instance, a three-life cycle tick, like *I.*

scapularis, after hatching a larva will attach and feed on a host. The larva will then detach and molt into a nymph before subsequently questing and feeding on another host (or the same host). The nymph will detach and re-emerge as an adult that will need to seek out another host. Through their feeding, ticks can carry, transmit, and spread pathogens to different hosts during their life cycle (Fig. 3).

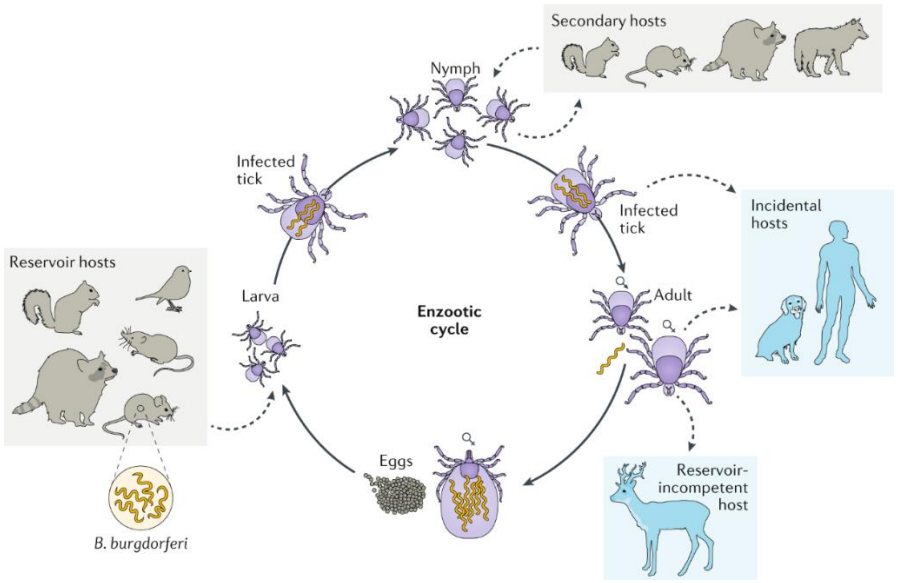


Figure 3. *Borrelia burgdorferi* and *I. scapularis* life cycle. Retrieved from Kurokawa et al. 2020. This image shows a three-tick host life cycle. It also shows the different hosts implicated in the spread of the pathogens. A tick can feed on reservoir, secondary, or incidental hosts during its life cycle. For example, a larva can acquire its blood meal from an infected reservoir host. A reservoir host helps maintain the pathogen in the population because they are more likely to be infected and to infect the pathogen when another vector feeds on them. After detaching and molting, the larva tick will become an infected nymph that will quest for a secondary host. The secondary host offers another chance of infection if the tick is not already infected. The infected nymph will hence infect this host, which could lead to the spreading of the pathogen when other ticks feed on the host.

After molting into an adult tick, it will find its blood meal from a human, dog, deer, or another large animal. Humans are incidental hosts, therefore if a tick feeds on them, the pathogen ends its cycle of spreading. Deer are incompetent hosts, therefore the *B. burgdorferi* will not be able to survive in this host and will not be transmitted to other ticks. After a successful bloodmeal is taken, an infected adult female will then lay eggs, when the larvae hatch, they will not be infected with *B. burgdorferi* as this pathogen is not transovarially transmitted. Overall, this figure shows the different factors involved in spreading a *B. burgdorferi* through the bloodmeal of ticks of three life stages to other hosts, which impacts transmission to more vectors.

Hard ticks are called hard ticks because they have a dorsal scutum, an anterodorsally sclerotized plate on their dorsal body, during all their life stages (except eggs) (Brites-Neto et al., 2015; Dantas-Torres et al., 2012; Ladislav et al., 2014; Sonenshine & Roe, 2014). The scutum covers the entire dorsum in adult males and only half in adult females. They do not have antennae and have four pairs of legs, except for larvae that have three pairs (Brites-Neto et al., 2015; Dantas-Torres et al., 2012; Sonenshine & Roe, 2014). Hard ticks also have a denticulate hypostome in their mouth and a sensory organ on the dorsal surface of one of the tick legs called Haller's organ (Brites-Neto et al., 2015; Sonenshine & Roe, 2014) . These features on the ticks' leg and mouth are important because they allow ticks to sense and attach to their host, respectively (Fig. 4). The ticks use denticles to anchor to the host's skin and Haller's organ to sense and localize the host (Ladislav et al., 2014; Sonenshine & Roe, 2014). Like many other ticks, hard ticks are small. *Ixodes scapularis*, a species in the hard tick family, are usually compared to a small poppy seed (measuring less than 0.039 inches) in their nymph life stage and are even smaller in their larvae stage (Fig. 4).

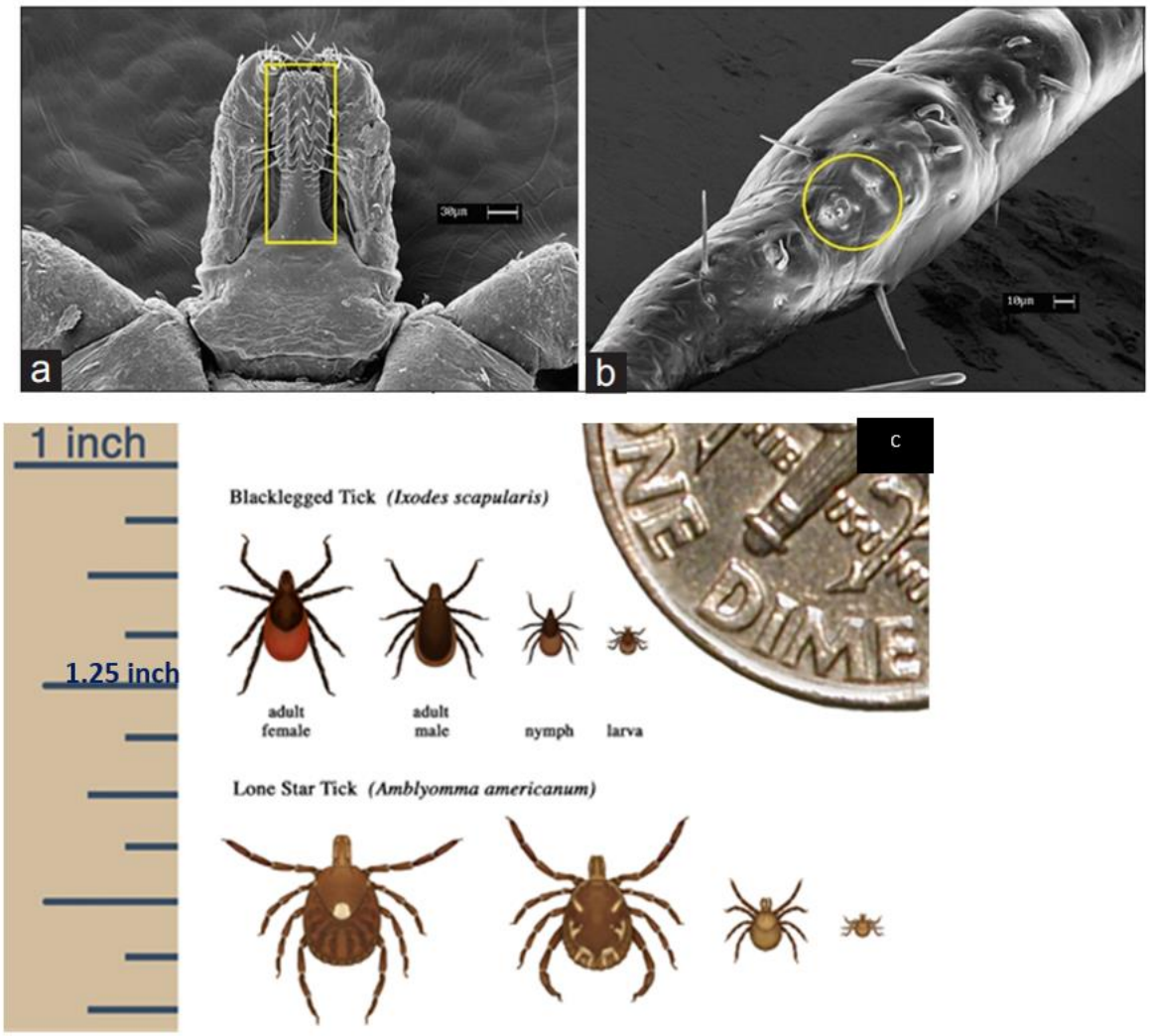


Figure 4. Tick morphology and size. A) shows the denticulate hypostome and B) haller's organ of a tick. Retrieved from Brites-Neto et al., 2015. C) shows the morphology and relative size of two species hard ticks. Retrieved from CDC 2022.

Ticks of the Ixodidae family are divided into 17 genera, with the most common genera including *Amblyomma, Dermacentor, Haemaphysalis, Hyalomma, Ixodes,* and *Rhipicephalus* (Estrada-Peña et al., 2018; Guglielmone & Robbins, 2018; Guglielmone et al., 2010). Hard ticks cause most of tick-borne infections and diseases observed in humans (Dantas-Torres et al., 2012). The most common tick species of health concern in the USA are *Amblyomma* spp., *Dermacentor* spp., and *Ixodes* spp. ticks. For instance, *Amblyomma* spp. ticks can carry and transmit several dangerous pathogens including *Ehrlichia* spp., *Rickettsia* spp., Southern tick-associated rash illness (STARI), Bourbon virus, and Heartland virus, while *Dermacentor* spp. are known to transmit Colorado tick fever, *Rickettsia* spp., and tularemia, and *Ixodes* spp. can transmit *Anaplasma phagocytophilum, Babesia* spp., *Borrelia* spp., and Powassan virus (Nathavitharana & Mitty, 2015; Nelder et al., 2016; Rochlin & Toledo, 2020). Different tick species may harbor and transmit diseases in different geographic locations. For instance, *I. ricinus* and *I. persulacatus* are known to transmit Lyme disease in Europe while *I. scapularis* and *I. pacificus* transmit it in North America (Boulanger et al., 2019).

Recent research studying the distribution of *Ixodes* spp. ticks in the USA found that *I. pacificus* is mainly located in Pacific coast states such as California and Oregon, while *I. scapularis* is primarily found in New York, Vermont, Maine, Pennsylvania, and other northeastern and mid-western states (Fig. 5) (Eisen et al., 2016; Hahn et al., 2016). The growing distribution of *Ixodes* spp. in the USA is one of the main reasons Lyme diseases and other tick-borne diseases have been expanding in the northeastern region of the USA. Biotic and abiotic factors, including climate change, forestation, host population, human behavior, etc., play a role in the expansion of tick spp. (Gilbert, 2021; Sonenshine, 2018). Various species of ticks of the Ixodidae reside in Pennsylvania. The five most common species in the state are *Amblyomma americanum*

Dermacentor variabilis, *Ixodes cookei*, *Ixodes scapularis*, and *Rhipicephalus sanguineus* (Pak et al., 2019). *Ixodes scapularis* is the most dominant tick species in the area, having surpassed *D. variabilis* in the 1990s, which had displaced the presence of *I. cookei* and *R. sanguineus* before the 1990s (Pak et al., 2019). *Ixodes scapularis* were predicted to continue in their expansion in the country due to current and future environmental conditions (Hahn et al., 2016).

Ixodes scapularis ticks are usually found in forested areas. A study evaluating the presence of *I. scapularis* ticks in suburban Maine found that the abundance of ticks was high in the shrub layer, deciduous litter, forest grasses, and moist-soil ferns (Lubelczyk et al., 2004). They are more likely to be found in areas considered to be animals' habitats, and they feed mainly on mammals, birds, and humans, which are their accidental hosts.

Figure 5. Estimated distribution of the main *Ixodes* ticks in the USA. A) shows the estimated distribution of the western blacklegged tick. B) shows the estimated distribution of blacklegged ticks in the eastern USA. Retrieved from CDC 2021.

1.4 Small mammals and rodents' implications in tick-borne pathogens

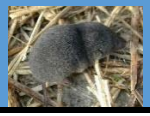
Multiple small mammals and rodents are known to be competent hosts for various tick-borne pathogens. For example, white-footed mice are competent hosts for *A. phagocytophilum, B. microti,* and *B. burgdorferi* while white-tailed deer are competent hosts for *Ehrlichia chaffeensis* (Hersh et al., 2012; Keesing et al., 2012). Some rodents and small mammals are competent hosts because they can assist in maintaining the pathogens in the environment through their ability to be infected and then proceed to infect tick vectors. During feeding, pathogens can infect hosts and then persist and magnify in the host until they are ingested by the next vector. Hence, the abundance of small mammals can help determine the density of infection in the tick population, which helps measure potential risk of infection in humans. A field study in Europe on the rodent's impact on *I. ricinus* activity found that the density of rodents was positively associated to the presence of nymphal ticks and the prevalence of infected *B. afzelli* ticks (Krawczyk et al., 2020). In the USA, the abundance of rodents like white-footed mice (*Peromyscus leucopus)* is associated with the high prevalence of Lyme disease observed in Northeastern and Midwestern states (Ginsberg et al., 2021; Larson et al., 2021; Ostfeld et al., 2014). Nevertheless, the prevalence of infection in small mammals, as well as the potential risk to other ticks and humans, relies not only on the abundance of the small mammals and rodents but also on the composition of the overall fauna of the area. The presence of more mice or shrews along with fewer reptiles in the north is one of the main reasons tickborne prevalence is greater in northern regions of the USA (Ginsberg et al., 2021). Moreover, the competency of hosts is not always similar. Not all small mammals are competent hosts for all pathogens, and when they are, their competency to a specific pathogen differs. For example, a study on competent reservoirs of *B. burgdorferi* found that while white-footed mice (*P. leucopus*), short-tail shrews (*Blarina brevicauda*), and eastern chipmunks (*Tamias*

striatus) were more likely to be infected and serve as competent hosts in this system, striped skunks (*Mephitis mephitis*) were not as likely to be infected (Brunner et al., 2008). In the same study, the infectivity of each host was measured. It was found that white-footed mice had high infectivity, eastern chipmunks and shrews had an intermediate level of infectivity, and white-tailed deer and squirrels had a low infectivity (Brunner et al., 2008). Due to its ability to maintain *B. burgdorferi,* white-footed mice are considered to be a reservoir host of the pathogen.

The state of Pennsylvania has a wide range of animals including multiple species of mammals and birds. It has 66 species of mammals (Carnegie Museum of Natural History, 2011), including diverse small mammals and rodents that are known as competent hosts for tick-borne pathogens such as the short-tail shrews, white-footed mice, etc. Therefore, *I. scapularis* can feed from multiple hosts with the potential to harbor and maintain *B. burgdorferi* and other tickborne pathogens (Table 1).

Table 1. List of common small mammals in Pennsylvania. This table describes different species of small mammals in Pennsylvania and their known potential competency. The competency was based on a review of competency by Ginsberg et. al (2020) from field study and laboratory study. Pot com = Potential competency, D = diurnal, N = nocturnal, * laboratory study. Small mammal characteristics were retrieved from Fergus (2010) and articles on the Pennsylvania Game commission.

Common Name	Scientific Name	Habitat	Breeding Season	D or N	Pot com
Deer Mouse 	*Peromyscus maniculatus*	Field, brush, woods	Mar-Oct	N	1-6%
White Footed Mouse 	*Peromyscus leucopus*	Shrubs, woods, fields, pastures, ravines, buildings	Mar-Oct	N	75%
Virginia Opossum 	*Didelphis virginiana*	Wherever water and food shelter exist	Jan-Oct	N	3%
Norway Rat 	*Rattus norvegicus*	Streams, rivers, marshes, fields, and city buildings, sewers, and dumps	All Year	N	72% *
Eastern Chipmunk 	*Tamias striatus*	Woods containing many logs, stumps, and nuts	Mar-Apr Jul-Aug	D	55%

Flying Squirrel 	*Glaucomys volans* *Glaucomys sabrinus*	Woods containing mature mast-producing trees and shrub species to provide food	Apr-May Jul-Aug	D	NA
Woodland Jumping Mouse 	*Napaeozapus insignis*	Cool, moist woods near streams	Jun-Aug	N, but venture on cloudy days	NA
Shrews 	*Blarina brevicauda* *Sorex* spp.	Woods, banks of small streams, tall grass, and brush	All Year	N, but can be active during the day	37%

1.5 Current work

Multiple studies have analyzed the prevalence and incidence of tick-borne pathogens in ticks from various parts of the country. The majority found that *B. burgdorferi* was the most prevalent pathogen circulating in tick populations in the USA (Barbour et al., 2009; Hutchinson et al., 2015; Prusinski et al., 2014; Schwartz et al., 2022; Simmons et al., 2020). The presence of *A. phagocytophilum* and *B. microti* were also reported, with the prevalence of *B. microti* ranking third overall in New York and Pennsylvania (Hutchinson et al., 2015; Prusinski et al., 2014; Schwartz et al., 2022; Simmons et al., 2020). In Pennsylvania, the infection rate of *B. burgdorferi* was lower in the western region than in the central and north central regions even though an increase was seen from the past (Simmons et al., 2020). However, the western region of the state showed an increase in Lyme disease and Babesiosis incidence (Eddens et al., 2019; Ingram & Crook, 2020). Also, it was found that there was a southwestward expansion in the proportion of cases of Lyme disease in Pennsylvania and an expansion from rural to non-natural areas (Eddens et al., 2019). This expansion in the vector and pathogen suggests that there is a need to evaluate ticks, hosts, and the most common tick-borne pathogens in western Pennsylvania. Current knowledge on *B. burgdorferi* in the state indicates that there is still a need for more work investigating the prevalence of these pathogens in hosts. Even though the incidence of these diseases is rising in the state, there is limited work conducted in the state to evaluate the prevalence of the pathogens in host reservoirs of tick-borne pathogens. Currently, there are some studies that look at the presence of *B. burgdorferi* in Pennsylvania, but they usually only focus on the presence of the pathogens in *I. scapularis* ticks. These studies are showing that the *B. burgdorferi* is the most prevalent pathogen in ticks in the state (Hutchinson et al., 2015; Schmidt et al., 1999; Schwartz et al., 2022) and that the prevalence of this pathogen in urban areas is

relatively similar to its prevalence in non-urban areas (Simmons et al., 2020). Research on competent and reservoir hosts that could participate in the maintenance of *B. burgdorferi* in western Pennsylvania is minimal. The prevalence of the pathogen is poorly researched in small mammals, hence limiting the possibility to evaluate the potential risk of infection in humans.

Understanding the prevalence of *B. burgdorferi* in the western region of the state allows for the evaluation of the potential risk of human infection in an area where the incidence and number of cases have been increasing. We currently know that infected nymphs and adult tick density are important for determining infection risk to humans. With small mammals and rodents, it was found that the abundance of mice and chipmunks during the previous two years was a strong predictor for the risk of Lyme disease to humans during the current year (Ostfeld et al., 2006). We know that the tick burden on small mammals can differ from one host species to another. For instance, it has been found that larvae are more likely to be found on white footed mice while nymphs are more likely to be found on chipmunks (Schmidt et al., 1999). Another work discusses how the risk of disease in humans is dynamically linked to changes in the abundance of small mammal hosts known to infect ticks (Levi et al., 2012).

Data on host infection rate and their distribution in Western Pennsylvania would be a valuable tool in prevention and control of Lyme disease in the area. We expect that the prevalence of infection will be high in white-footed mice, eastern chipmunks, and shrews. Moreover, we expect the presence of these pathogens will not differ between urban, rural, and suburban areas since the pathogens have been expanding in major cities, following the tick prevalence pattern observed in city and non-urban areas of western Pennsylvania. Finally, the tick burden will be the highest on animals that are known to be competent hosts for tick-borne pathogens. Larval tick burden will be higher on white-footed mice than on any other species.

2.0 METHODS

Sample locations were chosen based on suitable tick and small mammal habitats, distance from the city center, and area of the public park or land (Fig. 6). Habitats suitable for both ticks and small mammals usually are herbaceous woodlands that contain tall grass or brush and are usually covered with leaves. Two parks deemed suitable for tick and mammal sampling were in urban areas: Riverview Park (RVP) and Schenley Park (SCH), two parks in suburban areas: Boyce Mayview Park (BOM) and Crooked Creek Horse Park (CCH), and two parks in rural areas: State Game Lands 232 (SGL232) and State Game Lands 42 (SGL42). Grids (60 x 100 m) were established at each of these six locations in 2021 and were visited from May-August in 2021 and 2022. The grid sites were visited in a bi-weekly manner where three parks from different community types were visited during a week and the other three parks were visited the next week (Table 2). The grids at each site covered a total area of 6000 m^2 and flags were placed at each 10 m node (Fig. 7). Although the grids covered the same size area, due to differences in the topography at two sites (SCH and RVP) the grids were slightly different compared to the others. In RVP, the length of the flag lines was made shorter in certain areas and longer in others to allow the surface to be covered. At SCH, the grids site was divided into two grids (30 x 100m) found in two locations of the park.

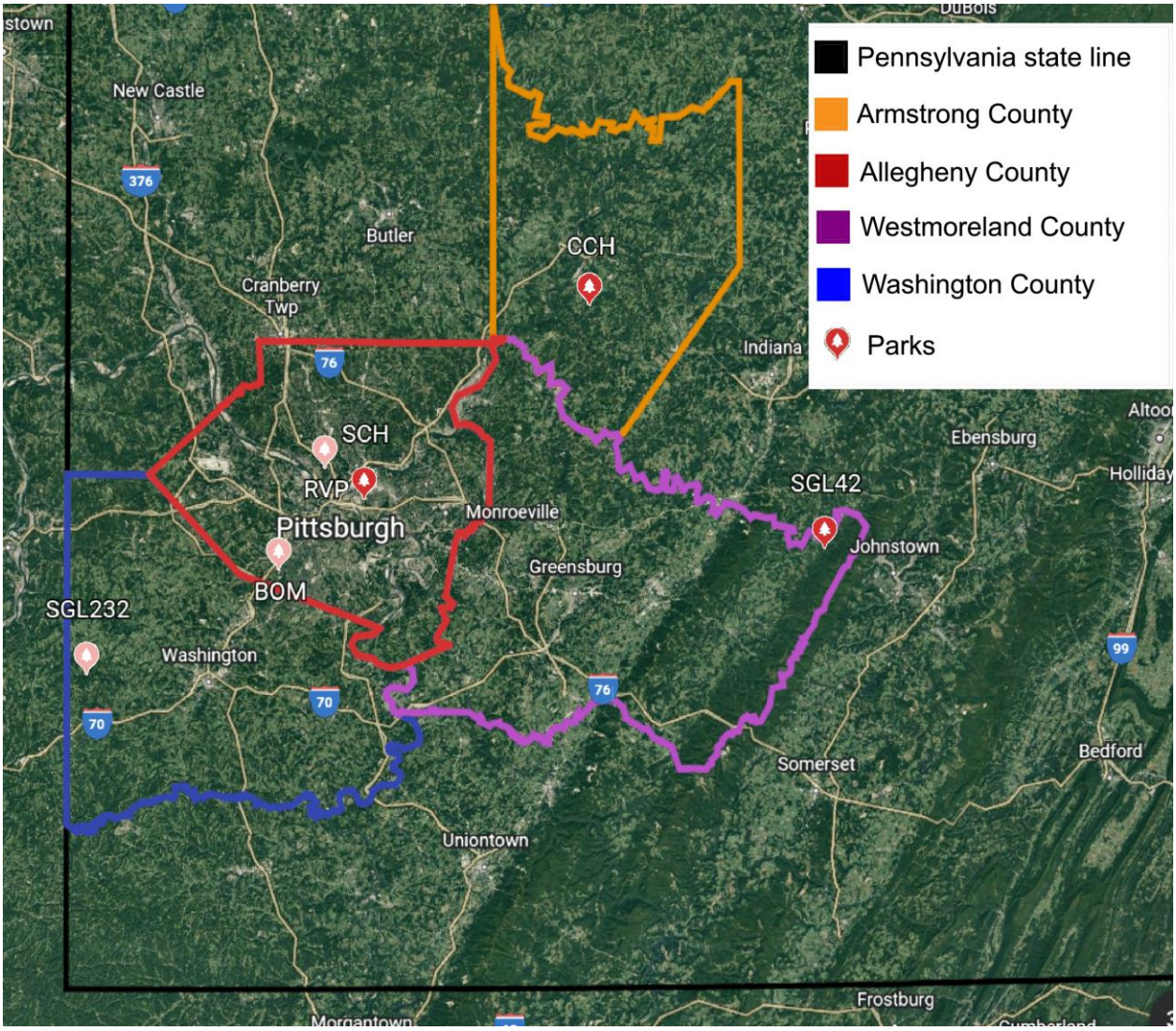


Figure 6. Grid sites located in southwestern Pennyslvania. This map shows each county's boundaries and the parks visited in each. Placemarks of parks of the same color were visited in the same week.

Table 2 . Information on visited grid sites. The table shows the different grid sites that were visited each week. The sites that were visited during the same week are found in the same week column (A, B). The grids sites were in various parts of southwestern Pennsylvania and in different community types. C/Type = Community type.

	Grid site name	County	C/ Type	GPS coordinates
Week A	Riverview Park, RVP	Allegheny	Urban	40.4833488, -80.0208987
	Boyce Mayview Park, BOM	Allegheny	Suburban	40.3324037, -80.11013
	State Game Lands 232, SGL232	Washington	Rural	40.1790707, -80.4799273
Week B	Schenley Park, SCH	Allegheny	Urban	40.4349436, -79.9470179
	Crooked Creek Horse Park, CCH	Armstrong	Suburban	40.7299866, -79.5043756
	State Game Lands 42, SGL42	Westmoreland	Rural	40.3872558, -78.9910352

Figure 7. Standard set up of grids. Each grid was set to cover 6000m^2. The structure of each grid site was set to follow this model unless the landscape prevented the setup. This grid represents the grid at BOM, SGL42, SGL232, and CCH

2.1 Ticks and small mammals sampling

All the tick and small mammal sample collection occurred with the Pennsylvania Game Commission's authorization under their special use permit for scientific studies. All small mammal procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee (IACUC). Data and samples were obtained while ensuring the well-being of the collectors and the small mammals.

Questing ticks were collected from the environment using a white corduroy drag cloth measuring one square meter, stopping every 10 m (each node on the grid) to remove attached ticks.

Tick life stage and species were identified and recorded. For each 100 m line, the removed ticks were placed in a 1.5 ml microcentrifuge tube containing 95% ethanol. Clear packing tape was used to collect larval ticks when more than 20 larvae were present on the drag cloth.

A capture-mark-recapture method was used to ensure that individual animals could be monitored for pathogens throughout the field season. Live Sherman traps measuring 7.62 cm by 8.89 cm by 22.86 cm (H.B. Sherman Traps, Inc. Tallahassee, FL) were placed close to the flags at each grid site. Each week, baits made of peanut butter, oats, and sunflower seeds were set at sunset over two consecutive nights. Species, age, sex, and body measurements were recorded for each captured animal. In addition, feces, ear tissues, blood samples, and ticks found on the host were collected so they could be analyzed for pathogens. To collect morphological traits and samples, the small mammals were immobilized using the scruffing technique except for chipmunks and flying squirrels which were instead restrained by holding tight on their shoulders. Ultra fine tipped forceps were used to remove ticks from the host and an ear punch biopsy was used to remove a 2 mm ear tissue from each individual. Ear punches were not collected on short-tailed shrews due to their lack of pinnae; instead, neck tissues were collected on dead shrews. All tools used in the field were disinfected before use on each animal. The ticks and ear tissues collected were placed in individual 1.5 ml microcentrifuge tubes containing 95% ethanol. Blood samples were collected from the submandibular (facial) vein. For each captured host, 100-150 µl of the blood was placed in a BD Microtainer serum separator tube (Fisher Scientific, Hampton, NH) and 2-3 drops of blood were placed on a Whatman FTA classic collection card (Fisher Scientific, Hampton, NH). The serum collection tubes were spun for 5 minutes with a mini centrifuge while in the field. After processing, each animal was released at the point of capture. Animals that were either found dead in the traps or that died during processing were frozen at -20°C and kept for further analysis in

the lab. All the samples were kept at room temperature except for serum tubes, feces, and dead animals, which were preserved at -20°C or -80°C upon return to the lab each day.

2.2 DNA extraction

DNA was extracted from all samples and were processed using Qiagen products (Qiagen DNeasy Blood and Tissue kit and QIAcube HT DNA extraction 96-well plate robotic workstation) and followed the manufacturer's protocols for each sample type. *Ixodes scapularis* nymphs collected during the drag were placed in individual tubes containing 95% ethanol. After removing the alcohol and drying the nymph, the tube containing the dried nymph was dipped into liquid nitrogen to facilitate crushing using a clean pestle. Lysis buffers and proteinase K were added to each sample, and they were incubated at 56°C overnight to ensure complete digestion. The samples were then extracted using a robotic workstation, Qiagen QIAcube HT DNA extraction system, and following the manufacturer's protocols. Each ear tissue was dried and placed in a dry empty microcentrifuge tube, lysis buffer and proteinase K were added in the appropriate amounts, and the samples were incubated overnight at 56°C. DNA was then extracted from tissue samples using the QIAcube HT and manufacturer's recommended protocol. A total of three 3 mm punches were removed from the blood blot cards and placed in individual microcentrifuge tubes. Lysis buffer and proteinase K were added, and samples were incubated at 56°C for an hour while vortexing every 20 min to ensure the cells were released from the paper. Another lysis buffer was added, and the samples were incubated at 70°C for 10 min vortexing every 5 min. DNA was then extracted from the blood samples using the QIAcube HT and manufacturer's recommended protocol.

The concentration and quality of DNA from each extraction plate was verified by randomly selecting and testing five samples using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific).

2.3 Pathogen screening

The DNA extracted from ticks and ear tissue were screened for the presence of *B. burgdorferi* using a quantitative real-time PCR (qPCR) assay using a Vii7 A machine (Applied Biosystems, ThermoFisher Scientific, Waltham, WA, USA). The assay was designed to identify a specific 69bp sequence of the *B. burgdorferi* 16S rRNA gene (Barbour et al, 2008; Tufts & Diuk-Wasser, 2018). All samples were screened in duplicate using the specific forward primer 16S-rRNA-F: 5'-GGC GGC ACA CTT AAC ACG TTA G-3' with the reverse primer 16S-rRNA-R: 5'-GCT GTA AAC GAT GCA CAC TTG GT-3' and the dye-labeled probe: 6FAM-TTC GGT ACT AAC TTT TAG TTA A-MGBNFQ. The reaction was completed under the following conditions of 95°C for 20 seconds followed by 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds (Tufts & Diuk-Wasser, 2018).

As previously described, a qPCR standard for each pathogen was constructed (10^6 to 1 gene copies) and used as a positive control during screening (Tufts & Diuk-Wasser, 2018). Positive control samples received from the Pennsylvania Department of Environmental Protection were also used to evaluate screening detection. For each run, quantity values, including the cycle threshold (Ct) values, were calculated by the machine.

2.4 Statistical analysis

The number of the small mammals and *I. scapularis* ticks were counted for each site and each session, and the proportion of larvae and nymphs per host was determined. The proportion of ticks was calculated by dividing the number of collected ticks of a specific life stage of host species to the total collected ticks of the similar life stage. The density of the host was measured by dividing the number of individuals by the surface area of the grids. The prevalence of infection in small mammals was calculated by dividing the number of infected samples to the total number of collected samples. We calculated the prevalence by species and then by sites. Samples collected from seven shrews were removed from our dataset due to limited information on hosts. Fisher's Exact test was used to determine the significant difference between the proportion of individuals infected and not infected with *B. burgdorferi* between years. One way ANOVA test was conducted to look at the difference in infection prevalence between the different species of animals and sites. Data management and Statistical analysis were conducted using Excel and Graphpad Prism 9. A p value of <0.05 was considered significant.

3.0 RESULTS

A total of 913 and 829 questing nymphs were collected from all six grid sites in 2021 and 2022, respectively. The total number of ticks collected on small mammals was 3918 in 2021 and 2111 in 2022. A total of 208 and 282 individual mammals from all grid sites were collected in 2021 and 2022, respectively. Captured hosts were from seven different species in 2021 (white-footed mice, shrew, Eastern chipmunk, flying squirrel, weasel, meadow vole, Virginia opossum) and six different species in 2022 (white-footed mice, shrew, Eastern chipmunk, flying squirrel, woodland jumping mice, rat) (Fig. 8).

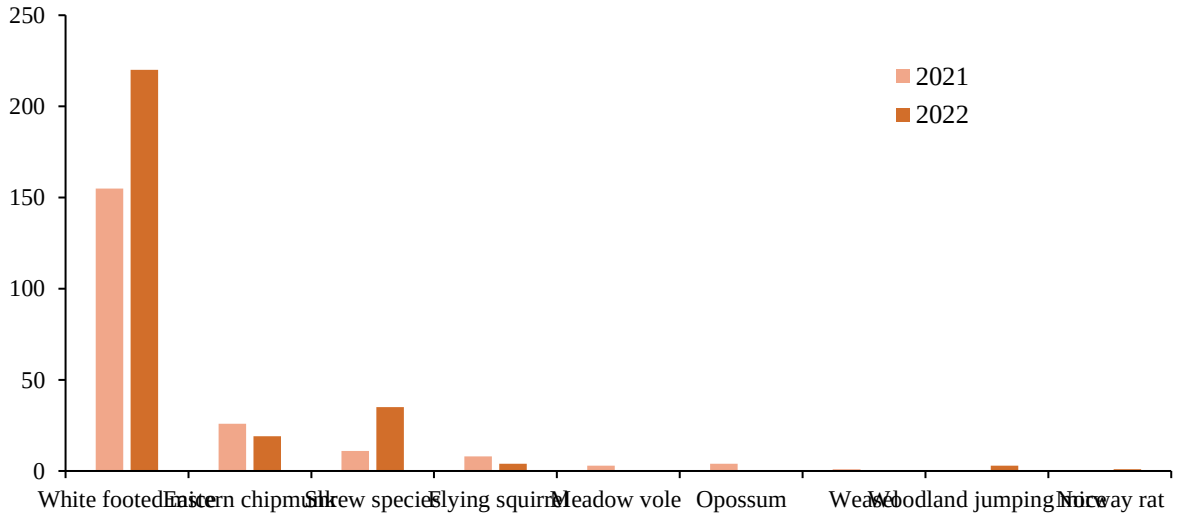


Figure 8. Number of individual hosts during 2021 and 2022. This graph shows the number of individual hosts collected during the two years.

`To better visualize the abundance of each host species during the two years, we measured the proportion of each species of collected species. This was measured by dividing the number of individuals collected of a specific species divided by the total number of collected species during the year (Fig. 9). In 2021 and 2022, the highest proportion of collected animals belonged to white-footed mice.

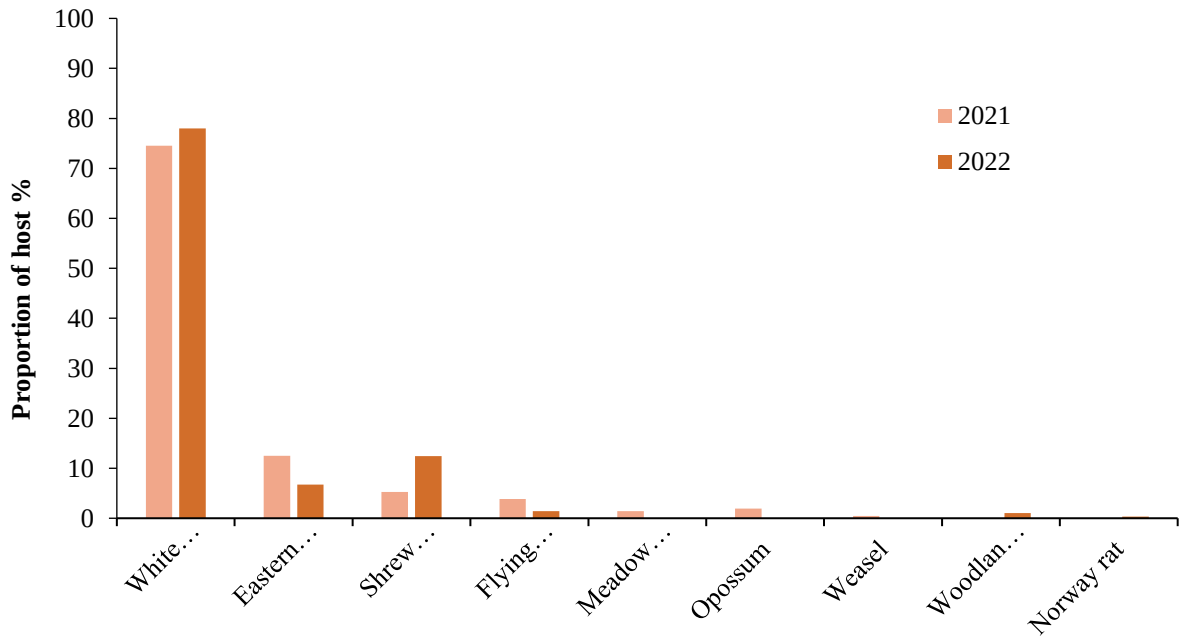


Figure 9. The proportion of each host species during each year. This graph allows us to better visualize the abundance of small mammals during the two trapping years.

3.1 Host presence across the sites

In 2021, white-footed mice (*n* = 155) were the most collected animal at all the sites. These small mammals were caught in high numbers at Schenley Park (SCH), which is a park located in an urban area. Eastern Chipmunks (*n* = 26), shrews (*n* = 11), flying squirrels (*n* = 8), opossum (*n*

= 4), meadow vole (*n* = 3), and weasel (*n* = 1) were also captured but were not collected at all trapping sites (Fig. 10). Some of the small mammals were captured once during field season and others multiple times.

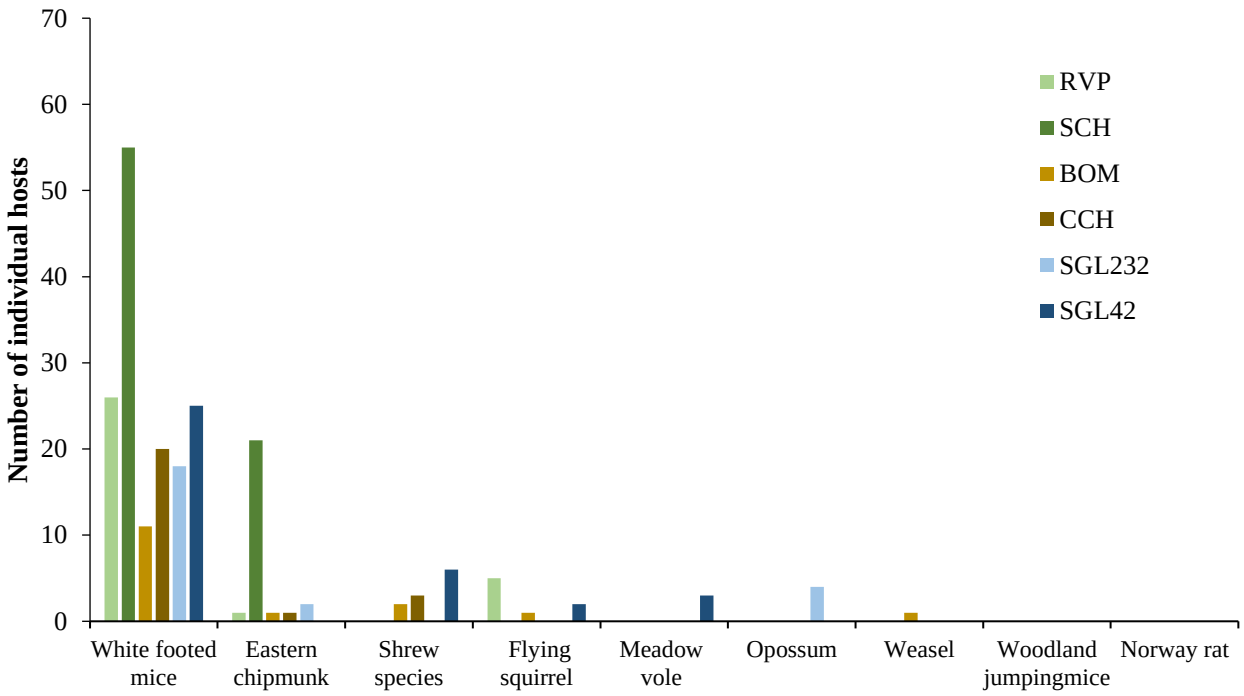


Figure 10. Number of individual hosts collected at each site in 2021. This graphs shows the numbers of hosts that were collected for each encountered species in 2021.

To better visualize the abundance of each host species in 2021, we measured the proportion of each species per site. Most of the collected small mammals were white-footed mice (about 74%). SCH was the site where we collected the most mammals (about 37%) with about 26% white-footed mice and 11% eastern chipmunks. Out of all the collected hosts, only about 7% were

collected from BOM with about 5% white-footed mice and the rest being eastern chipmunks, shrews, flying squirrels and weasel (Fig. 11).

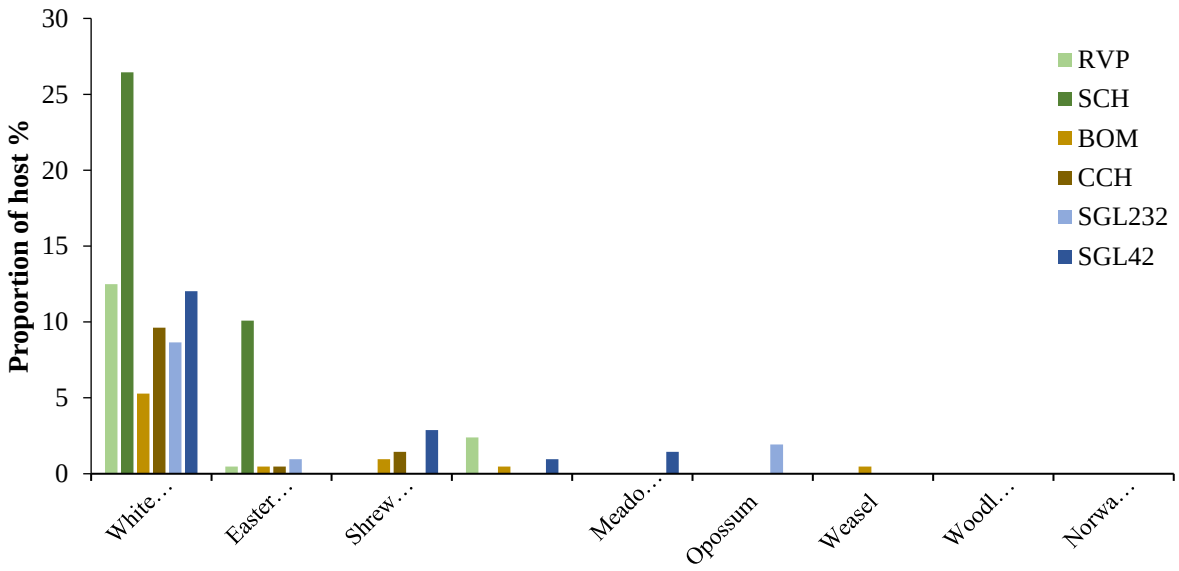


Figure 11. Proportion of host per site and species in 2021. The graph enlightens us on the proportion of a specific species were collected at specific sites out of the collected small mammals in 2021.

In 2022, the same trend was observed, with the highest number of animals sampled being white-footed mice (*n* = 220). However, during this trapping year, most of these mice were collected from CCH, followed by SGL232, and SCH. Shrews (*n* = 35) were the second most collected animals, followed by eastern chipmunks (*n* = 19), flying squirrels (*n* = 4), woodland jumping mice, and rat (*n* = 1) (Fig. 12). Some of the small mammals were captured once during field study and others multiple times.

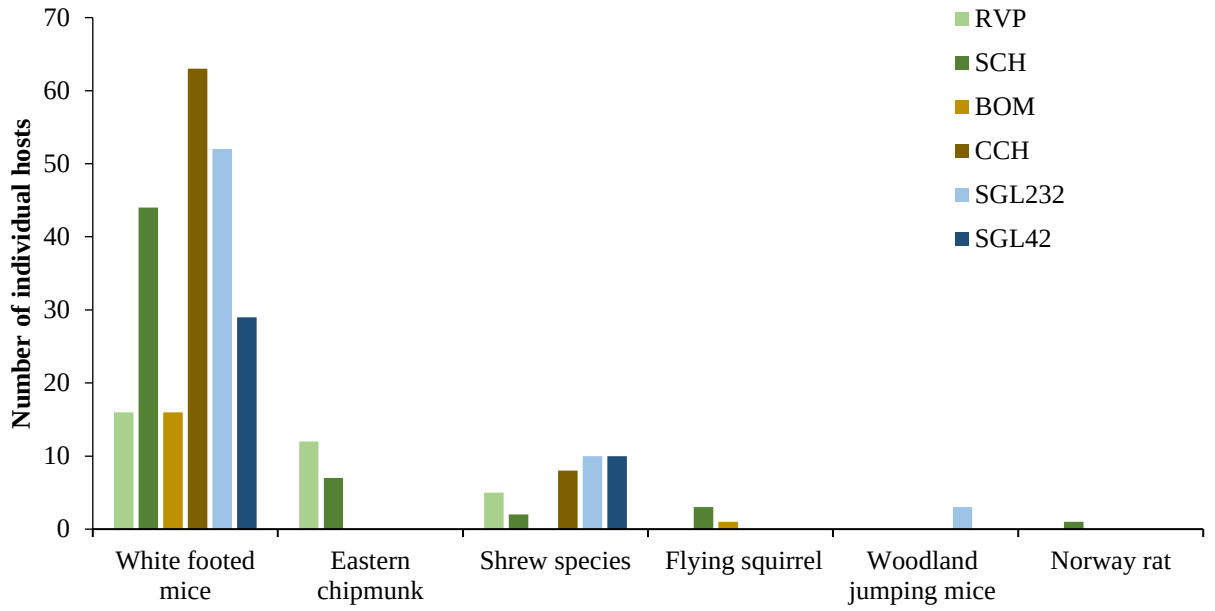


Figure 12. Number of individual hosts collected at each site in 2022. This graph shows the number of hosts that were collected for each encountered species at each site.

We also calculated the proportion of each species per site. The proportion of rodents collected was the highest at CCH. Out of all the collected animals, the white-footed mice were the most collected at CCH (about 22%) (Fig. 13).

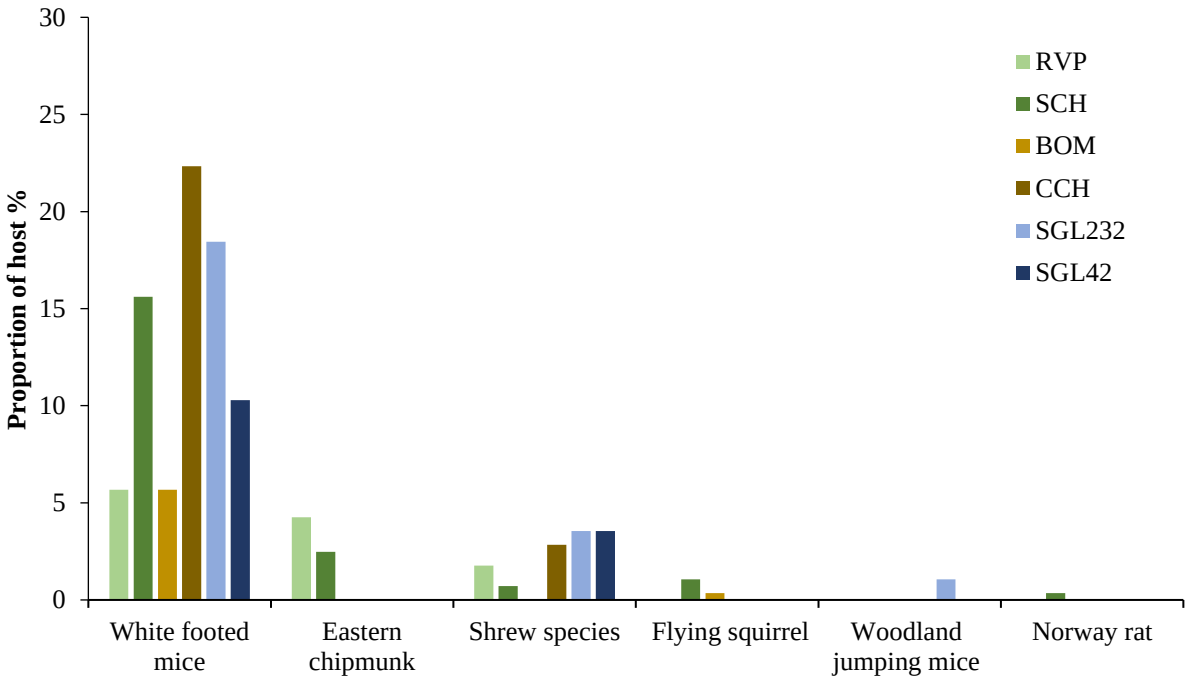


Figure 13. Proportion of hosts per site and species in 2022. The graph shows how many percent of a specific species was collected at a specific site out of the collected small mammals in 2022.

To further understand the abundance of these animals, host density was calculated per trapping site (Table 6, Appendix A). In 2021, host density was highest for white-footed mice and eastern chipmunks (Fig. 14). The difference between the density of white-footed mice (WF) and all the other species was not significant ($p < 0.01$). The density of white-footed mice was the highest at SCH (91.7 WF per ha), RVP (43.3 WF per ha), and SGL42 (41.6 WF per ha), two urban areas and one rural area. The density of white footed mice was least at BOM (18.3 WF per ha) and SGL232 (3.0 WF per ha), a suburban and rural area. No significant difference was observed

between the sites (p = 0.7567). Eastern chipmunks were the second most prominent host at all sites (Fig. 14), with the highest density seen at SCH (3.5 eastern chipmunk per ha).

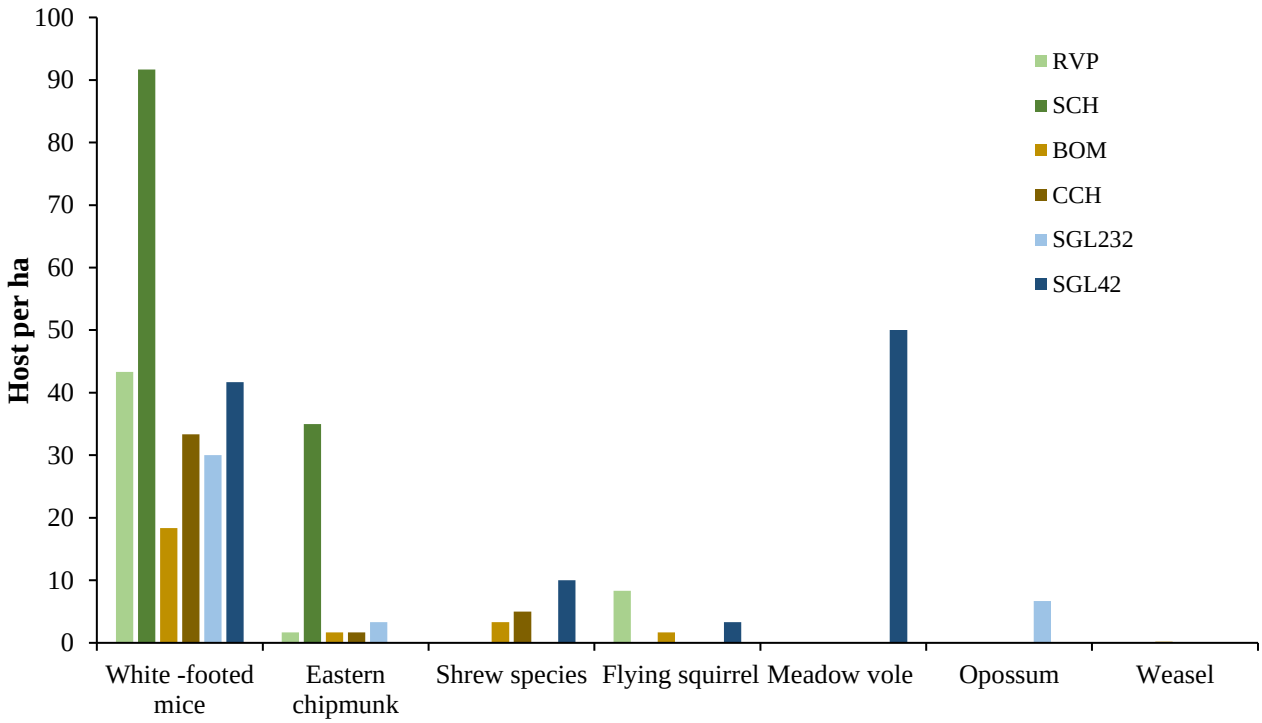


Figure 14. Host density of small mammals in 2021. The density of each species is expressed by the number of hosts per hectares (ha). One hectare is equal to 10000m^2.

In 2022, white-footed mice also showed the highest density compared to all other hosts sampled (Fig. 15, Table 6). The difference in the density of white-footed mice and with all the other species was significant (p<.001). The density of white-footed mice was the highest at CCH (105 WF per ha), SGL232 (86.7 WF per ha), and SCH (73.3WF per ha), a suburban, rural, and urban area. Shrews had the second highest host density of all species at each site except for BOM. The difference in the density of shrews with other species was only significant compared to the

white-footed mice (p<.001). The density of shrews was higher in rural areas compared to urban and suburban areas (Fig. 15). The difference in the density of shrews at each site was not significant.

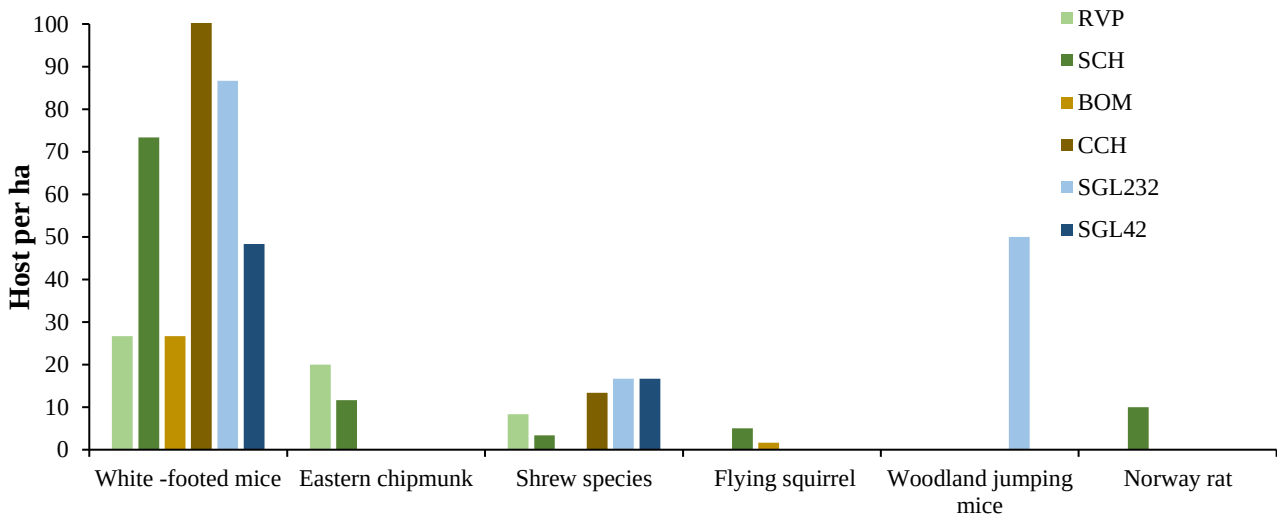


Figure 15. Host density of small mammals in 2022. The density of each species is expressed by the number of hosts per hectares (ha). One hectare is equal to 10000m^2.

3.2 The proportion of ticks on small mammals.

To understand the potential for pathogen transmission by *I. scapularis* ticks to each of the small mammals sampled, the proportion of ticks collected on each species was calculated. The proportion of ticks collected on each species of the small mammals varied with more ticks originating from white-footed mice at SCH (larvae = 2053, nymphs = 16) (Table 3). In 2021, from white-footed mice, we collected ≈ 97% of larvae out of all the collected larvae and ≈ 92% of

nymphs out of all the collected nymphs (Fig. 16). Out of all the collected larvae ≈ 3% were collected from chipmunks and ≈ 7% of all the collected nymphs were retrieved from them. The proportion of ticks collected on flying squirrel was minimal. No ticks were collected from opossum, shrews, meadow vole, or weasels.

Table 3. Number of ticks collected on each small mammal species. *n* = number of hosts, L = larvae, N = nymph. WF = white-footed, E chipmunk = Eastern chipmunk

2021	**RVP**			**SCH**			**BOM**			**CCH**			**SGL232**			**SGL42**		
Host	*n*	L	N	*n*	L	N	*n*	L	N	*n*	L	N	*n*	L	N	*n*	L	N
WF mouse	26	357	2	55	2053	16	11	80	8	20	212	2	18	399	45	25	579	30
Shrew	0	0	0	0	0	0	2	0	0	3	0	0	0	0	0	6	0	0
E chipmunk	1	1	0	21	111	7	1	0	0	1	0	0	2	12	20	0	0	0
Flying squirrel	5	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0
Weasel	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Meadow vole	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
Opossum	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0

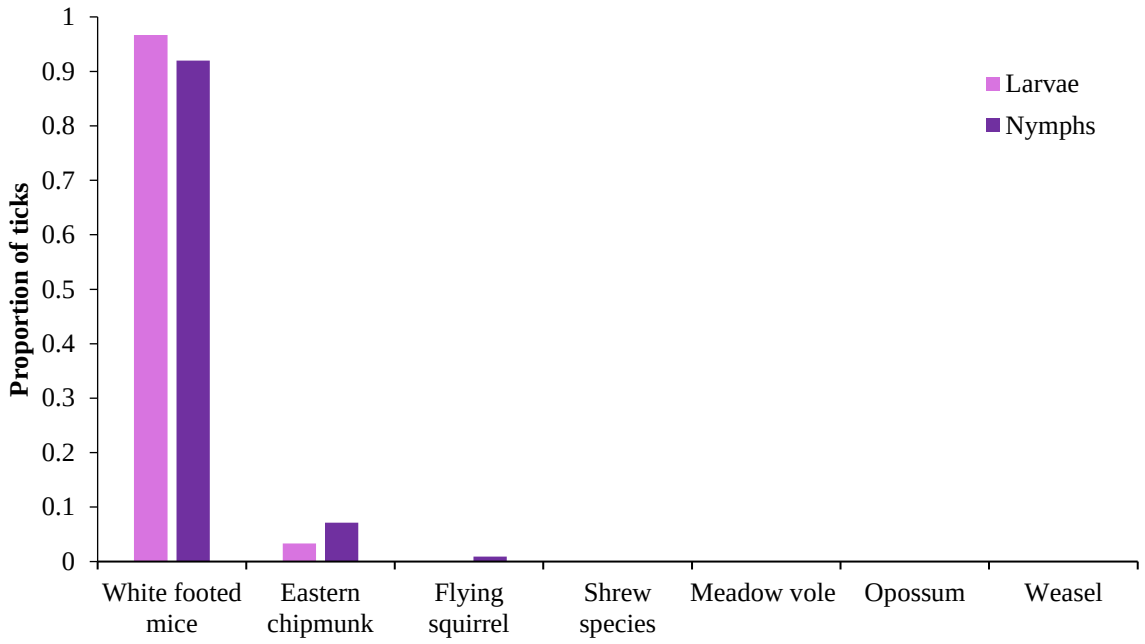


Figure 16. Proportion of ticks per host species in 2021. The proportion of larvae and nymphs collected from each rodent.

In 2022, the proportion of ticks was the highest in white-footed mice followed by woodland jumping mice and eastern chipmunks (Fig. 17). The proportion of larva collected out of all the larva was ≈ 98%, increasing from 2021. Most ticks collected on white-footed mice were collected at CCH (larvae = 368, nymphs = 11, Table 4). The remaining 2% of collected larvae were collected from the other small mammals. Out of all collected nymphs, ≈ 72% of all the collected nymphs were from white-footed mice. However, the proportion of nymph collected on white-footed mice decreased from 92% in 2021 to 72% in 2022. Out of all collected nymphs in 2022, ≈ 9% were collected from eastern chipmunks and 17% from woodland jumping mice. No ticks were found on shrews.

Table 4. Number of ticks collected on small mammal species. *n* = number of hosts, L = larvae, N = nymph. WF = white-footed, WJ = woodland jumping, E chipmunk = Eastern chipmunk

2022	RVP			SCH			BOM			CCH			SGL232			SGL42		
Host	*n*	L	N	*n*	L	N	*n*	L	N	*n*	L	N	*n*	L	N	*n*	L	N
WF mouse	16	84	3	44	368	11	16	131	4	63	367	14	52	0	0	29	0	0
Shrew	5	0	0	2	0	0	0	0	0	8	0	0	10	0	0	10	0	0
E Chipmunk	12	3	12	7	111	7	0	0	0	0	0	0	3	5	6	0	0	0
Flying squirrel	0	0	0	3	0	0	1	0	1	0	0	0	0	0	0	2	0	0
WJ mice	0	0	0	0	0	0	0	0	0	0	0	0	3	11	33	0	0	0
Rat	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3	0	0

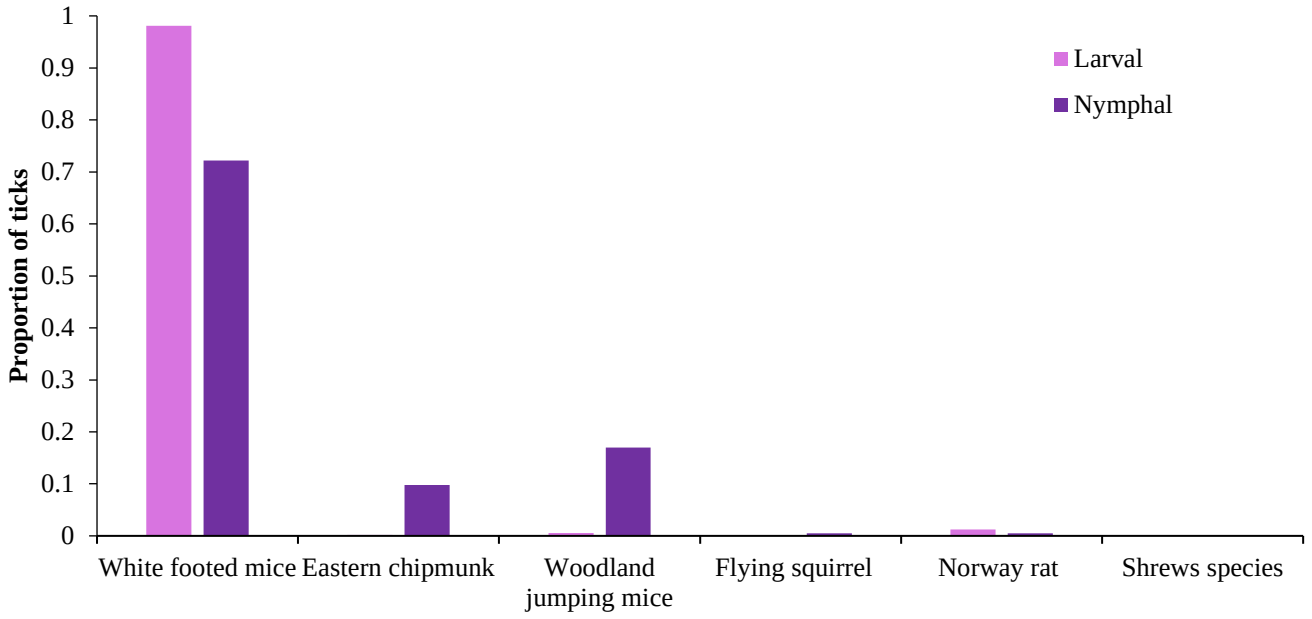


Figure 17. Proportion of ticks per host species in 2022. The proportion of larvae and nymphs collected from each rodent is shown.

3.3 Prevalence of *Borrelia burgdorferi*

The overall infection prevalence of *B. burgdorferi* from all small mammals sampled was significantly higher in 2021 (43.18%) compared to 2022 (35.44%) ($p = 0.029$). An ANOVA analysis was conducted to evaluate the difference in prevalence between sites during each year. In 2021, SGL232 had significantly higher prevalence of *B. burgdorferi* compared to CCH ($p = 0.003$) and RVP ($p = 0.002$). In 2022, RVP *B. burgdorferi* prevalence was significantly higher compared to BOM ($p = 0.035$).

To compare the infection prevalence at each visited site during the two years, a Fisher's Exact test was used (Fig. 18). The difference in prevalence between 2021-2022 was significant for RVP ($p = 0.013$), BOM ($p = 0.015$), and SGL232 ($p = 0.025$). The observed differences were not significant at SCH ($p = 0.42$), CCH ($p = 0.82$), and SGL42 ($p = 0.27$).

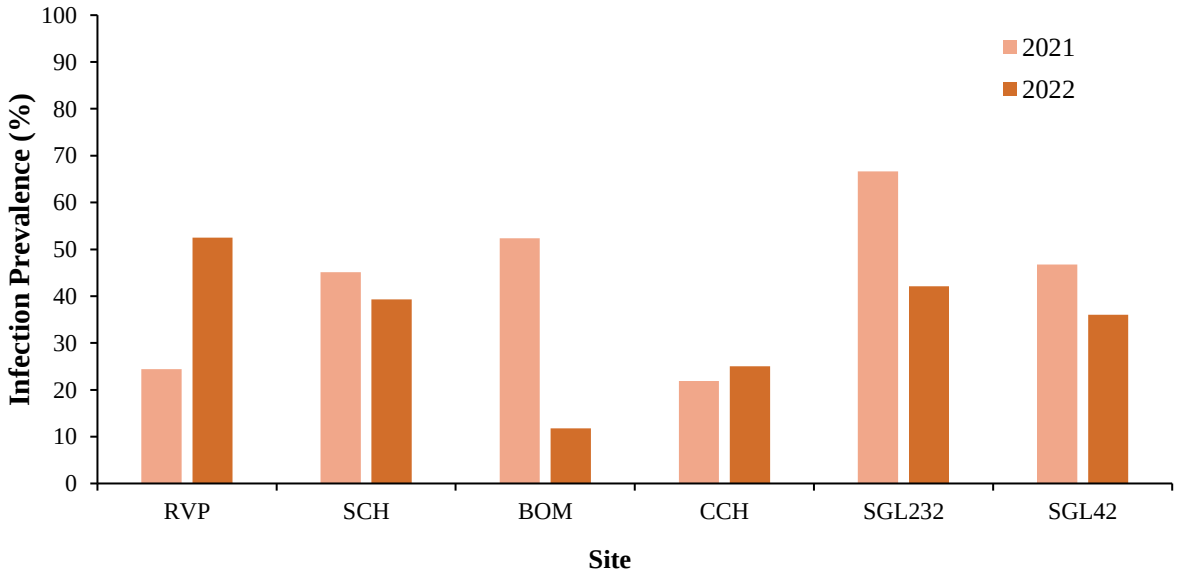


Figure 18. *B. burgdorferi* infection prevalence in small mammals at each site between 2021 and 2022. This graph compares the prevalence of infection in 2021 and 2022 at the six study sites.

3.3.1 Difference in infection prevalence in 2021 and 2022 by host species

The prevalence of *B. burgdorferi* infection in white-footed mice and eastern chipmunks decreased from 2021 to 2022 while it increased for shrews (Fig. 19). To compare, the prevalence between species a Kruskal-Wallis test was completed. We found that the difference in the prevalence of the pathogen was significant among the species ($p = 0.048$). A Fisher's Exact test was used to look at the difference in the prevalence within each species for two years. The difference between the prevalence of 2021 and 2022 was not significant for white-footed mice ($p = 0.109$), eastern chipmunks ($p = 0.062$), shrews ($p = 0.54$), or woodland jumping mice ($p > 0.99$).

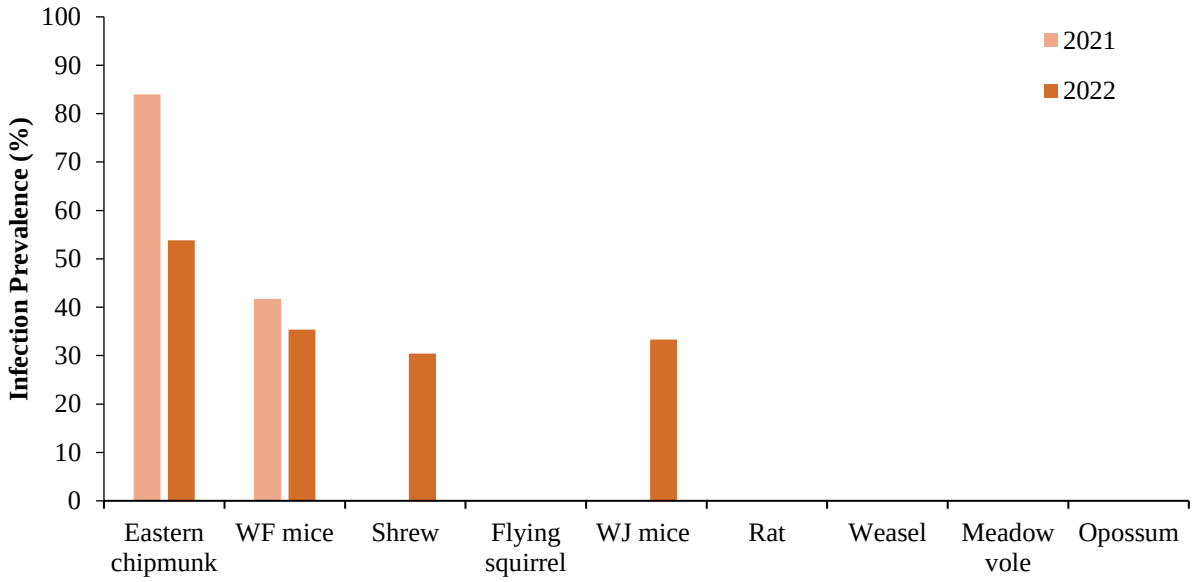


Figure 19. *B. burgdorferi* infection prevalence in species group at each site between 2021 and 2022. This figure compares the prevalence of infection in 2021 and 2022 for each host species.

Table 5. Infection prevalence per site per species. All the boxes in grey represent sites where small mammals were not collected.

	BOM	RVP	SCH	CCH	SGL232	SGL42	
WF mice	50.0	27.5	39.5	21.42857	65.5	49.3	
Shrew				0.0			
Eastern Chipmunk	0.0		80.0	100.0	100.0		
Flying squirrel		0.0					2021
WJ mice							
Rat							
Opossum					0.0		
Meadow vole						0.0	
	BOM	**RVP**	**SCH**	**CCH**	**SGL232**	**SGL42**	
WF mice	12.5	50.0	41.0	24.8	42.9	39.5	
Shrew		0.0	0.0	33.3	36.4	14.3	
Eastern Chipmunk	0.0	71.4	33.3				
Flying squirrel	0.0		0.0				2022
WJ mice					50.0		
Rat							
Opossum							
Meadow vole							

3.3.2 Difference in infection prevalence between sites of different community types

The prevalence of infection was further investigated between sites of the same community types. The prevalence of infection between urban and rural sites was compared. Fisher's Exact test was used to compare infection prevalence between sites of the same community types during the different years (ex. urban 2021 vs. urban 2022), and it was used to compare the prevalence of infection between sites of different community types within a year (ex. urban 2021 vs. rural 2021). From 2021 to 2022, the infection prevalence increased in urban sites, however not significantly (p

= 0.641), and the prevalence significantly decreased in rural sites (p = 0.050). In 2021, the prevalence of the pathogen was significantly higher in rural than urban sites (p = 0.047). In 2022, the prevalence of the pathogen was lower in rural than urban sites although not significantly (p = 0.6115).

The prevalence of infection between urban and suburban sites was compared. From 2021 to 2022, the infection prevalence was not significantly different at urban sites (p = 0.641), or at the suburban sites (p = 0.144). In 2021, the prevalence of *B. burgdorferi* was not significantly different between suburban and urban sites (p = 0.430). However, in 2022, the prevalence of the pathogen was significantly lower in suburban than urban sites (p = 0.006).

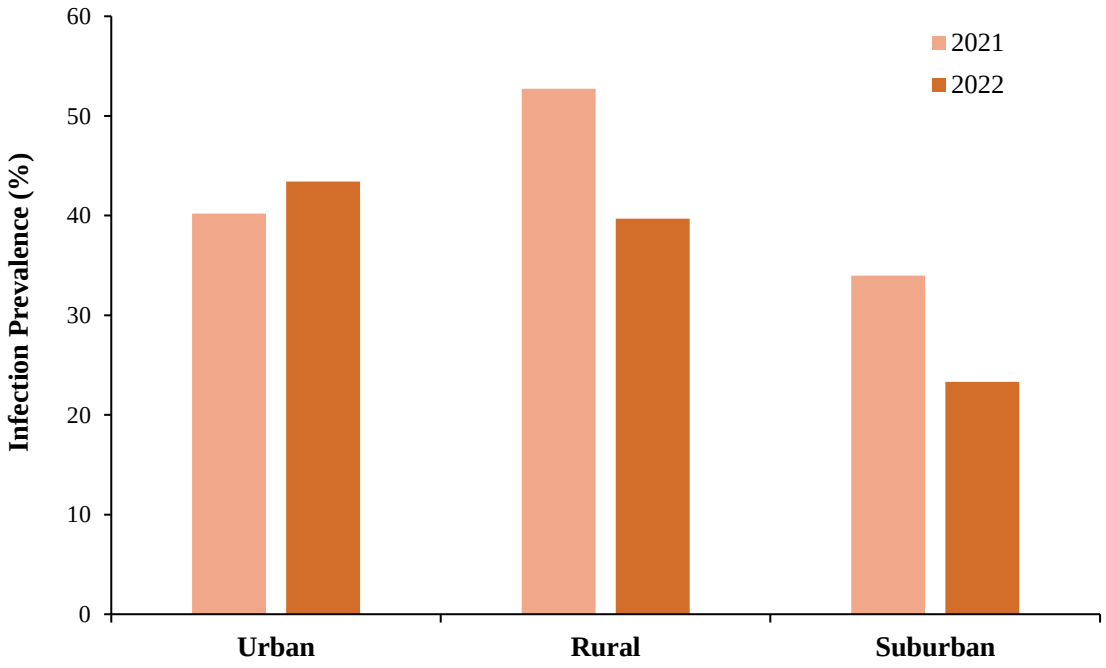


Figure 20. Comparison of the prevalence of *B. burgdorferi* in urban sites compared to rural and suburban sites between 2021 and 2022 field seasons.

4.0 DISCUSSION

4.1 Characteristics of small mammals

The number of questing nymphs and ticks collected on rodents decreased from 2021 to 2022. This decrease in questing could have resulted from a variety of reasons such as using less effective dragging techniques, climatic factors, or more, but it could also just be due to there being a smaller number of nymphs in 2022. Interestingly, more individual small mammals were collected in 2022 despite the decrease in nymphs, however there were less different groups of species in 2022 compared to 2021. Among the small mammals, meadow voles, Virginia opossums, flying squirrels, and woodland jumping mice were the least collected although they have all been collected by other researches (Brunner et al., 2008; Hersh et al., 2012; Rocco et al., 2020; Schmidt et al., 1999). They represent a small percentage of the total number of animals collected and continual surveying of these small mammals in the study sites around the state will allow us to better understand their occurrence and the potential competency to tick-borne pathogens that they each possess. Other animals, including shrews, eastern chipmunks, and white-footed mice are the mammals where we collected the most ticks. More total ticks were collected from white-footed mice in the two years than from any other animal, and the provided information is crucial because the proportion of collected larva and nymphs on white-footed mice indicates that they are getting more opportunities to infect and to be infected than the other small mammals. In other words, a mouse could potentially be infected by a nymph and subsequently infect a larval tick while feeding. Another animal that was one of the most abundant in the population is the shrews. No ticks were collected from them since they were usually encountered dead in the traps. Shrews have a fast

metabolism, and they stress very easily in small spaces, leading to behavior such as hyperactivity that increases their body temperature and leads to their death (Platt, 1974; Ressing, 2008). Eastern chipmunks also had some larvae and nymphs collected on them with more collected nymphs than larvae (Fig. 14-15). Finally, although we only collected three woodland jumping mice, these small mammals had about 17% of all collected nymphal ticks, which was higher than the proportion of collected nymphs on eastern chipmunks in 2022.

4.2 Borrelia burgdorferi

The infection prevalence of *B. burgdorferi* was significantly higher in 2021 (43.18%) than in 2022 (35.44%). However, infection prevalence in the small mammals from our study was higher than from small mammals from central Pennsylvania (21%) (Rocco et al., 2020). *Peromyscus leucopus*, white-footed mice had a prevalence of more than 30% each year. The prevalence of infection was the highest even when infection of the white-footed mice was calculated against all the collected mammals. This rodent is known to be a competent reservoir for *B. burgdorferi* and is usually shown to have a high infection prevalence for the pathogen (Brunner et. al, 2008; Simmons et al., 2019; Rocco et.al, 2020; Larson et. al, 2021). One explanation for the high infection prevalence observed in *P. leucopus* is that they tend to feed large numbers of immature ticks compared to other mammalian hosts in the environment (Fig. 17-18). While other mammal species are competent for *B. burgdorferi* (i.e. shrews and eastern chipmunks) (Lobo et al., 2013) they were less abundant in the environment and they fed fewer nymphs and larvae (Fig. 17, Table. 3). Therefore, white-footed mice are more important for maintaining tick-borne pathogens in the environment in western Pennsylvania. Eastern chipmunks also showed a high number of infected

individuals and fed more nymphs than larvae during the two years of our study, potentially infecting them. The trend of more nymphs feeding was also observed in other studies (Schmidt et al., 1999; Tsao et al., 2021). Field data and mathematical modeling on small mammals indicates that eastern chipmunk's relative abundance can positively influence the prevalence of nymphal ticks and even the risk for potential human infection (Ostfeld et al., 2006; Vuong et al., 2017). Recent studies conducted on islands in Michigan demonstrated that alternative hosts, like eastern chipmunks or other medium or small mammals, can help maintain the presence of *B. burgdorferi* (Sidge et al., 2021). Eastern chipmunk's infection may have played an essential role in the prevalence of *B. burgdorferi* at CCH and SGL232 in 2021 and RVP in 2022. They increased the overall percentage of infections due to their high prevalence percentage at those sites (Table 5) even though there were not as many eastern chipmunks collected compared to other species at those sites (Fig. 9-12). Although the increase in prevalence was not significant, it is possible that most of the nymphs feeding on the individual hosts were infected since nymphs have more chances to be infected than larvae. Woodland jumping mice (WJ) had a 33.33% prevalence when only three samples were tested. The high prevalence could be attributed to the high proportion of nymphs feeding on them. They are rarely cited as a typical host for *B. burgdorferi*, but they were found to be highly infected during our investigation, and in another study conducted in the Atlantic Canadian wildlife (Zinck & Lloyd, 2022). Woodland jumping mice could potentially be an overlooked competent host. Nevertheless, it is important to acknowledge that the high prevalence could also be because the infectivity of the small mammal is compared to each species rather than the entire population. When the prevalence of infection of woodland jumping mice was measured against all the collected species, the prevalence of *B. burgdorferi* in woodland jumping mice was less than a percent (Table 7, Appendix A). The other collected animals with a low count had only

uninfected individuals (Fig. 16). Small mammals like opossums and flying squirrels are considered as poor reservoir hosts (Halsey & Miller, 2020). Surprisingly, due to the low collected numbers of meadow voles, they also had a prevalence of zero even though they are known to be competent hosts (Ginsberg et al., 2021; Zinck & Lloyd, 2022). Unlike meadow voles, shrews had a prevalence of 30% in 2022. Although it is known to be a competent host for *B. burgdorferi* (LoGiudice et al., 2003; Vuong et al., 2017), the prevalence of the pathogen in this group of species went from zero to 30% between the years. The increase could be due to an increase in the number of collected shrews and an increase in their density (Fig. 14-15), indicating that changes in the composition of the species could lead to more or less infection.

The change in the population of collected animals' composition during the study period could be the reason for the change in prevalence of the pathogen at RVP and CCH (even though the difference is not statically significant). These two sites had the highest density of white-footed mice and eastern chipmunks in 2022. The presence of more hosts means that there will be more opportunities for ticks to feed from those hosts and potentially infect them or get infected.

Change in density was also observed at SGL232 and SGL42. From 2021 to 2022, the density of white-footed mice and shrews increased while a decrease in the density of eastern chipmunks was observed at SGL232, and flying squirrels' density increased at SGL42. These changes in species density may affect *B. burgdorferi* infection prevalence if there are higher densities of non-competent hosts in the environment, which may lead to fewer infected ticks and less pathogen transmission to more competent hosts (Ostfeld & Kensing, 2012). The change at SGL42 lead to a decrease in infection prevalence since the presence of incompetent hosts increased in the area. The change in host diversity at SGL232 could lead to a rise in the prevalence of the pathogen since we observed an increase in some competent hosts (Ostfeld & Kensing, 2012). In

this work, a decrease in prevalence was observed during the two years. This could be because the observation on the change in the community of the study sample does not mean that the change is reflected at grid sites. Long-term investigations at each site are needed to better understand the trends in infection prevalence. In addition, other abiotic factors such as temperature, humidity, and rain could play a role in the differences observed between the change in infection prevalence and host density.

The abundance of hosts like mice and eastern chipmunks during the previous two years was a strong predictor for the risk of Lyme disease to humans during the current year (Ostfeld et al., 2006). White-footed mice and chipmunks were denser in non-rural areas than rural areas (Table 6). In addition, *B. burgdorferi* at SCH, CHH and SGL42 each year was relatively similar even though the highest prevalence shifted from rural to urban areas (Fig. 18). A significant difference in the prevalence between the sites in 2021 was mainly observed between non rural areas (SCH, BOM) and a rural area (SGL232). However, in 2022, the difference between rural and suburban areas was not significant. When the prevalence of infection was compared between all urban sites against rural and suburban sites (Fig. 20), it was observed that the prevalence at urban sites was becoming like the prevalence at rural sites. A study conducted in regional Pittsburgh found that tick density and infection prevalence remained the same among urban and rural sites (Simmons et. al, 2019), however they only studied the prevalence of the pathogen in *I. scapularis*. We observed an increase in prevalence of *B. burgdorferi* infection in small mammal species in all the urban sites (SCH and RVP), and one of the suburban sites (CCH) of western Pennsylvania, suggesting a potential risk to humans in urban areas. This research shows that the density of small mammals and the diversity of small mammals such as WF mice, eastern chipmunks, and shrews could potentially impact the prevalence of *B. burgdorferi.*

4.3 Limitations and future investigations

While this study helped advance our understanding of tick-borne pathogens in small mammals in western Pennsylvania, there were several limitations. For example, more than one species of *Peromyscus* mouse may be present at our field sites: *P. leucopus* (white-footed mouse) and *P. maniculatus* (deer mouse). In our study, mice were identified using key identifier traits (body measurements, coat color pattern, etc.); however, it is difficult to distinguish these two species morphologically and molecular identification would be useful to distinguish the two species. Like the white-footed mouse, the deer mouse is a competent reservoir host of tick-borne pathogens, such as *B. burgdorferi* and *B. microti* (Larson et al., 2021). In fact, a recent study in the Midwest found that an individual deer mouse was two times more likely to be infected with *B. burgdorferi* than a white-footed mouse (Larson et. al, 2021). Early studies have shown the morphological differences in the external pelage, tail size, and body weight of each mouse species can be used to discriminate between the two species (Machtinger & Williams, 2021; Brusseo et al., 1999). However, there is still a possibility of confusion because of individual variation and variations related to the geographical area they belong (Holmes et al., 2019). Molecular and biochemical tests such as melt curve assay, can be run to determine if our captures contained both species of mouse. Also, other studies comparing the prevalence of tick-borne pathogens between the two species of *Peromyscus* could enlighten us on the prevalence of the pathogen in the state of Pennsylvania. In addition, more investigation of the prevalence of *B. burgdorferi* and other tick-borne pathogens such as *B. microti* is still needed. A recent work found that the prevalence of *B. microti* was higher than the prevalence of *B. burgdorferi* in research conducted in central Pennsylvania (Rocco et al., 2020). Therefore, looking at these two pathogens would give a clear view of the presence of the pathogen in the western area of the state. Although shrews are known

to be the competent hosts for *B. burgdorferi*, no ticks were collected on any shrew during the data collection. While some shrews were caught alive and checked for ticks, most shrews were found dead in traps. Therefore, it is possible that ticks feeding on the shrews detached from the animal before we were able to collect them. Future work should find another approach to collect ticks in this group of small mammals while considering their fast metabolism.

Another limitation of this project was the method used to trap small mammals. Even though we collected some diurnal animals, trapping was safely conducted to collect animals that are nocturnal or active at night, such as white-footed mice, woodland jumping mice, and shrews. Therefore, our study was limited and did not fully sample animals active during the day like the flying squirrels and eastern chipmunks. Another project could be implemented to ensure that diurnal animals are also targeted.

Furthermore, our trapping methods were passive. Although certain animals like white-tail deer and raccoon were observed, tissue and tick samples were not collected from them, missing knowledge on their prevalence for the pathogens studied. This work gives us an idea of different small mammals that are competent hosts of *B. burgdorferi* in western Pennsylvania. However, it only represents some of the possible small mammal hosts since we could not possibly collect them all. For instance, we did not collect red and gray squirrels, which are local animals of the state and have been studied in other research on small mammals (Anderson et al., 2006; Ginsberg et al., 2021). In addition, this work lacks information on tick-borne pathogens in medium to large mammals like striped skunks, raccoons, woodchucks, and eastern cotton rabbits, which have also been identified as a host for *B. burgdorferi*. Future researchers should explore the prevalence rate and competency of tick-borne pathogens in medium and large mammals to further understand their impacts in spreading the pathogens.

Although our study gives us an idea of infection of *B. burgdorferi* in small mammals for two years, the prevalence of infection was not significant in certain instances because of the small sample size. We only discussed the prevalence of the pathogens for two years. More work is needed to increase our sample size and better visualize the area's infection. Finally, potential infection risk to humans was suggested based on current knowledge of the spread of the pathogens. However, the risk of human infection is better assessed as a measurement. Future studies should use mathematical models or regression analysis to determine and compare infection in small mammal hosts and the real risk of infection in humans in western PA.

4.4 Public health implications

The One health approach shows us that human health is impacted by the health of the animals that share their environment. Small mammals like the white-footed mice and eastern chipmunks play an important role in the maintenance of *B. burgdorferi* in this state as well as other states. Understanding the prevalence in small mammals can help us not only know about the dynamics of their bacterial infections, but also about the potential risk it poses to humans. The findings of this study could be a useful tool in preventive and control measures against Lyme disease.

5.0 CONCLUSION

This study investigates the prevalence of *B. burgdorferi* in small mammals of Western Pennsylvania. The findings of the work elucidate that some species of small mammals are likely to be infected with *B. burgdorferi* in the study areas, and that infection prevalence in urban areas could be as likely as in rural areas. The white-footed mice, which are known to be reservoir hosts of *B. burgdorferi*, were significantly the most abundant species collected and had one of the highest prevalence during the two years. Other small mammals like eastern chipmunks and shrews were also found with a high prevalence of infection. The presence and the proportion of infection of *B. burgdorferi* in white-footed mice and other small mammals indicates that the pathogen can be maintained and could pose a risk of infection in humans living in the area. The perceived risk of infection in humans is imminent not only in rural areas but also urban areas. More research is still needed to further our knowledge of the tick-borne pathogens in Western Pennsylvania.

APPENDIX A

Table 6. The density of each host species at different sites in 2021 and 2022. The table shows the density of each host species at each site during the two years.

	2021						2022					
	RVP	**SCH**	**BOM**	**CCH**	**SGL232**	**SGL42**	**RVP**	**SCH**	**BOM**	**CCH**	**SGL232**	**SGL42**
WF	43.33	91.67	18.33	33.33	30.00	41.67	26.67	73.33	26.67	105.00	86.67	48.33
EC	1.67	35.00	1.67	1.67	3.33	0.00	20.00	11.67	0.00	0.00	0.00	0.00
S	0.00	0.00	3.33	5.00	0.00	10.00	8.33	3.33	0.00	13.33	16.67	16.67
FS	8.33	0.00	1.67	0.00	0.00	3.33	0.00	5.00	1.67	0.00	0.00	0.00
MV	0.00	0.00	0.00	0.00	0.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00
OP	0.00	0.00	0.00	0.00	6.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WE	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WJ	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	50.00	0.00
NR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00	0.00	0.00	0.00	0.00

Table 7. Infection prevalence per species for all collected small mammals. This table shows the percentage of infected species out of all the collected species during the year.

	Prevalence of infection 2021	Prevalence of infection 2022
White-footed mice	37.21%	31.44%
Eastern Chipmunks	5.97%	1.80%
Shrews	0	1.80%
Woodland jumping mice	0	0.257 %

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