Investigating Macro- and Microparasite Coinfection Dynamics in White-Footed Mice (*Peromyscus leucopus)*

by

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Coinfections in both humans and animals can lead to changes in the duration of infection, susceptibility to other parasites, and may affect the host's symptoms and treatment effectiveness. Infection with a macroparasite may allow for the establishment of microparasites due to an elicited immune response. Therefore, investigating the inhibitory or facilitative responses to macro- and microparasite infection in hosts is important for understanding host and community-wide health in natural populations. Variations in mammalian diversity play a role in parasitic abundance and persistence. This can be shown as an inverse relationship between human health and biodiversity. Each section of the digestive tract of white-footed mice (*Peromyscus leucopus*) were individually analyzed to identify the presence of macroparasites. PCR was then performed to identify each macroparasite to the species level. For microparasite identification, DNA was extracted from the collected ear tissue samples. Genomic DNA samples were then screened for the presence of *Borrelia burgdorferi* using a specific real-time quantitative PCR. Our findings showed no significant correlation between coinfection and a macroparasite only infection along with male and female mice infected with a macroparasite from either of the two sites. Our data suggests that there are substantial differences in geographical locations in terms of infection with or without a parasite, macroparasite infection, and *B. burgdorferi* infection. We found a significant difference in mice infected with only *B. burgdorferi* between the two geographical locations. In terms of seasonal variation, no significant differences were observed between the collection months in either site for

any of the infection groups. Examining coinfection of helminths and *B. burgdorferi* in natural rodent populations has shown that infection with a macroparasite may not lead to coinfection of a microparasites. This study demonstrates that seasonal variation and sex did not influence coinfection in *P. leucopus*, contrary to previous findings. However, this study does support previous research suggesting that variation in mammalian diversity plays a role in parasitic abundance and persistence. Further research into multiple parasitic infections and their influence on their host is needed to better understand the influence on natural populations and how human health and disease risk are affected.

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Preface

I want to start by thanking my committee members, Dr. Joshua Mattila and Dr. Jessica Stephenson for their guidance, support, and time during this process. I am grateful to be mentored by Dr. Danielle Tufts throughout my time as a graduate student at the School of Public Health. I want to thank her for her never-ending support during personally difficult times in my education. She was able to encourage and believe in me during this process when I personally could not, of which I am grateful for. My gratitude also goes out to the students in Dr. Tufts lab. Kelly Schenk, Deanna Dailey, Josee Kahambwe Kumba, and EJ Young provided me with support and encouragement while I worked through this project. I would also like to thank the 2016 field research teams who captured the mice used in this project. This project was partially funded by the American Society of Mammalogists Grants-in-Aid of Research. Lastly, I must thank my family for their unwavering support throughout my degree.

1.0 Introduction

The ubiquitous nature of parasites and their ability to affect their host species directly and indirectly at various levels of organization in natural populations has allowed for considerable research on host-parasite interactions (Ezenwa 2016, Bellay et al 2018, Stokke et al 2018, Karvonen et al 2019, Halliday et al 2020, Cabrilo et al 2018, Toro-Londono et al 2019, Sallinen et al 2023, Ramsay and Rohr 2023). While extensive research has been completed on the effects of parasitic infection within a host, until recently, few studies have focused on the relationship between multiple parasitic species infection in a host and how these coinfections affect host health (Lass et al 2012, Vaumourin 2015, Ezenwa 2016, Ramsay and Rohr 2023). Coinfection by various parasites, macroparasites or microparasites, is a common occurrence in natural populations (Lass et al 2012, Fenton 2008, Johnson and Hoverman 2012, Ezenwa 2016, Ramsay and Rohr 2023). Macroparasites are multicellular organisms, which include nematodes, cestodes, and trematodes, and do not multiply within their definitive host (Nunn et al 2014). Microparasites, which include bacteria, viruses, fungi, and protozoa, are parasites that replicate inside their definitive host (Nunn et al 2014). As the name implies, microparasites are small in size, have short generation times, and can create immunity to re-infection in their host (Nunn et al 2014).

Hosts can acquire infections with these types of parasites in numerous ways including through contaminated water or food sources, ingesting parasites from infected fecal matter, or from an infected vector (Vaumourin et al 2015). Parasites are known to have a strong immunomodulatory effect on their host species (Ezenwa 2016, Maizels and Yazdanbakhsh 2003, Grencis 2015). Coinfections may occur when infection by one parasite inhibits the host immune response thereby facilitating the ability of another parasite to invade (Vaumourin et al 2015,

Hoarau et al 2020). The ubiquity and immunomodulatory effects of parasites can have on their host sets the scene for potentially strong interactions with co-occurring infections (Ezenwa 2016, Salgame et al 2013). Such infections in both humans and animals can lead to changes in the duration of infection, further susceptibility to other parasites, and may affect the host's symptoms and treatment effectiveness (Vaumourin et al 2015, Petney and Andrews 1998, Ezenwa and Jolles 2011).

1.1 Macro- Microparasite Interactions

Investigating helminth-microparasite coinfections has allowed for further research into the interactions between the parasites and how that interaction can influence the host (Wilson and Cotter 2013). The macro- microparasite facilitation hypothesis states that infection with a macroparasite allows for the establishment of microparasites, intra- or extracellular, due to an elicited immune response (Wilson and Cotter 2013). The adaptive immune response in animals uses distinct pathways to control intracellular microparasites versus extracellular macroparasites. There are two distinct pathways used by the immune response, in which a Th1 response is elicited for microparasites and a Th2 response is elicited from macroparasites, both of these pathways are cross regulated (Jolles et al 2008). Macro- and microparasites can interact competitively or facilitatively in which they are able to modify the other's transmission efficiencies, virulence, or by removing the host from a shared susceptible pool (Jolles et al 2008). One potential mechanism for parasite coinfection facilitation is that helminth infection may induce a Th2 cytokine immune response in the host which sequentially downregulates the Th1 cytokine response, which is associated with combating microparasitic infections, and may allow microparasites to invade the

host (Lass et al 2012, Ezenwa et al 2010). This concept has been demonstrated in gastrointestinal helminths which significantly increased disease progression and infection rates in individuals infected by malaria and HIV (Lass et al 2012, Fenton 2008). Additionally, mathematical models have shown that coinfection of a microparasite and a macroparasite can lead to an increase in community stability among the parasites and the host, a reduction in the reproduction ratio in microparasites, and an increase in transmissibility in microparasites that leads to a decrease in the reproduction ratio of macroparasites (Fenton 2008). Therefore, investigating the inhibitory or facilitative responses to macro- and microparasite infection in hosts is important for understanding host and community-wide health in natural populations.

1.2 *Peromyscus leucopus* and Macroparasites

An array of different species of birds, fish, reptiles, and mammals can serve as hosts for parasites. Among mammalian species, white-footed mice (*Peromyscus leucopus*) are among the most widespread small rodents in North America and are commonly found in the northeastern parts of the United States (Dhawan et al 2018, Andre et al 2017). Common macroparasites found in white-footed mice include *Hymenolepis microstoma*, *Pterygodermatites peromysci*, and *Baylisascaris procyonis* (Macnish et al 2003, Luong and Hudson 2012, Vandergrift 2008). *Hymenolepis microstoma,* commonly known as the bile duct tapeworm, is an intestinal parasite that lives in the bile duct and small intestine and its definitive hosts include mice, rats, and hamsters (Macnish et al 2003). *Pterygodermatites peromysci* in the eastern United States, is a common gastrointestinal nematode that inhabits the small intestine of small mammals and *P. leucopus* is known to be the natural host (Luong and Hudson 2012). Previous field studies have shown

seasonal variation of infection, where coinfection of seven species of helminths in *P. leucopus* was observed, of the seven *P. peromysci* was the highest in the month of July while the lowest prevalence was observed in November (Vandergrift 2008). *Baylisascaris procyonis* is an emerging wildlife zoonosis of public health significance (French et al 2020). It is parasitic roundworm with *P. leucopus* being the most common intermediate host as well as being common in raccoons (Beasley et al 2013). The adult stage of the parasite is known to infect mammals but not cause any clinical manifestations however, infection with the larval stage of this parasite can lead to neurological disease in the animals, as well as humans (French et al 2020).

1.3 *Peromyscus leucopus* and Microparasites

Peromyscus leucopus are also the main reservoir host of several tick-borne pathogens, the most common being the spirochetal bacterium *Borrelia burgdorferi*, a causative agent of Lyme disease in the US (Vannier et al 2015). In the eastern US, *Ixodes scapularis* ticks are the primary vectors for *B. burgdorferi* (Eisen et al 2017). Ticks generally have a 2-year life cycle in which the eggs hatch in the spring, molt into larvae by summer, then by next spring they molt into nymphs, and, finally, by the fall they molt into adults, to molt into each different life cycle the tick will first need to take a blood meal (Eisen and Eisen 2018). Larvae and nymphs generally feed on any available host in the environment (including mammals, reptiles, and birds), especially *P. leucopus* (Schmidt and Ostfeld 2001). Infected nymphs may transmit pathogens to susceptible mice during feeding, and infected mice in turn transmit pathogens to larvae during bloodmeal uptake (Eisen and Paddock 2020). Tick-borne pathogens are thus maintained in this cycle between immature tick life stages and *P. leucopus* mice (Eisen and Paddock 2020). In order to keep up with the changing

environmental conditions caused by climate change many species have been forced to alter their geographic range, which may expose them to new or a greater diversity of pathogens. This geographic expansion is a cause for concern because it may result in an increase in tick-borne pathogen transmission such as Lyme disease into naïve areas (André et al. 2017).

1.4 Abundance and Density of Competent Hosts and Vectors in the Environment

Different environmental and habitat landscapes may influence variations in host diversity and composition. Variations in mammalian diversity also plays a role in parasitic abundance and persistence, in which parasite loads may decrease in the presence of more suitable host species than when only few hosts are present. This poses that the diversity of the environmental community can mediate infection levels and disease. For instance, the loss of biodiversity may indirectly promote an increase in disease (Civitello et al 2015, Johnson and Thieltges 2010). This can be shown as an inverse relationship between human health and biodiversity in which an increase in biodiversity leads to a reduction in pathogen abundance and human disease risk (Huang et al 2019). Most pathogens infect multiple hosts that range in their ability to obtain and transmit various pathogens by supporting reproduction and survival (Huang et al 2019, Schmidt and Ostfeld 2001). Compared to the presence of reservoir competency, the potential of a species to support and transmit pathogens, species, if there is an increase in other host species into the community, the pathogen can become less abundant and less likely to persist in the environment (Huang et al 2019, Randolph and Dobson 2012). On the other hand, there are those that criticize this concept and consider it to be idiosyncratic and to only occur under certain conditions (Huang et al 2016). The belief that noncompetent hosts in the community can reduce pathogen transmission, through

different kinds of mechanisms has been criticized (Huang et al 2016). Those against this concept say that the prevalence and abundance of infection may respond differently to the changes in species diversity and that disease dilution is a scale-dependent phenomenon and may not operate at all scales (Huang et al 2016). Competent hosts are relatively resilient to local species loss, whereas low competent hosts are comparatively vulnerable and usually occur in more diverse communities (Huang et al 2016, Randolph and Dobson 2012, Schmidt and Ostfeld 2001). However, there are also criticisms of this concept in that particular species in highly diverse communities can have positive effects on disease risk and that the negative relationship between local extinction risk and reservoir competence is still controversial (Huang et al 2016). There are those that support this idea and those that argue against it but there is a general agreement that the change in competent hosts and vectors may not be solely driven by species diversity. It could also be influenced by the association between diversity, the specific identity, and relative abundance of competent or incompetent hosts in the community (Huang et al 2019).

In the case of tick-borne diseases, such as Lyme disease, an assumption is made that as biodiversity declines there is a loss of competent hosts and therefore an increase in *B. burgdorferi* abundance is observed (Randolph and Dobson 2012). The spread of *B. burgdorferi* through the northeastern United States can be attributed to fluctuating weather patterns due to climate change, host distribution patterns, and reservoir competency (Couper 2021).

To date, no research has been conducted on the interactions between macroparasitic coinfections with *B. burgdorferi*. Appropriate locations to undertake such an experiment are in the New England area because of the high incidence of Lyme disease and abundance of *P. leucopus* mice and macroparasites. Connecticut and Block Island, Rhode Island are ideal locations to investigate the influence of mammalian diversity and parasite community on coinfection

dynamics. Connecticut is located on the mainland and supports various ecosystems in which mammalian diversity is at much higher levels when compared to Block Island which constitutes low mammalian diversity and is mainly composed of *P. leucopus* and white-tailed deer, *Odocoileus virginianus* (Huang et al 2019). Therefore, my hypothesis for this research is that *P. leucopus* mice with higher abundance of macroparasite infection will also be infected with microparasites. Here, we will i) investigate the presence of macro- and microparasitic infections in *P. leucopus* mice, ii) characterize the differences in parasitic diversity and abundance in *P. leucopus* from different geographical distributions, seasonal variations, and infections in male and female mice, and iii) characterize the relationship of co-infected *P. leucopus* with both micro- and macroparasites. The goal of this research is to address several questions: Does infection with a macro/micro parasite facilitate coinfection with a different macro/micro parasite in *P. leucopus*? Are there differences in parasite diversity or abundance in *P. leucopus* from different geographical distributions? Are there seasonal variations in parasite infections among *P. leucopus* in different regions? Do parasite combinations differ between male and female mice?

2.0 Methods

2.1 Study Sites

The white-footed mice used in this project were previously captured in Connecticut and Block Island, Rhode Island from April 26 - May 2, July 23 - 31, and October 25 - 28, 2016. These months were chosen due to the tick life cycle with April/May being early in the season before nymphs are active, July being during peak nymphal activity, and finally October is late in the season where tick numbers would be low, but animals would still be active (Schmidt and Ostfeld 2001). In Connecticut, two sites were sampled: Lake Gaillard (LG) (41°22'25.3"N, 41°22'25.3"W) and Old Lyme (OL) (41°22'21.5"N, 72°20'37.6"W). Connecticut is characterized by high mammalian diversity with the community being made up of numerous species, such as American black bears (*Ursus americanus*), bobcats (*Lynx rufus*), coyote (*Canis latrans*), gray foxes (*Urocyon cinereoargenteus*), opossum (*Didelphis virginianus),* groundhogs (*Marmota monax*), raccoon (*Procyon lotor*), striped skunk (*Mephitis mephitis*), white-footed mice (*Peromyscus leucopus*), and white-tailed deer (*Odocoileus virginianus*) (Pearman-Gillman et al. 2020). The annual high and low temperatures in Connecticut are 16.1°C and 6.7°C, respectively. The average annual rainfall is 47.11 inches (US Climate Data 2023).

On Block Island, two sites were sampled: the Block Island National Wildlife Refuge (BR) (41° 12' 41.3"N, 71° 34' 30.7"W) and Rodman's Hollow (RH) (41°09'25.2"N, 71°35'22.9"W). Block Island is located 23 km off the southern coast of Rhode Island and is 25.9 km^2 in size. The island is characterized by low mammalian diversity with the community being made up of muskrat (*Ondatra zibethicus*), the Block Island meadow vole (*Microtus pennsylvanicus provectus*), house

mouse (*Mus musculus*), Norway rat (*Rattus norvegicus*), white-footed mice (*P. leucopus*), and white-tailed deer (*O. virginianus*) (Huang et al 2019). The annual high and low temperatures on Block Island are 14.4°C and 7.2°C. The average annual rainfall is 39.73 inches (US Climate Data 2023).

2.2 Sample Collection

Sherman live traps were used to capture small mammals at each location and were baited with a mixture of peanut butter, oats, and sunflower seeds (Tufts and Diuk-Wasser 2018). Traps were opened at dusk and checked at dawn to prioritize capturing nocturnal animals especially *P. leucopus* mice. Trapping occurred for two consecutive nights at each site during each trapping month. Mice were removed from a trap and given an unique identification number, morphological characteristics (sex, age, weight, and body measurements) were documented, and the number of larval and nymphal ticks from the ears and body were recorded. All *P. leucopus* mice were euthanized, necropsied, and the heart, lungs, kidneys, liver, spleen, and gastrointestinal tract were placed in separate tubes and flash frozen in liquid N_2 in the field and long-term stored at -80ºC in the lab. All animal procedures were approved by Columbia University Institutional Animal Care and Use Committee (AC-AAAL6470). For this study, only adult male and female mice were used; juvenile, pregnant, or lactating mice were not included.

2.3 Macroparasite Screening and Identification

The digestive tract of each individual was thawed in warm water for 5 min, then the stomach, small intestine, and caecum were separated into individual Petri dishes in a 0.9% saline solution to prevent drying and rupturing of macroparasites. Each organ was then individually analyzed underneath the microscope for at least 30 min to identify the presence of any macroparasites. The number of macroparasites found in each section of the digestive tract for each host was also quantified. Macroparasites were stored in 1.5 ml microcentrifuge tubes containing 100% ethanol to preserve the DNA of each macroparasite. Tubes were appropriately labeled with the mouse identification number, what organ the macroparasites were collected from, and the date, for preservation and subsequent analysis. After successfully processing each sample, DNA was extracted from each helminth by hand using the DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's protocol. PCR was then performed using nematode-specific identification primers targeting the 18S small subunit rRNA gene: forward primer G18S4 (5'-GCT TGT CTC AAA GAT TAA GCC-3'), reverse primer 18P (5'-TGA TCC WKC YGC AGG TTC AC-3'), and an alternative internal reverse primer 26R (5'-CAT TCT TGG CAA ATG CTT TCG-3') (Floyd et al 2005, Blaxter et al 1998). A MiniAmp^{TM} Thermal Cycler (Applied Biosystems®, ThermoFisher Scientific, USA) was used with cycling conditions of 95 °C for 2 minutes, followed by 35 cycles of 95 °C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute, and lastly, 72 °C for 10 minutes with the samples being held at 4°C indefinitely. Successful PCRs (~2Kbp fragments or 500bp when using the internal reverse primer on a 1% agarose gel) were sent for Sanger sequencing by Eurofins Genomics to allow for identification of each macroparasite to the species level.

2.4 Microparasite Screening and Quantification

For microparasite identification, an ear punch biopsy was taken from *P. leucopus* mice and placed into 1.5 ml tubes containing 100% ethanol and stored at room temperature until processing. DNA was extracted from the collected ear tissue samples using the DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's protocol. Genomic DNA samples were then screened for the presence of *B. burgdorferi* using a specific real-time quantitative PCR (qPCR) (Barbour et al 2009). A specific primer (16S-F: 5′-GGC GGC ACA CTT AAC ACG TTA G−3′, 16S-R: 5′-GGC GGC ACA CTT AAC ACG TTA G−3′) and probe (6FAM-TTC GGT ACT AAC TTT TAG TTA A-MGBNFQ) combination was used to target the 16S rRNA region of the bacterium (Huang et al 2019). A ViiA 7 real-time PCR system (Applied Biosystems®, ThermoFisher Scientific, USA) was used with cycling conditions of: 95 °C for 20 seconds, followed by 40 cycles of 95 °C for 3 seconds, and 60 °C for 30 seconds.

2.5 Statistical Analyses

For analysis, individuals were divided into four groups: a control group (no infection with either type of parasite), infected with only macroparasites, infected with only microparasites, and coinfected with both macro- and microparasites. Infection prevalence of macro- and microparasite coinfection in *P. leucopus* was calculated by dividing the number of mice infected by the number of processed mice. Statistical analyses were performed using R statistical software. Assessments between sites from each of the two locations, coinfection prevalence between males and females as well as coinfection prevalence between Connecticut and Block Island were compared using

Fisher's Exact Test. Lastly, infection prevalence was compared between the 3 months of capture, April, July, and October using One-way ANOVA. Two-tailed t-test was used to compare coinfections and infections with at least one macroparasite.

3.0 Results

3.1 Uninfected *Peromyscus leucopus* Mice

No significant difference was observed (p = 0.3649) when comparisons were made between the two collection sites, Old Lyme and Lake Gailliard, in Connecticut which allowed us to combine these two sites for further analysis. No significant difference was observed (p = 0.1638) when comparisons were made between the two collection sites, Rodman's Hollow and Block Island National Wildlife Refuge, in Block Island. This allowed us to combine these two sites for further analysis. The goal of this study was to compare geographical locations, Connecticut and Block Island, so we decided to combine Rodman's Hollow and Block Island National Wildlife Refuge because they are close geographically. Characteristics of *P. leucopus* mice are provided in Table 1 and mice collection data for the four sites is provided in Table 2.

Of the 288 available samples, 211 *P. leucopus* mice were used and processed for this study (Table 3). From Connecticut, 101 mice were processed and from Block Island 110 mice were processed (Table 3). Of the 101 mice collected from the sites in Connecticut, 31 were completely uninfected with either a macroparasite or *B. burgdorferi* (Table 3). Of the 110 mice collected from the sites in Block Island, Rhode Island, 20 were not infected with either a macroparasite or *B. burgdorferi* (Table 3).

To characterize the differences in parasitic diversity and abundance in *P. leucopus* from different geographical locations statistical similarities were first made between Connecticut and Block Island. Mice from Connecticut were found to be significantly less infected compared to mice on Block Island (p = 0.0357) (Table 3).

Differences were assessed in uninfected male and female mice from each location. No significant differences in prevalence were found between uninfected male and female mice in Connecticut ($p = 0.1328$). In addition, no significant differences were observed between uninfected male and female mice in Block Island ($p = 0.6231$).

Evaluations on whether there are any seasonal variations in infection among the mice in Connecticut and Block Island were also made. No significant differences were observed between the three collection months in Connecticut and Block Island, $p = 0.9431$ and $p = 0.0812$, respectively.

Table 1. Characteristics of *Peromyscus leucopus* mice collected from Connecticut and Block Island. The weight, body length, tail length, and left foot length are shown as ranges to include male and female mice. Juvenile, pregnant or lactating mice were not included in this study.

Demographic	Connecticut	Block Island
Age		
Adult	99	110
Sex		
Male	49	66
Female	50	44
Weight (grams)	10 - 54	9 - 31
Body Length (cm)	71 - 100	80 - 111
Tail Length (cm)	31 - 89	56 - 90
Left Foot Length (cm)	17 - 21	19 - 21

Table 2. *Peromyscus leucopus* collection counts from the two collection sites in Connecticut, Lake Gaillard (LG) and Old Lyme (OL), and Block Island, Block Island National Wildlife Refuge (BR) and Rodman's Hollow (RH). Counts include only adult male and female white-footed mice. Juveniles, pregnant, and lactating mice where not included in this study. The number of infected individuals includes infection with a macro- or microparasite.

Connecticut	No. Collected	No. Infected
LG	54	21
OL	45	27
Total	99	48
Block Island		
BR	39	24
RH	71	33
Total	110	57

Table 3. *Peromyscus leucopus* mice uninfected, infected with only macroparasite(s), infected with *B. burgdorferi* (microparasite), and coinfected with both macro- and microparasites. Male and female mice from Connecticut (CT) and Block Island, RI (BI) collected from April, July, and October. Proportion and prevalence shown in percentages.

CT	Processed Samples		Uninfected (%)		Macroparasite Only (%)		Microparasite Only (%)		Coinfected (%)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
April	16	10	9 (56.3)	2 (20.0)	3 (18.8)	5 (50.0)	3 (18.8)	5 (50.0)	0 (0.0)	0 (0.0)
July	23	20	4 (17.4)	7 (35.0)	4 (17.4)	7 (35.0)	4 (17.4)	7 (35.0)	14 (60.8)	3 (15.0)
October	10	20	6 (60.0)	3 (15.0)	2 (20.0)	6 (30.0)	2 (20.0)	6 (30.0)	1 (10.0)	4 (20.0)
BI										
			Male	Female	Male	Female	Male	Female	Male	Female
April	22	9	3 (13.6)	2 (22.2)	9 (40.9)	5 (55.6)	5 (22.7)	0 (0.0)	5 (22.7)	2 (22.2)
July	20	15	3 (15.0)	3 (20.0)	2 (10.0)	5 (33.3)	3 (15.0)	1 (6.7)	12 (60.0)	6 (40.0)
October	24	20	5 (20.8)	4 (20.0)	15 (62.5)	10 (50.0)	1 (4.2)	1 (5.0)	3 (12.5)	5 (25.0)

3.2 Prevalence of *Peromyscus leucopus* Mice Infected with Only Macroparasites

To characterize the differences in parasitic diversity and abundance in *P. leucopus* infected only with at least one macroparasite from different geographical locations, statistical similarities were first made between Connecticut and Block Island. Significance was observed between mice infected with only a macroparasite from Connecticut when compared to Block Island ($p = 0.0302$), in which mice from Block Island were more likely to be infected with a macroparasite (Table 3 and Figure 1).

We assessed if there were any differences in macroparasite only infection among male and female mice from Connecticut and Block Island. No significant differences were found between males and females infected with only a macroparasite when compared to those not infected with only a macroparasite in Connecticut ($p = 0.0705$) (Table 3). No significant difference was found between males and females infected with only a macroparasite when compared to those not infected with only a macroparasite in Block Island ($p = 0.588$) (Table 3 and Figure 1).

We evaluated if there were any seasonal variations in macroparasite only infection among the mice from the two collection locations. No significant difference was found when comparing the three different months for either Connecticut or Block Island, $p = 0.7542$ and $p = 0.1124$, respectively (Table 3 and Figure 1).

Helminth quantification was completed for each infected mouse from the stomach, small intestine, and caecum. In male mice from Connecticut there were low numbers of parasite load in males when compared to female mice (Table 4 and Figure 2). On the other hand, only one worm was found in the caecum of a female mouse in Connecticut compared to much higher numbers in male mice (Table 4). In Block Island, only one worm was found in the caecum of a male mouse compared to Connecticut where the highest number of pinworms found in male mice (Table 4 and

Table 5). There were much higher parasite loads found in the stomach and small intestine from male and female mice in Block Island compared to mice in Connecticut (Table 4 and Table 5).

Macroparasites from each section of the digestive tract were identified to the species level. Of those found in the stomach for both Connecticut and Block Island, the species found was *Aonchotheca putorii* (Table 6). Only *Aonchotheca putorii* macroparasites were found in the stomach of *P. leucopus* mice in this study. The number of *A. putorii* in male and female mice from Connecticut ranged from 1-15 and 1-70 with an average parasite load of 6 and 21.17 per infected animal, respectively. In Block Island the number of *A. putorii* in male and female mice ranged from 1-100 and 1-153 with an average parasite load of 25 and 36.1 per infected animal, respectively. The species of helminth found in the small intestine include *Pterygodermatites peromysci*, *Capillaria gastrica*, and *Hymenolepis microstroma* (Table 6). The number of *P. peromysci* in male and female mice from Connecticut ranged from 1-6 and 1-7 with an average parasite load of 1.8 and 2.5 per infected animal, respectively. In Block Island the number of *P. peromysci* in male and female mice ranged from 1-4 and 1-3 with an average parasite load of 1.69 and 1.9 per infected animal, respectively. The number of *C. gastrica* in male and female mice from Connecticut ranged from 1-4 and 1-5 with an average parasite load of 1.5 and 2.7 per infected animal, respectively. In Block Island the number of *P. peromysci* in male and female mice ranged from 1-13 and 1-30 with an average parasite load of 3.63 and 4.7 per infected animal, respectively. *Hymenolepis microstoma* was found in three male mice collected from Block Island in July with each having only one. The number of *A. tetratera* in male mice from Connecticut ranged from 1-18 with an average parasite load of 4.57, only one parasite was found in one female mouse. In Block Island, only one *A. tetratera* in a male mouse, no parasites were found in female mice.

Table 4. Quantification of macroparasites found in each section of the digestive tract, stomach, small intestine, and caecum, for male and female *P. leucopus* collected from Connecticut (CT). Prevalence of infection shown as percentage. Shown are the total number of macroparasites collected from all infected hosts from that specific month from a specific section of the digestive tract. Total counts of helminths for each organ are shown in bold at the bottom of the table.

CT	Male			Female		
	Stomach	Small Intestine	Caecum	Stomach	Small Intestine	Caecum
April	0 (0.0)	5 (11.1)	4 (12.5)	70 (55.1)	9 (15.5)	0 (0.0)
July	18 (100)	39 (86.7)	23 (71.9)	47 (37.0)	22 (37.9)	1 (100)
October	0 (0)	2 (4.4)	5 (15.6)	10 (7.9)	27 (46.5)	0 (0.0)
Total	**18**	**45**	**32**	**127**	**58**	**1**

Table 5. Quantification of macroparasites found in each section of the digestive tract, stomach, small intestine, and caecum, for male and female *P. leucopus* collected from Block Island, RI (BI). Prevalence of infection shown as percentage. Shown are the total number of macroparasites collected from all infected hosts from that specific month from a specific section of the digestive tract. Total counts of helminths for each organ are shown in bold at the bottom of the table.

BI	Male			Female		
	Stomach	Small Intestine	Caecum	Stomach	Small Intestine	Caecum
April	156 (18.6)	15 (11.9)	1 (100)	30 (4.6)	7 (5.83)	0 (0.0)
July	407 (48.7)	54 (42.9)	0 (0.0)	249 (38.3)	53 (44.2)	0 (0.0)
October	272 (32.6)	57 (45.2)	0 (0.0)	370 (57.0)	60 (50.0)	0 (0.0)
Total	**835**	**126**	**1**	**649**	**120**	**0**

Table 6. Species of helminth identified in the stomach, small intestine, and caecum of *P. leucopus* male and female mice from Connecticut and Block Island, RI. Sanger sequencing was used to properly identify the helminth to the species level.

	Species Found
Stomach	*Aonchotheca putorii*
Small Intestine	*Pterygodermatites peromysci, Capillaria gastrica, Hymenolepis microstroma*
Caecum	*Aspiculuris tetratera*

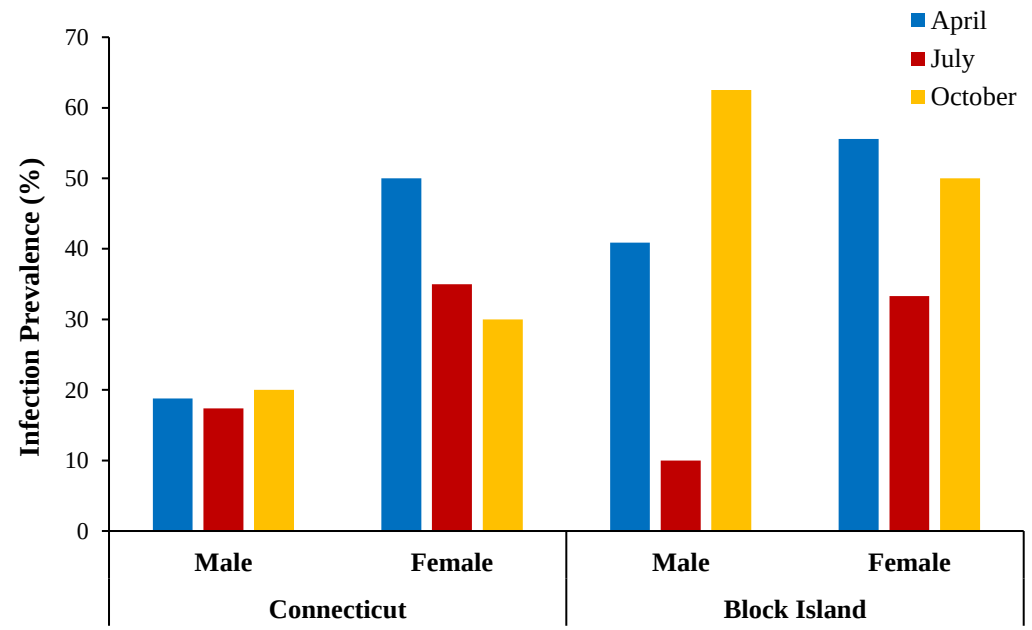

Figure 1. Macroparasitic infection prevalence, shown as percentages, in male and female *P. leucopus* mice from the months of April, July, and October from Connecticut and Block Island, RI.

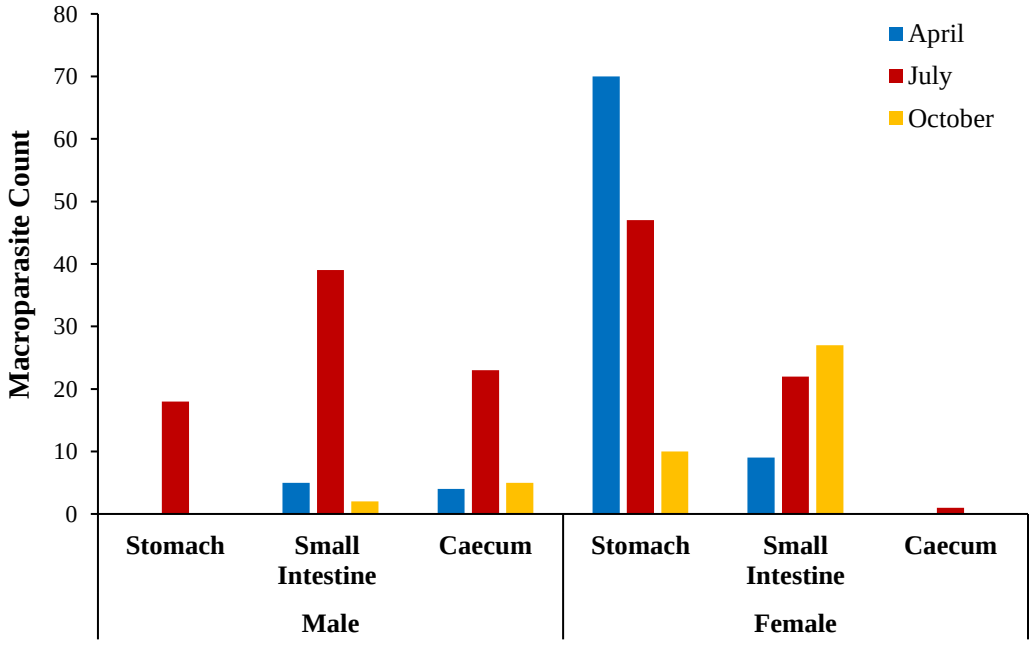

Figure 2. Macroparasite counts in the digestive tract, stomach, small intestine, caecum, of male and female *P. leucopus* collected from Connecticut. Shown are the total number of macroparasites collected from all infected hosts from that specific month from a specific section of the digestive tract.

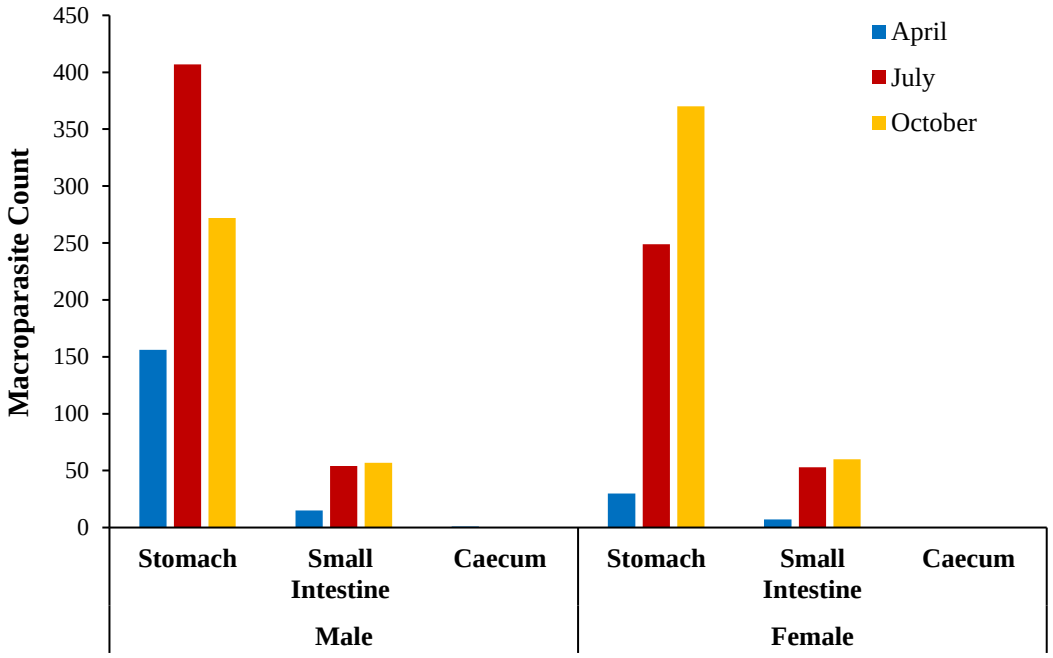

Figure 3. Macroparasite counts in the digestive tract, stomach, small intestine, caecum, of male and female *P. leucopus* collected from Block Island, RI. Shown are the total number of macroparasites collected from all infected hosts from that specific month.

3.4 Prevalence of *Peromyscus leucopus* Mice Infected with *Borrelia burgdorferi* Only

To characterize the differences in parasitic diversity and abundance in *P. leucopus* infected with only *B. burgdorferi* from different geographical locations, statistical similarities were first made between Connecticut and Block Island. We found that mice from Connecticut had significantly higher infection with only *B. burgdorferi* compared to mice from Block Island (p = 0.0335) (Table 3 and Figure 4).

We assessed if there were any statistical differences in *B. burgdorferi* only infection among male and female mice from Connecticut and Block Island. Significantly more females were infected with *B. burgdorferi* only compared to males in Connecticut (p = 0.0479), in which more females were infected. No significant difference was found between male and female mice who were only infected with *B. burgdorferi* on Block Island (Table 3 and Figure 4).

We evaluated if there were any seasonal variations in *B. burgdorferi* only infection among the mice from the two collection locations. No significant differences were observed when comparing the three different months in Connecticut as well as Block Island, p = 0.9460 and p = 0.7994, respectively (Table 3 and Figure 4).

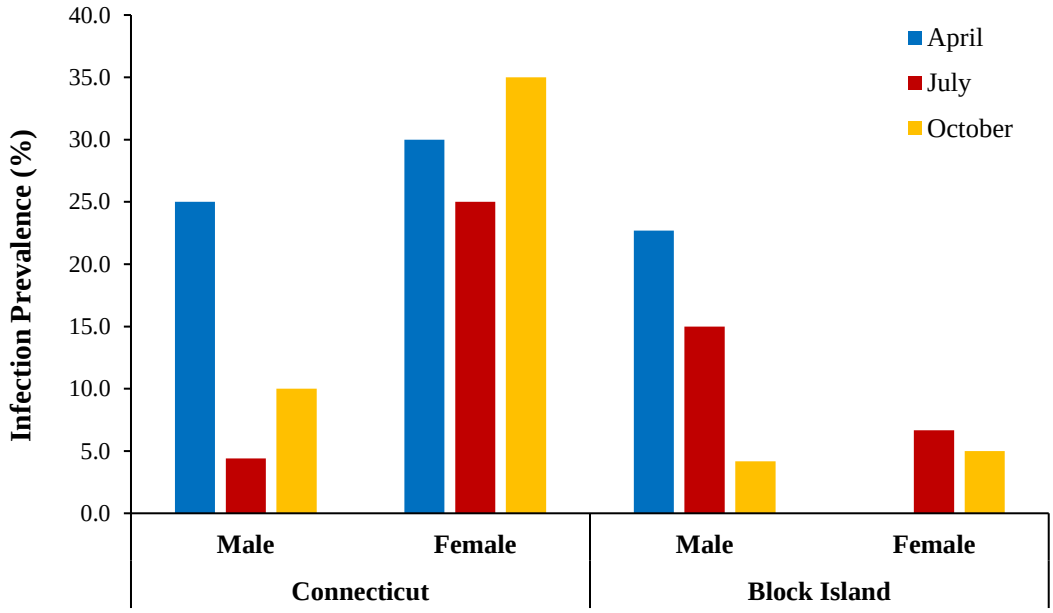

Figure 4. *Borrelia burgdorferi* infection prevalence, shown as percentages, in male and female *P. leucopus* mice from the months of April, July, and October from Connecticut and Block Island, RI.

3.5 Coinfection with Macroparasite(s) and *Borrelia burgdorferi* in *P. leucopus* Mice

The relationship of co-infected *P. leucopus* with both macro- and microparasites from Connecticut and Block Island were characterized. In terms of coinfection in Connecticut, there were 22 mice that were infected with at least one macroparasite and *B. burgdorferi* however, no significant difference was found ($p = 0.7225$). For coinfection in Block Island, there were 33 mice that were infected with at least one macroparasite and *B. burgdorferi*, but no significant difference were observed ($p = 0.3826$) (Table 3 and Figure 5). There was also no significant difference found when comparing coinfected individuals from Connecticut and Block Island against each other ($p = 0.2126$).

The presence of coinfection among the male and female mice from each of the two locations were also made. In Connecticut, males were marginally significantly more coinfected compared to female mice ($p = 0.0559$). No significant differences were observed between coinfected male and female mice from each Connecticut and Block Island, $p = 1$ (Table 3).

Evaluations on whether there are any seasonal variations in coinfection among the mice in Connecticut and Block Island were additionally made. No significant difference was found when comparing the three different months, April, July, October, in Connecticut ($p = 0.3119$). When comparing the collection months in Block Island, no significant differences were found ($p = 0.2514$) (Table 3).

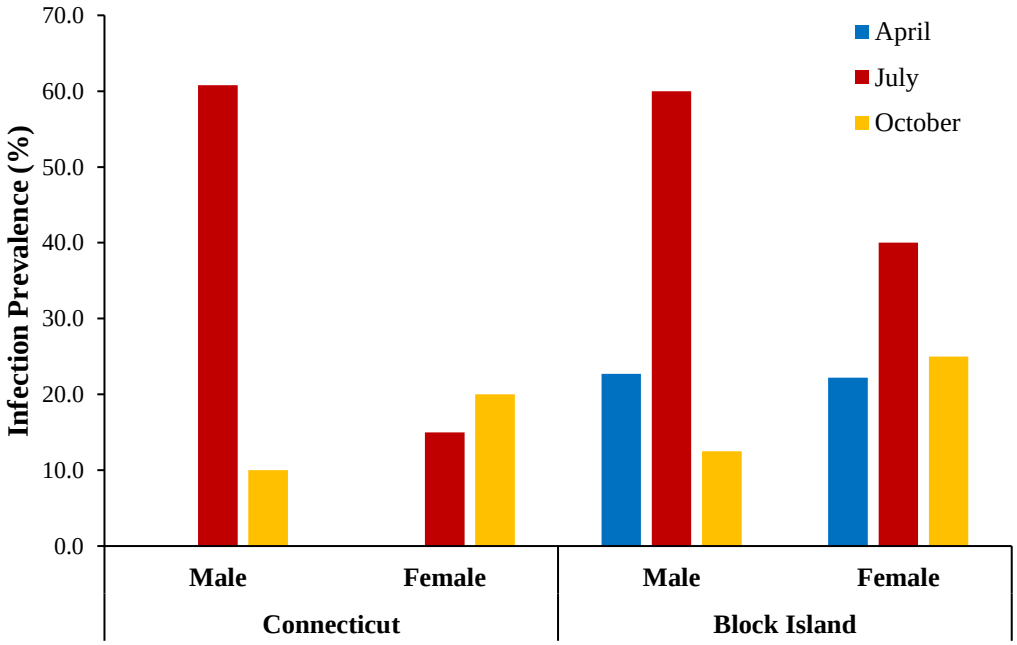

Figure 5. *Borrelia burgdorferi* and macroparasite coinfection prevalence, shown as percentages, in male and female *P. leucopus* mice from the months of April, July, and October from Connecticut and Block Island, RI.

4.0 Discussion

In this study, we investigated coinfection of *P. leucopus* mice by macro- and microparasites, specifically helminths and *B. burgdorferi*, respectively. This was done by investigating the presence of macro- and microparasitic infections, characterizing the differences in parasitic diversity and abundance in *P. leucopus* from different geographical distributions, seasonal variations, and between male and female mice.

We hypothesized that *P. leucopus* mice with a macroparasite infection will also be infected with microparasites. However, our findings showed no significant correlation between coinfection and a macroparasite only infection from either Connecticut or Block Island. This suggests that if a mouse is found to have a macroparasitic infection they are not more susceptible to a microparasitic infection compared to mice that have no infection.

In terms of comparisons between male and female mice infected with at least one macroparasite no significant difference was observed from either of the two locations, Connecticut, and Block Island. Previous studies have found a suppressive effect of testosterone on the immune system in males and that reproductive success outweighs immunity (Cabrilo et al 2018). This goes against our findings as they show that, in terms of infection prevalence, the females were observed overall to have higher numbers of at least one macroparasitic infection than the males in Connecticut and Block Island.

Our data suggests that there are substantial differences in geographical locations in terms of infection with or without a parasite, macroparasite infection, and *B. burgdorferi* infection between Connecticut, which has high mammalian diversity, and Block Island with a much lower mammalian diversity. This relates back to the concept that parasitic loads may decrease in the

presence of more suitable hosts species than when only a few hosts are present. The current study supports this argument as parasitic loads were observed to be higher in Block Island than Connecticut. This observation is also true of mice that were infected with only *B. burgdorferi* in Connecticut and Block Island. These findings have implications for human disease risk as those living in locations with lower biodiversity, Block Island, may be at a higher risk of pathogen infection (Huang et al 2019).

We found a significant difference in mice infected with only *B. burgdorferi* between the two geographical locations. The two geographical locations, Connecticut and Block Island, also relates to the concept of the influence of competent hosts, vector abundance, and density have on the environment in terms of pathogen spread and disease risk. As *I. scapularis* is the primary vector of *B. burgdorferi* if competent hosts decrease an increase in *B. burgdorferi* abundance is observed (Randolph and Dobson 2012). In Connecticut, female mice showed a significantly higher infection prevalence in *B. burgdorferi* compared to males; however, no significant differences were observed among male and female mice on Block Island. These results are contrary to previous studies that have shown a suppressive effect of testosterone on the immune system in which it would be assumed that males would have higher levels of infection (Cabrilo et al 2018).

In terms of seasonal variation, no significant differences were observed between April, July, or October in Connecticut or Block Island for any of the infection groups. In regard to macroparasite infection, our findings go against previous studies in which a higher prevalence of macroparasite infection was observed in the months of July, with the lowest numbers being from October (Vandergrift 2008). However, the Vandergrift (2008) study only looked at coinfection with other macroparasites and did not consider a possible infection with a microparasite and how that may or may not have influenced their results. While our results did not show any significant

differences, we did observe both male and female mice collected from April to have the lowest numbers in terms of positive infection for both Connecticut and Block Island. The month of July was observed to have higher numbers of macroparasitic infections for both Connecticut and Block Island compared to April and October. These findings could be because there was not a large enough sample size for this study to result in significant findings.

5.0 Limitations and Future Direction

Some potential limitations with this research may include mice being infected with other tick-borne pathogens that we did not screen for, such as *B. microti*, *A. phagocytophilum*, and viruses. Mice may also be infected with other microparasites that are not tick-borne but may still have an impact on macroparasites, such as viruses, protozoa, fungi, or bacteria. Additionally, some macroparasites that live in different tissue types (lungs, heart, bladder, etc.) were not investigated and will have gone undetected. Infection with other such pathogens in different areas of the body could lead to differences in the susceptibility of *P. leucopus* mice to obtain or clear infection with *B. burgdorferi* and/or a gastrointestinal helminth. The order of infection is also a limitation of this research because we were unable to tell whether the mice were infected with a macroparasite or a microparasite first and how the order of infection could facilitate coinfection (Ramsay and Rohr 2023, Karvonen et al 2019, Billet et al 2020, Clay et al 2019). Mice are able to recover from *B. burgdorferi* infection; therefore, they could have been coinfected with macro- and microparasites but were able to clear the microparasitic infection before the mice were euthanized in the field. Another limitation of this study is the sample size, a larger sample size may have increased the strength of our data.

Despite these potential limitations, we believe valuable knowledge can still be obtained about the role macro- and microparasites play in facilitating or inhibiting infection within a host and this experiment will pave the way for future laboratory and field experiments. Additional mathematical models, such as a generalized linear mixed model could be used for further analysis in which sex, location, seasonality, and parasite infection can be used as response variables. A possible future experiment could investigate how the microbiome of the host is affected in the

presence of coinfection with macroparasites and/or microparasites. Another future experiment could involve lab manipulation in which mice are infected with macro- and microparasites at different time points and specific order and timing of infection are analyzed. This experiment could involve infecting the mice with macroparasites before microparasites and vice versa and development/clearance of infection as well as host health can be studied.

As stated previously, Lyme disease is of growing concern for public health as the range of tick-borne diseases has greatly expanded over the years thereby raising the risk of transmission from infected ticks to humans. Macro- and microparasite coinfection are highly prevalent in *Peromyscus leucopus*, and a high burden of helminth infection may influence the success of pathogen transmission by repeated tick feeding and the immune control of tick-borne pathogens, such as Lyme disease, due to the cross regulation of the two immune response pathways elicited by helminth and microparasite infection. Therefore, helminth coinfection could affect the efficiency of transmission of *B. burgdorferi*, the causative agent of Lyme disease and thereby affect public health.

6.0 Conclusion

Considerable research on host-parasite interactions has been conducted due to the omnipresent nature of parasites and their ability to affect their hosts species directly and indirectly in natural populations. Until recently, few studies have focused on the relationship between multiple parasitic species infection in a host and how these coinfections affect host health. Examining coinfection of helminths and microparasites such as *B. burgdorferi* in natural populations has shown that infection with a macroparasite may not lead to coinfection of a microparasites. This study demonstrated that seasonal variation and sex did not influence coinfection in *P. leucopus,* contrary to previous findings. However, this study does support previous research suggesting that variation in mammalian diversity plays a role in parasitic abundance and persistence. In particular, the loss of biodiversity, a change in the number of competent hosts and vectors, may indirectly stimulate an increase in disease. Further research into multiple parasitic infections and their influence on their host should be conducted to better understand the influence it may have on natural populations and how that affects human health and disease risk.

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