

INVESTIGATING THE PATTERNS OF COINFECTIONS
WITH GASTROINTESTINAL HELMINTHS
AND SIN NOMBRE VIRUS
IN DEER MICE

by

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ABSTRACT

Zoonotic pathogens are infections in wildlife that are transmittable to humans. In natural settings, most wild animals host multiple species of parasitic organisms and other zoonotic pathogens. These parasites may interact and increase host susceptibility to secondary infections including zoonotic agents. Thus, understanding the parasite community of wild animals is important from ecological and public health perspectives, since parasites may be increasing the risk of transmission of zoonotic pathogens to humans in close association with wildlife. The purpose of this thesis was to identify the helminth parasites and to document patterns of coinfections between helminths and Sin Nombre virus (SNV) in deer mice (*Peromyscus maniculatus*) from two distinct ecoregions in Utah. I utilized a long-term database collected over seven years (2003-2009) along with necropsy of freezer-archived deer mice to identify patterns of coinfection between helminths and SNV. In year 2006, I found that SNV prevalence negatively correlated with helminth infection. This result suggests that one infection provides protection against the other. I sought to further elaborate on this study by live-sampling deer mice in a peridomestic habitat in Emigration Canyon, Utah from June 2010 through August 2010. I found

similarities between the helminth communities in this study, but I found an additional species *Trichuris peromysci*. Due to low SNV prevalence, I did not observe SNV / helminth coinfections. Finally, I designed a method to study tradeoffs between mounting a humoral antibody response to SNV antigen and bacterial killing capacity of serum in deer mice. I injected treatment mice with SNV nucleocapsid antigen while control mice received vehicle injection. Both the treatment and control mice significantly increased bacterial killing post injection; there were no significant differences between groups post injection. This suggests there is no tradeoff between mounting a humoral antibody response and the ability to kill bacteria. In summary, this was the first study to consider the role of parasite coinfections on the emerging viral pathogen, Sin Nombre virus. Since parasites can increase susceptibility to secondary infections, it is important for researches to investigate coinfections instead of focusing on a single parasite species.

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CHAPTER 1

INTRODUCTION

All animals at some time point during their lives are exposed to parasitic organisms. It is now accepted that most wild animals simultaneously host multiple parasite infections at any given time (1). For example, helminths are parasitic organisms commonly found in the gastrointestinal tracts of many species of wild animals, including mammals (2). Previous research demonstrates that infections with gastrointestinal helminths can increase susceptibility to secondary pathogen challenges (3-5). From a public health perspective, it is important to investigate the patterns of coinfections between helminths and viral zoonoses due to the frequent overlap of humans and wildlife. One area currently understudied in the literature is coinfections between gastrointestinal helminth infections and *Hantaviruses*. Only one study to date examined this and it finds a positive association between helminths and Puumala hantavirus (6). Thus, the overall goal of this thesis is to identify the primary gastrointestinal helminths and patterns of coinfections between helminths and Sin Nombre virus in populations of wild rodents from two distinct regions in Utah.

The study species for this thesis is the deer mouse (*Peromyscus maniculatus*), one of the most abundant nocturnal rodents across North America (7). Deer mice host an array of parasitic organisms including gastrointestinal helminths (8) and Sin Nombre virus (SNV), the etiological agent of human hantavirus infections in the United States (9). Previous studies hypothesize the virus is transmitted among rodents during aggressive interactions (10) and subsequently transmitted to humans in mouse excrement (11). Moreover, ecological factors such as deer mouse density (10, 12, 13), species diversity (14, 15), vegetative structure (16, 17), and anthropogenic disturbance (18) correlate with viral prevalence. Few identify, however, the impacts of helminths on infection with SNV (6). Helminth parasites have immunological effects on their hosts by stimulating a costly immune response and by increasing susceptibility to secondary pathogens (19-21). It is important to document the patterns of coinfections between these two different types of parasites since one type of infection may change host susceptibility to the other types of infection.

In Chapter 2, I described the gastrointestinal helminths of deer mice and patterns of coinfections with SNV and helminths. I utilized archived deer mice that were collected over a seven-year span from the Great Basin desert in Utah. This was the first study to look for patterns between these two infections. I identified six species of gastrointestinal helminths that infect deer mice in the Great Basin desert. In the year with the largest sample size (2006), deer mice with helminth infections had a lower prevalence of SNV infection compared to mice without helminth infections. These results suggest infection with

gastrointestinal helminths may decrease the likelihood of acquiring an SNV infection and this protection may be context dependent.

In Chapter 3, I identified the helminth community composition and prevalence of SNV in deer mice in a peridomestic setting. I utilized both field and snap trap capture approaches at a site in Emigration Canyon, Utah. In the field study, I followed a population of deer mice to determine if there were temporal changes in helminth infections by using a modified McMaster fecal egg flotation method (22). In conjunction with the field study, I also snap-trapped individuals to identify helminths. Similar species of helminths were found in the Great Basin, with the exception of a new nematode species, *Trichuris peromysci*. I found a female sex bias in helminth infection and intensity in snap-trapped animals. I also tested deer mice for antibodies against SNV to determine if the virus was present in this habitat and found that 2.8% of mice tested positive for antibodies but coinfections with helminths and SNV were not identified.

Finally, in Chapter 4 I challenged deer mice with SNV nucleocapsid antigen to determine if induction of an adaptive immune response would suppress the innate immune response. In this experiment, I characterized the ability of deer mouse serum to kill bacteria as a measure of innate immune function both pre and post antigen challenge. Both the treatment and control animals responded to injection by significantly increasing their bactericidal activity. There were no significant differences between the treatment and control groups. This result suggests there are no tradeoffs between mounting a humoral response to an antigen (adaptive) and ability to kill bacteria (innate).

Conclusions

These studies demonstrate the importance of considering the role of coinfections between helminths and SNV in deer mice. Parasites have the ability to modulate the host immune responses and impact host immunity (3-5). Thus, identifying the patterns of coinfection between parasites and zoonosis are important from a public health perspective since an increasing number of humans are living in close association with wildlife. Further work further elucidating the immunological impacts of gastrointestinal helminths on SNV is needed to verify the impacts of parasites on the prevalence of a viral pathogen.

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CHAPTER 2

HELMINTH INFECTION NEGATIVELY CORRELATED WITH VIRAL PREVALENCE IN A WILD RODENT

Abstract

Most wild animals host parasite infections at some point during their life. Although animals simultaneously host many parasites, most studies examine the effects of a single parasite species. The goal of this study was to identify the gastrointestinal helminths of deer mice (*Peromyscus maniculatus*) and to identify patterns of coinfection between helminths and Sin Nombre virus (SNV). Parasites were isolated from the intestinal tracts of mice (N=98) that had been frozen until dissection. An enzyme-linked immunosorbent assay was used to determine SNV infection status. We identified six species of helminths and found an overall prevalence of 68% in deer mice. SNV prevalence was 22%. For the year 2006, we found that mice with helminths had a lower prevalence of SNV infection compared to mice lacking infections. The results imply that helminth infections may provide protection to a host, although direction of protection needs further elucidation.

Introduction

All organisms are subject to infection by parasites. Parasitism is a highly successful strategy that is reflected by the enormous degree of parasite diversity identified to date (1). Most animals are generally coinfecting with multiple species of parasites at any given point during their life (2, 3). The few studies that have looked at the effects of parasite coinfections on hosts have had mixed results in terms of how a primary infection influences susceptibility to secondary infections (4-6). That is, coinfections have been shown to both benefit and harm the host. For example, African buffalo (*Syncerus caffer*) are more susceptible to bovine tuberculosis when infected with a strongyle nematode compared to buffalo lacking nematode infections (5). Alternatively, viruses of field voles (*Microtus agrestis*) can have both positive and negative effects on the hosts, either increasing or decreasing the probability of acquiring a secondary infection (6). The outcomes of interacting parasites appear to be context-dependent, highly variable, and dependent on species interactions (7, 8). Thus, more research is needed to fully understand the complexity and unpredictability of parasite coinfection since these interactions may play an important role in the transmission of zoonotic pathogens.

We examined patterns of coinfections between helminths and a virus in deer mice, *Peromyscus maniculatus*. Deer mice are ubiquitous across North America (9) and are the primary reservoir of Sin Nombre virus (10), the etiological agent of Hantavirus Pulmonary Syndrome in humans, with an associated mortality rate of 36% (11). Additionally, they host other parasites

including intestinal helminths (12-15). Thus, deer mice are excellent candidates in which to study the occurrence of macro and microparasite coinfections. The primary objectives of this study were to identify the gastrointestinal helminths of deer mice and to document the patterns of helminth coinfections, as well as helminth/SNV coinfections. We predicted that coinfections with gastrointestinal helminths and SNV were more common than predicted by chance alone, since helminths have been shown to increase host susceptibility to viral infections in other wildlife systems (4, 5).

Methods

Deer mouse sampling

For this study, we utilized archived deer mice collected during a long-term ecological survey of small mammals. The survey was conducted from 2003 to 2009 in the Great Basin Desert, west of the Tintic Mountains, Juab County, Utah. The deer mice represented the incidental mortalities that occurred over 96,940 trap nights of collection. Trapping, animal handling, and sample processing details can be found elsewhere (16, 17) and were approved by the Institutional Animal Care and Use Committee at the University of Utah (IACUC numbers # 08-02012 and 11-01007).

Helminth identification

Frozen deer mice (N = 98; 40 female, 58 male) were thawed in a BSL-2 laminar flow hood. Mice were pinned to a dissection tray and sprayed with 90% ethanol. The body cavity was opened with dissection scissors and the intestinal

tract, from the lower esophagus to the anus, was removed. The intestines were then placed into conical tubes containing 90% ethanol for a minimum of 24 hours to inactivate any viable SNV (Jason Botten, personal communication). The intestinal tract (stomach, small intestines, cecum, colic spiral, large intestines) was slit open and carefully examined under a dissection microscope to detect parasites. Contents were filtered through a 150-micron sieve (VWR International, Radnor, Pennsylvania 19087, USA) and reexamined a second time for parasites. If helminths were present, location in the gut was noted. The parasites were removed and placed in preservation fluid (9 parts 70% ethanol: 1 part glycerol) and were then identified to species by J M Kinsella, an expert parasitologist. Voucher specimens were deposited in the U.S. National Parasite Collection, Beltsville, Maryland under accession numbers 102724 to 102728.

Detection of SNV antibodies

Enzyme-linked immunosorbent assays (ELISAs) were used to test for SNV specific IgG antibodies (18). Since SNV infection is chronic (19, 20), infected deer mice produce antibodies against the infection for life, making ELISAs a reliable measure of infection status. The majority of the archived mice in this study had previously been tested for SNV antibodies with blood samples collected during the long-term ecological study (17, 18). Animals that died prior to processing or blood collection were tested with ELISA using homogenized heart and liver tissue collected at time of dissection. We validated the tissue ELISA by running sera samples from five deer mice infected with SNV in conjunction with the collected homogenized tissue. ELISAs were conducted in a

BSL-2 facility at the University of Utah following the methods of (21).

Statistical analyses

For each parasite species, we calculated prevalence (number of individuals with infection / total number of individuals sampled X 100), mean infection intensity (total number of each worm species / number of infected individuals with that worm species). The data were analyzed across all years as well as year 2006 alone, since 58% of the samples were collected in the year 2006 (Table 2.1).

We tested whether coinfections with helminths and SNV were more common than predicted by chance alone based upon the observed prevalence of helminths and SNV. The calculated expected values were: All years 14.6 % coinfecting, Year 2006: 15.6% coinfecting. Pearson Chi square analyses were used to determine if there were differences in SNV prevalence based upon helminth infection status. Since helminth distributions were aggregated, nonparametric Wilcoxon rank sum tests were used to determine if mean parasite intensities differed by sex. All statistical analyses were conducted in JMP 9 (SAS Institute, Cary NC) and considered to be significant if $P \leq 0.05$.

Results

Gastrointestinal helminths

A total of 67 out of 98 deer mice were infected with gastrointestinal helminths, which were found inhabiting all anatomical locations of the gut.

Table 2.1: Prevalence of gastrointestinal helminths and Sin Nombre virus by sampling year.

	2003	2004	2005	2006	2007	2008	2009
No. Mice sampled	7	2	15	57	3	0	14
SNV prev. %	1.4	0	13.3	22.8	33.3	0	28.5
Helminth prev. %	42.8	100	73.3	68.4	66.6	0	71.4

Prevalence of all helminth species combined was 68.3% in all years and 68.4% for year 2006 (Table 2.1). Six species of helminths were found in the intestinal tracts of deer mice. Five were nematodes: *Protospirura numidica* (PRNU; Seurat), *Syphacia peromysci* (SYPE; Harkema), *Pterygodermatites peromysci* (PTPE; Lichtenfels), *Aspicularis americana* (ASAM; Erickson), *Gongylonema peromysci* (GOPE; Hall), and one was a cestode: *Hymenolepis* sp.(HYME). The most common helminth was *P. numidica* (31%) and the rarest species were *Hymenolepis* sp. and *G. peromysci*, which were each found in only one deer mouse (Table 2.2). The majority of deer mice (51%) harbored a single species of helminth, while 17% harbored coinfections (Figure 2.1).

SNV prevalence and coinfection with helminths

A total of 21 deer mice tested positive for SNV antibodies. SNV prevalence ranged from 13.3% in year 2005, peaked at 33.3% in year 2007, and averaged 21.4% for all years (Table 2.1). There were no differences in SNV infection with respect to sex for the two subsets (all years: $\chi^2 = 1.659$, $df = 1$, $P = 0.19$; year 2006: $\chi^2 = 0.09$, $df = 1$, $P = 0.76$). There was no difference in SNV prevalence in animals with and without helminth infections across all years (all years: $\chi^2 = 1.785$, $df = 1$, $P = 0.18$; Figure 2.2) and, we found that 12.2% of deer mice were coinfecting with SNV and helminths (expected 14.6%). In contrast, during 2006, SNV prevalence of deer mice without helminth infections was 2.5x greater than individuals with worm infections ($\chi^2 = 3.865$, $df = 1$, $P = 0.05$; Figure 2.2), and we

found that only 10.5% of deer mice were coinfecting with SNV and helminths (expected 15.6%).

Discussion

The main objectives of this study were to identify the primary species of gastrointestinal helminths that infect deer mice and to document the patterns of coinfections with helminths and SNV. We predicted that coinfections with gastrointestinal helminths and SNV are more common than predicted from the prevalence of worm infection and SNV infection. The data did not support the hypothesis. We found that the majority of individuals hosted either helminths or SNV. Few individuals were coinfecting with both of these parasites simultaneously (12.2% in all years, 10.5% year 2006). We found that deer mice with helminth infections, collected in year 2006, had lower SNV prevalence than individuals lacking a helminth infection.

Helminths of deer mice

We found six species of helminths throughout the gastrointestinal tracts of deer mice. Moreover, most individuals hosted single infections with a small number of individuals harboring multiple helminths. Infection prevalence was similar to other studies in *Peromyscus* (12, 22-24). For the nematode *Pterygodermatites peromysci*, for example, Vandegrift and Hudson (25, 26)

Table 2.2: Prevalence and mean intensity of gastrointestinal helminths in the mice sampled (N = 98). 95% confidence intervals are reported for prevalence and all mean intensities are \pm standard error. No. Infections = number of infected mice.

	PRNU	SYPE	PTPE	ASAM	HYPE	GOPE
Prevalence	31.6 (23-41%)	24.4 (17-33%)	14.2 (8-22%)	11.2 (6-18%)	2.04 (0.5-7%)	2.04 (0.5-7%)
Gut location	Stomach	Cecum	Small	Colic spiral	Small	Stomach
Intensity	10.0 \pm 2.0	43.5 \pm 14.1	1.5 \pm 0.1	6.2 \pm 1.8	1 \pm 0	7.5 \pm 4.5
No. Infections	31	24	14	11	2	2

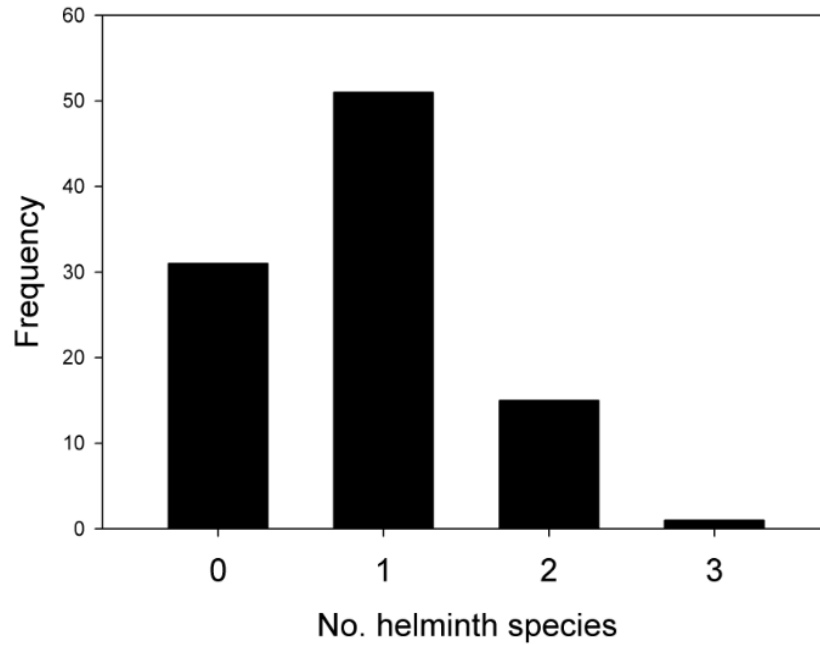


Figure 2.1: Frequency of gastrointestinal helminth infections in deer mice. The majority of animals (51%) hosted a single species of helminth and only 17% were coinfecting.

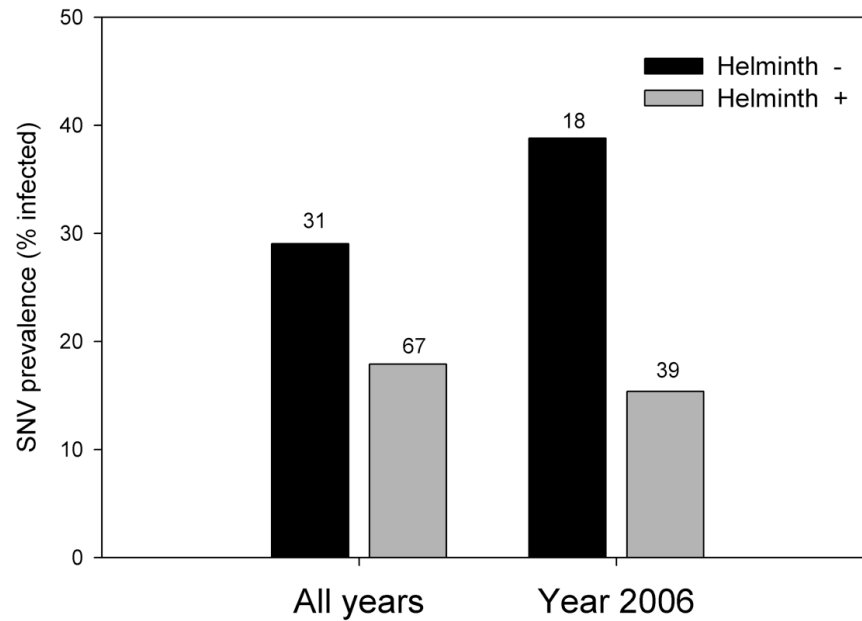


Figure 2.2: Prevalence of SNV infection in deer mice with and without helminth infection. Mice with worm infections had a lower prevalence of SNV in the year 2006 only ($\chi^2 = 4.284$, $df = 1$, $P = 0.05$). Numbers above the bars represent sample sizes.

found infection prevalence in white-footed mice (*Peromyscus leucopus*) ranged from 6.9 to 30% across a multi-year study and peaked at 52% in a different study. Additionally, Smith and Carpenter (24) found the prevalence of *Pterygodermatites peromysci* ranged from 30.4 to 56.5% in deer mice inhabiting different Channel Islands in California. We found infection prevalence of 14.28%, within the variation of previous studies.

Two factors may have biased the helminth prevalence observed in this study. First, the sample consisted of incidental trap mortalities. If an infection with helminths is costly to deer mice, either from directly decreasing nutrient availability or indirectly from the energetic cost of the immune system (27), infected mice may need to alter their foraging behavior to meet increased energetic demands. This may result in infected mice being trapped more frequently than uninfected mice, thus biasing the sample towards animals with greater parasite prevalence.

Second, high amounts of seasonal precipitation in 2005 (17) increased vegetation at the field sites (28) and may have increased the abundance of intermediate hosts during 2006. An increased vegetation structure may permit an increase in the abundance in the intermediate hosts (insects), thus increasing helminth prevalence. Moreover, two of the three most common parasites in this study (*Protospiruria numidica* and *Pterygodermatites peromysci*) have complex life cycles that require insect intermediate hosts for their transmission (12, 29). These results suggest that insects are common in the diets of deer mice at the

study sites and that high levels of precipitation and increased vegetative structure may indirectly influence the prevalence of helminth infections.

Pattern of coinfection between helminths and SNV

In 2006 only, prevalence of SNV was lower in deer mice with helminth infections compared to animals lacking infections. The sample acquired in 2006 was the largest of any single year in the study and also represents the majority of all individuals (58%) in the study, thus permitting detection of this pattern.

Alternatively, it is possible that the protective effects of helminth infection against SNV are context dependent. Transmission of SNV requires direct contact between individuals and the interaction between individuals is in part a function of density (30), which was twofold higher in 2006 compared to all other years (17). If helminths modify host behavior (e.g., decreased aggressiveness) or cause morbidity, helminth infected mice may be less likely to engage in behaviors that promote SNV transmission, in turn, leading to a decrease in SNV prevalence. This suggests helminth infections may confer protection against SNV or SNV may protect against helminths. We will focus on mechanisms of helminths providing protection against SNV.

Immunological theory predicts that a host should not be able to successfully defend itself against a simultaneous attack from microparasites and macroparasites. Due to the cross-regulatory nature of T lymphocytes (31), it is possible that helminth infection may “prime” the immune system by increasing other immunological defenses. For example, complement protein, a molecule of innate immunity, can recognize many different pathogens, including viruses and

helminths (32, 33). If a helminth infection up-regulates complement, there could be a decrease in the probability of a host acquiring an SNV infection.

Second, helminth infections could elicit behavioral changes in the host that reduce the probability of a secondary infection. Several studies have shown that harboring parasites imposes energetic costs on the host (27) or increases host stress (34). These demands may change the behaviors of deer mice. For example, hosting helminths during years of high conspecific density may negatively impact deer mice and lead to their inability to successfully defend territories and, therefore, decrease the likelihood of contracting an SNV infection due to decreases in aggressive encounters. This would hold true if helminth infection could decrease aggressiveness. Such a behavior change could reduce the probability of SNV infection through decreased contact rates or a reduction in aggressive encounters (35-38).

Another possible mechanism for lower SNV prevalence in helminth-infected deer mice would be dilution of SNV prevalence by juvenile mice (17). SNV is more common in older mice (39) compared to juvenile mice. In 2006, the mouse population was undergoing a large expansion (20-25 mice per hectare) and there were more juveniles in the population. Despite the shift in age structure of the population, SNV prevalence in the population at large was high (25% prevalence SNV in spring of 2006). Moreover, average body mass of the mice in this study did not differ between year 2006 and all other years combined (All except 2006 = 18.20 g, S.E = 0.65; Year 2006 = 17.35, S.E. = 0.56; ANOVA $P = 0.32$), thus lower SNV prevalence in helminth-infected mice of 2006 is not

due to dilution by juveniles. This result supports the hypothesis that helminth infection may provide protection against SNV.

Implications

The results imply that infection with helminths may provide protection to deer mice by decreasing their susceptibility to SNV. It is equally plausible, however, that SNV may provide protection against helminths. Studies manipulating either SNV or helminths are needed to determine the direction of protection. Although these effects might only apply to years of high mouse densities, this could positively impact humans since SNV outbreaks are linked to years of high deer mouse densities (36, 39). In a broader sense, this study highlights the importance of considering the effects of multiple parasite infections in disease ecology studies and how parasite infections may manipulate host behavior, immunological defense, and infection dynamics of zoonotic pathogens.

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CHAPTER 3

GASTROINTESTINAL HELMINTHS OF DEER MICE (*PEROMYSCUS MANICULATUS*) FROM A PERIDOMESTIC HABITAT IN EMIGRATION CANYON, UT

Abstract

Parasites directly cause detriment to a host by utilizing host energy for their own growth and survival, and they cause detriment indirectly by stimulating a costly immune response by the host. Helminth parasites can also increase host susceptibility to secondary pathogens (e.g., viruses). The first goal of this study was to identify gut helminths infecting deer mice in a peridomestic habitat using both live capture and destructive sampling methods. The second goal of this study was to test deer mice in the field study for the presence of Sin Nombre virus (SNV) antibodies, a viral zoonosis that poses a threat to humans inhabiting peridomestic locations, and to document patterns of coinfections between helminths and SNV. We live-trapped deer mice (*Peromyscus maniculatus*) in peridomestic habitats in Emigration Canyon, UT and snap-trapped deer mice inside human dwellings. We found eight species of helminths with an overall infection prevalence of 35.8% in field study and 44.1% in the snap-trap study.

A gender bias in helminths prevalence was found in the field study where reproductive males had higher helminth infection prevalence compared to reproductive females. Yet female mice shed a significantly greater number of parasite eggs per gram for *Trichuris peromysci* compared to males. A gender bias was also found in the snap trap study, with females showing significantly higher helminth prevalence and intensity compared to males. Deer mice expressed an SNV infection prevalence of 2.8% and lacked coinfections of SNV and helminths. In summary, deer mice in Emigration Canyon host helminths but have low levels of SNV infections compared to other peridomestic habitats. We did not find any coinfections between helminths and SNV; however, extensive long-term studies may better estimate the risk of SNV transmission to humans and to elucidate patterns of coinfections between helminths and SNV.

Introduction

Parasitic organisms acquire resources needed for survival at the expense of their hosts, which results in several forms of host detriment. Parasites recognized by the immune system initiate an immune response (1-6) that is energetically costly to the host (6-13). The parasites that withstand the immune response may establish a chronic infection and increase host susceptibility to secondary pathogen challenges (14-17). Such coinfections increase the risk of pathogen transmission to humans living in close association with wildlife carriers (18, 19). For example, Sin Nombre virus (SNV) is a zoonotic pathogen carried by deer mice (*Peromyscus maniculatus*) and the etiological agent of Hantavirus

Pulmonary Syndrome in humans (20, 21), which has an associated mortality rate of 36% (22). Hantavirus infections occur when humans are exposed to aerosolized mouse excreta (20), with a major proportion of transmission occurring in human dwellings (20, 21,23-26). It is of great importance for humans living in close association with wildlife to understand the infection patterns between parasites and other zoonotic pathogens and to therefore better assess the risk of infection.

In this study, we investigated the parasitic helminth community infecting deer mice (*Peromyscus maniculatus*) inhabiting a peridomestic habitat in Emigration Canyon, Utah. This habitat provides possibly greater food availability and shelter compared to sylvan habitats. In addition, the majority of SNV infections in humans are acquired in peridomestic habitats (24-26), they are therefore important habitats to study from a human health perspective.

Deer mice naturally host a variety of parasitic organisms including intestinal helminths (27-31) and SNV (21). We followed a population of deer mice to identify temporal changes in helminth infections. Temporal changes in helminths may arise due varying transmission strategies, either direct (host to host) or complex (host to intermediate host), of helminths or seasonal changes in intermediate hosts (29, 31). Temporal changes may also be due to the duration of helminth infections (chronic vs acute), however, this information is not known for deer mouse specific helminths. We also sought to determine if demographic factors (e.g., sex and reproductive condition) were important to helminth infection status. Moreover, snap-trapped deer mice were provided to us from

homeowners, which we dissected for second measure of helminth prevalence. Mice were also screened for SNV infection to estimate the prevalence of the virus and to document patterns of coinfections between helminths and SNV.

Methods

Study sites

This study was conducted on private property in Emigration Canyon, Salt Lake County, UT. Predominant vegetation in the canyon was Gambel oak (*Quercus gambelii*) interspersed with residential homes and paved roadways. Four trapping locations, a minimum of 150 meters apart, were established within close proximity (≤ 10 meters) of residential homes. Deer mice were live-trapped using standard small mammal traps (H.B. Sherman Co. Florida) from the first week of June 2010 through the third week of August 2010 (11 weeks). Traps were baited with rolled oats and peanut butter and set two consecutive nights at dusk and checked shortly after sunrise each morning. The trapping layout was a grid design with approximately 5 meters between each trap and 20 meters between transects. Due to differences in property size and field personnel, trapping intensity varied weekly. Sampling effort was estimated using trap nights (number of traps set X number of days sampled) and calculated for each week (Table 3.1).

Animal processing

Data recorded for each individual were sex, weight, and reproductive condition. Mice were weighed using Pesola® scales (± 0.1 g; Baar, Switzerland), individually marked with ear tags (National Band and Tag Co., Kentucky)... Females were classified as reproductive if vaginas were perforate, or if they were pregnant or lactating, and males if testes were descended. Fecal samples (approx. 0.2 g) were collected from each individual and placed in tubes containing 10% neutral buffered formalin to deactivate SNV and preserve helminth eggs. Additionally, blood (approx. 150 μ l) was collected from each individual and stored at -80°C until assayed for SNV using an Enzyme Linked Immunosorbent Assay (ELISA). After all animals were processed, they were released at the point of capture and all traps were sprayed with a 10% bleach solution. The Institutional Animal Care and Use Committee at the University of Utah approved procedures in this study (IACUC numbers # 08-02012 and 11-01007).

Snap-trapping by homeowners

Snap traps were set by a homeowner inside their residence that is in close proximity to one trapping location of the field study. These samples were used to estimate helminth infections using a second trapping method. During the field study, only six rodents were snap trapped inside the home, thus the impact of mouse removal was negligible. The majority of deer mice were snap-trapped after the cessation of the field study in August. Samples were immediately

Table 3.1: Trapping effort and the capture success of deer mice for the field study. Total captures is the number of deer mice captured each week. Percent recapture is the percent of total captures that were trapped previously and used as an estimate of the proportion of mice that are residents. Trap nights represent overall sampling effort each week.

Week	1	2	3	4	5	6	7	8	9	10	11
Total captures	10	17	25	22	21	24	26	20	18	26	15
Trap nights	88	88	136	120	136	168	168	168	240	240	168

frozen after death and stored at -4°C until necropsied for intestinal helminths.

Parasite identification

We used two distinct methods to identify the gastrointestinal parasites in this study: a modified McMaster fecal floatation and animal necropsy. The McMaster method is a common, nondestructive method for identifying and quantifying helminth infections in ecological studies (32-36). Fecal pellets (N = 131 individuals) were removed from the collection tubes and weighed (0.01 g) using an analytical balance. Feces were mashed in 1.0 ml of 1:1 zinc sulfate flotation solution (ZnSO_4 dissolved in distilled H_2O) for every 0.1 grams of feces, filtered through a $50\ \mu\text{m}$ sieve, and pipetted into the chamber of the McMaster slide (Hawksley, Lancing, Sussex). Each sample rested at room temperature for 5 minutes to allow the parasite eggs to float to the counting surface. The number of eggs floating in each sample was counted using a microscope at 100x magnification. We calculated the mean parasite eggs per gram for each individual as a measure of parasite burden (36, 37). Slides and sieves were soaked in a 10% bleach solution and vigorously rinsed with distilled water after each use to prevent contamination of subsequent samples. Egg morphology was compared to eggs isolated from dissected helminths to verify species identification.

Snap-trapped deer mice (N = 68) were thawed in a BSL-2 laminar flow hood. Mice were pinned to a dissection tray and sprayed with 90% ethanol. The body cavity was opened with dissection scissors and the intestinal tract, from the

lower esophagus to the anus, was removed. The intestines were then placed in conical tubes containing 90% ethanol for a minimum of 24 hours to inactivate any viable SNV (Jason Botten, personal communication). Each anatomical location of the intestinal tract (stomach, small intestines, cecum, colic spiral, large intestines) was slit open and carefully examined under a dissection microscope to detect parasites. Contents were filtered through a 150-micron sieve (VWR International, Pennsylvania) and re-examined a second time for parasites. If helminths were present, anatomical location in the gut was noted. The parasites were then removed, placed in preservation fluid, and identified to species by J. M. Kinsella, an expert parasitologist.

Statistical analysis

For the field study, data from all sites were combined and analyzed. For all helminth species, we calculated helminth prevalence (number of mice with helminth infections/ total number mice sampled X 100), and helminth eggs per gram (EPG) ($\text{number of eggs} \times \text{total vol. soln. (mL)} / ((0.3 \text{ vol. chamber (mL)}) \times \text{feces (g)})$). A one-way ANOVA was used to compare mean EPG (log transformed) by sex and this analysis was restricted to the two most common parasite species, *Pterygodermatites peromysci* and *Trichuris peromysci*. A Pearson Chi square test was used to determine if there were differences in the distributions of helminth infections by sex and reproductive condition of deer mice.

For the snap trap study, we calculated helminth prevalence and mean infection intensity per species (sum of helminth species / number of indiv. with that particular species). A Pearson Chi square test was used to determine if there were gender differences in infection prevalence and a Kruskal-Wallis H-test to test for differences in helminth intensities. All statistical analyses were conducted in JMP 9 (SAS Institute, Cary, NC) and differences were considered to be significant if $P \leq 0.05$.

Results

Gastrointestinal helminths in fecal samples

A total of 131 unique deer mice were captured over 1,720 trap nights (Table 3.1). Overall prevalence of helminth eggs in the feces was 35.8% (Table 3.2). We identified six nematode species: *Protostrongylus numidica* (PRNU; Seurat), *Syphacia peromysci* (SYPE; Harkema), *Pterygodermatites peromysci* (PTPE; Lichtenfels), *Aspicularis americana* (ASAM; Erickson), *Heligmosomoides vande-grifti* (HEVA; Kinsella), *Trichuris peromysci* (TRPE; Chandler) and one cestode: *Hymenolepis* sp. (HYME). The most prevalent species identified in the feces was *Pterygodermatites peromysci* (12.2%) and the least prevalent species were *Aspicularis americana* and *Syphacia peromysci* (1.5%). Several other helminth eggs were found, but we were unable to identify them to species level. We observed a pattern showing that parasites with complex transmission strategies were absent from the early weeks (1-5) of sampling but appear in mice feces between week 5 and week 11 (Figure 3.1). Two of the common parasites,

Table 3.2: Demographic data on the gastrointestinal helminths in the field and snap trap studies. The 95% confidence interval is provided for both the field and dissection helminth prevalence (% infection). Mean helminth eggs per gram (EPG; range) is reported for the field study. Infection intensity (\pm standard error) is for the snap-trap study. Gut location of each species was determined using dissection data.

Species	Location	% Inf. feces	EPG feces	% Inf. Dissection	Infection intensity
ASAM	Colic	2.3 (0.7-6.5)	767.7 (0-1900)	7.4 (2-17)	3.4 \pm 2.3
GOPE	Stomach	0	-	1.5 (0.1-9)	2.00
HEVA	Small	3.8 (1.6-8.6)	206.8 (0-350)	8.8 (3-18)	4 \pm 1.4
HYME	Small	1.5 (0.4-5.4)	5944.2 (0-11881)	5.9 (1-15)	1.00
PRNU	Stomach	3.1 (1.2-7.6)	11368.2 (0-44066)	1.5 (0.1-9)	1.00
PTPE	Small	12.2 (7.7-18.9)	655.9 (0-2628)	2.9 (0.5-11)	3 \pm 1.0
SYPE	Cecum	1.5 (0.4-5.4)	57.1 (0-60)	11.8 (5-22)	43.3 \pm 18.69
TRPE	Cecum	11.5 (7.1-18.0)	1438.0 (0-4556)	2.9 (0.5-11)	1.5 \pm 0.5
UNK	-	7.6	-	4.4	-
Overall prevalence		35.8		44.1	
No. inf. mice		47		30	

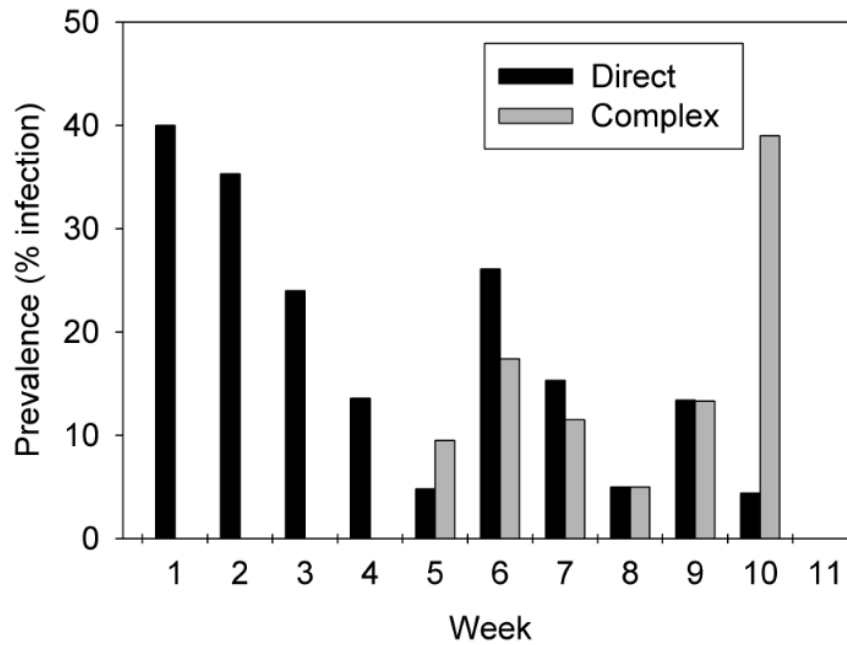


Figure 3.1: Prevalence of gastrointestinal helminths by transmission strategy in the field study. Parasites can be directly transmitted from host to host (ASAM, HYME SYPE, TRPE; see table 2.1) or have complex life cycles that require intermediate hosts (PTPE and PRNU).

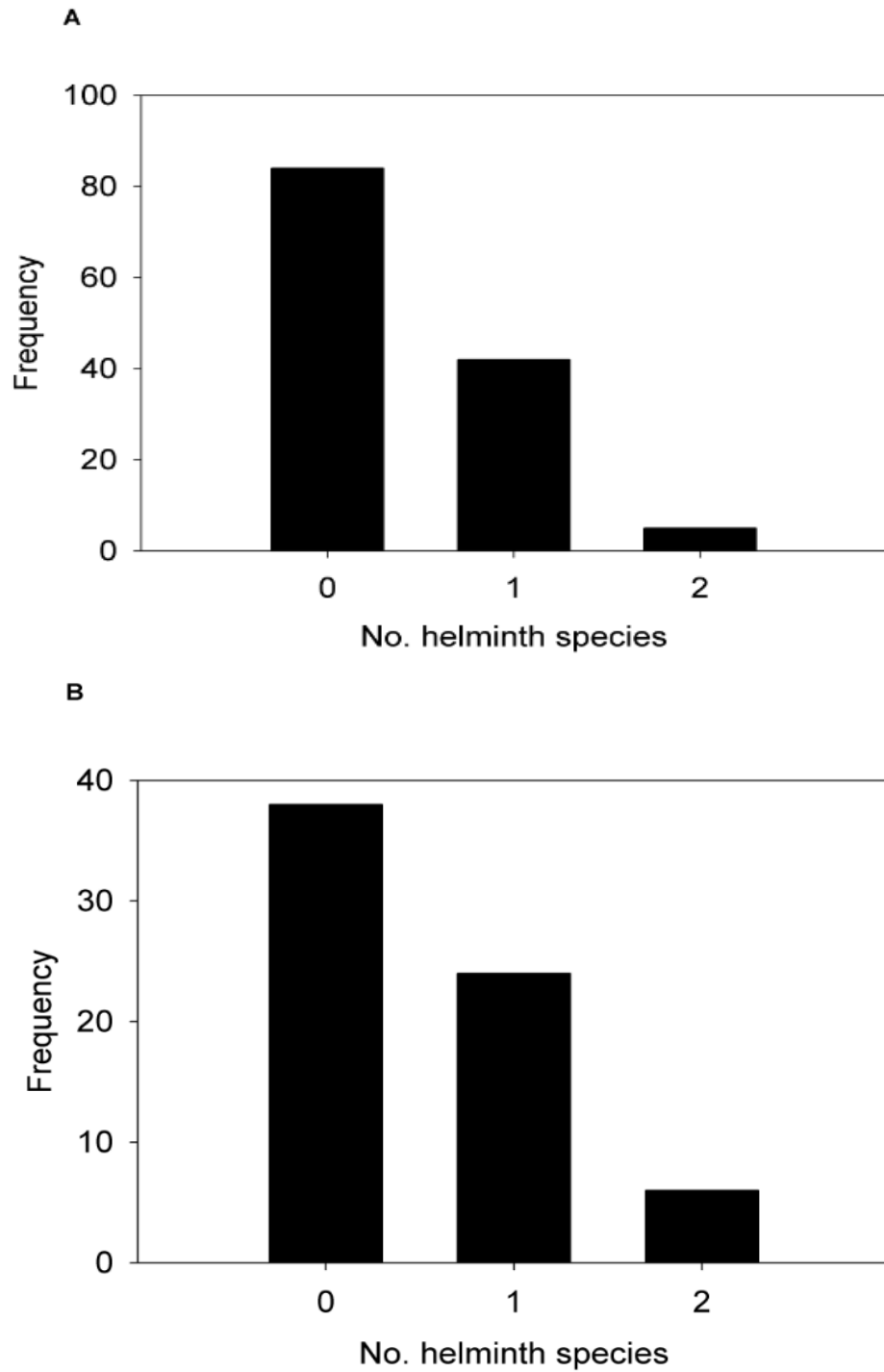


Figure 3.2: Frequency distribution of gastrointestinal helminth infections in deer mice from the field (A) and snap trap (B) studies. The majority of mice harbored single infections while few individuals were coinfecting. No animals hosted more than two species.

PRNU and PTPE, require passage through intermediate hosts to complete their lifecycles. Most deer mice (89.4%) were infected with a single species of helminth while only 10.6% of animals were coinfecting (Figure 3.2a).

There was no relationship between sex and helminth infection ($\chi^2 = 1.61$, $df = 1$, $P = 0.20$). However, we found that helminth prevalence was 1.8 times higher in reproductive (mean = 0.595; $\chi^2 = 6.27$, $df = 1$, $P = 0.01$) than non-reproductive deer mice (mean = 0.404). When the analysis was performed by sex, there was no trend for females ($N = 71$; $\chi^2 = 1.69$, $df = 1$, $P = 0.16$). This trend was driven by reproductive males, which had 1.3 times greater helminth prevalence (mean = 0.560, $N = 60$; $\chi^2 = 6.93$, $df = 1$, $P < 0.01$) compared to non-reproductive males with helminths (mean = 0.440).

EPG varied widely between species and ranged from 57.1 EPG for *S. peromysci* up to 11,368.2 EPG for *P. numidica* (Table 3.2). There were no differences between EPG and sex for *P. peromysci* ($F_{[1,14]} = 0.0$, $df = 1$, $P = 0.99$). Female deer mice, however, had higher mean EPG for *T. peromysci* ($F_{[1,14]} = 3.19$, $df = 1$, $P = 0.01$) compared to males.

Gastrointestinal helminths in dissections

A total of 68 deer mice were snap-trapped, with an overall helminth infection prevalence of 44.1%. We found gastrointestinal helminths inhabiting all anatomical locations of the gastrointestinal tract, although species exhibited high fidelity to a region of the gut (Table 3.2). We found the same eight species of nematodes and cestodes as in the fecal analysis, but we found one additional

nematode species, *Gongylonema peromysci* (Hall). The most prevalent species of helminth in the dissections was *S. peromysci* (11.8 %) and the least prevalent species were *P. numidica* and *G. peromysci* (1.5%; Table 3.2).

Most deer mice (80%) hosted single species of helminth while only some (20%) hosted dual helminth infections (Figure 3.2b). Female deer mice were more likely to be infected with helminths ($\chi^2 = 7.77$, $df = 1$, $P < 0.01$) than males. Additionally, female deer mice had higher mean helminth intensities (mean male = 2.56; mean female = 12.40; $H = 6.02$, $df = 1$, $P = 0.01$) compared to male deer mice.

Prevalence of SNV antibodies

A total of 106 deer mice were sampled to test for SNV antibodies. Three out of 106 deer mice tested positive for antibodies against the virus (2.8%). All mice that tested positive were adults that were greater than 15 grams in weight. No juveniles (< 15 grams) tested positive for antibodies. We did not find any mice with helminths and SNV coinfections (expected 1% mice to be coinfecting, or one mouse).

Discussion

Humans residing in peridomestic habitats run the risk of exposure to wildlife zoonoses. Previous studies have shown that concurrent parasitic infections increase host susceptibility to secondary pathogens (14-17), thus we were interested in the frequency of coinfections involving helminths and SNV in

deer mice residing in a peridomestic setting. The first objective of this study was to identify the gastrointestinal helminths of deer mice in Emigration Canyon, UT. We used two distinct methods to characterize the helminth community infecting deer mice. A field method was used to follow mice to determine if the helminth community varied temporally and to identify if helminth prevalence and intensities varied with sex and reproductive condition, since sex biases in helminth prevalence exist in other systems (35). We also dissected deer mice snap-trapped by homeowners to estimate helminth prevalence and intensity.

We found helminths in both studies with an overall prevalence of 35.8% in the field study and 44.1% in the snap trap study. There was temporal variation in helminth infection in the recapture study with the appearance of indirectly transmitted helminths at week five. We found a female sex bias in helminth prevalence and intensity in the snap trap study. Also, we found higher female parasite shedding rates for *T. peromysci* in the field study. We found a sex bias in helminth prevalence in the field study where reproductive males had higher helminth prevalence than reproductive females.

The second objective was to screen all mice for the presence of Sin Nombre virus antibodies and to determine infection prevalence of SNV in a peridomestic habitat. Moreover, we were interested in determining if SNV infection was correlated with helminth infection. Overall infection prevalence of SNV was 2.8%. We did not document any coinfections between helminths and SNV.

Comparison of helminths

Two common methods for documenting helminth infections in wildlife include the non-invasive McMaster fecal flotation method and destructive sampling methods such as snap trapping with necropsy. These two methods were utilized to identify the helminth community of deer mice in a peridomestic setting. Overall prevalence was 35.8% in the field study and 44.1% in the dissection study and relatively few animals were coinfecting with multiple helminths. Snap trap studies are highly effective for identifying helminths infecting rodents since they identify non gravid parasites that would otherwise be missed by fecal analysis. Previous studies have shown that helminths shed eggs following a rhythmic pattern (38), thus we may have underestimated their prevalence.

Similar helminth species were found between these methods; however, the stomach parasite *G. peromysci* was not seen in the field study. The helminth species in this study were similar to those found in deer mice in the Great Basin desert (Chapter 2). However, we found a roundworm, *Trichuris peromysci*, which was absent in the previous study but has been documented in the Bonneville Basin of Utah (39).

Temporal variation in helminth species

Parasitic helminths have differing transmission strategies and can be transmitted directly from host to host or indirectly by utilization of intermediate hosts. During the first five weeks of trapping, we only found eggs of directly

transmitted parasites (ASAM, HYME SYPE, TRPE) in the feces of the deer mice (Figure 3.2). Around week five, however, there was an increase in the prevalence of helminths that require passage through intermediate hosts to complete their life cycles (PTPE and PRNU). Many trophically transmitted helminths that utilize intermediate hosts exhibit a temporal increase in prevalence from summer into fall (28, 40-43). For example, the prevalence of the nematode *Pterygodermatites peromysci* (PTPE) increases seasonally in mice due to increases in both abundance and age of the intermediate camel cricket hosts (*Ceuthophilus* spp.) (31). Moreover, studies conducted in Utah have shown the prevalence of *Protospiruria numidica* increases seasonally, with the majority of deer mice infected with this nematode occurring in the fall (28). This trend is often attributed to an increase of insects in the diets of deer mice (28, 31, 41, 44). The seasonal increase in prevalence of trophically transmitted parasites in our research is in concordance with previous work and suggests an increase in use of insects as a food source for deer mice in early to late summer.

Sex bias in helminth prevalence and intensities

There is a trend that male hosts tend to exhibit higher parasite prevalence and intensities compared to females in species ranging from birds (45-47) to mammals (48-50). In the snap trap study there was a female bias in helminth infection prevalence. Female biases in infections occur (51, 52) and may arise for several reasons. The energetic cost of lactation and reproduction (8, 53, 54) may lead to decreases in immune investment, thus putting females at risk for

acquiring helminths. With an increase in energetic demands, females may be required to forage more to increase energy intake and, consequently, their exposure to parasites increases. Furthermore, if females selectively feed on insects to meet their energetic demands, they increase their risk of exposure to trophically transmitted helminths (28, 40-43, 55).

Female deer mice infected with *Trichuris peromysci* shed a significantly greater amount of eggs in their feces compared to infected males. Since our study was conducted during seasons when deer mice are reproductive, we speculate the energetic cost of lactation (8, 53, 54) or tradeoffs between immunity and reproduction (7, 10, 56) may be responsible for the increased egg shedding rate of this parasite in females. For example, studies conducted in big horn sheep have shown that fecal egg counts increase in lactating females (57, 58). We did not observe a gender bias trend for *Pterygodermatites peromysci*. Thus, increases in helminth fecal eggs counts are species dependent.

In the field study, the prevalence of helminths was 1.7 times greater in male deer mice that were reproductive compared to reproductive females. This trend, however, was not observed generally across the dataset. These results are in concordance with the literature that shows males have higher parasite prevalence than females (45-50). This pattern could be the result of increased susceptibility to helminths or increased exposure. Male-biased infections are often attributed to higher levels of testosterone (59, 60). Testosterone acts as an immunosuppressant in numerous species (47, 49, 60, 61). Thus, it is possible

that high levels of testosterone during the breeding season may make male deer mice more susceptible to helminth parasites.

Increases in testosterone can alter the behavior of male mice and potentially increase the risk of exposure to parasites (48). For example, Gear et al. (2009) showed that increases in testosterone (by receiving a testosterone implant) increased the connectedness between male white-footed mice (*Peromyscus leucopus*), which increased the transmission risk of parasites (62). In the present study, reproductive male deer mice may harbor more helminths due to differences in parasite exposure or decreases in immune function. Further research is warranted to untangle the potential effects of testosterone and reproductive condition on helminth infections in deer mice.

SNV in Emigration Canyon

Prevalence of SNV was low in Emigration Canyon compared to other studies conducted in peridomestic habitats (24, 25). Studies by Kuenzi and colleagues (2001) have reported SNV prevalence from 20 to 25% in peridomestic habitats (25). The vegetation type in the Kuenzi and colleagues (2001) study was quite different (sagebrush) from this study and might account for low SNV prevalence in this study (63). For example, Mills and colleagues (1997) estimated prevalence of SNV across different biomes. They found SNV prevalence varied from 17% in pinyon juniper habitats to 4% in chaparral (63). The authors attributed the variation in SNV prevalence to differences in mouse movements in each biome. The sampling sites in this study were chaparral with

the predominate vegetation composing on scrub oak (*Q. gambelii*), thus prevalence of SNV is similar to their findings. We did not find any animals coinfecting with helminths and SNV in this study

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CHAPTER 4

TESTING FOR TRADEOFFS WITHIN THE IMMUNE SYSTEM OF A WILD VERTEBRATE

Abstract

It is commonly understood that tradeoffs exist between the immune system and other physiological processes. Tradeoffs within components of vertebrate immune systems, however, are less understood. The objective of this study was to test if tradeoffs occur between the innate and adaptive immune systems of wild vertebrate. To test this, we measured the ability of deer mice (*Peromyscus maniculatus*, N = 20) to kill bacteria *ex vivo* before and after the stimulation of a humoral immune response. To elicit the adaptive immune response, we injected the nucleocapsid antigen from Sin Nombre virus, and then measured the subsequent innate immune response using bacterial killing assays. We found that both the antigen and vehicle injections increased the bacterial killing capacity post antigen injections compared to pre-injection values. There was no significant difference between treatment and vehicle controls. The data suggests that there is no tradeoff between mounting a humoral antibody response and the ability to kill bacteria.

Introduction

Nearly all animals at some time point in their lives are subjected to infection by parasites and pathogens. Pathogens and parasites have placed strong evolutionary pressure on the host to develop immune defenses that reduce the fitness costs of infections (1-4). Immune defenses can be costly (5-10) and investment in immune defenses has consequences on other physiological processes (10-15, reviewed in 16). Recent investigations show that tradeoffs exist between immunity and other energetically expensive processes like reproduction (17, 18) and lactation (19). Tradeoffs could exist within the immune system and few studies have directly investigated this possibility (12, 20).

The immune system is a complex set of interconnected mechanisms that are typically divided into two arms known as the innate and adaptive immune systems. The innate immune system provides the host with the first line of defense against invading pathogens. It is nonspecific and includes, but is not limited to, natural antibodies, phagocytic cells (e.g., macrophages), and opsonizing molecules (e.g., complement proteins) (21). In contrast, the adaptive immune system is pathogen-specific and includes both B and T lymphocytes (21). Investment in one branch of the immune system could retard investment towards the other branch. Previous work by Martin II and colleagues showed that eliciting a cutaneous wound has negative impacts on delayed-type hypersensitivity reactions to dinitrofluorobenzene in female white-footed mice

(12). These challenges were localized to the skin of the host. Thus, it is important to build upon these findings and examine if tradeoffs exist systemically.

The goal of this study was to determine if there was a tradeoff between the two arms of the immune system. Specifically, I investigated whether there was a tradeoff in mounting an innate immune response after an adaptive response had already been mounted. We tested if induction of an adaptive immune response, stimulated by an antigen challenge, would suppress the innate immune response (bacterial killing ability) during times of high antibody production in deer mice (*Peromyscus maniculatus*). In this study, we challenged deer mice with the nucleocapsid antigen from Sin Nombre virus and measured their ability to kill *Escherichia coli* using bacterial killing assays. We selected these two immune measures for the following reasons. First, deer mice are the reservoir species of Sin Nombre virus (22), a pathogen that causes 36% mortality in reported cases in humans (23). The use of SNV NA_g is a novel method to study the immune response of SNV infection in the laboratory because it eliminates the need for BSL-4 and outdoor research facilities (24). Second, bacterial killing assays provide an excellent measure of innate immune function and are relevant to the ability of organisms to respond to pathogens (25-30). Finally, these immune measures and challenges are relevant to free-living deer mice since they have evolutionary experience with both the antigen and bacterial challenges.

Methods

Animal care

All mice in this study were obtained from a breeding colony established at the University of Utah. Mice (N =14) were bred under standard conditions, following guidelines for establishing a *Peromyscus* breeding colony using wild caught founders (31). All mice used in this study were first generation animals (F1).

Deer mice (N = 30; all virgin, ca. 12 months old) were individually housed in standard mouse cages outfitted with aspen bedding and *ad libitum* access to food (Harland Tekad 8604) and filtered tap water. Animals were housed in a long day (16 hours light, 8 hours dark) light cycle at $22.5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for the duration of the study to simulate photoperiods during the reproductive season (12). The Institutional Animal Care and Use Committee at the University of Utah approved the procedures used in this study (IACUC numbers # 08-02012 and 11-01007).

Bacterial killing assays

We used bacterial killing assays to measure the ability of deer mouse serum to kill *Escherichia coli*. Bacterial killing assays are the gold-standard measure of innate immune function and provide a relevant measure of the ability of a host to respond to a bacterial pathogen (25-30). The killing of *E. coli* requires both complement proteins and natural antibodies. We followed the methods of French and colleagues (2010) and French and Neuman-Lee (2012) (29, 30). Briefly, tryptic soy agar (30 g tryptic soy agar in 750 ml diH₂O) was

autoclaved and dispensed into petri plates using sterile techniques in a laminar flow hood. The plates were then placed in a refrigerator upside-down overnight. A lyophilized pellet of *E. coli* (*E*^{powder}TM: Microorganisms #0483E7, MicroBioLogics, St. Cloud, MN) was activated in 40 ml of 1M sterile phosphate buffered saline (PBS) and incubated at 37°C for 30 minutes. Bacterial stock was diluted in sterile PBS to achieve a bacterial concentrate of 50,000 bacteria per ml. Serum was diluted (1:20 ratio) in glutamine enriched CO₂ independent media. Twenty µl of bacterial solution were added to each serum sample, vortexed and incubated for 30 minutes at 37°C. Positive controls (20 µl bacteria solution and 200 µl media) and negative controls (220 µl media) were also vortexed and incubated. After incubation, samples and controls were vortexed, 50 µl of each was plated using a sterile bacterial spreader on the center of a petri dish in duplicate. Plates were covered and sealed, placed upside-down in an incubator at 37°C for 12 hours. After incubation, the number of colonies was counted and killing capacity ((mean colonies per each sample / mean colonies of pos. cntl.) X 100) were calculated relative to the positive control.

Antigen injections

Truncated Sin Nombre virus nucleocapsid antigen (25 µg; SNV NAg) was emulsified in Incomplete Freund's Adjuvant (IFA) using 2 cc glass syringes with 18 gauge emulsification tubes. The emulsification was mixed at a 1:1 ratio (500 µl SNV Ag plus 500 µl IFA) and delivered approximately 25 µg of antigen per injection. Emulsions were mixed for approximately five minutes to ensure the

formation of a stable emulsion. Experimental injections (N = 20 mice) were injected subcutaneously with 50 μ l of emulsion bilaterally near the base of the tail using 24 gauge hypodermic needles. All injections were performed between 08:00 and 13:00 hours. Needles were left under the skin for about five seconds to ensure no emulsion leaked out from the injection site. The site of injection was cleaned with 95% ethanol prior to injections. The needles were sterilized with 95% ethanol after each injection. Control animals (N = 10) were injected with 50 μ l of sterile 1% phosphate buffered saline emulsified in IFA (1:1 ratio). All mice (N = 10 control; N = 20 experimental) received booster injections 21 days post-injection to elicit a strong antibody response to the antigen.

Blood collection and processing handling

Blood was collected from each individual at two distinct time points. Mice were sampled prior to injections to obtain baseline measurements of bacterial competence. Four weeks after the initial injection with antigen, mice were sampled to obtain measurements post injection bactericidal competence after challenged with either the antigen or a sham treatment. Approximately 75 μ l of blood were collected from the retro orbital sinus of each individual using sterile capillary tubes. The blood was allowed to clot for 30 minutes at 22.5°C inside a sterile laminar flow hood. Clots were removed from each tube and samples were spun at 2,500 rpm at 4°C for 30 minutes (Microfuge 22R centrifuge, Beckman Coulter, California). Plasma was removed and immediately frozen in two aliquots at -80°C until used in the immunological assays.

Sin Nombre virus antibody detection

We used an Enzyme Linked Immunosorbent Assay (ELISA) to detect SNV antibodies in serum samples. ELISAs were conducted in a BSL-2 facility at the University of Utah following the methods of (32). Briefly, 96-well plates (BD Falcon, BD Biosciences, MA) were coated with 100 μ l of SNV nucleocapsid antigen (1:400 dilution in PBS; Shountz Laboratory, University of Northern Colorado) in a milk diluent buffer and incubated at 4°C for a minimum of 24 hours. After 24 hours, plates were washed four times with 180 μ l of ELISA wash. Next, the samples and controls were diluted 1:50 in milk diluent and added to each plate in duplicate, and then incubated for 1 hour at 37°C. After incubation, plates were washed four times with 180 μ l of ELISA wash then 100 μ l of secondary antibody (10 μ l of peroxidase labeled anti- *Peromyscus* antibody diluted 1:1000 in milk buffer; KPL Laboratories, MD) was added to each well and incubated at 37°C for 1 hour. Plates were washed four times with 180 μ l of ELISA wash and then 100 μ l of ABTS solution was added to all wells and incubated for 30 minutes at 37°C. Immediately following incubation, absorption was measured for each sample (405 nm; Power Wave HT, BioTek,VT). Absorption values three times greater than the negative control were considered positive (33). Positive ELISA results verified the adaptive immune response occurred to the antigen.

Statistical analysis

A repeated measures ANOVA was used to determine if there were significant differences (\pm relative standard error) in bacterial killing ability between treatment and control groups. All statistical analyses were conducted in JMP 9 (SAS Institute, Cary, NC) and differences were considered to be significant if $P \leq 0.05$.

Results

Verification of immune response to antigen treatment

Six out of 20 deer mice (30%) injected with the IFA SNV NAg emulsion responded to the treatment (data omitted for nonresponders; Table 4.1). Animals were considered to test positive if optical density (OD) values post injection were greater than 1.14 OD thus indicating the initiation of an adaptive immune response and subsequent generation of antibodies against SNV (Table 4.1). This positive cut-off point was determined by multiplying the negative control value (0.383) by three (33). The geometric mean OD for animals that tested positive was 3.26.

Bactericidal killing capacity

Baseline bacterial killing ability was not significant ($F_{[1,14]} = 1.25$ $P = 0.28$) between control and treatment (Table 4.2). Bacterial killing increased from pre to post injection ($F_{[1,14]} = 17.27$ $P < 0.01$; Figure 4.1). However, there was no

significant difference between groups (control: = 59.2% \pm 21.9%; treatment = 69.7% \pm 20.0%; $F_{[1,14]} = 0.72$ $P = 0.40$; Figure 4.1; Table 4.2).

Discussion

The immune system of a host must respond appropriately to challenges from pathogens and parasites. A host has a limited amount of resources in the form of energy to dedicate to immunity. Several studies have highlighted tradeoffs between immune function and other physiological processes (17-19). We sought to identify whether there were tradeoffs between the innate and adaptive immune systems of deer mice. Specifically, we were interested in any tradeoffs between mounting an antigen (adaptive, humoral) response and the bactericidal killing ability of deer mouse serum (innate). Six out of the 20 treatment mice (30%) responded to the antigen challenge. Both the treatment and control groups responded to the injections and significantly increased the bacterial killing ability. However, there were no significant differences between the treatment and experiment groups. Thus, the results suggest there are no tradeoffs between the adaptive and innate immune systems of deer mice.

Variation in immune response

Despite strong selection for increased immunity against pathogens (1-4), a significant amount of variability in immunological measures of wild organisms exists (34). In our study, there was variability in both the ability of the mice to respond to the antigen challenge and in their ability to kill bacteria

Table 4.1: Enzyme-Linked Immunosorbent Assay (ELISA) results pre- and post antigen injections. ID = animal ID, Trmt = treatment, Cntl = control, + = positive ELISA result, – = negative ELISA result.

ID	Group	Pre inj. OD	Post inj. OD	ELISA
163	Trmt	0.234	4.01	+
164	Trmt	0.208	3.873	+
168	Trmt	0.296	3.442	+
172	Trmt	0.205	4.079	+
186	Trmt	0.148	3.963	+
194	Trmt	0.15	1.404	+
179	Cntl	0.195	0.271	–
180	Cntl	0.168	0.258	–
184	Cntl	0.123	0.318	–
185	Cntl	0.21	0.237	–
188	Cntl	0.194	0.216	–
189	Cntl	0.173	0.258	–
192	Cntl	0.159	0.165	–
193	Cntl	0.149	0.172	–
197	Cntl	0.168	0.275	–
I	Cntl	0.221	0.352	–
POS Cntl =	4.124			
NEG Cntl =	0.383			
Cut-off for +	1.149			

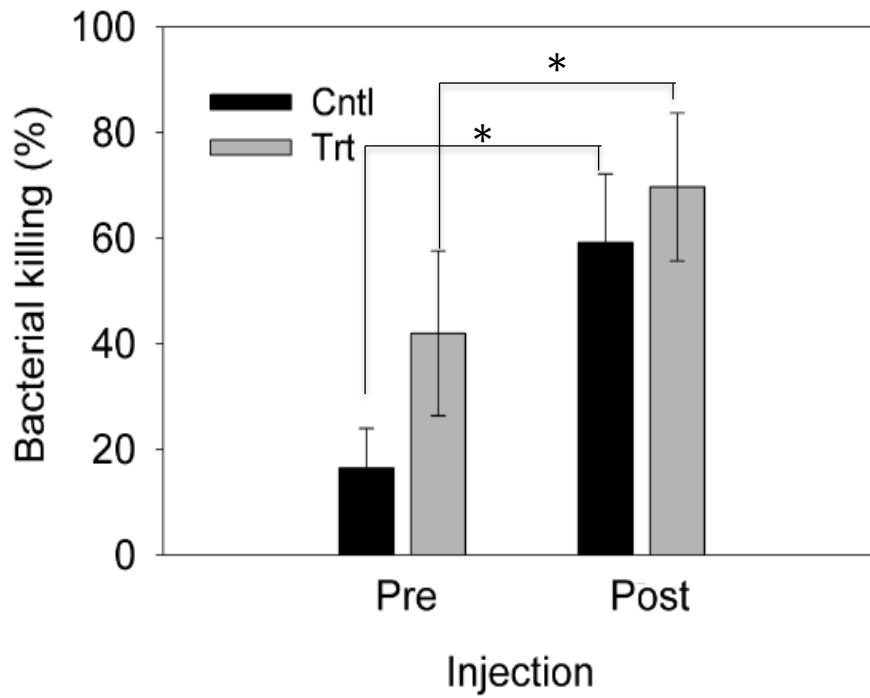


Figure 4.1: Bacterial killing competency (% bacteria killed relative to positive control) for deer mice pre- and postinjection. Experimental injection significantly increased the bacterial killing capacity of deer mice ($F [1,14] = 17.27 P < 0.01$). Trt = treatment, Cntl = control. Asterisks indicate significant differences ($P < 0.05$).

Table 4.2: Percent bacteria killed values for deer mice pre- and postinjections. Difference was calculated by subtracting the post injection killing values from pre injection values. Negative killing values were converted to zeros. ID = animal ID, Trmt = treatment, Cntl = control, % killed = percent of bacteria killed relative to the positive control.

ID	Group	% Killed pre inj.	% Killed post inj.	Difference
163	Trt	0	15.3	15.3
164	Trt	27.2	98.2	71
168	Trt	0	40	40
172	Trt	68.2	87.6	19.4
186	Trt	90	77.1	-12.9
194	Trt	65.9	100	34.1
179	Cntl	0	0	0
180	Cntl	0	0	0
184	Cntl	56.8	96.5	39.7
185	Cntl	0	61.2	61.2
188	Cntl	9.1	73.5	64.4
189	Cntl	0	6.5	6.5
192	Cntl	40.9	100	59.1
193	Cntl	0	94.7	94.7
197	Cntl	59.1	87.1	28
I	C	0	73.5	73.5

(see Tables 4.1 - 4.2). The majority of deer mice (70%) did not respond to the antigen injections, which could be due to a couple reasons. First, it is possible that animals did not receive an effective injection due to improper technique (e.g., improper injection, emulsion stabilization) and thus a response would not be generated. Moreover, if a stable emulsion of the antigen and IFA was not achieved, the antigen may be released rapidly and cleared before sufficient presentation and initiation of the humoral immune response (35). Second, the antigen used in this study was a truncated version of the full-length nucleocapsid antigen. The truncated antigen (15kD) contained the dominant epitope recognized by the B cells in natural infections (Jason Botten, Personal communication). The full-length antigen (56kD) has been verified to generate antibodies. This is the first time a truncated version has been used to generate the same response (Jason Botten, Personal communication). Thus, the truncated antigen may have not been adequate to generate a detectable response in some mice. Moreover, since most molecules are poor immunogens (35), a follow up study using the full-length antigen may provide an increase in humoral immune activation and produce greater success in initiating an adaptive immune response in deer mice.

We also found a considerable amount of variation in the baseline bacterial killing ability of deer mice in this study. In general, the use of wild animals, instead of their inbred congeners, captures the natural variation in immune responses seen in the wild (20, 36, 37). In this study, we used first generation young derived from wild-caught deer mice captured at the study sites of chapter

three in Emigration Canyon. Other studies have also documented intraspecific variation of bactericidal competence in birds and mammals (12, 25, 27). For example, studies conducted in wild house mice have documented variability in immune responses between individuals (36). The variation between individuals is often attributed to either genetic composition (38-40) or environmentally-induced differences due to varying pathogen exposures (41, 42).

Tradeoffs within the immune system

Bacterial killing increased in both the control and antigen treated groups post injection. However, there were no significant differences in bacterial killing detected between treatment and control groups. This result suggests that the increases in bacterial killing were solely due to the IFA adjuvant and that the IFA primed the bacterial killing ability of deer mice. Studies show that IFA has the ability to stimulate humoral immunity to raise antibody titer (43). However, previous work has only been completed with adjuvant antigen emulsions not adjuvant only scenarios. Since the immune system is highly interconnected, it is possible that differences might arise in an alternative measure of immune function.

Although there was no difference in bacterial killing ability of the antigen treated animals versus the control animals, the increase in BKA of both groups post injection suggests that there is not a tradeoff between the adaptive and innate immune systems in this experimental system. Previous studies both in the field and laboratory show mixed results. For example, bacterial killing capacity of

Peromyscus melanophrys was negatively related to antibody production to keyhole limpet hemocyanin (KLH; a novel antigen), while *P. maniculatus* increased bacterial killing in response to KLH injections (12). Moreover, a study of free ranging African buffalo demonstrated that buffalo naturally infected with bovine tuberculosis had higher bacterial competence of whole blood (20). Alternatively, a study in Brazilian free-tailed bats showed a negative correlation between bacterial killing and a swelling response to phytohaemagglutinin (PHA) (42). In this study we found no evidence for tradeoffs. If there were, we would have expected a decrease in BKA in both groups post injection. One difference between this study and previous work is that deer mice have evolutionary experience with the antigen challenge. Thus, the differences documented in the previous studies may result due to the novel aspect of the antigen (e.g., KLH and PHA).

The immune system of vertebrates is composed of the innate and adaptive arms and is highly interconnected (21). Although we did not find direct tradeoffs between the innate and adaptive immune systems, there were impacts on other cells of the immune system. In conjunction with this study, white blood cell counts were conducted to measure stress response to the SNV antigen challenge. Deer mice challenged with the SNV NA_g had increases in monocytes and decreases in lymphocytes as measured by blood smears (Disney et al; unpublished data). Studies conducted in wild African buffalo (*Syncerus caffer*) show that animals with lower lymphocyte counts have higher BKA competence, which suggests polarization of the adaptive immune response (20). Stimulation

of the humoral immune response requires T helper cells of the Th2 lineage (44). Additionally, monocytes are precursor cells of antigen presenting cells (e.g., dendritic cells and macrophages) that migrate to infection sites (21). Thus, the increase in monocytes over controls may be due to the foreign SNV antigen. Future studies using whole blood, with emphasis on multiple measures of immune function (45), may elucidate differences between the controls and antigen challenged mice.

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