

Interactive Effects of Nematode and Bot fly Parasitism on the Survival of White-footed (*Peromyscus leucopus*) Mice

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Abstract

The white-footed mouse, *Peromyscus leucopus* (Rafinesque), is the most abundant rodent in Northern America. These mice are frequently studied in laboratory and field settings due to their tendency to harbor many zoonotic pathogens. There is interest in factors that influence the survival of these mice; parasitism is one such factor that is known to impact their survival (Burns, Goodwin and Ostfeld, 2005). An ecto-parasite known as *Cuterebra fontinella*, or bot flies, has curiously been shown to cause a significant increase in survival. We set out to determine if bot flies alter mouse survival in Pennsylvania, and further, to investigate if this effect is mediated, in any way, by the removal of gastrointestinal parasites, which have also been shown to influence mouse survival in Pennsylvania. We monitored bot fly infection rates and mouse survival in 12 populations. Half (6) of these populations were provided with anthelmintic treatments which cleared nematode infections. This study uses generalized linear models to determine if botfly infections alter mouse survival and if this impact is mediated in any way by nematode removal.

Key words: *Peromyscus leucopus*, white-footed mice, bot fly, nematodes

Introduction

Peromyscus leucopus (the white footed mouse) is a rodent native to North America (Rafinesque). These rodents are known to be reservoirs for several tick-borne and zoonotic pathogens, such as arboviruses (Hardy, 1994) and *Borrelia burgdorferi* (Burgdorferi), to name a few (Vandegrift and Hudson, 2009). The most studied is Sin Nombre Hanta virus, which was identified as the etiological agent of the 1993 human outbreaks in the four corners region of the United States (Childs et al). This pathogen is transmitted to humans by inhalation of aerosolized urine and feces of these rodents who often invade houses garages and sheds. They also play a major role in the life cycle of *Borrelia burgdorferi*, the spirochete responsible for Lyme disease (Burgdorferi). Because *P. leucopus* are important reservoirs of zoonotic agents, much subsequent attention has been paid to their population dynamics and their parasite communities (Wolf 1996,

Jones Ostfeld, Falls, Fryxell). This led to the discovery of arboviruses (Hardy, 1994) and lymphocytic choriomeningitis virus (Ralph, 1999) and several bacterial pathogens such as *Borrelia burgdorferi* (Burgdorferi), *Anaplasma phagocytophilum* (Magnarelli et al., 2006) and *Francisella tularensis* (Eisen et al., 2008). Although transmission of these zoonotic diseases is rare, understanding the factors that facilitate spillover to humans is critically important.

It is well known that the population dynamics of *P. leucopus* are unstable; the mice population can shift to be as much as 16 times more abundant than their typical population level (Wolff). The occurrence of these population shifts are important to monitor; a shift in the population could cause an increase in human exposure to zoonotic diseases, such as Lyme disease, due to an increase in rodent-human contact (Ostfeld et al, 1996). The survival of *P. leucopus* is an essential topic to study because the potential for population instability among *P. leucopus* and other small mammals could ultimately lead to higher accounts of zoonotic diseases. Parasitism and food availability are factors that may potentially influence the survival of *P. leucopus*.

Furthermore, a 1996 study on the population fluctuations of sympatric mast-eating rodents, such as *P. leucopus*, *P. maniculatus* (deer mice), and *Tamias striatus* (eastern chipmunks), examined their relationship between food availability; it specifically focused on how the production of acorn mast influences population fluctuations (Wolff, 1996). This study utilized a 14-year dataset to establish a correlation between that the population dynamics of small rodents and acorn production (Wolff, 1996). The results of this study showed a positive relationship between acorn production and rodent density the following year ($r^2=0.79$). Additionally, the results showed that a large production of acorns in the fall also increased the survivorship of rodents (Wolff, 1993).

Numerous studies have examined the effect of gastrointestinal and ecto-parasites on the population dynamics of *P. leucopus*. However, most studies have sought to evaluate what role gastrointestinal parasites play in the instability of small mammal populations and have also closely observed the effect of parasitic infections in *P. leucopus* through manipulative studies where mice were given an anthelmintic or non-anthelmintic treatment. As discussed by Vandegrift et al. (2008) Parasitism, more specifically in regards to the *P. leucopus* species, is suspected to impact midsummer breeding hiatus. Studies have been conducted to determine if parasites, food availability, or a combination of the two, plays a role in breeding patterns of *P. leucopus*. Evidence from Vandegrift et al. (2008) suggest removal of parasites through anthelmintic treatments resulted in a reversal of the midsummer breeding hiatus, whereas increased habitat quality did not alter the vital rates of mice. Through these studies, seven distinct gastrointestinal parasites have been known to infect *P. leucopus*.

A 2009 study conducted by Vandegrift and Hudson looked at the role of these seven gastrointestinal parasites, *Pterygodermatites* (Ricklefs), *Syphacia peromysci* (Harkema), *P. Peromysci* (Lichtenfels), *Mastophorus muris* (Gmelin), *Capillaria Americana* (Read), *Heligmosomoides vangebriti* (Durette-Desset and Kinsella) and *Brachylaima peromysci* (Reynolds), to evaluate how they affect *P. leucopus*. This study examined the impact gastrointestinal parasites have on the population dynamics of these mice. The results of this study suggest that gastrointestinal parasites may play a role in the dynamics of these mice because infection is associated with reduced breeding and has little effect upon mouse survival (Anderson and May 1978). The result was tested more formally in 2008 by Vandegrift and Hudson, where they examined how anthelmintic treatment affected the reproductive output of *P. leucopus* (Vandegrift et. al. 2008). By manipulating the GI parasite burden. The results showed

that both sexes were positively affected by the anthelmintic treatment and an increase in body condition, growth rate and survival was observed (Vandegrift et. al.). This causation suggests that gastrointestinal nematodes have an impact on *P. leucopus* by reducing their reproductive output (Vandegrift et. al.). In addition to gastrointestinal parasites, *P. leucopus* are also susceptible to ecto-parasites such as fleas, ticks and *Cuterebra fontinella* (bot flies) and these too may have an impact on the survival of these mice.

A bot fly is an obligate parasite, meaning it requires a host to complete its life cycle. They are common in eastern deciduous forests and this species relies on mice to complete its life cycle (Cramer and Cameron, 2006). The bot fly life cycle begins with bot fly eggs being laid in the host habitat. Egg hatching occurs as a response to an increase in temperature, which also indicates a nearby viable host (Catts, 1982). The bot fly larva then enters the host through the nostril, mouth or an open wound and begins migrating to the inguinal region, or groin, of the host (Cramer and Cameron, 2006). Once the larva implants itself in the inguinal region of the host, the development lasts for typically one month (Cramer and Cameron, 2006). Studies conducted on bot flies display counterintuitive results: this warble fly tends to increase mouse survival, unlike the results observed in the studies focusing on the effect of gastrointestinal parasites.

A 2005 study focused on how bot flies affect the population dynamics of *P. leucopus* and revealed that mice who were infected with bot flies were living longer than those who were not infected (Burns, Goodwin and Ostfeld, 2005). This study discovered that the effects of bot fly parasitism on an individual-level, such as persistence and reproduction, can extend into population-level parameters. The data collected from this study was consistent with previous studies that have been done in the field and laboratory setting (Burns, Goodwin and Ostfeld, 2005). The data suggested that bot fly parasitism may enhance the survival of infected mice due to the fact that mice with a bot fly parasitism experience constricted movement, hence causing these mice to remain on the trapping grid longer or avoid predation (Burns, Goodwin and Ostfeld, 2005).

However, many researchers suspect that bot fly parasitism imposes a reproductive cost on individual *P. leucopus* and thus their population growth, yet the cost exhibited by these mice has been difficult to document (Cramer and Cameron, 2006). For example, although the reproductive organs of infected *P. leucopus* had less mass, upon examination, it appeared that infected males and infected females exhibited similar reproductive functioning to their uninfected controls (Cramer and Cameron, 2006).

Additionally, some studies have analyzed the movements of *P. leucopus* in regards to bot fly parasitism and sought to examine the effect bot flies have on the movements of individual mice. The study found that the movements of uninfected males and females differ and are closely related to the size of their specific home range (Cramer and Cameron, 2009). More specifically, similar laboratory studies found that bot fly infected animals were less active and spent less time doing specific locomotor activities (Smith, 1978). These laboratory studies have allowed researchers to consider how reduced movement of *P. leucopus* and *P. maniculatus* as a result of bot fly infections may affect these mice in their natural habitats. However, field studies discovered that bot fly infestations did not have an apparent effect on the movement of male and female *P. leucopus*. These conflicting results counter the assumed knowledge that bot flies inflict some sort of cost on their hosts (Cramer and Cameron, 2009). The difficulty of documenting the cost of bot fly parasitism on hosts has led several researchers to conclude that a bot fly-host interaction may not be parasitism at all (Cramer and Cameron, 2006).

The purpose of our study was to test if gastrointestinal parasite removal would alter prevalence and or intensity of bot flies infecting mice. It also was set up to examine whether bot flies reduce survival and if there are any interactive effects of botflies and nematodes. To evaluate these questions, we analyzed the data collected from these 6 treated and 6 control populations between 2011 and 2013.

Materials and Methods

Experimental design and trapping habitat

The field grids containing the live capture traps used to capture the mice in this study were set in the Appalachian Highlands of central Pennsylvania. Traps were checked triweekly beginning in spring and ending during the fall. Sunflower seeds were used to bait the traps; each trap was set in the evening just before dark and checked again the following day. The traps were checked routinely and maintained often, any damaged traps were replaced.

Anthelmintic treatment and vital rate estimates

There were 12 total trapping grids used in this study: 6 control grids and 6 experimental grids. The experimental grids were treated with an anthelmintic treatment called Levamisole Hydrochloride, which was administered to the mice by the food placed in the traps. Data was collected on 9,190 mice. Baseline demographics and density levels were obtained by trapping each site twice before anthelmintic treatments were administered. Once captured the mice were processed on site; data on body length, body mass, sex, body condition (noting any wounds or scars), and breeding status were collected and recorded. The male mice were determined to be in breeding condition if their testes had descended, whereas females were examined to determine if they were lactating, pregnant or had a perforate vagina. Animals captured in more than one trapping session were considered residents. Mice were identified using Trovan (EIDAP, Sherwood Park, Alberta, Canada) which are transponder tags that are inserted subcutaneously into the scruff of the neck of the mice during processing.

Dissections

The gastrointestinal tract of incidental mortalities was dissected. Dissections occurred in order to collect data on the internal parasites present in each mouse. Mice were pinned to a dissection tray and sprayed with 70% ethyl alcohol to lyse any lingering viruses. The body cavity was cut open with scissors; the gut (stomach to the anus) was placed in the water bath to prevent the gut from drying out. The gut was cut into approximately 5 cm pieces; the tract was open longitudinally and carefully examined under a dissecting microscope and any gastrointestinal parasites present were recorded, removed, collected and preserved. Each collected worm was identified and counted to measure the density and prevalence of nematode infection.

Statistical analysis

Binomial generalized linear models (GLMs) were used to determine what factors influence botfly prevalence and intensity. Using stepwise backward selection, the best predictors of each response were chosen and variables with $P < 0.05$ were retained. To illustrate the survival of *P. leucopus* infected and uninfected with bot flies, 4 graphs were generated based on the statistical results obtained.

Results

For this experiment, we set out to identify if there is any interaction between gastrointestinal nematodes and bot fly infections that may increase survival of *P. leucopus*. The number of individually sampled resident mice was 9,190 and for each mouse, we recorded data including breeding status, reproduction, and the absence or presence of nematodes and bot flies. Survival was measured as days known alive. The Levamisole Hydrochloride anthelmintic treatment was successful at reducing the nematode infections of mice on the experimental grid (Fig. 1). This confirms that our manipulation of nematodes was successful and means we can address further questions regarding the potential interactions with botflies.

We wanted to measure the survival of mice infected with nematodes before looking at whether or not the interaction between bot fly and nematode parasitism has an effect on the survival of *P. leucopus*. There was no significant difference between the survival of female mice

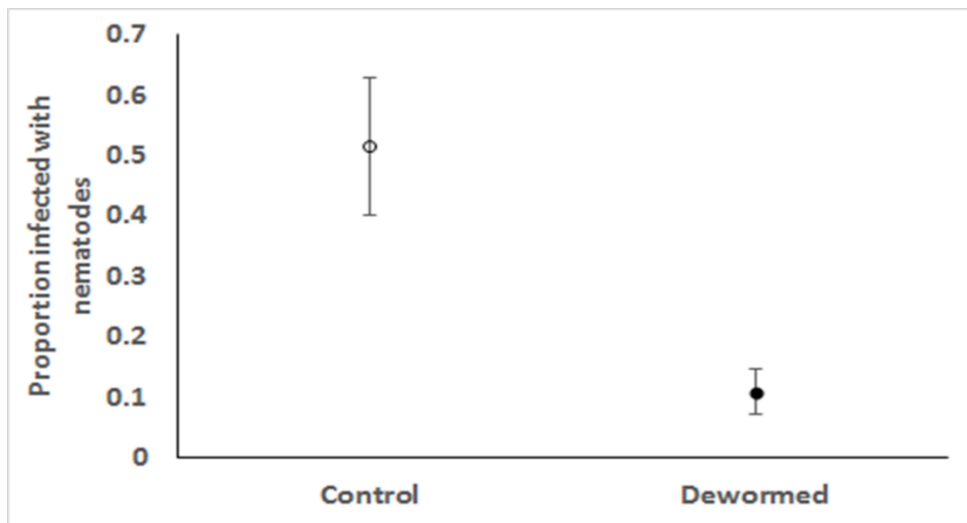


Figure 1. Proportion of Mice Infected with Nematodes for the Control and Dewormed

on the control grids and dewormed grids ($P>0.05$). The mice on the control grid lived on average 97.2 days whereas dewormed female mice lived 98.2 days. In contrast, there was a significant difference between the survival of male mice infected or uninfected with nematodes ($P<0.05$). Male mice on the control grid lived 93.8 days while dewormed male mice lived on average 102.7 days (Fig. 2).

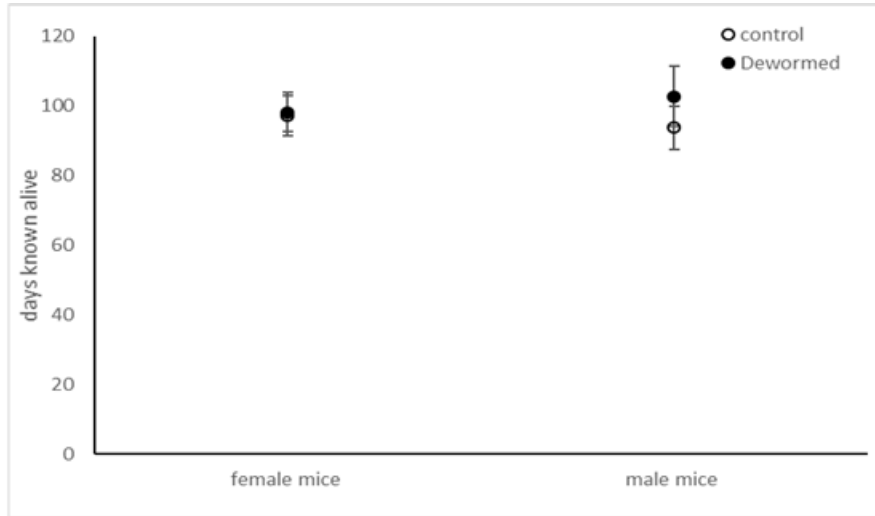


Figure 2. Survival of Female and Male Mice Infected With Nematodes

Figure 3 depicts the average survival of mice on dewormed and control populations as well as whether or not the mice were infected with botflies. Although there was no difference in days known alive between mice with and without botfly infections, among those with botfly infections, those mice that were also dewormed survived longer than those from the control populations. There was no difference in average survival for dewormed mice (47.4 days) and the control's (54.4 days) survival. However, mice on the control grids without botflies survived 54.4

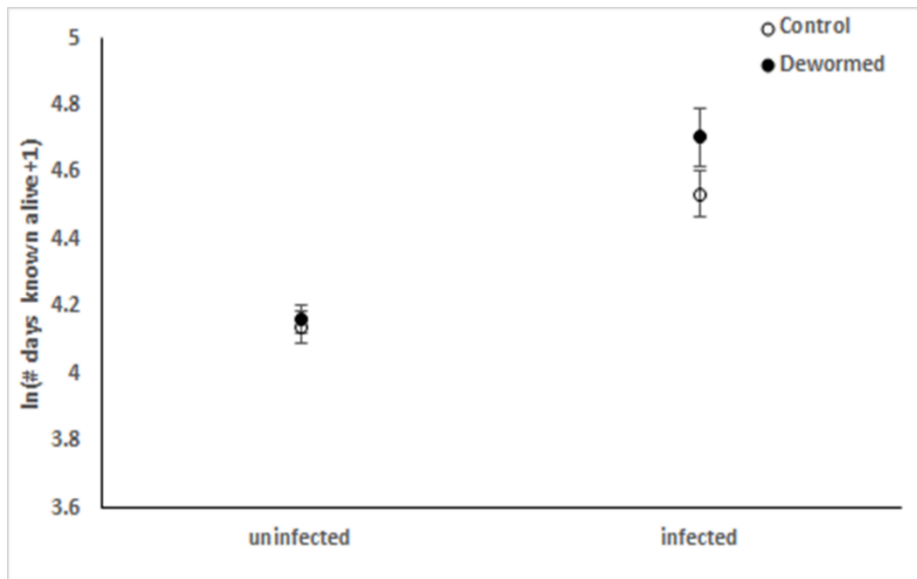


Figure 3. Survival of Male and Female Mice Infected and Uninfected with Bot Flies

The x-axis illustrates bot fly uninfected mice and bot fly infected mice for the control and dewormed groups.

days on average whereas mice with botflies on the control grid survived 95.5 days, which displays an increase in survival (Fig.3). Of those mice that were infected with botflies, dewormed mice survived significantly longer at 143.3 days relative to 47.4 days survived by

dewormed mice uninfected with bot flies (Fig.3). It is also clear from this figure that botfly infections greatly increase the lifespan of these mice.

In Vandegrift, Raffel, and Hudson (2008), deworming was reported to influence survival in male *P. leucopus*, but not in females. Here, sex remained a significant variable in the model for predicting days known alive and so we analyzed the sexes independently. The results of their 2005 experiment research were confirmed here with dewormed males surviving about 30.8 days longer than untreated males, (Fig. 4) There was no difference between dewormed (87.6 days) and control populations (88.6 days) in the survival of male bot fly infected mice.

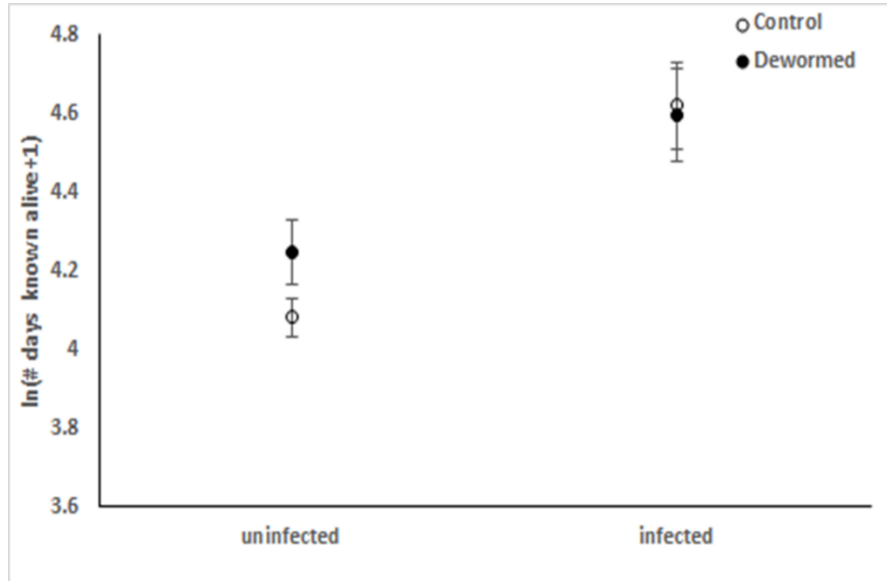


Figure 4. Survival of Male Mice Infected and Uninfected with Bot Flies

The x-axis illustrates bot fly uninfected mice and bot fly infected mice for the control and dewormed groups.

In light of these interesting sex effects, we decided to determine if our interaction between worm removal and botfly infection may have influenced the survival of one sex or another. Here, we found that survival of male mice was only increased by deworming when they were not infected by botflies. Survival did not differ when the males became infected with botflies. However, the pattern observed is the opposite in female mice. Dewormed females without botflies did not survive longer than control (wormy) female mice. Dewormed females that had botflies survived significantly longer (51 days) when compared to bot infected but wormy females from the control population. (Fig. 5).

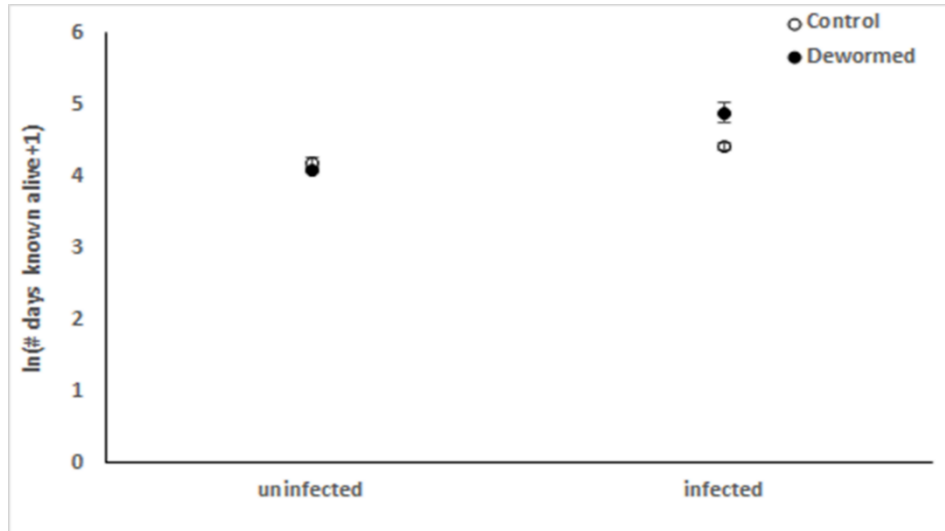


Figure 5. Survival of Female Mice Infected or Uninfected with Bot Flies

The x-axis illustrates bot fly uninfected mice and bot fly infected mice for the control and dewormed groups.

Discussion

An ideal experimental design would involve manipulation of both worms and botflies whereby we would be able to specifically remove only botflies from mice. Currently, a medicine of this type does not exist. Ivermectin has been utilized in trials of this type, but it is not parasite specific, which makes it impossible to examine only the impact of botflies as this broad spectrum treatment would also alter flea, tick, mite and worm prevalence. Another improvement to this ideal factorial design would be to have populations that are infected with additional worms and additional botflies; we can push the pendulum in both directions and potentially gain more information about the tensions that shape the observed dynamics. Although these are strong, well supported population-level results, further experimentation (as described above) is warranted and would be beneficial to our understanding of the forces that drive the instabilities in the dynamics of *P. leucopus*. As discussed these instabilities likely impact the exposure of humans to the vast array of known and unknown zoonotic pathogens these mice can harbor. Therefore, there is an opportunity to increase public health in the human population by disseminating messages warning of high density mouse years and how to safely handle and dispose of mice caught within the home.

Burn's study focused on the role bot fly parasitism played in the reproductive output of *P. leucopus*. The findings of this study suggested that mice infected with bot flies were surviving longer. Burns and Ostfeld suspect that these trends may be the result of a life-history trade off. Coupling these findings with the earlier findings from Vandegrift and Hudson, which showed nematodes impact mice survival, we set out to test if there were interactive effects between worms and bot flies which might further influence *P. leucopus* survival.

An apparent difference between the survival of dewormed and non-dewormed bot fly infected *P. leucopus* was observed. These findings however were not substantial for male bot fly

infected mice and were isolated to female bot fly infected mice. Dewormed bot fly infected male mice only lived 118.4 known days alive in comparison to 127.9 known days alive for bot fly infected mice on the control grid, which is about an 8% increase in survival. Furthermore, dewormed bot fly infected female mice averaged 162.0 known days alive, whereas non-dewormed bot fly infected female mice survived on the control grid for 110.9 days known alive. Dewormed bot fly infected mice displayed a 46% increase in survival than non-dewormed bot fly infected mice. This difference indicates an interaction between nematode removal and bot fly parasitism on the survival of female *P. leucopus*.

Although these findings suggest that survival of *P. leucopus* may be affected by the interactions of bot flies and nematodes, more research needs to be conducted to further explore these findings. In order to strengthen this study and obtain concrete results a manipulative experiment of bot flies would be ideal. While this experiment did manipulate nematodes, by treating groups of mice with anthelmintic treatment, this study does not fully explore the interaction between bot flies and nematodes because the mice were not experimentally infected with bot flies.

Given these limitations an ideal and more successful future study would consist of both experimentally manipulating bot flies and nematodes. To do so *P. leucopus* would need to be dewormed using an anthelmintic treatment and infected with bot flies. Additionally, an effective study would contain a factorial design with 3 grids where mice were only dewormed, 3 grids where mice were only infected with bot flies, 3 grids where mice were both dewormed and infected with bot flies and lastly 3 control grids.

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