Parasite community dynamics in dewormed and worm-infected Peromyscus leucopus populations

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Abstract

Peromyscus leucopus, white-footed mice, are reservoirs and hosts for numerous human diseases, including hanta virus, arboviruses, and bacterial pathogens, which are transmitted via zoonotic spillover. In order to control spillover, it is important to study and predict population dynamics within natural mice populations. An important aspect of mouse survival and therefore disease transmission is the mouse's role as a host for parasites. Mice are often co-infected with various types and intensities of parasites, each of which interacts with the host. We experimentally removed nematodes from the tested mouse population to view the effect on the number and intensity of other parasites, as well as the effect on the overall body condition and fecundity of the mice. This information will provide a greater understanding of the relationship between parasitism and population dynamics, and ultimately help prevent and control future zoonotic diseases.

Introduction

Within the past 30 years, 87 new species of human pathogen have emerged at an alarming rate of about 3 introduced every year (Woolhouse 2009). However, the scientific community has completely failed to predict the pathogen type, time, or place of these emergences. Moreover, pathogens rarely exist in isolation; many host organisms are simultaneously co-infected with multiple pathogen species. Pathogen interactions can increase or decrease transmission, virulence, and emergence rates, while also altering host and pathogen populations via effects on host fitness and population dynamics (Seabloom et al. 2015).

Nonetheless, current research emphasizes a one-host one-pathogen paradigm instead of the more common co-infection of multi-host pathogens (Yakob et al. 2013).

This research project focuses on parasite interactions in a widespread peri-domestic rodent, the White-Footed Mouse (*Peromyscus leucopus*) (Rafinesque 1818). We chose this species because the history of pathogen emergence tells us the most common pathogen type to emerge are viruses and about 60% of these viruses are zoonotic (jumping from animal to man) (Woolhouse 2009). Among animal groups, rodents serve as reservoirs to most of these new pathogens. For example, it is a reservoir host and vector for many zoonotic pathogens, such as hanta virus (Childs et al., 1991), arboviruses (Hardy 1994), and various bacterial pathogens, including its large role in Lyme disease (Donahue, et al. 1987). In addition, *P. leucopus* is the most abundant rodent in the densely populated northeast of the United States. Therefore, *P. leucopus* is a compelling choice to investigate co-infections and gain a better understanding of how this specific pathogen community may aid in the prediction or prevention of the next emerging infectious disease.

Much of the research on the drivers of population instability in *P. leucopus* populations has focused upon bottom-up factors like the mast of Oak trees (*Quercus spp.)*. Indeed, acorns play a major role in mouse population growth via increasing breeding and survival (Ostfeld, Jones & Wolff 1996; Wolff 1996). This has led some authors to speculate that they can predict the number of human Lyme disease cases based upon the amount of acorns present in the environment two years prior (Ostfeld, Jones & Wolff 1996). However, the factors driving the decline phase in *P. leucopus* remain unknown. Gastrointestinal parasites can destabilize host population dynamics (Anderson and May 1978). For example, nematodes drive the cyclic populations of Red grouse (*Lagopus scoticus*) in Northern England and Scotland (Hudson et al. 1999). *Pterygodermatites peromysci* is the dominant nematode parasite in the gastrointestinal tract of *P. leucopus* and in a 2005 deworming experiment, it was shown that *P. peromysci* diminished *P. leucopus* body condition and breeding in the summer months (Vandegrift et al. 2008). In another helminth manipulation experiment on *P. leucopus*, the reduction of nematodes increased the prevalence of other gastrointestinal parasites, including protozoans and cestodes (Pedersen and Antonovics 2013).

These results provide insight into parasite interactions and confirm that parasite-host dynamics require more than a single-host single-parasite experiment, because parasites may interact agonistically or antagonistically within a mouse's gut. It also provides an opportunity for further investigation -- because these two parasite removal studies utilized different deworming drugs. In the former, Levamisole hydrochloride (BOC Sciences) treatment removed only nematodes, while in the latter,Ivermectin (Merck & Co, Inc.) eliminated nematodes as well as other ectoparasites like ticks, fleas, botflies, and mites. Given these interesting results, we sought to determine if levamisole dewormer may have indirectly influenced the ectoparasites community of *P. leucopus* in our prior research effort.

Methods

Experimental design, trapping

In order to accomplish this, mice were trapped on 12 experimental grids in open hard-wood forest in central Pennsylvania USA. To insure independence, experimental grids were at least 250 m apart and each grid contained twelve-8 x 8 grids of multi-capture live traps, with

traps 10m apart. Traps were checked two consecutive days per week, every three weeks, from April to December.

Anthelmintic treatment, small mammal processing, dissections

Each grid was trapped twice before anthelmintic treatment in order to establish baseline demographic and density levels. Of the twelve grids, six grids were chosen at random and all of the mice caught on these six grids were given Levamisole Hydrochloride, an anthelmintic, upon each capture. The mice caught on the remaining six grids were provided with an equal amount of sterile water without wormer and were used as a control. Mouse sex, body length, tail length, body mass, body condition, and reproductive condition were recorded upon each capture. Body condition was determined from the residuals of a cubic spline fit of mass versus body length. Reproductive condition was determined by the presence of descended testes in males or whether the female was lactating, pregnant, or had a perforate vagina. The number of ticks and botflies were counted and the absence or presence of fleas were measured.

Mice were tagged and identified by Trovan transponder tags that were inserted into the scruff of the neck upon first capture. Animals that were captured repeatedly were considered residents of the area. Deceased mice were dissected to quantify gastrointestinal parasite infection burden. The gut (from above the stomach to the anus) was removed and cut. The tract was examined under a microscope and all gastrointestinal worms were removed, collected, and preserved. Worms were identified to species and counted to determine the prevalence and intensity of infection for each. This experiment was conducted with the approval of the Pennsylvania State Animal Care Committee (IACUC #16061, "Transmission Dynamics of Directly Transmitted Diseases in Wildlife Reservoir Hosts").

Statistical analyses

Generalized linear models (GLMs) were utilized to analyze response variables, and the best predictors of the response variables were determined via backward stepwise selection, keeping variables with $p < 0.05$. When the response variable was presence or absence of an infection, data were analyzed with binomial GLMs. For some analyses, mice were separated into groups based on mass, with juveniles being less than 16g, sub adults being 16-20g, and adults being 20g.

Results

The number of individual *P. leucopus* captured was 9,189. Recapture of these individuals represented over 24,000 captures in the 99,840 trap nights from 2011-2013. Based on the Jolly-Seber population size estimates, there was no influence of parasite treatment on the population density of mice both before and after the anthelmintic treatment (all $P > 0.05$).

The effect of treatment on parasites

To determine pretreatment differences in the response variables between treatment and control grids, we tested for the effects of parasites on the density, mass, and body condition of the mice. We also tested for the effects of parasites on the proportion of the population breeding before anthelmintic treatment. There were no significant initial differences between experimental and control grids for any of the response variables (all $P > 0.05$).

Following anthelmintic treatment, there was a significant difference between the prevalence of nematodes in the control and treatment populations. The treatment population had a significantly less prevalence of nematodes than did the control population (Fig. 1). There were no significant differences (all $P < 0.05$) in fleas, mites, mobile mites, bot flies, ticks on neck, or ticks on head, between control and experimental grids (Fig 2).

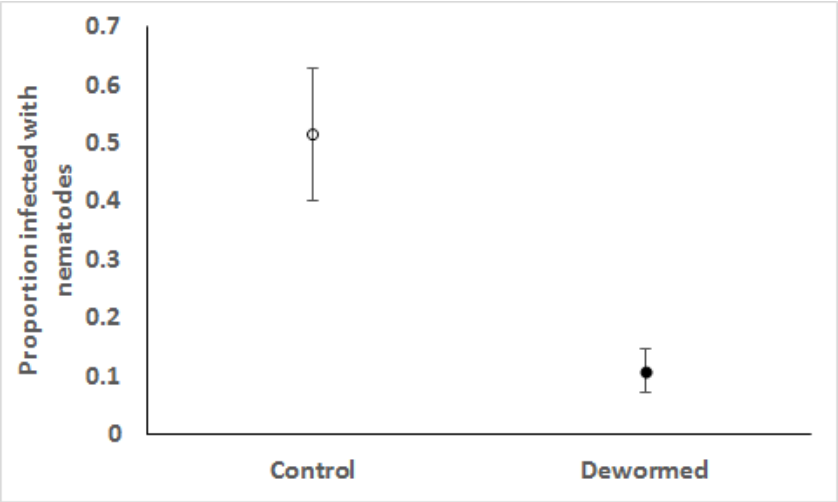

Fig 1: Proportion of mice infected with nematodes in the control vs. dewormed mouse populations.

Discussion

Although our results were not statistically significant, there are reasons to suspect it could have altered the community. In the 2013 de-worming experiment (Pederson and Antonovics 2013), the mice that had been de-wormed had a higher prevalence of protists and cestodes, or tapeworms. However, there were large differences between experiments in terms of space, duration and replication. Their experiment was in Mountain Lake, Virginia, USA, and was only conducted for one summer, while the experiment in Pennsylvania, USA, lasted over three years. They also only used 6 mouse populations, 3 control and 3 treatment, while our experiment used 12 mouse populations, 6 control and 6 treatment. The final and likely the most critical difference was that they used Ivermectin, which kills ectoparasites as well as nematodes, while we used Levamisole, which only kills nematodes.

Based on their results that de-wormed mice had more cestodes, or tapeworms, it is very likely the Ivermectin killed the ecto-parasites, including fleas, on the mice. When the mice groom themselves, they likely ingest these dead and dying fleas, and fleas are often intermediate hosts for tapeworms (i.e. dog and cat tapeworms, *Dipylidium caninum*). Further, the dewormed mice are better able to consume the fleas because the fleas are dying or dead. This could allow for extra time to groom off more fleas than other mice and therefore ingest more fleas than other mice. This may permit a higher likelihood of tapeworm infection; however, if the experiment ran longer, this increase in tapeworms would eventually fade because the flea population would be extinguished. Since we used Levamisole, no ectoparasites were killed, as shown by the similar prevalence and intensity of fleas, mites, mobile mites, and ticks on both the control and de-

wormed populations. Therefore, our treated mice evaded grooming and did not have an increase in cestode prevalence.

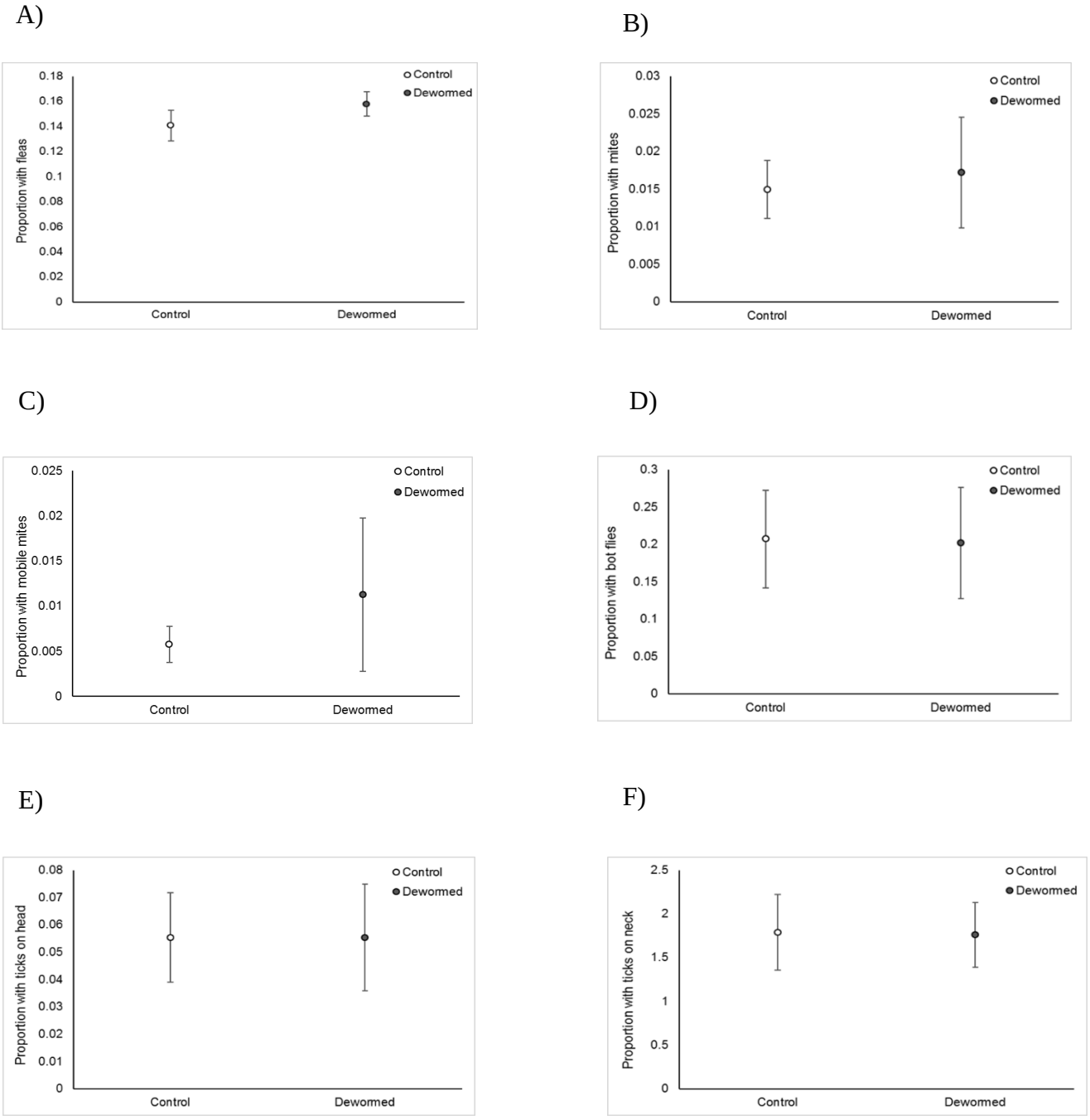

Fig 2: Proportion of mice with (A) fleas, (B) mites, (C) mobile mites, (D) bot flies, (E) ticks on head, and (F) ticks on neck in control vs. dewormed mice for all, female, and male animals in sites 2 and 3. Site 1, which has 3 grids, was not included due to the lack of yearly repetition from 2012-2014. Error bars indicated +/- SE of the grid means (n = 9 grids).

In the future it would be interesting to test the effects of different anthelmintic treatments on the prevalence and intensity of parasites within the same mouse population. It would be interesting to measure the effects of the various treatments on the mice survival, fecundity, and population dynamics. Another critical development that would aid the study of parasite community ecology would be the development of parasite-specific drugs. The nematode parasite community of the *P. leucopus* gastrointestinal tract has 7 members in Pennsylvania and we are completely unable to disentangle the impacts of each species, because we are unable to treat to remove individual species. This type of treatment likely has ramifications for selection of these species as well as those that are parasitic of the companion animals (dogs and cats) for which we have been altering parasite community dynamics for centuries. Only after some of the aforementioned issues are resolved will we be able to address these parasite community dynamics in a controlled and replicated way.

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