2021

Cost of Co-infection in Peromyscus leucopus Mice

Ananya Sagar

Costs of Co-infection in *P. leucopus* mice

A Thesis Presented

by

Ananya Sagar

To the Keck Science Department

Of Claremont Mckenna, Pitzer, and Scripps Colleges

In partial fulfilment of

The degree of Bachelor of Arts

Senior Thesis in Biology

Nov 20, 2020

Abstract:

Co-infections occur frequently across humans & wildlife, and can have effects on host health. Despite the prevalence of microparasite-helminth co-infections, majority of the research focuses either on singular infections or on concurrent infections in a laboratory setting. This fails to take into account the interspecific interactions between the multiple parasites and the host in natural settings. The interactions could be direct by resource competition, or indirect via the host immune system. In our research, we studied the relationship between nematodes and coccidia in their host, *Peromyscus leucopus* mice, at the Mountain Lake Biological Station, Virginia. We further evaluated the effects of deworming treatment on the mice to determine the underlying interactions of the parasites in their natural habitat. Our study indicates that although interactions are difficult to study without any perturbation, deworming treatment can be used as an effective tool to infer the mechanisms of parasite interactions. Our results from the cross-sectional analysis point to immune mediated interactions within the host, but this is complicated by our findings from deworming treatment analysis which suggest resource competition as the interaction. Overall, the major trends are consistent with previous literature on co-infections in mice species.

Acknowledgements:

First & foremost, I would like to thank Professor Sarah Budischak who has guided me throughout my research process and expanded my knowledge by helping me discover the field of disease ecology. I am grateful to her for giving me the opportunity to work in her lab, and for encouraging me to pursue this exciting research topic all the while being a supportive mentor even in these unpredictable times. I would also like to thank Professor Matthew Faldyn for taking the time out to provide me with insights on my thesis as my second reader and for helping me understand the foundations of biostatistics. Thank you both for the constructive feedback that has only improved my work. Additionally, I would like to thank my friends who have sat with me over countless nights on zoom studying and providing me with much needed distraction during these times. A final thank you to my family for always supporting me and pushing me to do my best, while placing an emphasis on my learning.

Table of Contents:

List of Figures:

Costs of Co-infection in *P. leucopus* mice

I) Introduction

Co-infections manifest when there is a simultaneous infection of two or more parasites within a host and are a common occurrence throughout the world. In fact, most organisms are infected by multiple parasites at once, and helminth co-infections alone are thought to be present in 800 million people worldwide (Hotez et al. 2007) . While co-infections in humans are prevalent throughout the world, they are exceedingly common in socio-economically disadvantaged or marginalized communities and in developing nations (Steinmann et al. 2010). In spite of the pervasiveness of these concurrent infections in humans and wildlife, a majority of the research conducted on parasites tends to focus only on singular infections (Fenton 2013). This fails to take into consideration the effect that co-infecting parasites could have on each other and the host. In fact, a meta-analysis conducted on co-infections in humans revealed that co-infections have significant effects on host health and tend to exacerbate infections (Griffiths et al. 2011) .

Previous studies have shown that there are consistent interspecific parasite interactions within a co-infected host which depend on many factors. Host immunity is one such factor that shapes the interactions of the parasite community while also being affected by co-infecting parasites (Pedersen and Fenton 2007, Graham 2008). Considering the role that the immune system plays in parasitic interactions, studying the costs of co-infection would allow us to improve the efficacy of our parasite control programs. Studying concurrent infections in the wild and conducting experiments involving removal of one of the parasite species would elucidate the relationships between these parasites. This would clarify the

potential ramifications of disease control procedures like deworming at the individual as well as population levels.

Recently, researchers have started delving into studying the basic interactions between multiple parasites within a host. Microparasites are usually smaller in size, cause transient infections and reproduce directly within the host, usually at high rates (most viral and bacterial infections). Conversely, macroparasites tend to be relatively larger, have more complex life cycles, multiply outside of the host, and the infections are more chronic in the sense that the host can get continually re-infected and accumulate macroparasites (e.g. most helminth infections). Studies show that these parasites can interact with each other directly, via resource competition or indirectly via the host immune system (Cox 2001, Graham 2008). In helminth-microparasite co-infections the immune responses are mutually inhibitory, because helminths induce a Th1 helper cell response whereas microparasites induce the Th2 helper cells response, and these immune cells have an antagonistic relationship (Yazdanbakhsh et al. 2002). This results in a positive interaction between helminths and microparasites since the host immune system can only direct its response towards one parasite, increasing the intensity of the other. Furthermore, helminths induce regulatory T cells which suppress immune function of the host, implying that helminths could facilitate microparasite infection (White et al. 2020) On the other hand, in resource competition, both parasites compete for the same resources within the host such as space and nutrition, which leads to a negative interaction between the two parasites. Thus, we see that the broad bottom-up and top-down ecological concepts can be applied to parasite populations and interactions within the hosts in terms of resource competition or immune interaction (Pedersen and Fenton 2007, Graham 2008).

Research on co-infection also indicates that parasite communities are not random assemblages, and that these interspecific-interactions within the host tend to be consistent, but need to be further studied (Lello et al. 2004, Knowles et al. 2013). These interactions in a co-infected host can affect the transmission and virulence of the disease – factors which would be different in the case of a singular infection (Alizon et al. 2013). Several studies have suggested that the inter-specific interactions between parasite species affect host organism susceptibility to other infectious diseases (Cattadori et al. 2007, Telfer et al. 2010, Mabbott 2018). Consequently, simply characterizing individuals as infected or uninfected by one parasite is not enough and does not give an accurate representation of what occurs inside the hosts body.

However, as a burgeoning field of study, we still lack a general understanding of when and under what circumstances to expect a strong interaction between different combinations of worms and microparasites (Ezenwa 2016). Multiple studies have indicated that we do not know much about the mechanistic processes behind the parasite interactions (Pedersen and Antonovics 2013, reviewed in Ezenwa 2016). Further research is needed because there is not enough information on these underlying mechanisms, and the parasite interactions themselves vary greatly based on which species is being studied.

Individual host heterogeneity needs to be analyzed too, because while general patterns have been observed when it comes to single parasite infections we don't know what happens when multiple parasites are involved. For instance a meta-analysis on the effect of sex hormones on immune function revealed that higher testosterone in males suppresses immune function causing males to have higher parasite loads than females (Foo et al. 2017) Another meta-analysis displayed how host body condition varies with seasonal changes, availability of resources and differences in age and sex and how this is further complicated by parasitic infection (Sánchez et al. 2018). These were conducted in single parasite studies and

we need to observe how sex, body mass, age, breeding status and other characteristics affect parasite loads of co-infected hosts.

Considering that a majority of the studies on co-infection so far have been carried out in laboratory settings, the effects of co-infection interactions in natural populations remain unclear with limited reliable methods to evaluate these interactions in natural settings (Fenton et al. 2010) . Therefore, future research must focus on quantifying co-infections in natural settings. Research indicates that not all interactions are equal, as some microparasites respond more strongly to helminth co-infections than others. This cannot be observed in laboratory settings where targeted pairs of parasites are studied and artificial introduction of infections is required, so experiments where the hosts are studied in nature can help clarify which co-occurring worms and microparasites interact strongly (reviewed in Ezenwa 2016). Environmental factors could also drastically change how hosts deal with co-infections; for instance, a study releasing mice into wildlife showed that the environment plays a huge factor in how susceptible hosts are to nematode infections and how they respond to the parasites immunologically (Leung et al. 2018). Conducting such projects in the hosts natural habitat would then reduce the need for artificial introduction of infections as well as the variability. In cases where one parasite is removed, the other factors affecting the hosts remain the same.

As aforementioned, a majority of the research on co-infections has been carried out in laboratories or has been observational with few experimental deworming studies in the wild (Fenton et al. 2010, 2014, Ezenwa 2016). Solely using observational approaches with correlation analyses are not sufficient to reveal the complex interactions of the parasites and must be supplemented with an experimental approach (Fenton et al. 2014). Determining whether co-infecting parasites interact by competing for host resources or through the host immune system in their natural environment is crucial for our understanding of co-infections

and for predicting outcomes of deworming programmes carried out in wildlife (Pedersen and Fenton 2007).

In our research, we aim answer some of these questions by looking at co-infection in rodents at the Mountain Lake Biological Station in Pembroke, Virginia. We will assess the parasite community within deer mice and quantify the correlation between coccidia and worm infection in their natural habitat. Additionally, we will also observe how experimentally removing one parasite via deworming affects the host and co-infecting parasite species. This will allow for a more thorough analysis than a simple observational study which may not reveal all the underlying interspecific parasitic interactions (Fenton et al. 2014). Ultimately, we intend on uncovering the mechanism by which coccidia and nematodes interact within *P. leucopus* mice and how deworming affects the infection status of the hosts.

Our objectives are three-fold, assessing:

1. Is there a relationship between coccidia and worm infection among the non-dewormed hosts? We predict a positive correlation between coccidia and worm infection if there is interaction via host immune system and a negative correlation if the parasites interact through resource competition (Fig 1).

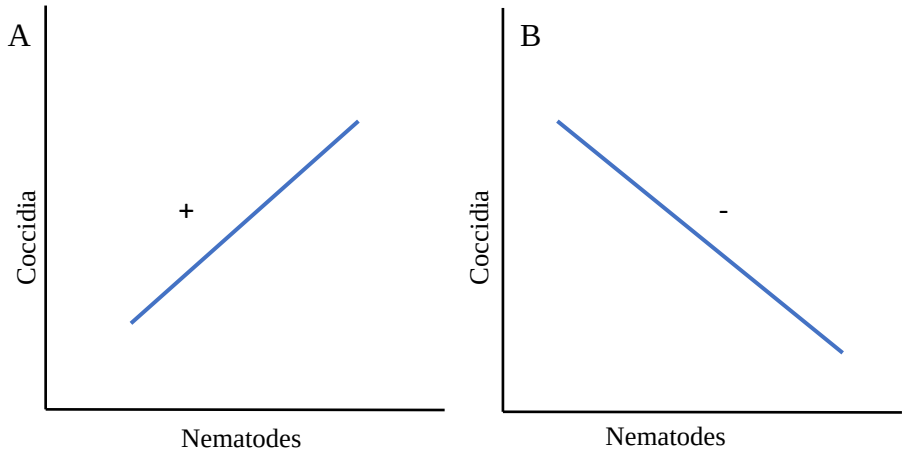

Fig 1. Predicted relationship between coccidia and nematodes in the case of (A) immune interaction and (B) resource competition

2. How does deworming affect the susceptibility towards coccidia infection and the intensity of infection? We predict that the coccidia intensity would be higher in the case of dewormed hosts if resource competition is present whereas lower if host immune system is involved in the parasite interaction (Fig 2).

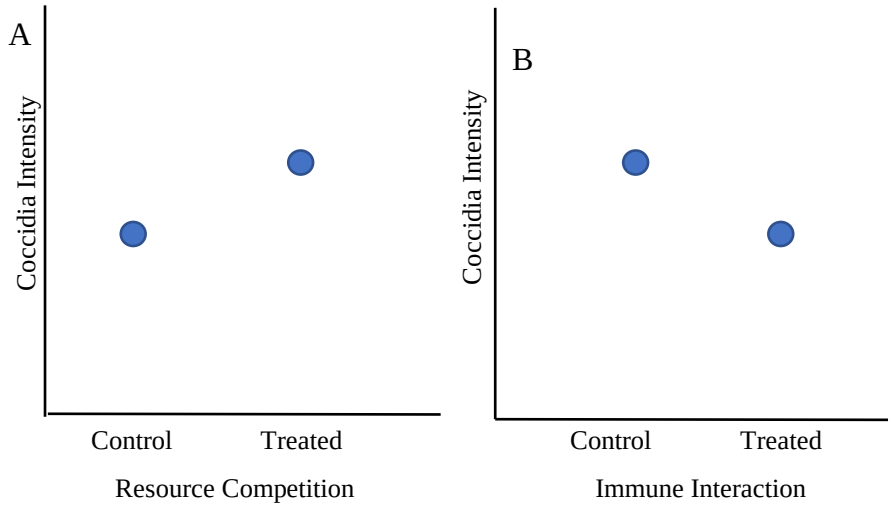

Fig 2. Predicted Effect of treatment on coccidia intensity based on hypotheses of resource competition and host immune interaction

3. How does effective deworming treatment affect the change in infection in individual host mice? We predict that in case of resource competition, the coccidia intensity would increase whereas with immune interaction it would decrease.

II) Methods:

2.1 Fieldwork:

This experiment was carried out on two species of deer mice - *Peromyscus leucopus* and *Peromyscus maniculatus* at the Mountain Lake Biological Station (MLBS) in Pembroke, Virginia (37º22'30.4" N 80º31'22.0" W). *Peromyscus* mice are model organisms and were used because they are easy to handle and trap, carry high parasite loads, and are affected by many non-lethal parasites. Mark-recapture sampling was conducted for three years for around three months from May up to September. Fieldwork was led by Dr. Sarah Budischak and Dr. Courtney Thomason (Ph.D.) assisted by a team of undergraduate and graduate students. The Institutional Animal Care and Use Committee approved the animal handling care protocol before the study commenced.

Four sites were selected for sample collection; Pond (PO), Spring (SP), Bear Cliffs (BC), and Hedwig (HW) (Fig 3). Two eight by eight array grids made up each site, which were then divided into sixty-four points and flags were used to separate each point, approximately 10 m apart. Trapping was done three days in a row at a site, and then followed up with another trapping session every two weeks. Traps were set up with some oats to lure the mice and rats into the traps. The traps were checked every morning and mice caught for the first time were given a metal ear tag for identification. Rodents that were caught two days in a row were released without any new treatments or measurements. The grid and location were recorded each time a rodent was caught. The rodents were also weighed and had body length measured. Ectoparasites presence such as fleas, botflies, ticks were recorded and additional information about sex, and reproductive status was recorded. Age was determined by observing the molting patterns of the mouse fur (Ezeonu 2019).

Deworming medication or a sham-control (sugar water) were orally administered the first time each rodent that was caught during every trapping session. Treatments were

assigned systematically to each new capture by alternating between deworming and control by rodent species to ensure randomization. The deworming treatment or sham-control were administered to each animal only once per trapping session. The control treatment consisted of a 5% sucrose solution whereas the deworming treatment included an Ivermectin and Pyrantel mix consisting of one gram of Eqvalan (1.87% ivermectin) and half a gram of Pyrantel mixed into 4 mL of deionized water. The final dosage of each treatment was weight-adjusted, with 2 μL of the treatment solution for each gram of body mass (Ezeonu 2019).

Fecal samples were collected from traps and the date, grid and trap number were recorded. The feces was weighed in microcentrifuge tubes by weighing the tube along with the sample and then subtracting the weight of an average empty microcentrifuge tube. This was done to standardize comparisons between fecal egg counts of the mice. The fecal samples were preserved with the help of formalin at room temperature for a minimum of 24 hours before being analysed to reduce the risk of spread of any disease.

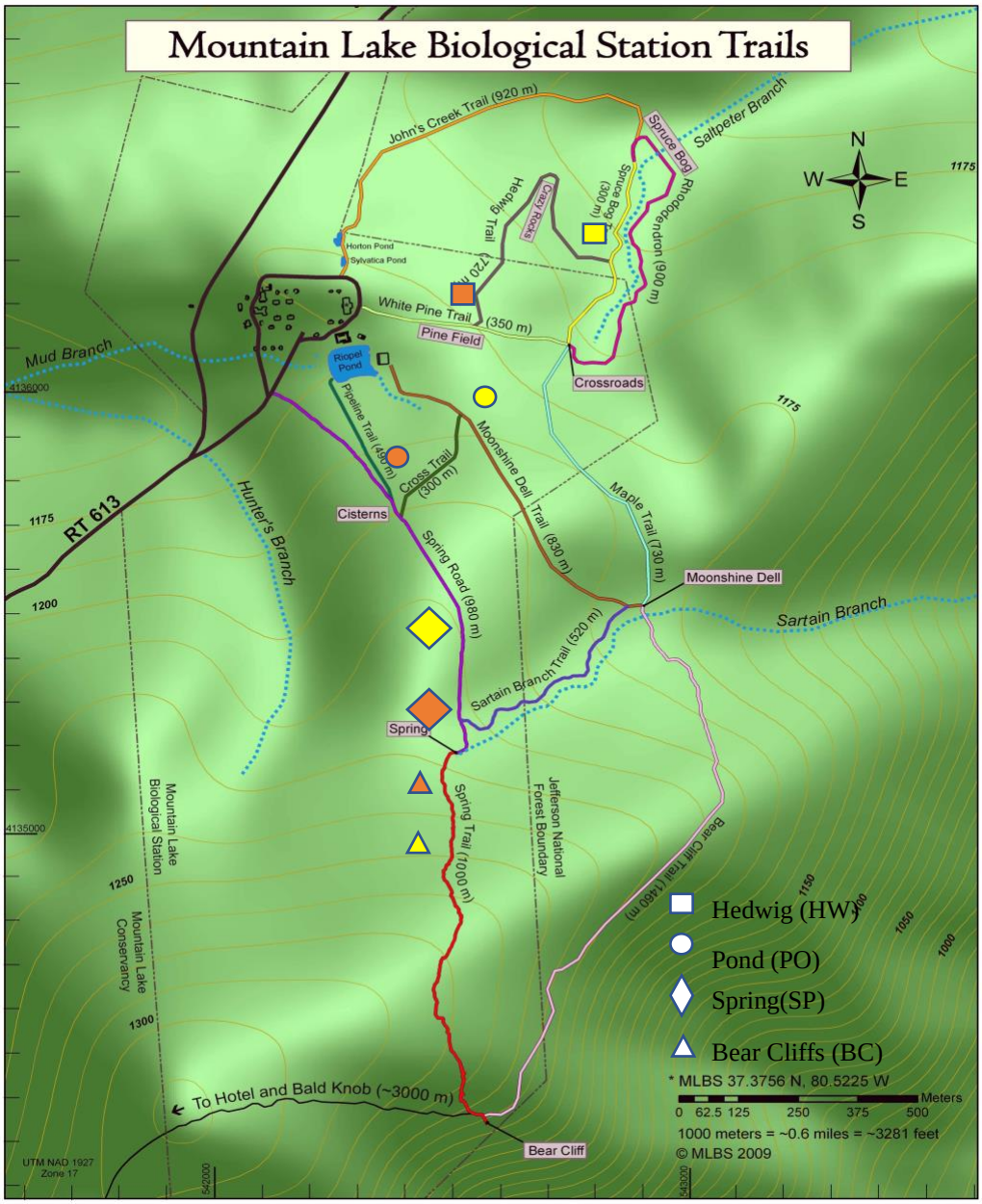

Fig 3. Map showing the trapping sites at Mountain Lake Biological Station, Virginia. Orange represents grid 1 and yellow represents grid 2 for each site.

2.2 Quantifying Parasite infection:

Fecal egg counts were conducted using a fecal flotation protocol to quantify the parasite species present in the hosts. The weighed fecal samples were grinded in the fecalyzer along

with a sodium nitrate saturated solution (fecasol) for about 90 seconds to separate the oocysts from the fecal solution. The saturated fecasol ensured that the oocysts floated to the top, leaving the denser fecal particles at the bottom. More fecasol was added to the fecalyzer till a meniscus was created at the top and a coverslip was placed on top of the meniscus for 15-20 minutes to allow the oocysts to collect at the surface. The coverslip was then transferred to a microscope slide and viewed under 20x and then 40x. The number of oocytes from the different parasite species were observed and recorded. Infection was detected by the presence of nematode or coccidia eggs in the fecal samples and infection intensity was measured as the number of parasite eggs per gram of fecal matter (Ezeonu 2019).

2.3 Data Analysis:

The dataset consisted of a total of 1442 captures, 1264 of which *Peromyscus. leucopus* mice. Since the number of *Peromyscus maniculatus* mice captures (171) was too low for detection of co-infection interactions given the low prevalence of the infections, we decided to proceed with data analysis using just the *P. leucopus* mice which included 582 individual mice. All statistical analyses were carried out using R Studio software (RStudio 2020).

A linear mixed effects model (LMM) was carried out to determine if there was any correlation between coccidia and nematode intensity, first in all the captures and then on just the control, untreated animals. We used this model to account for mixed and random effects. The tag identification number was used as a random effect to account for repeated measurements of individual mice. The sex of the mice was included as a fixed effect. The distribution of both the coccidia and nematode intensities were log transformed to approach a more normal distribution; and the linear model tests we used for analyses are also more

robust towards non-normality especially given large sample sizes. We also included deworming treatment as a co-variate.

We further used this linear mixed effects model to determine the impact of the effective deworming treatment on the coccidia and nematode intensities at the population level by comparing all the control and the dewormed mice. A generalized linear mixed effects model (GLMM) with binomial errors was run to evaluate the prevalence of the coccidia infection and nematode infection in the control and treatment groups. This model has been found to be more reliable in detecting interspecific macroparasitic interactions than correlation or comparative analyses both (Fenton et al. 2014).

Finally, for the strongest experimental evidence of co-infection interactions, we examined the change in coccidia and nematode infection after treatment in individual mice by comparing their first and second captures. We used linear models, with sex and age as covariates, to assess the magnitude of change in parasite egg counts for treated vs. control animals for both coccidia and nematode parasites.

III) Results:

There was no significant relationship between log coccidia intensity (eggs/g) and log nematode intensity (eggs/g) ($t_{240.926} = 0.765$, $p = 0.445$). Additionally, there was no significant effect of sex on nematode intenstiy ($t_{154.823} = 1.203$, $p = 0.231$; Fig 4).

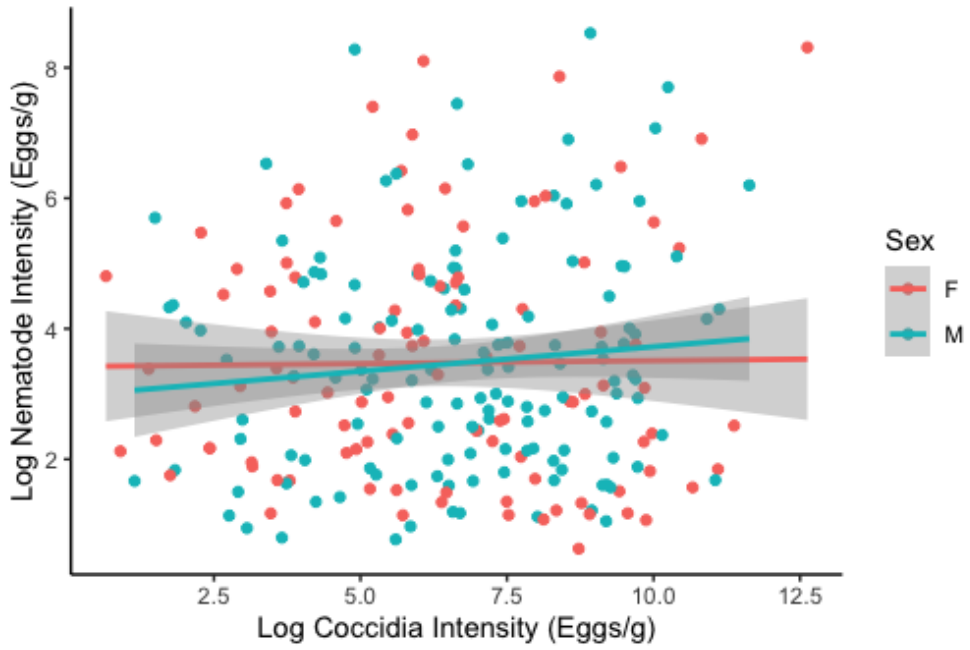

Fig 4. Relationship between log coccidia intensity (eggs/g) on log nematode intensity (eggs/g)

A test for interaction effects of the treatment status on the host coccidia and nematode intensity revealed no significant interaction either, although the plotted data seemed to display a weak trend of a slight decrease in nematode intensity as compared to coccidia intensity in the dewormed hosts ($t_{289.428}$ = -1.481, p = 0.140; Fig 5).

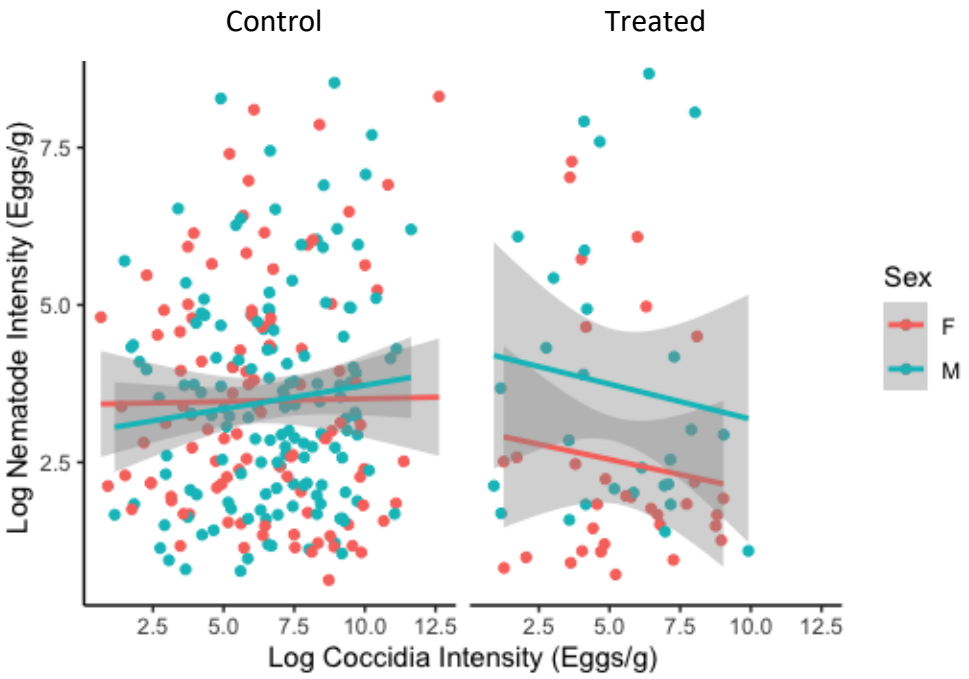

Fig 5. The relationship between log coccidia intensity (eggs/g) and log nematode intensity (eggs/g) taking into consideration the effective anthelminthic treatment.

The next analysis focused on the outcome of the effective deworming treatment on both the parasite intensities in the treated hosts. As expected, the log intensity of nematodes decreased significantly after the deworming treatment ($t_{401.435}$ = -2.137, p = 0.0332; Fig 6A). The anthelminthic treatment also led to a significant decrease in the log coccidia intensity of *P. leucopus* mice, which we predicted would occur in the case of parasite immune interaction ($t_{593.219}$ = -3.785, p < 0.001; Fig 6B).

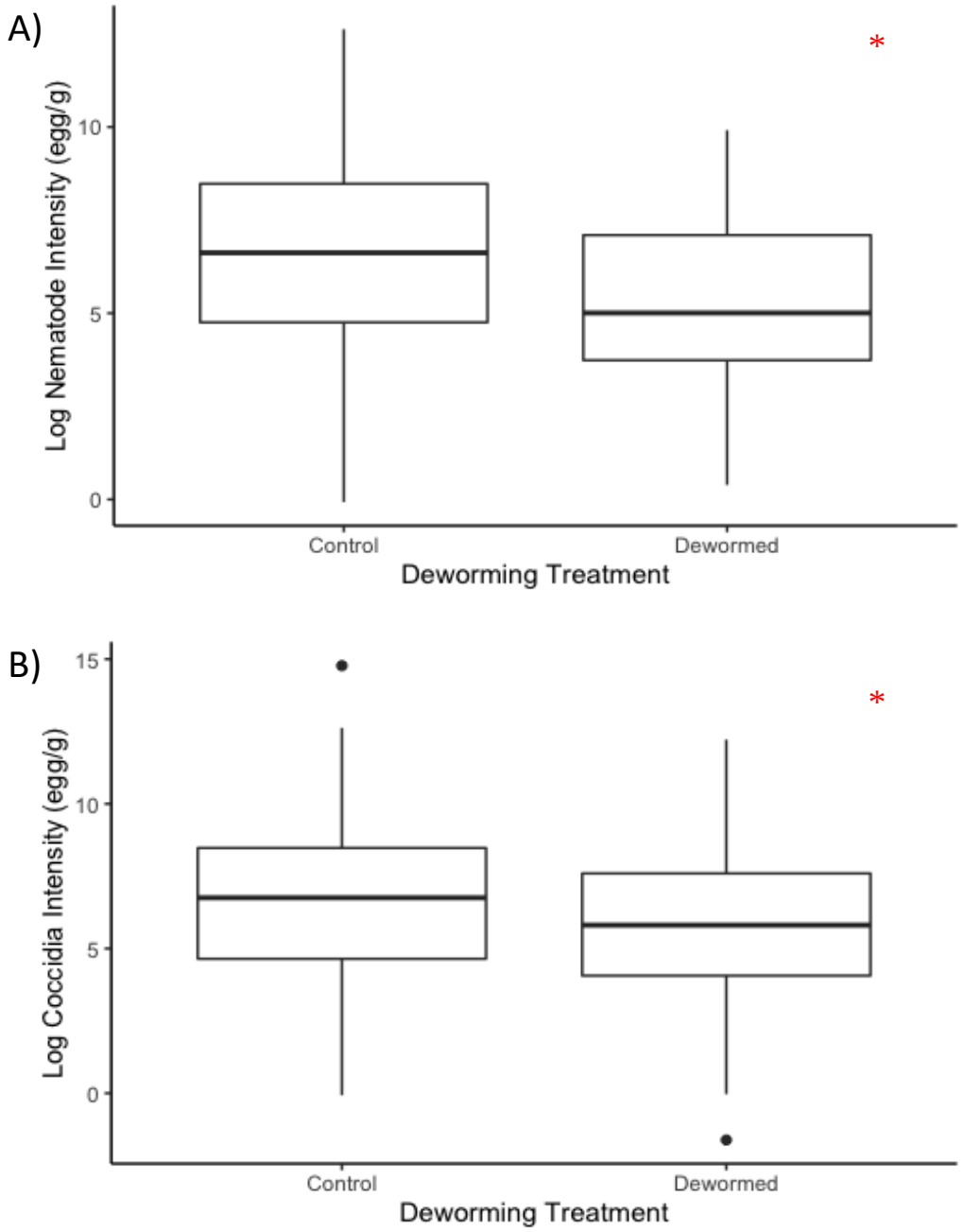

Fig 6. The effect of deworming treatment on (A) log nematode intensity (eggs/g) and (B) log coccidia intensity (eggs/g). Asterisks denote significant effects.

The generalized linear mixed model used for prevalence analysis displayed that the prevalence of coccidia was actually similar across the control and the treated groups. The treatment didn't have a significant effect on the presence or absence of coccidia in the mice

(z = -0.196, p = 0.844; fig 7A). It also did not have any significant effect on the prevalence of nematodes in the hosts (z = -1.293, p = 0.196; fig 7B).

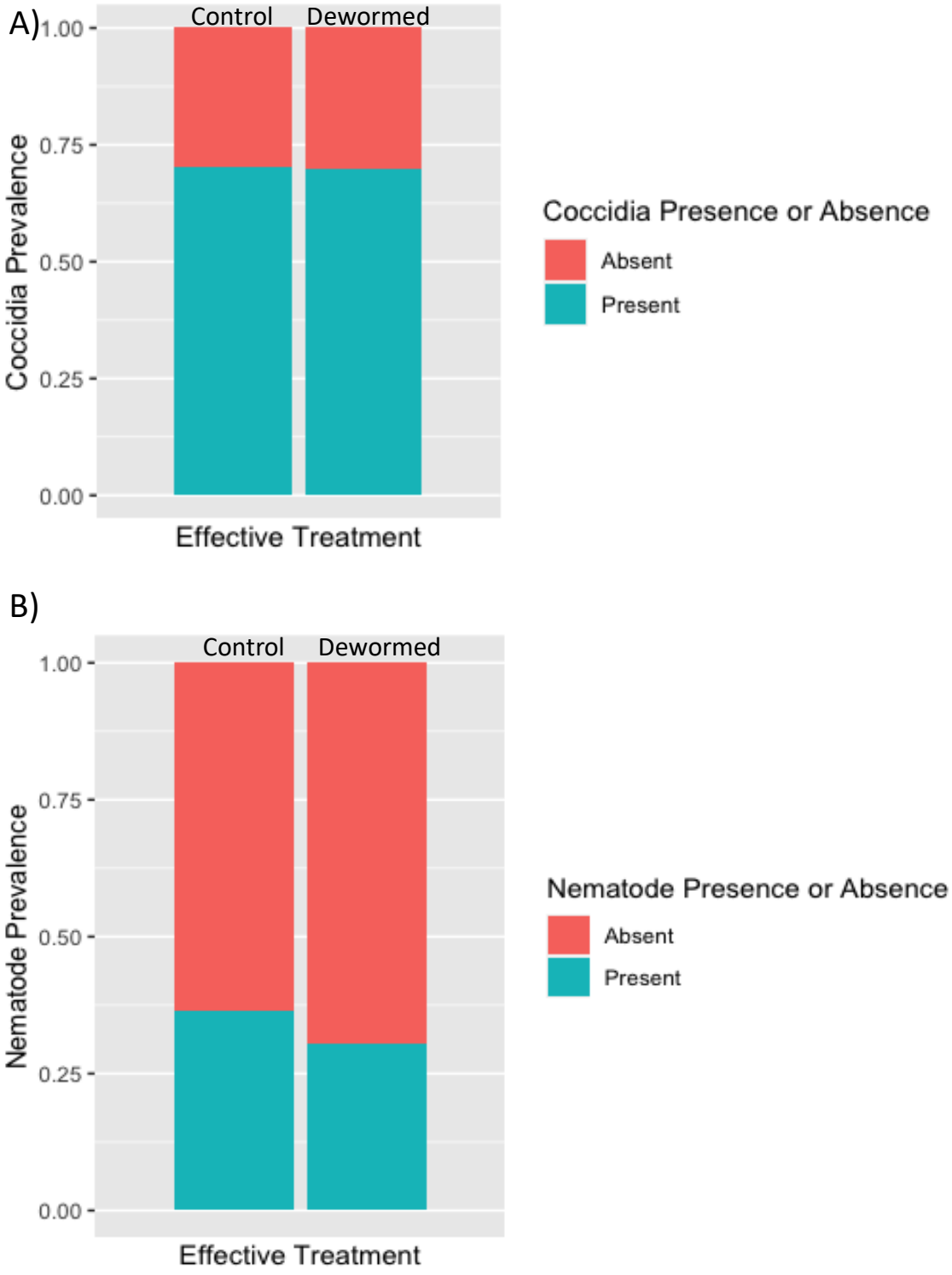

Fig 7. The effect of deworming on A) coccidia prevalence and B) nematode prevalence in *P. leucopus* mice.

Lastly, we conducted a comparative analysis on the individual mice that were treated with Ivermectin by looking at the change in infection post their first and second captures. There appeared to be an increase in coccidia intensity after anthelminthic treatment, although it was only marginally significant ($F_{1, 165} = 3.452$, $p = 0.06496$; Fig 8A). Predictably, there was a sharp decrease in nematode infection for individual mice upon deworming ($F_{1, 274} = 20.77$, $p < 0.001$; Fig 8B). Neither age nor sex had a significant effect on the change in nematode infection when included as covariates ($F_2 = 1.6974$, $p = 0.1851$; $F_1 = 0.0411$, $p = 0.8396$).

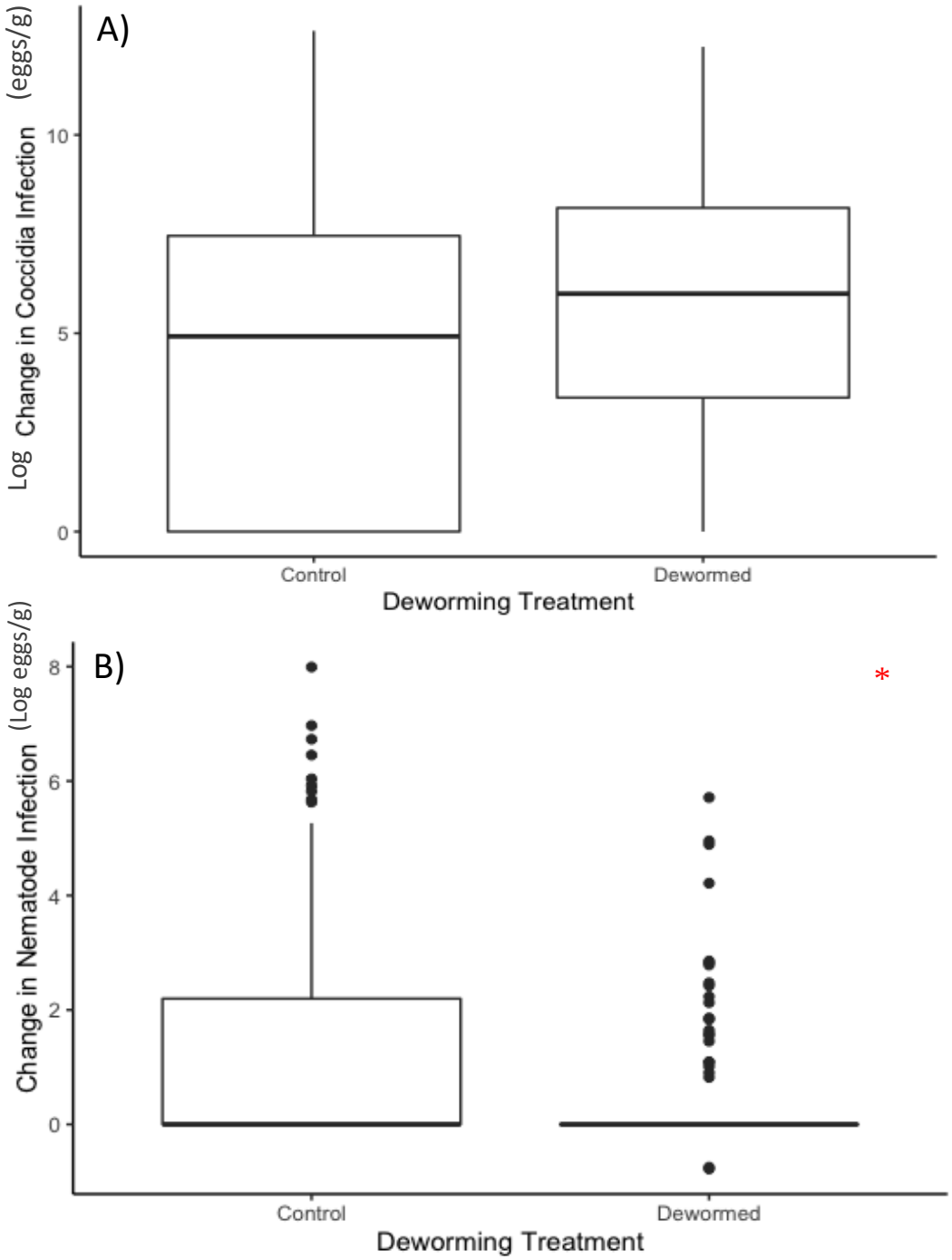

Fig 8. Effect of deworming treatment on changes in (A) coccidia infection and (B) nematode infection. Asterisks denote significant effects.

IV) Discussion:

This study evaluated the parasitic interactions in *Peromyscus leucopus* mice co-infected with microparasites and helminths. Our results indicated that the deworming of mice was effective in reducing nematode intensity, allowing us to use it as an experimental manipulation to observe the coccidia response. While there was no overall correlation between nematode and coccidia intensity in untreated mice, the dewormed hosts had significantly lower coccidia intensity at the population level. The prevalence of both coccidia and nematode intensity remained unchanged upon anthelminthic treatment. The pre- and post-treatment analysis for individual mice revealed a marginally significant increase in coccidia intensity with deworming, with no effect on prevalence.

The significant decrease in coccidia intensity in the dewormed mice as compared to the control mice at the population level could be explained by the host immune hypothesis. In this, the immune response induced by both parasites is antagonistic and a reduction in nematodes by deworming could lead to a decrease in coccidia intensity because the host immune system can direct its response more towards the microparasites. While a meta-analysis on lab experiments has shown that helminth suppression of the host immune response can affect microparasite intensity, multiple fieldwork studies have suggested resource competition as the main interaction among the parasites in rodents (Graham 2008, Reviewed in Ezenwa 2016). To investigate this further, immunological data could be collected and analyzed. This would aid in determining the underlying mechanisms of the parasitic interactions and could also provide context on how the host reacts to multiple parasites.

There was a marginal, but statistically nonsignificant, increase in coccidia intensity in the pre-/post-treatment analysis of individual mice. These results suggest resource competition as the underlying interspecific interaction between the parasites; worms &

coccidia compete for the hosts resources and deworming reduces this competition, thereby increasing coccidia intensity. This is consistent with previous deworming experiments conducted in wild mice co-infected with nematodes and microparasites where there was an increase in microparasite levels post-effective deworming (Knowles et al. 2013, Pedersen and Antonovics 2013, Thomason 2014). The actual prevalence of the coccidia infection did not change upon treatment, indicating that while the nematodes do not alter the host susceptibility to coccidia infection, they could affect the coccidia intensity. Previous research on nematode and *Eimeria* co-infection in wild wood mice supports this as well, wherein an anti-nematode treatment led to an increase in *Eimeria* intensity but not in susceptibility to the infection (Knowles et al. 2013).

The contrasting results from our population and individual level analyses suggest inconsistent parasitic interaction patterns with the former indicating resource competition and the latter, immune mediated interaction. These contradictory mechanistic patterns underscore that the parasitic interactions are context dependent and can change when we compare the population and individual level. This has been discussed in a theoretical framework designed by Fenton et. al. in which they acknowledge that interspecific relationships of parasites may switch from negative to positive or vice versa at different levels of study (Fenton 2008, Fenton et al. 2014). A potential explanation for this increase in coccidia intensity at the individual level and the decrease at the population level could be that the deworming reduces the resource competition for coccidia within the individual mice allowing them to proliferate. This higher coccidia intensity within an individual might affect the life span of the host or external behaviors which could reduce transmission across mice, thus reducing the intensity at the population level which we then detect in our analysis. This inconsistency could also be a consequence of the statistical models used for analysis itself, as it has been previously pointed out that even models that control for fixed & random effects cannot always predict

parasitic interactions which may not be linear (Fenton et al. 2014). The deworming treatment is unlikely to have had any direct effect on coccidia intensity as Ivermectin has been thoroughly studied and is extensively used in various experiments (Reviewed in Lumaret et al. 2012).

Future studies could include a larger samples size, capture and recapture rates, behavioral changes, and immunological data. There was not a large enough sample size of the *Peromyscus maniculatus* mice, preventing us from using them in our analyses and thus limiting a comparison of these effects across species. We were unable to measure the chronic effects of the deworming treatment as we didn't compare the changes in coccidia intensity over multiple recaptures of the treated mice. Hence, we are only able to discuss any acute effects of the treatment on the infection and cannot comment on the resistance of the parasites over time. In fact, the study on *Eimeria* and nematodes in wood mice found that the microparasite populations were resilient and recover from perturbations, such as deworming, quite quickly (Knowles et al. 2013). Thus, it would be beneficial to perform such a drug perturbation study looking at the more long-term effects; for the analysis we could also include time since deworming treatment as a covariate. Furthermore, it was also difficult for us to account for any behavioral changes the mice made, such as changes in foraging, mating etc., upon infection or treatment application – which in turn could affect the spread of infection.

Taken altogether, our study on microparasite-helminth co-infections in *Peromyscus* mice suggests some underlying interaction between the two parasites. Our analysis suggests that the interspecific parasitic interactions in the natural environment are based on multiple factors and more specifically, that anthelminthic treatment can have contrasting effects at different levels of study. Our experiment, carried out in the natural environment of the host and parasite species, emphasizes the fact that interspecific interactions are more complex than

those we observe in a laboratory setting and that drug treatments can have important population level consequences. Ultimately, this work highlights needed avenues for future research such as long-term drug perturbation studies and comparative studies across different species.

References:

Alizon, S., J. C. de Roode, and Y. Michalakis. 2013. Multiple infections and the evolution of virulence. Ecology Letters 16:556–567.

Cattadori, I. M., R. Albert, and B. Boag. 2007. Variation in host susceptibility and infectiousness generated by co-infection: the myxoma–Trichostrongylus retortaeformis case in wild rabbits. Journal of The Royal Society Interface 4:831–840.

Cox, F. E. G. 2001. Concomitant infections, parasites and immune responses. Parasitology 122:S23–S38.

Ezenwa, V. O. 2016. Helminth–microparasite co-infection in wildlife: lessons from ruminants, rodents and rabbits. Parasite Immunology 38:527–534.

Fenton, A. 2008. Worms and germs: the population dynamic consequences of microparasite-macroparasite co-infection. Parasitology 135:1545–1560.

FENTON, A. 2013. Dances with worms: the ecological and evolutionary impacts of deworming on coinfecting pathogens. Parasitology 140:1119–1132.

Fenton, A., S. C. L. Knowles, O. L. Petchey, and A. B. Pedersen. 2014. The reliability of observational approaches for detecting interspecific parasite interactions: comparison with experimental results. International Journal for Parasitology 44:437–445.

Fenton, A., M. E. Viney, and J. Lello. 2010. Detecting interspecific macroparasite interactions from ecological data: patterns and process. Ecology Letters 13:606–615.

Foo, Y. Z., S. Nakagawa, G. Rhodes, and L. W. Simmons. 2017. The effects of sex hormones on immune function: a meta-analysis. Biological Reviews of the Cambridge Philosophical Society 92:551–571.

Graham, A. L. 2008. Ecological rules governing helminth-microparasite coinfection. Proceedings of the National Academy of Sciences of the United States of America 105:566–570.

Griffiths, E. C., A. B. Pedersen, A. Fenton, and O. L. Petchey. 2011. The nature and consequences of coinfection in humans. Journal of Infection 63:200–206.

Hotez, P. J., D. H. Molyneux, A. Fenwick, J. Kumaresan, S. E. Sachs, J. D. Sachs, and L. Savioli. 2007. Control of Neglected Tropical Diseases. New England Journal of Medicine 357:1018–1027.

Knowles, S. C. L., A. Fenton, O. L. Petchey, T. R. Jones, R. Barber, and A. B. Pedersen. 2013. Stability of within-host–parasite communities in a wild mammal system. Proceedings of the Royal Society B: Biological Sciences 280:20130598.

Lello, J., B. Boag, A. Fenton, I. R. Stevenson, and P. J. Hudson. 2004. Competition and mutualism among the gut helminths of a mammalian host. Nature 428:840–844.

Leung, J. M., S. A. Budischak, H. C. The, C. Hansen, R. Bowcutt, R. Neill, M. Shellman, P. Loke, and A. L. Graham. 2018. Rapid environmental effects on gut nematode susceptibility in rewilded mice. PLOS Biology 16:e2004108.

Lumaret, J.-P., F. Errouissi, K. Floate, J. Römbke, and K. Wardhaugh. 2012. A Review on the Toxicity and Non-Target Effects of Macrocyclic Lactones in Terrestrial and Aquatic Environments. Current Pharmaceutical Biotechnology 13:1004–1060.

Mabbott, N. A. 2018. The Influence of Parasite Infections on Host Immunity to Co-infection With Other Pathogens. Frontiers in Immunology 9.

Pedersen, A. B., and J. Antonovics. 2013. Anthelmintic treatment alters the parasite community in a wild mouse host. Biology Letters 9:20130205.

Pedersen, A. B., and A. Fenton. 2007. Emphasizing the ecology in parasite community ecology. Trends in Ecology & Evolution 22:133–139.

RStudio Team (2020). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com

Sánchez, C. A., D. J. Becker, C. S. Teitelbaum, P. Barriga, L. M. Brown, A. A. Majewska, R. J. Hall, and S. Altizer. 2018. On the relationship between body condition and parasite infection in wildlife: a review and meta-analysis. Ecology Letters 21:1869–1884.

Steinmann, P., J. Utzinger, Z.-W. Du, and X.-N. Zhou. 2010. Chapter 2 - Multiparasitism: A Neglected Reality on Global, Regional and Local Scale. Pages 21–50 *in* X.-N. Zhou, R. Bergquist, R. Olveda, and J. Utzinger, editors. Advances in Parasitology. Academic Press.

Telfer, S., X. Lambin, R. Birtles, P. Beldomenico, S. Burthe, S. Paterson, and M. Begon. 2010. Species Interactions in a Parasite Community Drive Infection Risk in a Wildlife Population. Science (New York, N.Y.) 330:243–246.

Thomason, C. A. 2014. AN EXPERIMENTAL APPROACH TO UNCOVERING THE CONSEQUENCES OF PARASITE CO-INFECTION IN PEROMYSCUS:165.

White, M. P. J., C. M. McManus, and R. M. Maizels. 2020. Regulatory T-cells in helminth infection: induction, function and therapeutic potential. Immunology 160:248–260.

Yazdanbakhsh, M., P. G. Kremsner, and R. van Ree. 2002. Allergy, Parasites, and the Hygiene Hypothesis. Science 296:490–494.