It's all connected: Parasite communities of a wild house mouse population exist in a network of mixed positive and negative associations across guilds

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Abstract

1. Wild animal populations typically harbour multiple parasite species, which can interact in various ways depending on the species involved and the state of the host upon infection. While many pairwise parasite interactions and within-guild parasite communities have been characterised, understanding how an interaction network spanning multiple parasite groups might be mediated has been less commonly explored. 2. We aimed to characterise parasites associations across guilds in a wild population of a model species, allowing for comparisons with existing laboratory-based research, and better understanding of how any observed associations might manifest within the host. 3. We used cross-sectional data from an island population of the house mouse, Mus musculus domesticus, to identify associations between a broad range of parasite species, including blood-borne microparasites, arthropod ectoparasites, and gastrointestinal and hepatic helminths. 4. Every recorded species was found to exist within a framework of positive and negative associations, involving multiple between-guild associations, and with the under-studied helminth species Calodium hepaticum playing a central role. 5. This study highlights the need to account for as many infections as possible when studying naturally infected populations, due to the prevalence of inter-species associations. Various potential mechanisms, including immunological and ecological, are suggested to explain how these associations might occur. Comparisons with analogous laboratory research from the same species are explored. A need for longitudinal study to determine causality of interactions is highlighted.

Introduction

Parasitic infection in wild populations is ubiquitous, and coinfection with multiple parasite species is the norm. There has been a recent effort to explore within-host parasite communities through an ecological lens, rather than treating infections as isolated phenomena (Cattadori, Boag, & Hudson, 2008; A L Graham, 2008; Johnson, De Roode, & Fenton, 2015; Pedersen & Fenton, 2007; Seabloom et al., 2015). Predicting how parasite species will interact in individuals and populations coinfected with multiple species is difficult, and accounting for infection by ectoparasites, protozoan and helminth endoparasites, bacteria and viruses poses a significant challenge for disease ecologists. Different parasite guilds (here distinguished by size, life-cycle and niche as helminth macroparasites, single-celled microparasites and helminth macroparasites) impact their hosts in a variety of ways, causing qualitatively diverse types of immune responses e.g. Th1/2 immune polarisation in response to intracellular parasites and helminth macroparasites respectively(Jankovic, Sher, & Yap, 2001), and varying levels of pathology, from asymptomatic infections that approach commensalism, to more severe infections that cause morbidity or mortality. Understanding interactions between parasite species in coinfected hosts and populations is essential for understanding and predicting associated outcomes in the host. Laboratory infection models are useful in demonstrating the influence of coinfection upon the course

of infection and the role of host immunity. However, only recently have studies of wild populations begun to explore multi-species parasite communities incorporating multiple parasite types in naturally infected animals (Dallas, Laine, & Ovaskainen, 2019; S Telfer et al., 2010).

Parasites alter the environment within their host in a variety of ways, and these changes can influence the course of other infections. Interactions between parasite species can be mediated both through changes in a host's immune state (Chen, Louie, McCormick, Walker, & Shi, 2005; Andrea L Graham, Lamb, Read, & Allen, 2005; Su, Segura, Morgan, Loredo-Osti, & Stevenson, 2005), through direct ecological processes such as niche competition(Balmer, Stearns, Schötzau, & Brun, 2009; Bashey, 2015; Stancampiano, Gras, & Poglayen, 2010), or through a combination of these processes (A L Graham, 2008). They may also change between stages of a parasite's life cycle, due to changes in immune response and the niches exploited within the host (Duncan et al., 2012).

While the importance of parasite community structure in coinfected wild animals is increasingly well understood, coinfection studies have typically focussed on within-guild interactions (Lello, Boag, Fenton, Stevenson, & Hudson, 2004; Lutermann, Fagir, & Bennett, 2015; Sandra Telfer et al., 2010), or on specific pairwise interactions between species of different guilds (Brettschneider, Anguelov, Chimimba, & Bastos, 2012; Ferrari, Cattadori, Rizzoli, & Hudson, 2009; Knowles, Palinauskas, & Sheldon, 2010; Legesse, Erko, & Balcha, 2004; Nacher et al., 2002). Mutualist and competitive interactions between species pairs observed in wild populations include interactions between microparasites (Taylor et al., 2018), and between microparasites and helminths (Ezenwa, Etienne, Luikart, Beja-Pereira, & Jolles, 2010). Networks of positive and negative cross-species interactions have been observed in microparasites infecting wild field voles, *Microtus agrestis*(Sandra Telfer et al., 2010), and lions, *Panthera leo*(Fountain-Jones et al., 2019), in helminth communities of wild rabbits,*Oryctolagus cuniculus* (Lello et al., 2004), and in arthropod ectoparasite communities infecting wild sengis, *Elephantulus myurus* (Lutermann et al., 2015).

Cross-guild parasite communities have been more recently explored in wild populations of non-model species. Parasites of 22 rodent species in the Sonoran desert were shown to associate across tissue types and guilds, with the majority of associations being positive (Dallas et al., 2019). Here, negative associations were most commonly found between parasites which shared a physical niche, indicating that while positive associations may be governed by the state of the host, or overlap in transmission methods or distribution, negative associations primarily result from localised competition.

New bovine tuberculosis infections in wild African buffalo (*Syncerus caffer*) were found to shift cross-guild communities of parasites within the hosts, increasing taxonomic richness of parasite assemblages, shifting the overall taxonomic position and favouring parasites with specific functional traits (Beechler et al., 2019). These examples illustrate the importance of considering individual infections within a context of multi-parasites ecosystems. Characterising cross-guild interact networks in a wild population of a model species will allow our understanding of these associations to be more directly related to and informed by analogous co-infection studies performed in controlled laboratory settings.

Working towards holistic models which account for as broad a range of infections as possible will improve our understanding of parasite communities, and the factors affecting distribution of individual infections, and performing these assessments in model species will allow us to more easily connect this research to related laboratory studies. Here we use cross-sectional parasitological survey data from a wild population of the house mouse (*Mus musculus domesticus*) to explore associations among prevalence and intensity of infections from a broad range of infections, establish a network of positive and negative associations involving every observed parasite, and discuss potential mechanisms underlying these associations.

Materials & Methods

The fieldwork for this study was undertaken on the Isle of May (56.1833deg N, 2.5667deg W), in the Firth of Forth, Scotland. The island is a designated National Nature Reserve of around 45 hectares and consists of a mosaic of coastal grassland with patches of tall ruderal vegetation and frequent exposed bedrock. A feral population of house mice has been present on the island for over a century, and was supplemented by an

introduction of 77 animals from the Scottish island Eday in 1982 (Berry, Triggs, King, Nash, & Noble, 1991; Muir, 1885; Triggs, 1991). The population is assumed closed, with no migration. Due to the seasonality of predators (primarily raptors) on the island, including short-eared owls *Asio flammeus* during the winter, there is assumed to be very little predation pressure.

Eight trapping sessions (see Supplementary Materials) were carried out using a mixture of Ugglan and Longworth live-traps. Trapping effort consisted of transects of twenty traps set in different areas across the island. Trapping locations were selected in order to maximise the coverage of the island within the limits of the terrain, and allowing clearance for bird breeding sites (for a map of trapping locations see Taylor et al, 2019 (Taylor et al., 2019)). Traps were baited with mixed birdseed, provided with hay as bedding material, and checked frequently throughout the day.

Dissection

Following trapping, mice were euthanised in accordance with Home Office guidelines using increasing CO_2 concentrations. Death was confirmed by exsanguination. External physiological variables taken were: (i) body weight, the mass of the mouse in grams, and (ii) snout-vent length, the distance from the tip of the nose to the anus. These values were then used to calculate Scaled Mass Index, a measure of relative body mass which scales against a curve for the population as a whole, to be used as a proxy for animal condition (Peig & Green, 2009). The sex of the animal was also confirmed at this point.

Blood samples were obtained by pipetting during exsanguination, allowed to clot at room temperature for 1 hour, then stored at 4deg^{C} for another hour. The blood was then centrifuged at 13,500 RPM for 5 minutes, and resulting clots were retained and stored in liquid nitrogen for microparasite diagnostics, then kept at -80deg^{C} upon return from the field. The gastrointestinal tract was removed and stored at room temperature in 80% ethanol.

During dissection, the eyes were removed and stored in 10% buffered formalin. Later, the eye lenses were dissected out, dried and weighed, and the resulting measurements used to estimate age (Rowe, Bradfield, Quy, & Swinney, 1985). Eye lens mass is commonly used to estimate age as it grows continuously throughout an animal's lifespan (Augusteyn, 2008). When compared to body mass, body length, tail length, foot length and ear length in corn mice, eye lens mass proved the most reliable at discriminating age (Carreno, Brigada, Rosi, & Castro-Vazquez, 1990). Bodies were wrapped in paper roll until ectoparasite surveys could be conducted in order to prevent parasites from being lost.

Infection

To survey gastrointestinal helminth infection status, the stomach, caecum, small intestines and large intestines were dissected under a microscope and all parasites observed were visually identified and recorded, including both larval and adult forms. Two species of helminth worms were commonly found in the gastrointestinal tracts of the dissected animals: the whipworm *Trichuris muris* and the pinworm*Syphacia obvelata* . Also recorded was the tapeworm*Hymenolepsis sp.* , though this was rarely recorded (5 instances over 366 recorded mice) and was not included in further analysis.

To measure the level of infection by the hepatic helminth *Calodium hepaticum* (also known as *Capillaria hepaticum*), the liver was visually assessed during initial dissection and scored based on the level of decolouration (from 0 = no decolouration, to 5 = extensive discoloration).

To assess ectoparasite infections, following dissection, the fur of each mouse was examined under a dissecting microscope with all parasites identified using a visual key (key created at University of Nottingham, from photos by Laura Myhill), and total number of each species counted. As fleas rapidly move to and from the body, including after capture and after death, numbers are highly dynamic, and therefore only prevalence was recorded.

To record microparasite prevalence, DNA was extracted from the frozen blood clots using a 2-step extraction kit (Extracta DNA Prep, Quanta Bio Massachussetts, USA), and the resulting DNA used for PCR. The mi-

croparasites selected for detection were the apicomplexan protozoan *Babesia microti* (although in fact another closely related apicomplexan was detected instead – see below), the flagellate protozoan *Trypanosoma sp.* and the bacteria *Anaplasma phagocytilum* and *Bartonella spp.* (see Supplementary Materials). These parasites were chosen based on species and genera which are commonly detected across wild rodent populations (Duh, Petrovec, Trilar, & Avsic-Zupanc, 2003; Healing, 1981; Taylor et al., 2018).

Abundance measures of these parasites could not be reliably recorded, and so only presence/absence data from these species are used in analyses. Amplified DNA was run on a 2% agarose gel for 30 minutes at 100V, and visualised under ultraviolet light to confirm amplification. Identity of successfully amplified samples was confirmed through sequencing at an external company (Source Bioscience, Nottingham, UK) and comparison against reference sequences on BLAST (National Center for Biotechnology Information). Successfully amplified samples from *B.microti* primers were found to actually be from infections by another apicomplexan parasite, *Sarcocystis dispersa* . The identity of this parasite was further confirmed through PCR of an additional gene with *Sarocycstis* -specific primers, sequencing of PCR product as described above.

Infection by *C.hepaticum* was not recorded for the first 150 mice caught, and blood clot material for these mice was lost, so no *C. hepaticum* or microparasite data was available for 150 of 366 mice.

Statistical Analysis

All statistical analyses were performed in R version 3.5.3.

The presence of each parasite was used as the response variable in a series of binomial generalised linear mixed models (GLMMs). Ecological variables incorporated in each model were age, sex, Scaled Mass Index (SMI) as a measure of condition, captures per transect per day (used here as a proxy for population density), prevalence of microparasites, *T. muris* , *S. obvelata* , *C. hepaticum* and fleas, and the infection intensity of *T. muris, S. obvelata, C. hepaticum* and fur mites. Location and trapping session were included as random effects. Although it is likely that trapping session would have a significant bearing on parasite infection dynamics, the irregular spacing of each session means that any seasonal or inter-annual patterns of parasite distribution are unlikely to become evident, and so it was not included as a fixed effect.

For parasite species with measures of abundance (gastrointestinal helminths and fur mites), a series of negative binomial GLMMs were performed using abundance data, excluding uninfected individuals. As infection levels of *C. hepaticum* were measured on an arbitrary 1-5 scale of infection score, the '**clmm** ' function of the '**ordinal** ' package in R was used to create a cumulative link mixed model, taking the infection score as an ordinal factor. For this model, individuals with infection scores of zero were taken to be uninfected, and thus were excluded.

As there is missing data for microparasites and *C. hepaticum* infection, models in which these parasites were not the predicted variable were repeated with these species excluded to increase statistical power. Any significant relationships between pairs of parasites with complete datasets that are henceforth reported, are taken from these exclusionary models.

Models of infection level were subsequently simplified and selected through a model averaging process. The '**dredge** ' function was used to compare all possible sub-models, and those with $\Delta\text{AIC} < 2$ when compared to the "best model" (with the lowest AIC) were averaged using the '**model.avg** ' function (both from R package '**MuMIn** ') to find optimal models for each parasite species.

Due to the issues surrounding the use of Bonferroni corrections and statistical power in ecological data (Nakagawa, 2004), the models are not corrected for multiple testing, but all effects sizes are reported (Supplementary Materials).

Results

A summary of all parasite species recorded in the mouse population is shown in Table 1. Out of 366 recorded mice, 127 were uninfected with *T. muris* or *S. obvelata* (34.70%), 169 were infected with *T. muris* (46.17%) and 112 were infected with *S. obvelata* (30.60%). Of these mice, 42 (11.48%) were coinfected with both

parasites. Burden distributions for both species were found to approximate a negative binomial distribution, with infections greatly shifted towards low infection burdens, with rare individuals having dramatically higher loads.

The *T. muris* binomial mixed model found the prevalence of the parasite increasing significantly with the age estimate in days (z=6.038, $p<2x10^{-16}$, OR=1.026). There was no association observed, however, between age and parasite abundance (z=0.684, p=0.4941, OR=1.001), suggesting that once infection has established, the number of parasites established in the host remains fairly stable. The prevalence of *S. obvelata* was significantly negatively associated with the number of captures per transect per day (z=2.780, p=0.005, OR=0.613), implying a negative association between host population density and *S. obvelata* prevalence.

Most captured individuals (152 out of 261) showed some level of infection by *C. hepaticum* . The distribution of infection score in infected individuals is predominantly uniform. The prevalence (z=3.921, $p=8.82x10^{-5}$, OR=1.020) and infection intensity (z=3.163, p=0.00156, OR=1.011) of the parasite was positively associated with age.

No *Anaplasma sp.* or *Trypanosoma sp.* were detected by PCR in any of the mice. In 127 mice, no microparasites were detected. 123 mice were found to be infected with *Bartonella sp.* , and 18 were found to be infected with *Sarcocystis dispersa* . Of these mice, 7 were coinfected with both parasites.

Prevalence of *Sarcocystis sp.* was strongly positively associated with increasing age (z=2.487, p=0.0129, OR=1.0167). No ecological parameters were found to significantly impact *Bartonella sp.*prevalence.

Four genera of mite (*Myobia* , *Radfordia* , *Myocoptes*& *Trombicula*), and one species of flea (*Nosopsyllus fasciatus*) were found in the fur of the mice. Prevalence of fleas was found to be positively associated with age (z=2.552, p=0.0107, OR=1.007). Abundance of mites also showed a positive relationship with SMI (z=2.533, p=0.0113, OR=1.060) and age (z=4.659, $p=3.2x10^{-6}$, OR=1.006).

Parasite Interactions

The two common species of gastrointestinal helminths have a negative association, as evidenced by both the *S. obvelata* prevalence (z=2.468, p=0.0136, OR=0.207) and *T. muris* abundance (z=2.125, p=0.0336, OR=0.456) models. There was a strong positive association observed between *C. hepaticum* and *T. muris* , with the prevalence of the former significantly predicting that of the latter (z=3.558, p=0.00037, OR=4.272) and with *C. hepaticum* infection score predicting the abundance of *T. muris* (z=2.521, p=0.0117, OR=1.189). *C. hepaticum* prevalence also showed a positive association with *Bartonella sp.* prevalence, a relationship that is observed in the models for both species (*Bartonella sp.* model z=2.322, p=0.0203, OR=3.067. *C. hepaticum* model z=1.971, p=0.0488, OR=2.648). *Sarcocystis sp.* prevalence was negatively predicted by *C.hepaticum* prevalence (z=2.306, p=0.0211, OR=-1.815). The abundance of mites in the fur showed positive associations with fleas in the fur (z=2.22, p=0.0264, OR=1.811), and with the prevalence of *C. hepaticum* (z=2.421, p=0.0255, OR=2.384), predicting the prevalence of both. The network of parasite interactions seen in this population of *M. musculus* is shown in Fig 1.

Discussion

The most frequent variable determining infection prevalence and intensity was coinfection, highlighting a crucial role of parasite community structure in determining individual parasite species are distributed in this population. Every parasite species measured was associated with at least one other species, with many observed across guilds, confirming the importance of accounting for coinfection across multiple parasite groups when studying coinfected wild populations. Both negative and positive associations were observed, forming a complicated picture of parasite community structure, and providing a framework upon which mechanisms of parasite interactions might be further explored.

Immunity

Immune antagonism may explain negative associations between parasites susceptible to similar immune responses or environments.*C.hepaticum* shows negative associations with *S.dispersa* , which may be due

to Th1-type responses exhibited during early *C.hepaticum* infection (Kim, Joo, & Chung, 2007) negatively impacting *Sarcocystis sp.* infection.

Immune antagonism may also explain the negative association between *T.muris* and *S.obvelata* . Similar associations are observed in mice artificially infected with the pinworm *Aspiculuris teraptera* , which suffered reduced *T. muris* burdens (Keeling, 1961). The steady increase in *T. muris* prevalence associated with age and high prevalence of low-level infection in this population may be indicators of *T. muris* establishing chronic infections, which are characterised by immunosuppression of protective Th2 responses and polarisation to a Th1 environment in infection models (Artis, Potten, Else, Finkelman, & Grencis, 1999; Else, Hültner, & Grencis, 1992). This is in contrast to *T. muris* infections observed in other wild rodents, in which burdens increase with age (Behnke, Lewis, Zain, & Gilbert, 1999), and *Trichuris* distributions observed in humans, in which burdens spike during early life, then decrease to a steady low level (Bundy, Cooper, Thompson, Didier, & Simmons, 1987; Faulkner et al., 2002; Needham et al., 1992). *S. obvelata* prevalence shows no significant association with age, indicating that parasite clearance and possible reinfection could be the norm. Induction of Th2 responses by *S. obvelata* could disrupt the immunosuppressive environment established by *T. muris* , negatively impacting its abundance (Moreau & Chauvin, 2010).

Immune facilitation or suppression may contribute to the positive association between *C.hepaticum* and *Bartonella sp.*Polarisation to a Th2 environment observed during some stages of the *C.hepaticum* life cycle (Kim et al., 2007) could reduce protective immunity to microparasites, which are typically characterised by Th1-type responses (Arvand, Ignatius, Regnath, Hahn, & Mielke, 2001; Kabeya et al., 2007; Karem, Dubois, McGill, & Regnery, 1999). A positive association between *C. hepaticum* and *Bartonella elizabethae* was reported in the brown rat (*Rattus norvegicus*), though this was based upon an unusual increase in pathology observed in one coinfected individual (Kamani, Akanbi, Baneth, Morrick, & Harrus, 2013).

Peripheral impacts of immunomodulation could be playing a role in the association between *C.hepaticum* and *T.muris* . Jackson et al (2009) found positive associations between *C. hepaticum* and the intestinal nematode *Heligmosomoides polygyrus* in wild wood mice *Apodemus sylvaticus,* associated with reduced innate immune responses. In rats artificially infected with *C.hepaticum* , Th1/2 polarisation is observed, with IL-5 (Th2) increasing as the worms matured, and IFNγ (Th1) peaking during egg deposition (Kim et al., 2007). Systemic polarisation to a Th1 environment during *C. hepaticum* egg deposition could improve survival of *T. muris* . Moon et al (2017) observed a protective immune response to infection against *C. hepaticum* and a similar hepatic nematode *Clonorchis sinensis* in laboratory rats, characterised by eosinophilia and lymphocyte proliferation. The interaction observed in this population may, therefore, alternatively be mediated by an immunosuppressive effect on the part of *T. muris* , favouring *C. hepaticum* survival. (Kim et al., 2007).

Associations observed with ectoparasites are less well defined in this population, and less well characterised from laboratory models. Inflammation in the skin may contribute to protection against parasitic arthropods (Moats, Baxter, Pate, & Watson, 2016), and the dermal tissue has been recognised as a site of important immune activity (Kupper, 1990). Th2-type responses have been detected in response to ectoparasite infection (Burgess et al., 2010; Wikel & Alarcon-Chaidez, 2001), which may polarise away from the Th1-type response observed in early *C.hepaticum* infection, and contribute to a positive association between *C.hepaticum* and mite burden. (Kim et al., 2007).

Pre-existing immune environments within the host, determined by factors other than infection and not accounted for in this study, such as genetics, predispose individuals to susceptibility to or protection against infection. Cross-reactivity between antigens of different nematode species has been observed in both mice and humans (Lillywhite, Bundy, Didier, Cooper, & Bianco, 1991; Nieuwenhuizen et al., 2013; Roach, Wakelin, Else, & Bundy, 1988), and so modulations to the immune environment caused by one helminth may impact the survival of other species.

Ecological Effects

Both *T.muris* and *S.obvelata* are typically found in the caecum or proximal colon, so competition for resources physical space which the parasites occupy may contribute to the negative association observed between these

species. While *T. muris* shows plasticity in its site of establishment in caecectomised mice (Panesar & Croll, 1980), it obtains nutrients from the intraepithelial space of the gut lining while embedded (T. D. Lee & Wright, 1978; Panesar & Croll, 1980), whereas *S. obvelata* are typically found free in the lumen, meaning that competition for a physical niche is perhaps unlikely, and the grounds for antagonistic niche competition between these species is unclear. While *T. muris* and mature *C. hepaticum* exist in separate organs, the infective stage of *C. hepaticum* does pass through the gut wall, meaning that changes in gut tissue histology by one parasite may be beneficial to another.

Prevalence of the microparasite *Sarcocystis sp.* is negatively associated with infection with *C. hepaticum* . *Sarcocystis muriviperae* has been shown to form cysts in the liver of laboratory mice (Paperna & Finkelman, 1996), while mice experimentally infected with *S.dispersa* show histopathological changes in the liver parenchyma (Skárková, 1986). Any impacts upon the liver caused by*S. dispersa* mean it could potentially be in competition for a spatial niche with *C. hepaticum.*

Positive associations between mites and *C. hepaticum* could indicate a role for ectoparasites as indicators of condition, with endoparasitic infection causing reduced fur quality or reduced levels of grooming. Alterations in grooming behaviour have been recorded in rodents coinfected with *Toxoplasma sp.* (Queiroz, Viel, Papa, Lescano, & Chieffi, 2013), though whether this could result from helminth coinfection is unclear. Wild deer mice (*Peromyscus maniculatus*) show natural increases in ectoparasite prevalence, which are prevented by treatment with an antihelminthic (Pedersen & Antonovics, 2013). While mites of wild bank voles (*Myodes glareolus*) showed a positive association with both fleas in the fur and*Calodium spp.* (referred to as *Capillaria spp.*) dissected from the gastrointestinal tract, coinfected individuals had higher body mass in both instances, suggesting that these interactions may be immune mediated rather than a change in host condition (Perkins, White, Pascoe, & Gillingham, 2017). This is supported by the positive association observed between SMI and mite abundance in our study mice. Fur mite abundance was the only measure found to be associated with SMI in this study, bringing into question the significance of infection, and associated coinfection, on the fitness of the host. While this does not take into account reproductive fitness, that different aggregations of parasites within hosts do not seem to be strongly linked to host condition is notable.

Interactions between parasites could also arise from similarities or dissimilarities in transmission method, as might be expected from associations observed within guilds. All three helminth macroparasites observed here are typically transmitted orally (Flynn, 2007), a factor that could underpin positive associations observed between*T.muris* and *C.hepaticum* . The impact of life-cycle discrepancies may in turn interact with individual host behaviour e.g. more exploratory animals may expose themselves to more fleas, which infect hosts by detecting vibrations in the environment, while hosts which spend more time socialising may experience higher mite burden, as mites are typically passed directly between animals (Bordes, Blumstein, & Morand, 2007; Dizney & Dearing, 2013, 2016).

As the data were derived from cross sectional cull data it was not possible to infer direct causal links explaining associations between parasite species, and any observed relationships may potentially be mediated by some unaccounted-for variable. We have tried to mitigate this possibility by including a number of relevant physiological and ecological parameters in all models. The reported associations are therefore independent of possible confounding factors such as sex, age or season, and provide a robust framework from which the mechanisms of parasite interactions can be further hypothesised and tested.

Conclusion

Interactions among parasites in naturally coinfected animals are well-recorded. Incorporating multiple guilds of parasites in a comprehensive network has, however, been less commonly reported, particularly in model species. Every parasite surveyed in this wild host population was implicated in an association with at least one other parasite, with multiple cross-guild interactions observed. Interactions which may explain many of these associations are potentially immune-mediated, as most of the species pairs exhibiting associations exist within different niches. *C. hepaticum* and *T.* muris impart well-characterised changes to immune state in infection models which may be evident here. While potential pathogens such as viruses are not included in this

study, we have demonstrated the importance of accounting for coinfection when studying parasitic infection in wild populations, and the prevalence of cross-guild associations which may not be always accounted for during studies of natural infections. This point is highlighted by the prominence of associations between *C. hepaticum,* an often overlooked infection in wild rodent populations, and ectoparasites, bacteria, protozoa and helminths within other niches. The associations reported here are based on cross-sectional data; this work would be greatly improved with the addition of longitudinal data, to determine causality in associations, examine the significance of order of infection, and obtain a clearer picture of the mechanisms underlying coinfection dynamics.

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Data Availability Statement

Data available from the Dryad Digital Repository: doi.org/10.5061/dryad.c866t1g2z (Fenn et al, 2019).

Author Contributions

JF, SY, SG, AL, AM contributed to field work and dissection, JF, SY performed gut dissections, SG performed microparasite PCRs, JF, CT performed statistical analyses, JB, AM were responsible for general planning of the project JF, AW wrote the first draft of the manuscript with all other authors contributing to revisions.

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FIGURES

Figure 1.

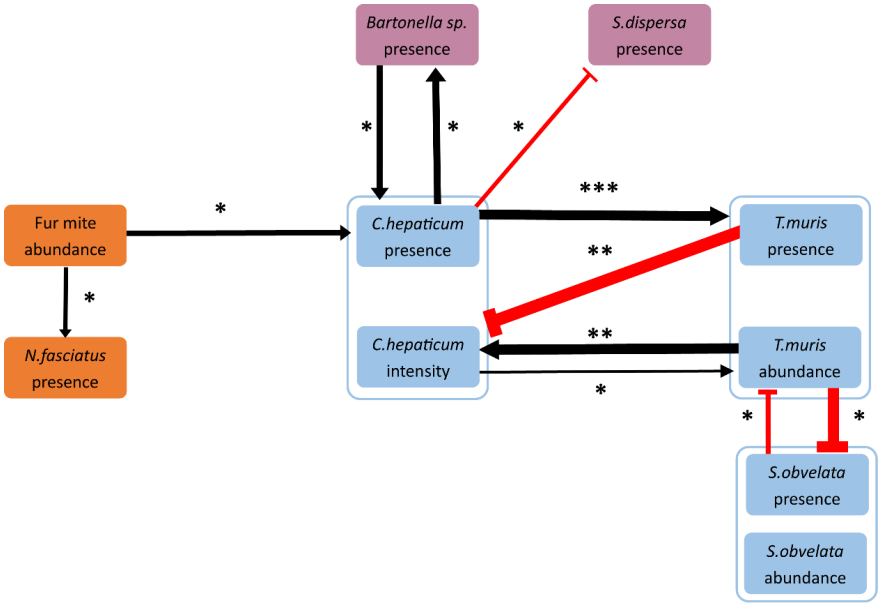

Fig 1. Network of positive and negative associations between parasite species, based on general linear models of prevalence, and where appropriate, parasite abundance (representing log values) or intensity. Blunt, red arrows indicate negative association and black, pointed arrows indicate positive association. The direction of each arrow indicates leads from the predictor variable to the output of the model. Thickness of the line is proportional to the odds ratio value of the association.

*p[?]0.05, **p[?]0.01, ***p[?]0.001

Table 1.

Parasite	Niche	Clade	Prevalence	Life Cycle Summary
Trichuris muris	Gastrointestinal Helminth	Nematoda	46.17%	Eggs embryonate in environment and hatch upon ingestion. First stage larvae establish in mucosa of colon and caecum, and moult until reaching maturity. Adult worms remain embedded in epithelium and release eggs with the host faeces. (Flynn, 2007; Panesar & Croll, 1980; Wakelin, 1969)
	Gastrointestinal Helminth	*Nematoda*	*30.33%*	*Direct – eggs embryonate on perianal region of host, and are ingested by another host from the perineum, or from contaminated food or water. Eggs hatch in the host, and adults reside in the lumen of the caecum and colon and release eggs with host faeces. (Flynn, 2007)*

Parasite	Niche	Clade	Prevalence	Life Cycle Summary
	Hepatic Helminth	Nematoda	58.23%	Eggs embryonate in environment and hatch in the intestine following ingestion. Infective larvae penetrate the mucosa and enter the liver via the hepatic portal system. Larvae develop into adults and reproduce, leaving gravid females in the liver. Eggs are released upon the host's death via necrophagy or decomposition of the host body. (Flynn, 2007; C. W. Lee, 2009)
Bartonella sp.	Bloodborne Microparasite	Bacteria	47.12%	Transmission occurs during feeding on the host by an arthropod vector (typically fleas and ticks), potentially from the vector's faeces. The bacteria enter the bloodstream, where they invade erythrocytes and reproduce intracellularly. Further transmission occurs during later bloodfeeding by the arthropod ectoparasites. (Harms & Dehio, 2012)

Parasite	Niche	Clade	Prevalence	Life Cycle Summary
Sarcocystis dispersa	Bloodborne Microparasite	Ampicomplexa	6.90%	*M.musculus* is the intermediate host, and becomes infected upon ingesting sporozoites, which disperse through the blood. Bradyzoite cysts develop in skeletal tissue, and complete their life cycle following predation of the host by owls. (Flynn, 2007; Kolářová, 1986; Skárková, 1986)
Nosopsyllus fasciatus	Arthropod ectoparasite	Fleas (Siphonaptera)	34.15%	Eggs are laid on the fur of the host, and fall into the environment where they hatch. Larvae moult and pupate, and jump onto the fur of the host when movement is detected, where reproduction occurs. (Flynn, 2007)
Radfordia sp., Myobia sp., Trombicula sp.	Arthropod ectoparasite	Mites (Acari)	84.42%	Parasite eggs hatch on the host, where they develop through larval and nymph stages to become adults. Reproduction occurs in the fur of the mice, and transmission occurs through direct contact between hosts. (Flynn, 2007; Friedman & Weisbroth, 1977)

Table 1. Table of all parasites detected in *M.m.domesticus* on the Isle of May, including recorded prevalence across all trapping sessions and a summary of the parasite's life cycle.