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Of mice and worms: are co-infections with unrelated parasite strains more damaging to definitive hosts?

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ABSTRACT

Intraspecific competition between co-infecting parasites can influence the amount of virulence, or damage, they do to their host. Kin selection theory dictates that infections with related parasite individuals should have lower virulence than infections with unrelated individuals, because they benefit from inclusive fitness and increased host longevity. These predictions have been tested in a variety of microparasite systems, and in larval stage macroparasites within intermediate hosts, but the influence of adult macroparasite relatedness on virulence has not been investigated in definitive hosts. This study used the human parasite *Schistosoma mansoni* to determine whether definitive hosts infected with related parasites experience lower virulence than hosts infected with unrelated parasites, and to compare the results from intermediate host studies in this system. The presence of unrelated parasites in an infection decreased parasite infectivity, the ability of a parasite to infect a definitive host, and total worm establishment in hosts, impacting the less virulent parasite strain more severely. Unrelated parasite co-infections had similar virulence to the more virulent of the two parasite strains. We combine these findings with complementary studies of the intermediate snail host and describe trade-offs in virulence and selection within the life cycle. Damage to the host by the dominant strain was muted by the presence of a competitor in the intermediate host, but was largely unaffected in the definitive host. Our results in this host–parasite system suggest that unrelated infections may select for higher virulence in definitive hosts while selecting for lower virulence in intermediate hosts.

1. Introduction

How do we control and combat infectious disease? This has been a question plaguing scientists for centuries with no clear solution, despite an increasing need for an answer (Holt and Dobson, 2006). A critical element that allows us to mitigate disease is our understanding of the factors that influence the virulence of parasites. Parasite virulence is the damage that a parasite does to its host (Combes, 2001). The virulence of a parasite has a genetic basis, in which some parasite genotypes inherently do more damage than others (Davies et al., 2001), but this trait has also been shown to exhibit variation in the presence of co-infecting parasites. For example, the combination of a highly virulent parasite and a low virulence parasite in the same infection does not always manifest in a higher overall virulence, and there is evidence that virulent strains can be suppressed in competitive interactions (Gower and Webster, 2005; Gleichsner et al., 2018).

Kin selection theory predicts that related parasites should cooperate when present in co-infections to decrease virulence and prolong host survival, thereby increasing inclusive fitness. Unrelated parasites in a co-infection are predicted to compete for a shared resource, increasing virulence to increase individual fitness. Theoretical studies have shown that parasite competition selects for higher virulence (May and Nowak, 1994) and support for kin selection theory predictions has been demonstrated empirically in a variety of parasite systems (see review by Gleichsner and Minchella, 2014). These studies have also revealed that the outcomes of parasite interactions are dependent on several factors beyond relatedness, including the virulence and infectivity of co-infecting parasites (Davies et al., 2002; Gower and Webster, 2005; Gleichsner et al., 2018), the susceptibility of the host (de Roode et al., 2004; Wegner et al., 2009), and even host age (Izhar et al., 2015). However, these studies have been limited to either microparasite systems or the examination of macroparasite interactions in an intermediate host.

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Many macroparasites have complex life cycles in which they use one or more intermediate hosts (usually an invertebrate such as a mollusk) to undergo asexual reproduction and development before infecting their final definitive host (usually a vertebrate). In definitive hosts, parasites develop into adults and reproduce sexually. The lack of studies of definitive host–intraspecific parasite interactions is especially important given that there is evidence of virulence trade offs from intermediate to definitive hosts, with highly virulent strains in the intermediate host exhibiting low virulence in definitive hosts (Davies et al., 2001). Whether parasite relatedness influences virulence in definitive hosts, and how parasite interactions at this level compare with those seen in intermediate hosts, is unknown.

The goal of this study was to determine whether the relatedness of parasites in a co-infection influences the success of parasites, and to determine the amount of damage they do to a definitive host. To test this, we used the trematode parasite *Schistosoma mansoni* and rodent definitive hosts. *Schistosoma mansoni* is a parasite responsible for causing schistosomiasis, a neglected tropical disease that impacts over 250 million people worldwide. This parasite uses aquatic snails of the genus *Biomphalaria* as its intermediate host. Within the snail, the trematode asexually produces cercariae, a free-swimming stage that infects vertebrate definitive hosts. The parasite develops into dioecious adult worms which inhabit the circulatory system, pair, and reproduce sexually. Eggs are released through the feces or become lodged in host tissues including the liver, causing pathology in the form of inflammation, enlarged liver and spleen (hepatosplenomegaly), hypertension and general increased morbidity.

This host–parasite system has been used to test kin selection predictions and to examine the influence of virulence on competitive interactions in its snail intermediate host (Davies et al., 2002; Gower and Webster, 2005; Thiele and Minchella, 2013; Gleichsner et al., 2018). These studies document support for kin selection theory predictions, with unrelated parasite infections having higher virulence (Davies et al., 2002; Gleichsner et al., 2018). Gower and Webster (2005) also concluded that co-infections between low and high virulence *S. mansoni* strains result in the suppression of the highly virulent strain. There is also evidence that virulence and transmission are decoupled in the intermediate host (i.e., higher parasite transmission does not yield higher virulence), but are coupled in the definitive host, and that there is a trade-off between high virulence at the intermediate host stage and in the definitive host (Davies et al., 2001). Our study seeks to further explore this system by testing the influence of parasite relatedness on virulence in the definitive host stage, using the same two laboratory lines of *S. mansoni* that have been used to examine this relationship in the intermediate snail host (Gleichsner et al., 2018). Based on kin selection theory, we predicted that unrelated parasite infections in the definitive host would have higher virulence (measured as higher liver to host weight ratios) and lower transmission success (lower infectivity of each strain and lower numbers of adult worms) compared with co-infections by related individuals.

2. Materials and methods

2.1. Experimental animals

We used NMRI and PR-1 *S. mansoni* parasite laboratory strains obtained from infected snails from the Biological Research Institute (Rockville, MD, USA). Both strains were originally collected from Puerto Rico (NMRI was collected in 1945 from infected school children and PR was collected in 1950 from infected snails). These strains have been extensively studied in our laboratory as well as throughout the research community and their ecology, genetics and life history are well described (Blank et al., 2010; Protasio et al., 2012; Thiele and Minchella, 2013).

Historically, we have documented that these two strains differ in their virulence, with PR being more virulent than NMRI (Thiele and Minchella, 2013). These strains were passed through *Biomphalaria glabrata* snails and BALB/cJ mice prior to use in this experiment. Laboratory mice are routinely used as definitive hosts for *Schistosoma mansoni* research, as a proxy for human infection (Oliver and Stirewalt, 1952; Fallon and Doenhoff, 1994; Pica-Mattocia and Cioli, 2004). In natural settings, this parasite infects humans and rodents as its definitive hosts. For this experiment, a total of 22 male 6 week old BALB/cJ mice were used as hosts for the PR strain only, NMRI only, or PR and NMRI strain combinations in this experiment. Each mouse was weighed at the beginning and end of the experiment. Growth was calculated by subtracting the initial weight from the final weight and was used as part of a measure of virulence.

All animals used in this experiment were cared for, maintained, and handled in accordance with Purdue University (USA) Animal Use and Care Committee standards (Protocol # 1111000225) which ensured that this research was conducted in an ethical and humane manner.

2.2. Experimental design

As in Gleichsner et al. (2018), we ensured that single strain (NMRI only or PR only) infections were comprised of related parasite individuals by first infecting snails with a single miracidium, sexing the resulting infections using the female-specific w 1 primer and PCR, and infecting mice with clonal male and female *S. mansoni* cercariae. The resulting progeny were then passed through snail intermediate hosts to obtain related cercariae for mouse infections. To obtain the *S. mansoni* cercariae, we placed infected *B. glabrata* snails into cell culture plate (Thermo Fisher Scientific, USA) wells with well water and exposed them to fluorescent light for 1 h to activate the release of cercarial parasite stages from the snail. PR- and NMRI-infected snails were kept separately to obtain PR only and NMRI only cercariae samples. Cercariae from all of the same strain wells were pooled to ensure a homogenous representation of gender and genetic material for that strain. We exposed each mouse to 150 *S. mansoni* cercariae via the tail immersion technique (Oliver and Stirewalt, 1952). One hundred and fifty cercariae were counted and placed into test tubes before immersing the tail of each mouse into a tube. Four mice were sham exposed to serve as uninfected controls, five mice were exposed to 150 PR cercariae each, five mice were exposed to 150 NMRI cercariae each, and eight mice were exposed to 75 PR and 75 NMRI cercariae each, to create single (related) and mixed (unrelated) strain infections. Mice were exposed to the cercariae for 1 h prior to removal and placement back into cages. Mice were marked with a labelling marker to track treatment groups over time and randomly assigned to cages.

At 6 weeks post-exposure, mice were euthanized and worms were removed via a modified perfusion technique (Valentim et al., 2009). Adult worms were counted to ascertain total worm burdens and assigned identifiers to determine the number of worms of each strain. Worms were placed in 70% ethanol (Thermo Fisher Scientific) and stored for DNA extraction and quantitative PCR (qPCR). Liver weight was recorded for each mouse. Each liver was rinsed and patted dry prior to weighing. Liver to total mass ratios were calculated by dividing the liver mass by the final mass of each mouse. Liver weight:total mouse weight was calculated as a measure of parasite virulence, given the pathology of this parasite (Nash et al., 1982; Davies et al., 2001) in which damage by the parasite increases liver mass while decreasing total host mass, thus increasing the proportion of the host's total weight that is

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