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Antibodies and coinfection drive variation in nematode burdens in wild mice

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ABSTRACT

Coinfections with parasitic helminths and microparasites are highly common in nature and can lead to complex within-host interactions between parasite species which can cause negative health outcomes for humans, and domestic and wild animals. Many of these negative health effects worsen with increasing parasite burdens. However, even though many studies have identified several key factors that determine worm burdens across various host systems, less is known about how the immune response interacts with these factors and what the consequences are for the outcome of within-host parasite interactions. We investigated two interacting gastrointestinal parasites of wild wood mice, *Heligmosomoides polygyrus* (nematode) and *Eimeria* spp. (coccidia), in order to investigate how host demographic factors, coinfection and the host's immune response affected parasite burdens and infection probability, and to determine what factors predict parasite-specific and total antibody levels. We found that antibody levels were the only factors that significantly influenced variation in both *H. polygyrus* burden and infection probability, and *Eimeria* spp. infection probability. Total faecal IgA was negatively associated with *H. polygyrus* burden and *Eimeria* spp. infection, whereas *H. polygyrus*-specific IgG1 was positively associated with *H. polygyrus* infection. We further found that the presence of *Eimeria* spp. had a negative effect on both faecal IgA and *H. polygyrus*-specific IgG1. Our results show that even in the context of natural demographic and immunological variation amongst individuals, we were able to decipher a role for the host humoral immune response in shaping the within-host interaction between *H. polygyrus* and *Eimeria* spp.

1. Introduction

Parasitic helminths can negatively affect individual health and impact the population dynamics of wild animals, livestock and humans (Charlier et al., 2015; Rose et al., 2014; Weinstein and Lafferty, 2015). For example, an estimated 39 million disability-adjusted life-years (DALYs) were lost between 1993 and 1994 due to four human intestinal helminth species (*Ancylostoma duodenale*, *Necator americanus*, *Ascaris lumbricoides* and *Trichuris trichiura*) (Stephenson et al., 2000), and population crashes in wild Soay sheep, Red grouse, and wild mice have been linked to helminth infections (Gulland, 1992; Hudson et al., 1998; Pedersen and Greives, 2008). Therefore, much effort has been devoted to study the immune pathways and cell types involved in resistance to important helminth infections (Sorobetea et al., 2018). However, most of this work has been carried out in well-controlled laboratory settings in order to minimise environmental variation and other potential confounding factors. Under natural circumstances, however, both animals and humans are rarely uniform in factors such as sex, age, behaviour, nutritional status and genetics (Charmantier and Garant, 2005; Turner et al., 2011; Nussey et al., 2012). Discrepancies between the reductionist approach of traditional immunological studies and the diversity of the real world make it difficult to extrapolate the role of many immune functions derived from controlled and homogenised laboratory studies, to predict their impact or importance for individual infection levels and health in the natural setting.

A major issue contributing to the difficulty in extrapolating from the laboratory to the field is the frequent use of immunologically naïve animals in laboratory experiments, which do not readily compare with wild animals that can face repeated and diverse parasite and pathogen challenges. This incompatibility has been highlighted by a recent study comparing the immune cell composition of laboratory-raised, pet shop and feral mice, showing that

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naïve laboratory mice immunologically resemble human newborns rather than adults, whilst normal immune development was found for both pet shop and wild mice (Beura et al., 2016). A recent review by Viney and Riley (2017) further emphasises the comparability issues between laboratory-raised mice and wild mice, with the immune system of the former consistently showing less activation and evidence of antigenic exposure compared with the latter across studies (Viney and Riley, 2017). The authors also point towards the stark over-representation of laboratory-based experiments compared with studies carried out in the wild (19,997 versus 26 publications in 2016; Viney and Riley, 2017). Added to that is the fact that prior or current coinfection with multiple parasite species is the norm rather than the exception (Petney and Andrews, 1998; Rigaud et al., 2010; Griffiths et al., 2011). These coinfecting parasites often interact with each other (Griffiths et al., 2014), either directly through parasite-induced toxins, tissue damage or physical interference, or indirectly through competition for shared host resources or the host immune system (Christensen et al., 1987; Cox, 2001; Pedersen and Fenton, 2007). This means that coinfection can impact an individual's ability to mount an effective immune response and control infection of a focal parasite. For example, it has been shown repeatedly that helminths cause significant morbidity/mortality when individually infecting a host, and are further associated with increased susceptibility of humans to HIV (Downs et al., 2017) and *Mycobacterium tuberculosis* (Lang and Schick, 2017). Hence, previous parasite exposure and (natural) coinfection are two key factors that can determine an individual's susceptibility to infection and the outcome of disease. However, few studies in wild animals have explored how heterogeneity in environment, host demography and coinfection affect the host's immune response (but see Ezenwa et al., 2010; Babayan et al., 2011; Pedersen and Babayan, 2011; Japp et al., 2017).

Studies measuring the immune response of naturally infected animals offer a valuable avenue to complement traditional immunology and fill the gap between evidence gathered from the laboratory and the wild. Wild wood mice (*Apodemus sylvaticus*) harbour a diverse parasite community comprising >30 different microparasite and macroparasite species with often >60% of individuals being coinfected by two or more parasite species (Knowles et al., 2013). In the wild, we previously found that the use of a broad spectrum anthelmintic drug (Ivermectin) reduced the prevalence of the most common helminth species, *Heligmosomoides polygyrus*, by approximately 70% (Knowles et al., 2013). However, we also found that Ivermectin-treated mice showed a subsequent 15-fold increase in the intensity of the coinfecting protozoan parasite *Eimeria hungaryensis* 1–3 weeks post-treatment (Knowles et al., 2013), suggesting a strong negative interaction between the two parasites. These parasites trigger opposing arms of the immune system (Th2 in the case of *H. polygyrus*, Th1 in terms of *Eimeria* spp.), making it an ideal study system to investigate the role of the immune response in mediating this interaction, in combination with the impact of demographic variation and coinfection on the potential interrelationships between the immune system and parasite infection.

We used cross-sectional data from wild-trapped *A. sylvaticus* populations to measure factors representing host demographics, coinfection and the host's immune response. Specifically, we measured two antibodies as a simplified read-out for host immune activity from each individual caught. From blood serum, we measured titres of *H. polygyrus*-specific IgG1 as an indicator of the magnitude of an anti-*H. polygyrus* immune response. This antibody is highly important in the immune response against *H. polygyrus*, as it reduces fecundity of adult female worms (Hewitson et al., 2011; Maizels et al., 2012; Reynolds et al., 2012) and correlates negatively with worm burden (Ben-Smith et al., 1999). Generally, resistance towards *H. polygyrus* in the laboratory is associated with a strong and quickly established Th2 type immune response, with mice that also show increased Th17 and/or Treg cell activity usually being more susceptible towards (re)infection (Maizels et al., 2012). Further, we measured the concentration of total faecal IgA from host faecal extracts. IgA is the most common antibody on mucosal surfaces and its production is triggered by a multitude of microbial stimuli (pathogenic and commensal), as well as different helminth species (Fagarasan and Honjo, 2003; Macpherson et al., 2012). It has previously been shown that IgA from caecal contents of chicken had high anti-schizont and sporozoite-neutralising activities with regard to infection with *Eimeria maxima* (Davis et al., 1978; Trees et al., 1989). Further, mice that were able to quickly clear *H. polygyrus* infections also had higher levels of parasite-specific IgA than slow-resolving mice (Ben-Smith et al., 1999; Behnke et al., 2003). As we measured total levels of IgA rather than parasite-specific levels, we consider the read-out of IgA to represent overall immune activation, i.e. high total IgA levels suggest current infection and/or accumulation of previous parasitic exposures with both *H. polygyrus* and *Eimeria* spp. Even though there are many other immune components involved during infection with either parasite (e.g. transcription factors, cytokines, different cell types), antibodies represent one of the downstream consequences of these immune cascades, and in the case of helminth infections, total faecal IgA levels have been shown to correlate with parasite-specific IgA levels in wild Soay sheep (Watt et al., 2016). Further, measuring these two immune effectors has proven to be feasible given sample collection under field conditions.

It is clear that the cause-and-effect relationships between immune read-outs and parasitic infections are often not as straightforward to define in the wild compared with controlled laboratory infection experiments. This makes it difficult to distinguish between immune effectors (i.e. is a cell type/molecule associated with reducing parasite load or clearance) and immune markers (i.e. is a cell type/molecule stimulated by the presence of an infection), both of which are not mutually exclusive. Here we aimed to analyse both possibilities, by first testing how host demographic factors, coinfection and the host's immune response drove variation in parasite burdens and infection probability, and then, in turn, investigating which factors predict parasite-specific and total antibody levels. Combining these two types of approaches allowed us to gain vital insights into the likelihood for either of the two possibilities, thereby allowing an unbiased approach to understanding cause-and-effect relationships between immune markers and parasite infections in the wild.

2. Materials and methods

2.1. Field experiment

In October 2013, wild wood mice (*A. sylvaticus*) were trapped in a mixed woodland in northwestern England (53°16′12.0″N, 3°01′48″W). Trapping took place over the course of five consecutive days, with 96 Sherman Live Traps (H.B. Sherman 5.08 × 6.35 × 16.51 cm folding traps, Tallahassee, FL, USA) being baited with grains, carrots and cotton bedding, and set up each day at dusk in three longitudinal transects. Each transect had 16 trapping stations (two traps per station), which were 10 m apart from each other. Transects were laid out parallel to each other, with 10 m space between transects. Traps were checked the following morning and we measured the following parameters of all non-gravid (pregnant or lactating) females and all males: body mass (g), body length (mm), sex, body condition (vertebral column and pelvic bones fat reserves, each scored from 1 to 5: 1 being bones sharp and easily distinguishable with no pressure applied, and 5 being

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