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Experimental manipulation reveals few subclinical impacts of a parasite community in juvenile kangaroos

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ABSTRACT

Large mammalian herbivores are commonly infected with gastrointestinal helminths. In many host species, these helminths cause clinical disease and may trigger conspicuous mortality events. However, they may also have subclinical impacts, reducing fitness as well as causing complex changes to host growth patterns and body condition. Theoretically, juveniles should experience significantly greater costs from parasites, being immunologically naive and undergoing a significant growth phase. The aims of our study were to quantify the subclinical effects of helminths in juvenile eastern grey kangaroos (*Macropus giganteus*), which commonly harbour large burdens of gastrointestinal nematodes and are susceptible to associated mass mortality during cold, wet conditions. We conducted a field experiment on a population of free-ranging kangaroos, removing nematodes from one group of juveniles using an anthelmintic treatment. We then compared growth parameters (body condition and growth rates) and haematological parameters of this group with an age-matched, parasitised (untreated) control group. Treated juvenile kangaroos had significantly higher levels of plasma protein (albumin) but, contrary to our predictions, showed negligible changes in all the other parameters measured. Our results suggest that juvenile kangaroos are largely unaffected by their gastrointestinal helminth burdens, and may be able to compensate for the costs of parasites.

1. Introduction

Gastrointestinal helminths commonly infect mammalian herbivores (Sykes, 1987). Helminth infections often have clinical impacts, causing disease and mortality (Holmes, 1987), but can also cause what has been termed 'subclinical' disease (Gunn and Irvine, 2003), inducing more subtle effects in the host. Subclinical impacts are well known in livestock: reductions in appetite and food absorption caused by helminth infections can decrease host fecundity and growth (Mejia et al., 1999), body condition (Loyacano et al., 2002) and alter metabolism (O'Kelly et al., 1988). In contrast, the effects of parasitism on wildlife have received far less attention, although there is mounting evidence that parasites can have similar negative impacts, reducing fitness (Watson, 2013) as well as causing complex changes to host physiology (Van Houtert and Sykes, 1996), behaviour (Scantlebury et al., 2007) and population dynamics (Hudson et al., 1992a; Albon et al., 2002; Stien et al., 2002). However, the types of impacts measured in livestock and wildlife often differ, due to the difficulties of studying natural host–parasite relationships and quantifying fitness consequences. In order to thoroughly investigate such effects, experimental manipulation is imperative. Field experimentation allows the actual costs of parasites on hosts to be investigated rigorously, eliminating many of the issues associated with extrapolating laboratory results onto free-living individuals or populations (Seitz and Ratte, 1991). Studies of fitness consequences in the wild typically focus on natural covariation between parasite load and fitness parameters, and so may be confounded by the inherent differences among individuals that can contribute to high parasite burdens. Consequently, it is often unclear whether changes in fitness parameters are due to heavy parasite burdens, or if these burdens result from other pre-existing factors related to fitness. Ecological host–parasite studies in wild animals have been mostly based on correlations, although there have been some field experiments in wild systems (e.g. Svalbard reindeer, *Rangifer tarandus platyrhynchus* (Stien et al., 2002), Soay sheep, *Ovis aries* (Gulland, 1992) and red grouse *Lagopus lagopus scoticus* (Hudson et al., 1992b)).

The subclinical effects of parasites can be extremely difficult to quantify in the wild. Ecologists tend to use body condition as an

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indicator of an animal's health and reproductive potential. Body condition essentially reflects available energy reserves (Green, 2001): an animal in good condition is assumed to have more reserves than one in poor condition (Schulte-Hostedde et al., 2005). Energy reserves can be quantified directly by measuring fat stores (e.g. amount of back or kidney fat (Riney, 1955)) or non-invasively using mass/size ratio indices of body condition, which attempt to determine the size of energy stores after correcting for structural body size (Schulte-Hostedde et al., 2005). Alternatively, haematological and serum biochemical parameters can be used to assess an animal's health. Although less commonly used in ecological studies of wildlife, haematological parameters may provide more sensitive information on the immediate physiological status of a host (Milner et al., 2003; Budischak et al., 2012). That is because parasite infections can alter haematological parameters directly through haematophagy (blood-feeding) and indirectly through activation of host immunity in response to infection or by limiting the digestion and absorption of essential nutrients, such as protein (Colditz, 2008). Red blood cell counts, haemoglobin and plasma protein concentrations can all be used to assess an animal's health and condition, and have been directly linked to performance and reproductive success (Moore and Hopkins, 2009). The combined application of both haematological and body condition indices may therefore provide greater insight into the subclinical effects of parasite communities on a host.

Juvenile mortality is commonly increased by infection (Schmidt et al., 1979), however evidence from livestock suggests that this age-class can also experience considerable subclinical effects such as reductions in body weight (Chiejina and Sewell, 1974), growth (Loyacano et al., 2002) and appetite (Kyriazakis et al., 1998). Despite the evidence from livestock hosts, it is unclear to what degree subclinical effects occur in juveniles of wildlife species. Theoretically, juveniles should experience significant costs when infected with parasites due to the nutritional deficits they cause and the costs of mounting an immune response (Colditz, 2008), and these effects should be particularly marked during early growth and development. Such effects are important to comprehend as it is well established that conditions early in life can have significant implications for survival and reproductive success as an adult (Metcalfe and Monaghan, 2001).

Most wildlife hosts harbour complex parasite communities (Bordes and Morand, 2011), and kangaroos (Marsupialia: Macropodidae) are known to support more species of parasites than any other group of mammals (Beveridge and Chilton, 2001). The eastern grey kangaroo (*Macropus giganteus*) carries a diverse fauna of gastrointestinal nematode parasites in its complex, sacculated forestomach (Beveridge and Arundel, 1979), with most species showing seasonal fluctuations, peaking in the winter months (Arundel et al., 1990). Most of these gastrointestinal nematodes are directly transmitted via ingestion (Sykes, 1987). Adult kangaroos do not appear to develop immunity to most of these nematode species (Arundel et al., 1979), and juveniles are susceptible to gastrointestinal parasitism, primarily from high burdens (400–1500) of the intestinal trichostrongylid nematode *Globocephaloides trifidospicularis* Juveniles can experience high mortality, coupled with declining haematocrit and plasma protein concentrations, in their first winter post-weaning between 14 and 20 months of age (Arundel et al., 1990). Populations of eastern grey kangaroos can reach high densities, and individuals are gregarious, forming mixed-sex, open-membership groups to forage and rest (Coulson, 2009), conditions that favour helminth parasite transmission (Altizer et al., 2003).

Eastern grey kangaroos are capable of breeding throughout the year, but most births occur between September and March, during the austral spring/summer months (Poole, 1983). Following a short gestation period and then an extended period of development in the pouch, young will exit permanently at around 320 days (Poole, 1975). Toward the end of pouch life, juveniles begin to forage on the pasture and are exposed to the infective stages of nematodes. Juveniles will continue to associate with and suckle from their mothers until over 18 months of age (Poole, 1975). The year following permanent pouch exit is the most critical for juvenile kangaroos, as they must undergo substantial growth. During this period, the average monthly weight gain is 1.4 kg for males and 0.9 kg for females (Poole et al., 1982). To sustain this growth, juveniles have around 1.8 times the energy requirement of mature, non-lactating females (Munn and Dawson, 2004). In addition, during this period of growth, individuals are immunologically naive (Arundel et al., 1990), and become infected by gastrointestinal parasites. Individual variability in body size increases following permanent pouch exit (Poole et al., 1982), suggesting that growth rate is a plastic trait that can be influenced by external factors.

We examined the effect of concomitant infection with multiple parasites on the growth, body condition and blood chemistry of one cohort of free-ranging juvenile eastern grey kangaroos, by manipulating parasite loads. We removed gastrointestinal parasites from a group of juveniles using an oral anthelmintic and then compared them with control individuals, with the expectation that control juveniles would show subclinical effects. We predicted that due to an increased availability of nutrients and energy resources, treated juveniles would have a greater growth rate and mass gain, and would increase their body condition relative to controls. We also predicted that there would be changes in haematological parameters, with decreases in red blood cell counts, haemoglobin concentration and haematocrit in control juveniles. Similarly, we expected that serum biochemistry would indicate subclinical effects, with decreased levels of total protein and albumin, and increases in levels of globulin.

2. Materials and methods

2.1. Study site

This study was conducted at the Anglesea Golf Club (38°24′S, 114°10′E) in southern Victoria, Australia, in 2012. The golf course covers an area of 73 ha and contains open, grassy fairways dominated by couch grass (*Cynodon dactylon*), separated by patches of woodland and shrubland (Inwood et al., 2008). The course is bordered by native heathy woodland to the north and west; kangaroos move freely between the course and native vegetation, as well as through residential properties in the south and east. Population surveys (following Inwood et al., 2008) at the time of the study showed that the population density of kangaroos at the site was approximately 2.0/ha (Cripps and Coulson, unpublished data). Potential predators at the site include the red fox (*Vulpes vulpes*) and domestic dogs (*Canis lupus familiaris*).

Post-mortem examinations of three juveniles found dead at the study site during 2010–2011, using standard methods (Beveridge and Arundel, 1979), revealed a diverse gastrointestinal parasite community. The stomach contained the strongyle nematodes *Rugopharynx macropodis* (intensity 8240–69,000 per kangaroo), *Rugopharynx rosemariae* (500, $n = 1$) and *Pharyngostrongylus kappa* (500–10,000). The small intestine contained *G. trifidospicularis* (31–73), levels well below those known to cause clinical impacts (Arundel et al., 1990). The large intestine contained two oxyuroid nematodes, *Macropoxyuris brevigularis* (40–3450) and *Macropoxyuris longigularis* (500–1050), and one strongyle nematode species *Macropostrongyloides baylisi* (50–9000). The bile ducts of two juveniles had the cestode *Progamotaenia festiva*. Examination of seven adults from the site revealed a further ten helminth species: *Labiosimplex kungi*, *Labiosimplex bipapillosus*, *Cloacina pelops*, *Cloacina*