The role of endemic infection in disease emergence

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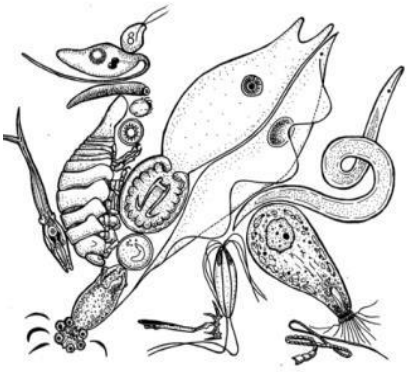

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Abstract

Human and animal populations are confronted by emerging microparasitic infections which pose a major threat to public health and the global economy. In natural conditions, emerging microparasites will encounter host populations that are already infected with common endemic macroparasites. Interspecific interactions between coinfecting parasites may alter the host immune response, the emerging parasite infection dynamics, the disease outcome and the efficacy of parasite control strategies. This thesis explores the role of macroparasites as potential suppressors or promoters of microparasite disease emergence. The potential impact endemic infections may have on disease emergence were explored experimentally using the model German cockroach host *Blattella germanica*, its endemic gut macroparasite *Gregarina blattarum* and the virulent microparasite *Steinernema carpocapsae*. First the effect of a hosts' endemic parasite burden on the immune response and secondly, susceptibility to infection were investigated (Chapter 2). These experiments revealed that the host immune response was altered by the endemic parasite burden but this had no effect on susceptibility to infection with the emerging microparasite. The impact of host endemic parasite burden on the quality and quantity of the emerging parasite transmission stages was then explored. Here, coinfection resulted in a reduced output of the epidemic parasite transmission stages compared to a single infection. Further, endemic parasites had a non-linear effect on the quality of the transmission stages of the emerging microparasite measured as lipid energy reserves (Chapter 3). Finally, the fitness cost of coinfection on the between-hosts transmission of the emerging parasite was explored. Experimental findings revealed that the disease spread of the microparasite within the host population was altered by the endemic parasite (Chapter 4). The findings from this thesis demonstrate the importance of considering macro- and microparasite coinfections, and that this, in turn, is pivotal to improving control strategies and ability to accurately predict epidemic outbreaks.

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Chapter 1

1.0 General Introduction

In Epidemiology, parasites are termed as endemic, when such parasites are permanently maintained within a geographic host population, without the need for re-introduction (Dicker et al. 2006). Many parasitic infections, for example, most gastrointestinal helminths are endemic, particularly in developing countries where adequate water and sanitation are lacking (Cappello 2004; Savioli and Albonico 2004). By contrast, epidemic refers to a situation where there is a sudden increase in the number of parasites in a given host population (Green et al. 2002; Angelini et al. 2008; Tuomilehto 2013; Piot et al. 2014). Some parasites (e.g. HIV, Rubeola) can be endemic under certain circumstances but shift to epidemic dynamics under other conditions, for example, in particular season (Bjørnstad et al. 2002; Van den Hof et al. 2002; Lancet 2004; Wallinga et al. 2005; Stone et al. 2007; Bharti et al. 2010). More generally, epidemics can occur when there is a recent introduction of a parasite into a population or when there is a change to the susceptibility of the hosts within a population (Dowell 2001). Owing to the diversity of parasites found under natural conditions, epidemic parasites will encounter hosts that are already infected with common endemic parasites (Fenton 2008). The outbreak of an epidemic may, therefore, be driven by interactions with endemic parasites that share the same host (Rynkiewicz et al. 2015; Susi et al. 2015; Busby et al. 2016). Yet, only a few research take into consideration, the effects that endemic coinfection may have upon epidemic transmission.

Under natural conditions, hosts populations are normally challenged by multiple parasite infections simultaneously (Cox 2001). Within-host interactions can occur between parasites, either directly (e.g. competition for space) or indirectly (e.g. via host immune interaction, Table 1). Such interspecific interactions may alter parasite dynamics, disease severity (Chen et al. 2005; Graham et al. 2005) and the efficacy of parasite control strategies (Lello et al. 2004; Steenhard et al. 2009). Both micro- and

macroparasites pose threats to health and can simultaneously infect a host (Fenton et al. 2008; Jolles et al. 2008; Ezenwa and Jolles 2011; Nunn et al. 2014). Emerging infectious diseases (EIDs), chiefly caused by microparasites (Cleaveland et al. 2001; Pepin et al. 2007; Morse 2012; Cunningham et al. 2017; Kelly et al. 2017), pose one of the greatest threats to human and animal health, and the global economy (Macpherson 2005; Prokop et al. 2010; Hotez 2015). EIDs caused by either microparasites (e.g. viruses, fungi, bacteria, protozoa) or macroparasites (e.g. helminths) are largely zoonotic in origin (Jones et al. 2008; Billinis 2013; Thompson 2013; VanderWaal and Deen 2018; Walker et al. 2018). The rising incidence of EIDs over the last few decades has increased our need to understand the dynamics of infectious diseases (Chyba 2004; Graham 2008; Jones et al. 2008; Thompson et al. 2010; Morens and Fauci 2013; Seltmann et al. 2017). Macroparasitic infections are also a major public health problem, particularly in most developing countries (Crompton 2000; O'Lorcain and Holland 2000; Wiria et al. 2012). Given the ubiquity of macroparasites and the rise in emerging infections (microparasites) in host populations, there is a high potential for interactions to occur between the two types of infection. This study explores the role of endemic infections, which are frequently macroparasites, as potential suppressors (antagonistic) or promoters (synergistic) of emerging infectious diseases.

Heterogeneity in host susceptibility to infection is a key driver of infectious disease dynamics within populations (Dwyer et al. 1997; Lloyd-Smith et al. 2005; Kilpatrick et al. 2006). Differences in host susceptibility have been attributed to factors such as host sex (Schmid-Hempel 2005) and age (Stear et al. 2000), or to differences in immune response (Leongson et al. 2013), environmental conditions (Krist et al. 2000), exposure risk (Woolhouse et al. 1997) and host contact rates (Clay et al. 2009). One parasite can also modify the host immune response, resulting in an altered susceptibility to a second infection (Rodriguez et al. 1999; Bandilla et al. 2006; Cattadori et al. 2007; Graham et al. 2007; Cattadori et al. 2008; Telfer et al. 2008; Telfer et al. 2010). Since emerging infections under natural conditions typically occur against a background of

existing endemic infections, understanding the effects of an existing infection on host susceptibility and parasite dynamics are key to accurately predicting the likelihood of an outbreak or epidemic spread within a host population. When parasites coinfect the same host, interactions may be direct (Fellous and Koella 2010; Randall et al. 2013; Griffiths et al. 2014) or indirect (Stewart et al. 1999; Graham et al. 2005; Lamb et al. 2005; Dzhivhuho et al. 2018). One form of indirect interaction involves parasites having regulatory effects that alter the host population dynamics (Rohani et al. 2003; Bottomley et al. 2005; Fenton 2008). The outbreak of rabbit hemorrhagic disease virus in the European rabbit, for example, can alter the dynamics of the infected host population, which compromises transmission of the myxoma virus by reducing the pool of susceptible host (Mutze et al. 2002). The interactions between parasites can result in either antagonistic or synergistic effects upon the host, which may have fitness implications for both host and parasite dynamics (Cox 2001). Antagonistic effects, for example, can occur when parasites compete for the same limited host food resource and/or share the same niche (space) within the host (Pedersen and Fenton 2007; Susi et al. 2015; Wilcox et al. 2015). The depletion of host resources by an existing parasite can impair the growth and survival of both parasites and the host (Budischak et al. 2012; Randall et al. 2013; Griffiths et al. 2014; Birger et al. 2015). A recent study (Budischak et al. 2018), for example, demonstrated that individuals coinfected with hookworm, a blood-sucking helminth, and the malaria parasite *Plasmodium vivax* had lower densities of *P. vivax* compared to singly infected individuals (without hookworm) and that deworming resulted in a 2.8 fold increase in *P. vivax* density. In other cases, antagonistic effects can occur when the initial parasite activates and modulates the host immune response against a second parasite (Abbas et al. 1996; Cox 2001; Telfer et al. 2008; Chen et al. 2013). Furthermore, cross-immunity may occur when the host immune response to a parasite is effective against an antigenically similar but different pathogen. For example, in mice, vaccination with Newcastle disease virus-based recombinant vaccine (rL H5) containing the A/H5N1 gene induced a cross-protective immune response against subtype influenza A/H1N1 virus (Yang et al. 2013).

Alternatively, one parasite can modulate the host immune response (synergistic effect) in such a way that the host's ability to mount an adequate immune response against another parasite species is impaired (Brady et al. 1999; Chen et al. 2005; Graham et al. 2005; Chen et al. 2006; Su et al. 2014). Prior infection of a host with one parasite can facilitate colonization of the host by another parasite (Lello et al. 2018). For example, immunosuppressed individuals infected with the human immunodeficiency virus experience increased susceptibility to secondary infections like tuberculosis and malaria (Toossi et al. 2001; Kamya et al. 2006; Nkhoma et al. 2012; Finney et al. 2013; Jallow and Mahdi 2018; Rodgers et al. 2018). Despite the considerable interest in coinfection, the diversity of parasites in natural conditions and the complexity of interactions (resource, immune or demography mediated) mean that it is challenging to predict the consequences of coinfection on diseases dynamics. There is therefore, a considerable need for studies that investigate these complex interactions between parasites and their effect on within-host infection and parasite dynamics.

Table 1: Examples of within-host parasite interactions

Mechanism of interaction	Interaction type (Positive - P or Negative - N)	Examples	References
Mechanical facilitation	Direct (P)	*Argulus coregoni* infection in rainbow trout increases susceptibility to *Flavobacterium columnare*	Bandilla et al. 2006
	Direct (P)	Herpes simplex virus creates an entry point for HIV in humans	McClelland et al. 2007
	Direct (P)	*Trichomonas vaginalis* increases the risk of HIV acquisition	Masha et al. 2019
	Direct (P)	*Plasmodium falciparum* increases susceptibility to non-typhoid *Salmonella* by increasing serum iron availability and impairing neutrophil	van Santen et al. 2013; Takem et al. 2014; Lokken et al. 2018

		function	
Interference competition	Direct (N)	Parasites produce bacteriocins to reduce the growth of competing species (*Photorhabdus* and *Xenorhabdus*)	Bashey et al. 2013
	Direct (N)	Reduced egg output of the barber's pole worm *Haemonchus contortus* during coinfection with *Ostertagia circumcincta*	Dobson and Barnes 1995
Resource competition	Indirect (N)	Indirect competition between parasites sharing the same host resources	Graham 2008; Randall et al. 2013; Ruiz-Daniels et al. 2013
Immune system	Indirect (P)	Immune suppression where a parasite reduces the efficacy of the host immune system and thus results in increased parasite load, disease severity	Su et al. 2005; Su et al. 2006; Nazzi et al. 2012; Van Geertruyden 2014; Lello et al. 2018
	Indirect (P)	Th1/Th2 trade-off: in the vertebrates, induction of Th1 pro-inflammatory response by an existing microparasite reduces the host ability to induce a Th2 anti-inflammatory response against a macroparasite and vice versa	Nacher et al. 2002; Resende Co et al. 2007; Hartgers et al. 2008; Salazar-Castanon et al. 2014
	Indirect (N)	Cross-immunity: Influenza A/H1N1 virus and influenza A/H5N1; Adenovirus and Hepatitis C virus; *Trichostrongylus colubriformis* and *Haemonchus contortus*	Yang et al. 2013; Lello et al. 2018; Agrawal et al. 2019

Parasites vary in the immune response they stimulate in the host (Maizels et al. 2012). In vertebrates, there are two arms of the T-helper (Th) cell immune response: the Th1 pro-inflammatory response most active against microparasites and a Th2 anti-inflammatory response against macroparasites (Abbas et al. 1996; Forsthuber et al. 1996). Both the Th1 and Th2 immune responses are co-regulatory and down-regulatory of one another. Whilst, some studies suggest that the Th1 immune response induced

against a microparasite tends to have a stronger influence in suppressing Th2 response during coinfection (Helmby et al. 1998; Santiago et al. 1999; Liesenfeld et al. 2004), the majority of studies suggest that, the interaction between macro- and microparasites will benefit microparasites where the Th2 response is stronger (Hartgers and Yazdanbakhsh 2006; Kamal and El Sayed Khalifa 2006; O'Neal et al. 2007; Resende Co et al. 2007; Salazar-Castanon et al. 2014).

Like vertebrates, invertebrates possess an immune system capable of responding to the presence of invading parasites. The majority of described invertebrate immune functions are innate, however, evidence of long-term regulation and immune priming has been documented (Hartman and Karp 1989; Moret and Siva-Jothy 2003; Schmid-Hempel 2005; Siva-Jothy et al. 2005). The invertebrate immune system broadly comprises humoral and cellular responses against both micro- and macroparasites (Hoffmann 1995; Cotter et al. 2004; Agianian et al. 2007). Hosts respond to the presence of macroparasites by activating haemocyte cells (controlled by the cellular response) which adhere to the invading pathogen, forming a capsule around it, in a process known as encapsulation (Ratcliffe 1985; Strand 2008). The formation of a capsule is accompanied by melanin production, where the invading parasite is enclosed by melanin-containing cells (Leonard et al. 1985; Rowley and Powell 2007). The host response that acts against a microparasite involves antimicrobial peptides and enzymes circulating in the haemolymph, for example, lysozymes and lectins which bind to bacteria causing disruption of their peptidoglycan wall (Leonard et al. 1985; Gillespie et al. 1997; Rolff and Reynolds 2009). Similar to the Th1/Th2 paradigm in vertebrates, some studies have shown the potential for trade-offs in the invertebrate host responses to different parasites and the consequences for the hosts' susceptibility to other parasites (Gillespie et al. 1997; Cotter et al. 2004; Siva-Jothy et al. 2005; Cotter et al. 2008). For example, in the field cricket *Gryllus bimaculatus*, the rate of cellular encapsulation and humoral antibacterial activity were negatively correlated, suggesting a potential trade-off between both components of the innate immune response (Rantala and Roff 2005). An improved understanding of this immune trade-off is crucial

371 if we are to predict how indirect interactions between parasites might alter parasite
372 dynamics and transmission.

373 *Why might endemic parasites alter emerging infections?*

374 Endemic macroparasites often survive within the host for a protracted period of time
375 and are aggregated in distribution within their host populations. Parasite aggregation is
376 broadly explained by the 20:80 rule, which states that the majority (approximately
377 80%) of the host individuals transmit or harbour only a few parasites, while a minority
378 (approximately 20%) are responsible for the majority of transmission (Shaw and
379 Dobson 1995; Woolhouse et al. 1997; Boag et al. 2001; Poulin 2013; Hupalo et al.
380 2014). Given that hosts within a population vary in existing parasite burden, we may
381 see different effects on the between-host transmission of an emerging parasite when
382 the host is coinfected with a low endemic parasite burden compared to a host co-
383 infected with a higher parasite burden. In other words, interspecific interactions within
384 a host are likely to vary dependent on the infection burden. Elevated immune
385 responses in heavily infected hosts, for example, could result in stronger interspecific
386 interactions compared to low infection (de Lope et al. 1998). An improved
387 understanding of the within-host mechanism of interactions and how it determines
388 parasite transmission is crucial for accurately predicting the outbreak of emerging
389 infections.

390

391 **1.1 Host-parasite model system**

392 The German cockroach *Blattella germanica* (Fig. 1.1a), and two of its parasites, the
393 endemic gastrointestinal protozoan *Gregarina blattarum* (Fig. 1.1b & c) and the
394 epidemic parasite, entomopathogenic nematode *Steinernema carpocapsae* (Fig. 1.1d)
395 provide a suitable system in which to explore the effects of an endemic macroparasite
396 upon potential disease emergence. The German cockroach is a common household
397 pest species in Europe and America. It is a highly fecund insect with a short

development time of about 2-3 months between egg stage and adulthood, this makes them easy to rear in large populations in the laboratory. This system has already been used successfully as a model to study host-population dynamics, life history and the transmission potential of an epidemic parasite during coinfection with an endemic macroparasite (Randall 2011; Randall et al. 2013). Here, this system was used to explore the density-dependent effects of an endemic parasite on the within-host and between-host dynamics of an epidemic microparasite.

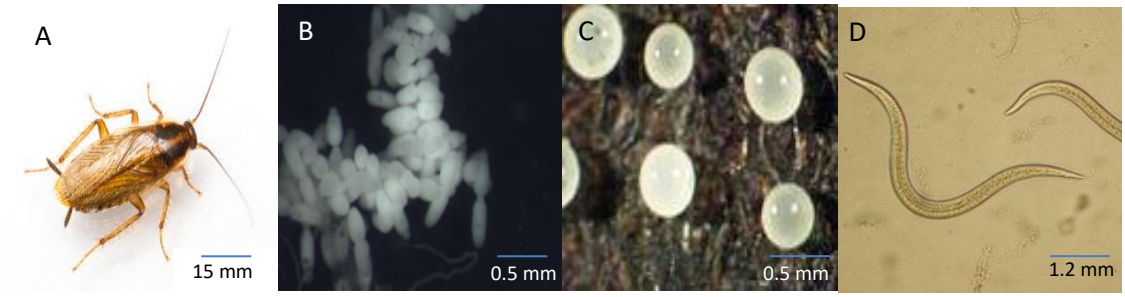


Figure 1.1: Model host-two parasite system: (a) Female German cockroach host, *Blattella germanica*, (b) the gut protozoan *Gregarina blattarum* trophozoite stage, (c) *G. blattarum* gametocyst stage containing infective oocysts, and (d) the *Steinernema carpocapsae* infective juvenile.

1.2.1 Model macroparasite *Gregarina blattarum*

Gregarines are primitive, apicomplexan parasites that inhabit the coelom, intestine and/or reproductive vesicles of many terrestrial and freshwater invertebrates (Levine 1988). These gregarines can occur in high densities and in some hosts can cause occasional blockage of the intestine, which results in growth reduction and host mortality (Åbro 1971). Gregarines are a diverse group of unicellular parasites that are continuously maintained in most invertebrate populations, including insects, i.e. they are endemic, as the persistence of environmental infective stages maintains the disease within the host populations (Clopton 2000; Desportes and Schrével 2013; Rueckert and Devetak 2017). Gregarines reside in the host gut where they absorb

nutrients (Randall 2011) and, in high densities, they can cause direct damage to the host gut tissue (Valigurova and Koudela 2005). It has been shown previously that *G. blattarum* depletes host lipid resources with negative consequences for survival and fecundity of the German cockroach, making this an ideal model parasite with which to investigate host resource competition during coinfection (Randall 2011). Transmission of *G. blattarum* into a new host occurs via oral ingestion of oocysts that have developed from gametocysts (Fig. 1.1c). The ingested oocysts (infective stage) each release eight sporozoites, which further develop to form trophozoites (reproductive stage, Fig. 1.1b). The trophozoites attach to the host gut (via the epimerite which is embedded into the intestinal epithelium) and feed on host food resources (Kuriyama et al. 2005). Two mature trophozoites pair to form a gametocyst which is subsequently surrounded by the oocyst wall and released from the gut in the host faeces. The released gametocyst sporulates to form oocysts and the infection cycle is completed when the oocyst is ingested by a cockroach. Gregarines show an aggregated distribution within their host, a typical characteristic of many macroparasites. Due to this distribution and their persistence in the host population, the gregarine *G. blattarum,* is referred to as an endemic macroparasite.

1.2.2 Model microparasite *Steinernema carpocapsae*

Entomopathogenic nematodes (EPNs) are extensively used as biological control agents against a diverse array of insect pests (Ehlers 2001; Lacey and Georgis 2012; Shapiro-Ilan et al. 2012; Pena et al. 2015; Cutler et al. 2017; Heve et al. 2017). These highly virulent parasites replicate within the host and are often used as biocontrol agents through introduction into naïve populations, where they display epidemic dynamics (Griffin 2012). The third larval stage, i.e. the infective juvenile (IJ) of *S. carpocapsae* (Fig. 1.1d), enters the host body cavity through natural openings such as the mouth, spiracle, cuticle and anus. Upon penetration of the host body cavity, these non-feeding IJs migrate to the haemolymph where they release the intestinal symbiotic bacterium

Xenorhabdus nematophilus, which multiplies rapidly causing septicaemia (Han and Ehlers 2000) and kills the insect host within 48-72 hours (Park and Kim 2000; Lewis et al. 2006). The IJs then proceed to feed on the bacteria and the decaying host tissue. The nematode undergoes a series of reproductive cycles within its host, the number of cycles being dependent on the available host resources (Kaya and Gaugler 1993). The depletion of host resources stimulates the nematode to switch its life-cycle to the production of larval stages. New IJs then emerge from the dead host in search of a new host to infect. It takes approximately 5 days, under laboratory conditions, from the point of host penetration to the emergence of the first IJs and this emergence period extends for approximately 2 weeks (Barbercheck 2015), peaking around 10 days post-exposure (Randall et al. 2013). When used as a biocontrol agent, IJs are applied in high numbers (Shapiro-Ilan et al. 2012), but only a fraction successfully penetrate a host due to the rapid decline of active IJs under field conditions (Griffin 2015). Evidence is equivocal regarding the role of IJ density on host mortality with some studies demonstrating a concentration effect (Legaspi et al. 2000; Siegel et al. 2004; Athanassiou et al. 2008; Memari et al. 2016), while others have found no effect of dose (Gaugler et al. 1990; Grewal and Richardson 1993; Tomalak 1994; Scheepmaker et al. 1998; Gouge et al. 1999; Menti et al. 2000; Morton and Del Pino 2007; Athanassiou et al. 2010; Steyn et al. 2019). In the case of *S. carpocapsae* used in the model system, studies have shown that there is no effect of dose on host mortality (Epsky and Capinera 1994; Gouge et al. 1999; Arthurs et al. 2004; Athanassiou et al. 2010; Therese and Bashey 2012; Tourtois and Grieshop 2015; Kajuga et al. 2018), highlighting that dose does not seem to be important. Nevertheless, all host were exposed to the same dose in the current study.

1.3 *Thesis outline*

The objectives of this thesis are to explore the role of macroparasite as potential suppressor or promoter of microparasite disease emergence, including whether immune bias by macroparasites leads to increased microparasite susceptibility and

transmission, or whether interspecific parasite competition for host resources might result in a reduced microparasite transmission and disease emergence. The interplay between the two potential outcomes (increased or decreased) on microparasite transmission was explored using the model cockroach system. The within-host interactions between coinfecting macro- and microparasites may vary depending on the intensity of infection, and this can have implications for the host immune response. **Chapter 2** tests the hypothesis that gregarine intensity-dependent reduction of host resources will result in immune modulation and that, in turn, this will increase host susceptibility to the epidemic parasite. The within-host interaction and infection dynamics were further examined in **Chapter 3**, which tests the hypothesis that where microparasites compete with gregarines for host resources, the output and quality (lipid provisioning) of microparasite transmission stages emerging will be inversely correlated with the burden of endemic gregarines in the host. **Chapter 4** explored the fitness cost of gregarines on the transmission of the epidemic microparasite at the host population level, by testing the hypothesis that epidemic spread within the host population will be dependent on the number of infective juveniles emerging from an infected host and the lipid provisioning of the transmission stages. Finally, **Chapter 5** summarises the data from each experimental chapter and considers the opportunities for future research.

2.0 Influence of endemic parasite infection intensity on the host susceptibility and immune response to *Steinernema carpocapsae*

2.1 Abstract

Simultaneous infection of a host by multiple parasite species is the norm under natural conditions. During coinfection, within-host interactions, either direct or indirect can alter the host's immune-competence and susceptibility to infection. Here, using a laboratory-reared population of German cockroaches (*Blattella germanica*), this study aimed to determine the effect of an endemic protozoan, *Gregarina blattarum,* on the host immune response and susceptibility to an entomopathogenic nematode, *Steinernema carpocapsae*. Activation of immune cells in the presence of a pathogen is energetically costly for the host and the amount of resources required for this can be further depleted by an existing infection. A reduction in the transmission potential of *S. carpocapsae* occurs when the host is coinfected with *G. blattarum* due to depleted host resources, which might also modulate the ability of the host to mount an effective immune response. It is hypothesized that the host lipid resource, which mediates the interaction between both parasites, would decrease in response to higher levels of gregarine infection and that this, in turn, would reduce the amount of resources available to mount an adequate immune response. Further, it is hypothesized that a reduced immune response during high levels of gregarine infection would increase susceptibility to *S. carpocapsae*. Groups of specific parasite free cockroaches were exposed to a range of gregarine gametocyst doses to establish different gregarine infection intensities, and subsequently monitored for the time of host death post-exposure to nematodes. The host cellular and humoral response during single and coinfection with *S. carpocapsae* were also quantified. In support of the hypothesis, the findings revealed that the production of phenoloxidase and lysozyme activity in the haemolymph was dependent on the intensity of the endemic infection but that there was no significant effect of host gregarine burden on the cellular encapsulation

response. Contrary to the hypothesis, host gregarine burden did not significantly impact susceptibility to nematode infection, although there was a trend where coinfected hosts with low gregarine burdens had the highest survivorship. In natural conditions, the aggregated distribution of endemic parasites means that hosts will vary in resource utilization, resulting in heterogeneities among individuals in their immune response to infection. Whilst the immune response to endemic infection did not alter host survivorship, upregulation in phenoloxidase and lysozyme activity could be effective against other pathogens. The findings highlight the need to consider the impact of density-dependent variation in resources in order to effectively predict which host population are likely to experience an epidemic outbreak.

2.2 Introduction

Endemic macroparasites (e.g. helminths and protozoans) are ubiquitous in nature (Shaw and Dobson 1995; Wang et al. 2000). In natural systems, parasites rarely occur in single infections, therefore, epidemic microparasites will inevitably encounter hosts already infected by endemic macroparasites (Fenton 2008). Macroparasites can compete directly with a microparasite for host resources (Griffiths et al. 2014) and space (Dobson and Barnes 1995). Alternatively, macroparasites can have indirect effects on host health by altering the host biology, for example through altering the host immune response (Petney and Andrews 1998; Cox 2001; Lello et al. 2004; Chen et al. 2005; Chen et al. 2006; Cattadori et al. 2008; Graham 2008; Beldomenico et al. 2009; Nunn et al. 2014; Salazar-Castanon et al. 2014; Su et al. 2014). The modulation of the host immune response can alter the intensity of infection (Pathak et al. 2012), disease severity (Chen et al. 2005; Graham et al. 2005) and duration of infection (Walzl et al. 2000; Pathak et al. 2012). Given the heterogeneity in endemic parasite intensities between hosts (Wanji et al. 2003; Brooker et al. 2006; Viney and Cable 2011), the interaction between macroparasites and the invading microparasites may also vary. A significant gap in knowledge is what effect different intensities of an endemic parasite

559 have upon the host susceptibility and immune response against an invading epidemic
560 parasite.

561 The ability of a host to respond to a foreign pathogen is a fundamental trait of all
562 organisms, including insects. The immune system requires sufficient energy stores to
563 maintain functionality (Chandra 1996; Lochmiller and Deerenberg 2000; Klasing et al.
564 2004; Martin 2nd et al. 2007), and in most cases, the immune responses are host
565 condition-dependent, i.e. immune responses are increased in hosts possessing greater
566 energy reserves compared to host with low reserves (Lifjeld et al. 2002; Siva-Jothy and
567 Thompson 2002). The stimulation of a host immune response results in increased use
568 of resources (e.g. increased metabolic rate) (Lochmiller and Deerenberg 2000), or a
569 trade-off in other life-history traits, such as reduced growth and reproduction (Sheldon
570 and Verhulst 1996; Brace et al. 2017; Miller and Cotter 2018). In vertebrates, studies
571 have demonstrated that activation of the immune system can result in elevated host
572 basal or resting metabolic rate (RMR) by up to 32% (Demas et al. 1997; Lochmiller and
573 Deerenberg 2000, Ots et al. 2001; Martin et al. 2003; Derting and Virk 2005; Eraud et
574 al. 2005; Martin 2nd et al. 2007). Similarly, in invertebrates, a significant increase in the
575 host metabolic rate has been demonstrated to be a consequence of immune
576 activation. In the cabbage white butterfly, *Pieris brassicae*, for example, the standard
577 metabolic rate (SMR) measured as CO2 production increased by 8% after an immune
578 challenge compared to controls (Freitak et al. 2003). In *Drosophila*, maintaining
579 hematopoietic cells (essential for cellular immunity) and the synthesis of humoral
580 antimicrobial peptides by host body fat results in a drain of host glycogen and
581 triglyceride store (Rera et al. 2012; Bajgar et al. 2015). When challenged by a pathogen,
582 there is an energetic cost associated with mounting an effective immune response
583 (Norris and Evans 2000; Raberg et al. 2002; Siva-Jothy and Thompson 2002; Klasing et
584 al. 2004; Rahnamaeian et al. 2015; Brace et al. 2017). It should be noted, however, that
585 energetic costs have not been shown in all studies, Bashir-Tanoli and Tinsley (2104)
586 demonstrated that, in *Drosophila melanogaster*, immune responses are not
587 energetically costly, instead, infection-induced anorexia reduces host resource

588 acquisition needed to support metabolism. Nevertheless, in most systems there
589 appears to be a strong link between maintaining, activating and deploying immune
590 components and in the absence of specific evidence from the cockroach system, we
591 make the assumption that costs exist.

592 In natural conditions, hosts are limited in available resources which can be depleted
593 further by the presence of parasitic infection (Tocque 1993; Tocque and Tinsley 1994;
594 Rivero et al. 2007; Frost et al. 2008; Randall et al. 2013). Differences in a host's
595 resource level can have a significant impact on host susceptibility to a pathogen (Boots
596 2000; Demas et al. 2003; McKean et al. 2008). Resource limitation can alter a host
597 immune response (Koski and Scott 2001), which in turn may affect susceptibility to
598 infection (Nasci and Mitchell 1994; Wale et al. 2017). Coinfecting parasites can interact
599 indirectly via the host immune system and this interaction can be dependent on
600 resource availability (French et al. 2009). Yet, there is a limited understanding of the
601 potential impact of depleted host resources (due to an existing infection) on the
602 susceptibility to a secondary pathogen.

603 Despite the increasing number of coinfection studies on the vertebrate immune system
604 (Lamb et al. 2005; Hoeve et al. 2009; Su et al. 2014; Beechler et al. 2015; Griffiths et al.
605 2015; Lehmer et al. 2018), there is a significant lack of research on the invertebrate
606 immune system. Invertebrate immune responses (Fig. 2.1) are chiefly innate consisting
607 broadly of both humoral and cellular components (Karp et al. 1994; Hoffmann 1995;
608 Braun et al. 1997; Cotter et al. 2004; Schmid-Hempel 2005; Siva-Jothy et al. 2005);
609 however, a form of adaptive immune response (memory and long term upregulation of
610 the innate response) has been demonstrated in some systems (Hartman and Karp
611 1989; Kurtz and Franz 2003; Moret and Siva-Jothy 2003; Korner and Schmid-Hempel
612 2004; Kurtz and Armitage 2006; Schulenburg et al. 2007). The innate humoral response
613 to invasion by a pathogen includes complement-like proteins, antimicrobial peptides
614 and the enzyme cascade that regulate melanin production, which is also involved in
615 wound healing (Soderhall and Cerenius 1998; Blandin and Levashina 2004; Theopold et

al. 2004; Imler and Bulet 2005; Cerenius et al. 2010). Pro-phenoloxidase, the precursor of the enzyme phenoloxidase (PO), is activated immediately an invading microbe is detected in the host haemolymph (Ebrahimi et al. 2014). Insect PO plays a vital role in initiating both components of the innate immune response. The main role of PO, however, is the conversion of phenols to quinones which are polymerised in the haemolymph to form melanin, a crucial step in melanogenesis (Soderhall and Cerenius 1998; Gonzalez-Santoyo and Cordoba-Aguilar 2012). The level of host haemolymph PO activity correlates with immune competence against a parasite (Nigam et al. 1997). The cellular response involves the production of protein or plasma, and haemocytes which bind to the invading pathogen and engulfs them by phagocytosis or the formation of a capsule around larger pathogens (Gillespie et al. 1997; Meister and Lagueux 2003; Strand 2008). The invertebrate immune responses towards microparasites includes: the production of antimicrobial peptides which are effective against bacteria and fungi (Dimarcq et al. 1990; Bulet et al. 1999); the action of lysozyme which causes breakdown of the peptidoglycan cell wall of bacterium and; the recognition and subsequent clearing of lipopolysaccharide cell walls of gram-negative bacteria by haemolymph protein (Jomori et al. 1990; Jomori and Natori 1991) or the glucan component of the fungal cell wall (Duvic and Söderhäll 1992; Ochiai et al. 1992; Lee et al. 2000). Both phenoloxidase and lytic activity are important components of the immune response indicative of host resistance to a microparasite (Adamo 2004). In vertebrates, the Th1 & Th2 which target micro- and macroparasites respectively (Fenton et al. 2008), are governed by trade-offs, such that upregulation of the Th1 response, results in downregulation of the Th2 response and vice versa (Fenton et al. 2008; Graham 2008). Evidence of a similar trade-off has been documented for invertebrates (Rantala and Roff 2005; Siva-Jothy et al. 2005), for example, bumblebees challenged by lipopolysaccharide obtained from bacteria surface displayed an increased antibacterial activity and a reduced phenoloxidase activity, suggesting a possible trade-off between both immune responses (Moret and Schmid-Hempel 2001). Yet, our knowledge of the insect immune response during coinfection remains limited.

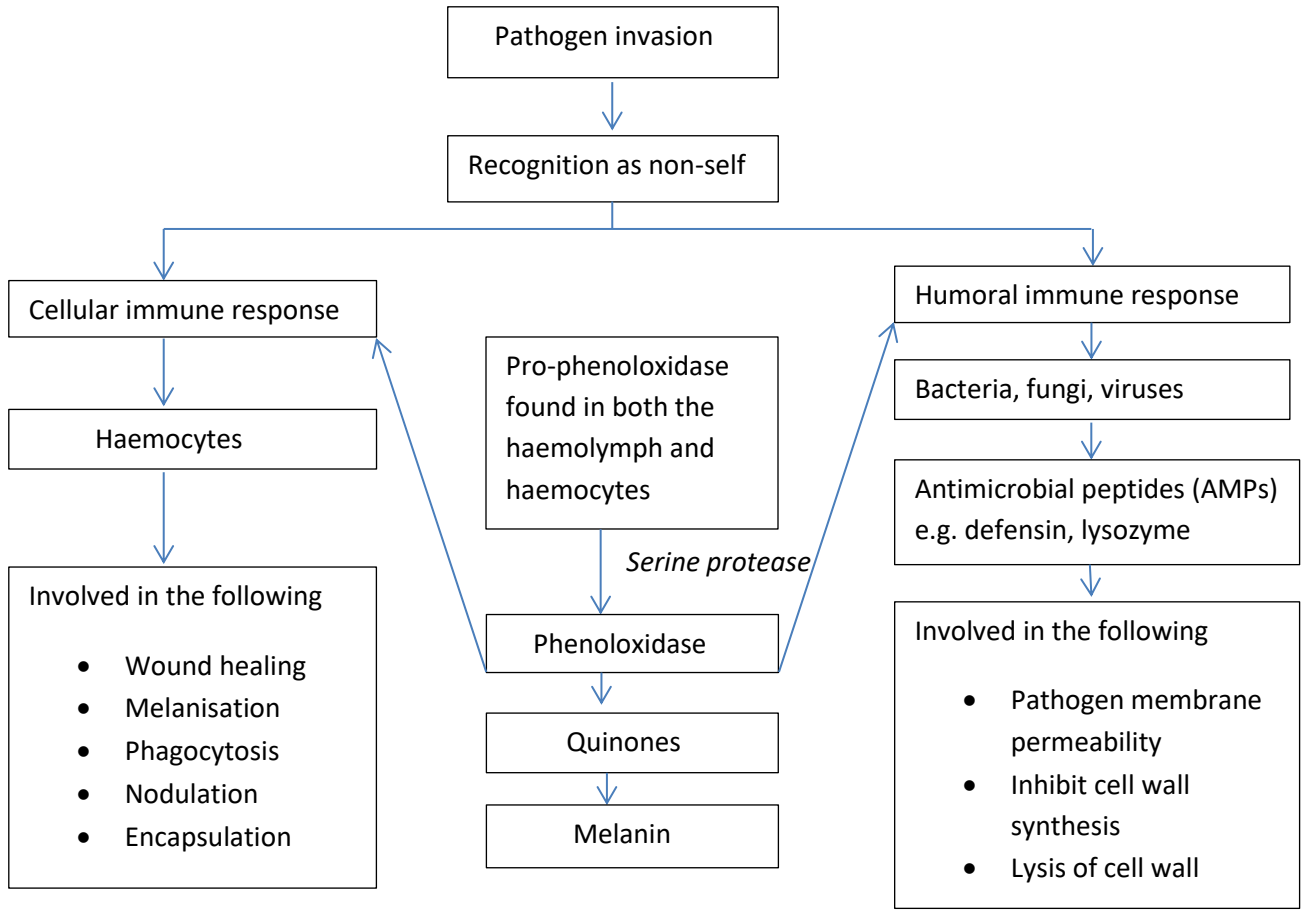


Figure 2.1: The insect innate immune response against an invading pathogen includes melanisation, encapsulation, phagocytosis and the production of antimicrobial peptides. Phenoloxidase plays a vital role in initiating the cellular and humoral response (adapted from Rao 2011).

In the current study, the effect of gastrointestinal macroparasite on the host immune response and susceptibility to a microparasite was investigated using the model host system, the German cockroach (*Blattella germanica*). Like other insects, this cockroach is host to a wide array of macro- and microparasites (Clopton and Gold 1996; Tachbele et al. 2006; Salehzadeh et al. 2007; Tilahun et al. 2012). In particular, the cockroach has a common, species-specific, endemic gastrointestinal parasite, the apicomplexan, *Gregarina blattarum*. Like many endemic pathogens, *G. blattarum* displays an aggregated distribution within the host population and causes relatively low virulence, reducing host lifespan and fecundity in an infection intensity-dependent manner under

669 population-level conditions (Fredensborg et al. 2004; Benesh 2011; Yamada et al.
670 2018). These cockroach hosts can also be infected with a generalist entomopathogenic
671 nematode (EPN), *Steinernema carpocapsae,* a highly virulent parasite with epidemic
672 dynamics in the host populations. This nematode species is used as a biological control
673 agent against a diverse array of insect pests (Koehler et al. 1992; Appel et al. 1993). The
674 nematode has a symbiotic relationship with the gut bacterium *Xenorhabdus*
675 *nematophilus*, which kills the insect host by causing septicaemia (Park and Kim 2000;
676 Lewis et al. 2006). A host must, therefore, respond to the nematode with a
677 macroparasite type response (e.g encapsulation) and the production of antimicrobial
678 peptides against the endosymbiotic bacterium. Both the endemic and epidemic
679 parasites in this system utilize host lipid resources (Fitters et al. 1999; Ciancio et al.
680 2001; Valigurova and Koudela 2005). The lipid resources used by the parasites are also
681 required by the host, in order to mount an effective immune response (Norris and
682 Evans 2000; Raberg et al. 2002; Siva-Jothy and Thompson 2002; Klasing et al. 2004).

683 In preliminary unpublished work, Randall (2011) demonstrated a potential trade-off in
684 the host encapsulation (anti-macro) and lytic immune (anti-micro) response in the
685 presence of gregarines in the German cockroach. Randall et al. (2013) also
686 demonstrated that the gregarine parasite depletes host resources and affects the
687 transmission potential of co-infecting nematodes. Since both parasites are dependent
688 on the host resources for survival and reproduction, a stronger drain on host resources
689 might be expected with increasing intensities of gregarine infection. It is hypothesized
690 that the density-dependent reduction in host lipid resource as a result of endemic
691 gregarine infection will, in turn, reduce host resources required to mount an adequate
692 immune response. Further, it is hypothesized that resource limitation in gregarine
693 infected hosts (Randall 2011), will result in immune modulation that increases host
694 susceptibility to an epidemic parasite because immune cell activation is energetically
695 costly for the host. As the endemic parasite elicits an encapsulation response and the
696 epidemic parasite an antibacterial response, immune bias by the gregarine
697 macroparasite might negatively affect the nematode that vectors the endosymbiont

but downregulate the microparasitic response against the Gram-negative bacterium (which ultimately kills the host by inducing septicaemia). It is proposed that the potential trade-off between the cellular encapsulation and antibacterial lysozyme activity will be altered with increasing endemic infection, which in turn increases host susceptibility to infection.

2.3 Materials and Methods

2.3.1 Host maintenance and nematode culture

Final stage nymphs were collected from specific parasite free (SPF) colonies maintained at Cardiff University School of Biosciences since 2007. These SPF cockroaches were created from an original stock purchased from Blades Biological Ltd., with the gregarine free nymphs obtained via oothecal sterilization and incubation (Muller-Graf et al. 2001). Cockroaches were maintained in 19-litre white plastic boxes coated with Fluon® to prevent escape. To avoid cross-contamination, gregarine and SPF cockroaches were reared in separate controlled temperature (CT) rooms, both set at 25±1˚C, 30% ±2% humidity and a 12 L: 12 D photoperiod. All experiments were conducted under these same environmental conditions. The cockroaches were fed ground dry dog food (Tesco complete dog food), dechlorinated water *ad libitium* and provided with stacked cardboard egg boxes for refugia.

Freeze dried Infective Juveniles (IJs) of the entomopathogenic nematodes (EPNs) *Steinernema carpocapsae* were donated by BASF Agricultural Specialities Ltd. For experimental infections (see sections b and c below), all infection arenas consisted of small round plastic pots (275 ml vol., 11.5 cm dia. x 7.5 cm height) coated with Fluon and lined at the base with 75g of dry sterile sand. A known nematode concentration (100 nematodes/ml) was created by adding the stock (freeze-dried) to distilled water. On to the sand substrate of the pots was carefully pipetted 8 ml of distilled water containing 100 *S. carpocapsae* nematodes/ml to give an even distribution across the sand surface. Cockroaches added to the infection arena died after 48-72 hours

726 exposure to nematodes. Cadavers of the dead cockroaches were then collected and
727 placed onto White's trap (White 1927), from which emerging IJs were collected after 5
728 days. The IJs emerging from the White's trap after a single passage through SPF
729 cockroaches were used for all experiments. To maintain nematode stock levels, new
730 infection arenas were created weekly and pipetted with a new dose of nematodes.
731 Cockroaches were added to these infection arenas and they died after 48-72 hours
732 exposure to nematodes. Cadavers were collected and placed on White's trap to isolate
733 the infective juveniles.
734
735 *2.3.2 Gregarina blattarum culture and experimental cockroach infection*

736 Cockroach frass (excreta and food) containing *G. blattarum* gametocysts was collected
737 from gregarine infected colonies and soaked in a petri dish containing deionized water
738 for approximately one hour to soften the faeces and allow for easy removal of
739 gametocysts with tweezers. Using a dissecting microscope (GX Optical XLT-101),
740 gametocysts appearing translucent and similar in size (ca. 40 μm dia., a subsample was
741 measured using an ocular micrometer) were collected into a petri dish containing
742 distilled water. For the susceptibility study, newly developed adult male and female
743 cockroaches (N=180) were grouped (N= 10 per group) into plastic rearing pots (275 ml
744 vol., 11.5 cm dia. x 7.5 cm height) with perforated lids. These pots were divided into six
745 treatment groups (N=3 replicate pots, i.e. 30 cockroaches per treatment). Two
746 treatment groups were left unexposed to gregarines and were used as negative
747 (uninfected) and positive (infected only with nematodes) controls. Remaining
748 cockroaches were exposed to one of four treatments (i.e. 5, 10, 15 or 20 gametocysts),
749 with the gametocysts placed on 0.063g of ground dog food (Tesco complete) contained
750 within the lid of a 150 μl Eppendorf tube. Cockroaches were then fed water and food
751 *ad libitium* in the same rearing pot, maintained at 25±1˚C, 30% ±2% humidity and a 12 L
752 : 12 D photoperiod for 10 days to allow for the development of trophozoites in the host
753 gut. For the immunity measures, newly developed female SPF cockroaches (N=840)
754 were grouped (N=10 per group) into plastic rearing pots and divided into four

755 treatment groups (N=21 replicate pots, i.e. 210 cockroaches per treatment). Two
756 treatment groups were exposed to low or high levels of gregarine parasite intensity by
757 exposing the cockroaches to either 10 or 20 gametocysts respectively. The remaining
758 cockroaches left unexposed to gregarine gametocysts served as negative and positive
759 controls. The 21 replicate pots were further divided and used for the three immunity
760 measures (PO, encapsulation response and lysozyme activity). All experiments were
761 conducted under the same environmental conditions described above.

762 *2.3.3 Coinfection*

763 *Susceptibility study*

764 To determine the effect of endemic gregarine infection on host susceptibility to
765 nematodes, the gregarine treatment groups and positive control group (N=30) were
766 exposed to *S. carpocapsae* IJs in an infection arena for 6 hours. This time period for
767 exposure was chosen to provide variation in the cockroach time to death; preliminary
768 experiments indicated that this 6 hours exposure time resulted in approximately 80%
769 of cockroaches dying over 10 days post-exposure, whereas longer exposure resulted in
770 100% mortality within the same timeframe. After exposure, cockroaches were
771 removed, housed in clean colony pots (Cater For You Ltd), fed dry dog food and water
772 *ad libitium* and monitored for 10 days. Negative controls (N=30) treated in the same
773 manner were exposed to sand, moistened with distilled water only, for a duration of 6
774 hours before removal and maintenance for 10 days. The number of cockroach deaths
775 and time to death (to the nearest hour) were recorded. Host deaths that occurred
776 overnight (between 7pm-8am) were recorded as occurring at the first check at 8 am.

777 *2.3.4 Host immunity measures*

778 *(i) Nematode coinfection*

779 Each cockroach from the gregarine treatment groups and positive control group were
780 carefully placed in a 1.5 ml Eppendorf tube (1 cockroach per Eppendorf) with a
781 perforated lid. The Eppendorf tubes were fitted with carefully cut filter paper strips (60

x 45 mm) and dampened with 5 µl distilled water containing 100 *S. carpocapsae* nematodes/ml. After two hours of exposure, all cockroaches were placed in recovery pots for 20 hours before haemolymph samples were extracted. This time period of 20 hours was chosen as a preliminary study indicated host death occurs from 23 hours post-exposure to nematodes. The negative controls (NC, N=230) were treated in the same way but exposed to filter paper strips dampened with sterile distilled water.

(ii) Haemolymph sampling

Cockroaches from each treatment group (single, coinfected and controls) were collected, anaesthetized using CO_2 and maintained on ice. After swabbing with 70% ethanol to remove any contaminants, the hind limb of each cockroach was cut and 3 cockroaches per replicate were placed into a 1.5 ml Eppendorf tube with a perforated base (made with a sterile dissecting probe) inside a 15 ml falcon tube and stored on ice to prevent haemolymph coagulation. Haemolymph accumulated at the base of the 15 ml falcon tube after centrifugation at 3500 rpm for 5 min at 4°C to prevent coagulation and activation of enzymes.

(iii) Phenoloxidase (PO) assay

To measure the quantity of PO enzyme in the pooled haemolymph for controls, the two gregarine infected treatments and those coinfected with nematodes (N=5 replicates per treatment), 8 µl of haemolymph was added to 400 µl ice-cold phosphate-buffered saline (pH 7.4) in a 1.5 ml sterile Eppendorf tube. The sample was frozen to disrupt the haemocyte membranes. Triplicate 100 µl samples of the defrosted haemolymph-PBS mixture were added to a microtiter plate containing 100 µl of 20 mM L-DOPA (Sigma-Aldrich) and incubated at 25°C for 30 min (Wilson et al. 2001). The absorbance of the haemolymph-PBS and L-DOPA solution was measured at 490 nm on a temperature-controlled microplate reader (Tecan infinity M200 Pro). PO activity was measured spectrophotometrically as the formation of dopachrome with a minute interval for 30 min (Cotter et al. 2004). The slope during the linear phase of the reaction was used to determine the quantity of PO enzyme.

(iv) *Encapsulation response*

The innate anti-macroparasitic encapsulation response of cockroaches exposed to 0, 10 and 20 gregarine gametocysts was measured by assessing the level of melanisation and cell deposition around a flattened 2 mm long, ca. 0.3 mm width nylon filament sterilised in 70% ethanol for 20 min (method adapted from Cotter et al. 2008). The nylon filament was flattened using a bench vice so that a 2-dimensional image could be used to assess immune cell deposition. Cockroaches from all treatment groups (N =60 per treatment) were anaesthetized with ice and the abdominal plates swabbed with 70% ethanol and then sterile PBS to remove any contaminants. A single nylon filament was inserted between the 5^{th} and 6^{th} sternite using sterilized forceps. Cockroaches from each treatment were grouped and placed in recovery pots (Cater For You Ltd) with food and water *ad libitum.* All cockroaches were dissected after 24 hours to extract the nylon implants. Implants from each host were individually stored in Eppendorf tubes containing 0.5 ml PBS solution for 24 hours at 4°C. The implants were placed on absorbent tissue to remove excess PBS fluid before images were captured on a microscope slide using a bright field microscope (Meiji macro-imaging system) with an Optem Zoom 125 micro-inspection lens. Photographed images were analysed in ImageJ® for Windows (Rasband 2016). Implant total area was measured in 2-dimensions using the same HSB (Hue: 0-255, Saturation: 2-210, Brightness: 0-255) colour threshold. The total area of pigments on the implant was also measured using the same HSB (Hue: 0-245, Saturation: 70-96, Brightness: 0-255) to determine the percentage of encapsulation. All images were analysed using the same set scale (747.01 pixels/mm).

(v) Anti-bacterial activity

Lysozyme antibacterial activity was measured using a turbidimetric method (Drayton and Jennions 2011), 2 µl of haemolymph was added to 8 µl Phosphate Buffered Saline (PBS, pH 7.4) and frozen to induce cell lysis. The thawed haemolymph-PBS sample was added to 90 µl of *Micrococcus lysodeikticus* solution (3 mg/ml PBS, Sigma-Aldrich) in a

838 96 well microtiter plate (CELLSTAR®). The plate was loaded into a temperature
839 controlled (30°C) Tecan infinity M200 Pro microplate reader (Tecan Life Sciences,
840 Switzerland) and the absorbance read at 490 nm for 30 min. Haemolymph samples
841 from all groups (single and gregarine coinfected) and control (PBS and *M. lysodeikticus,*
842 without haemolymph) were included on the same microtiter plates. Each treatment
843 group had 10 replicates. The rate of change in optical density for the samples was
844 calculated as the sample slope minus the control slope. The obtained values were
845 multiplied by -1 for ease of interpretation (a greater value indicates more lysozyme
846 activity in the haemolymph; Drayton and Jennions 2011). This simple assay detects
847 changes in the turbidity caused by the enzymatic activity of the lysozyme against the
848 cell wall of *M. lysodeikticus* used as a substrate (Moreira-Ludewig and Healy 1992).

849 *2.3.5 Statistical analysis*

850 All statistical analyses were conducted using the R statistical programming software
851 v.3.2.2 (R Core Team 2015). As the gregarine parasite distribution amongst the hosts
852 was aggregated, the bootstrapped mean abundance of trophozoites in the cockroach
853 gut was calculated, to give a more accurate representation of the true mean and
854 provide an estimate of the 95% confidence intervals, for each level of gametocysts
855 exposure. This was achieved by randomly sampling a single host from each of the three
856 replicate populations (as there was substantial variation between the replicates) and
857 calculating the sample mean. The process was repeated 10,000 times and the overall
858 mean of the replicates and its 95% confidence interval is reported. A non-parametric
859 (Kendall's tau) correlation was conducted to assess the association between the
860 abundance of trophozoites for each level of gametocysts exposure.

861 *Does phenoloxidase response differ with gregarine exposure?*

862 A Generalised Linear Model (GLM) with Gaussian error distribution and identity link
863 function was used to assess the effect of host gregarine exposure on the activation of
864 phenoloxidase in the haemolymph. The dependent variable was transformed prior to
865 analyses using natural log (LN(x+1)), resulting in a normal distribution of the residuals.

The host gregarine exposure was treated as a categorical variable (0, 10 or 20 gametocysts) and included in the model as an independent term. The F test statistic was used to assess the effect of host treatment on the amount of haemolymph PO (with $p<0.05$ taken as significant for all tests). Where host treatment was found to be significant, differences between the category levels were assessed using the R package "contrasts" (Max Kuhn 2016).

Does the host gregarine exposure alter lysozyme activity?

A General Linear Model (GLM) with Gaussian error distribution and identity link function was used to assess the effect of the host gregarine exposure on the baseline level of lysozyme activity (antibacterial response) in the haemolymph. The baseline lysozyme activity was transformed prior to analysis using natural log, while the host gregarine exposure (low and high infection levels) was treated as a categorical variable and included in the model as an independent term. The F test statistic was used to assess the effect of host gregarine exposure on the lysozyme activity. Where the host treatment was found to be significant, differences between the category levels were assessed using the R package "contrasts" (Max Kuhn 2016).

Does the host gregarine exposure impact the encapsulation response?

A GLM with Gaussian error distribution and identity link was used to assess the effect of host gregarine load on the proportion of melanisation (anti-macroparasitic response) on an artificially inserted nylon filament. The proportion of melanisation was transformed prior to analysis using arcsine, while the gregarine load was treated as a categorical variable and included in the model as an independent term. The F test statistic was used to assess the effect of host gregarine exposure on the encapsulation response.

Does the host gregarine exposure impact susceptibility to Steinernema carpocapsae?

Having confirmed that no deaths occurred during the 10-day experimental period in the negative control group, this group was removed from subsequent analysis. The effect of gregarine exposure (independently and pooled gregarine exposure) on the survival of cockroaches, controlling for host sex, was assessed by conducting a Kaplan-Meier analysis using the Survdiff of the R package "survival" (Therneau 2015). A Cox proportional hazard model was created to assess the simultaneous risk of death between the treatment groups, with the gregarine load and sex added as covariates. The effect of host gregarine exposure on the susceptibility to *S. carpocapsae* was assessed using a binomial generalized linear model (GLM) and logit link function. The time taken for death of individual cockroaches (used as a proxy for susceptibility) to occur and the censor (i.e. 1=observed death, 0=unobserved death during the study period) was included in the model as the dependent variable while gregarine load and host sex were treated as categorical variables and added as independent terms. The effect of gregarine exposure and host sex on susceptibility to nematodes was assessed using the Chi test statistic and associated p-value (with $p<0.05$). Subsequently, a Cox proportional hazard model using the ssizeCT.default of the package "powerSurvEpi" (Qiu et al. 2012) was used to assess whether there was sufficient power to detect differences between the treatment groups.

2.4 Results

Gregarine exposure correlated positively (tau = 0.67) with the number of trophozoites in the cockroach gut (Fig. 2.2).

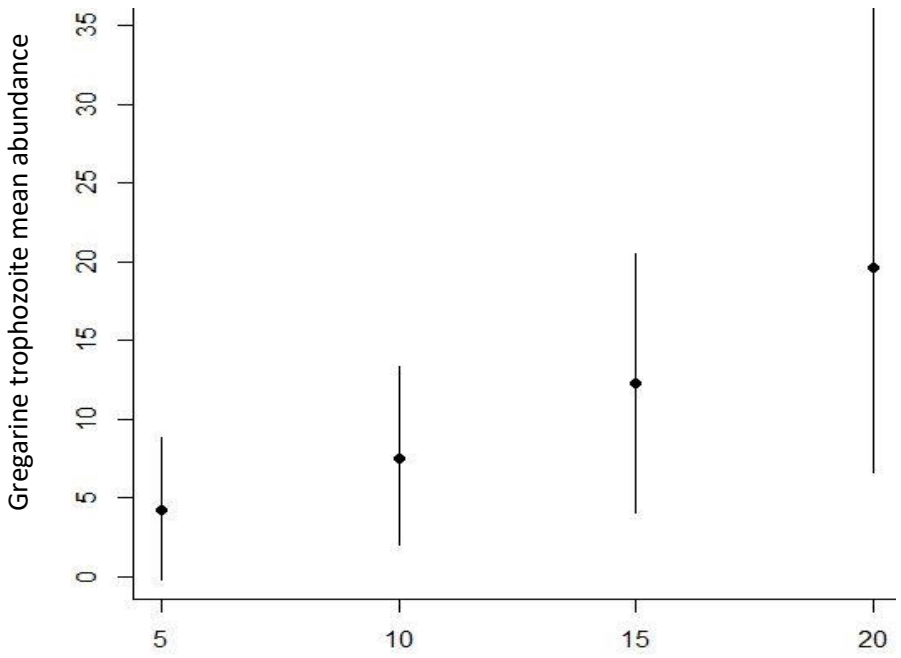


Figure 2.2: Gregarine trophozoite abundance in cockroach host over the range of gametocyst exposure levels. Circles denote bootstrapped means and dotted lines denote 95% confidence limits of the mean.

Effect of host gregarine exposure on phenoloxidase levels

The level of phenoloxidase activity in the haemolymph was significantly associated with the host gregarine exposure ($F_{5,906}$=12.75, p<0.001). All cockroaches infected with *G. blattarum* alone had significantly higher phenoloxidase compared to coinfected groups (Fig. 2.3).

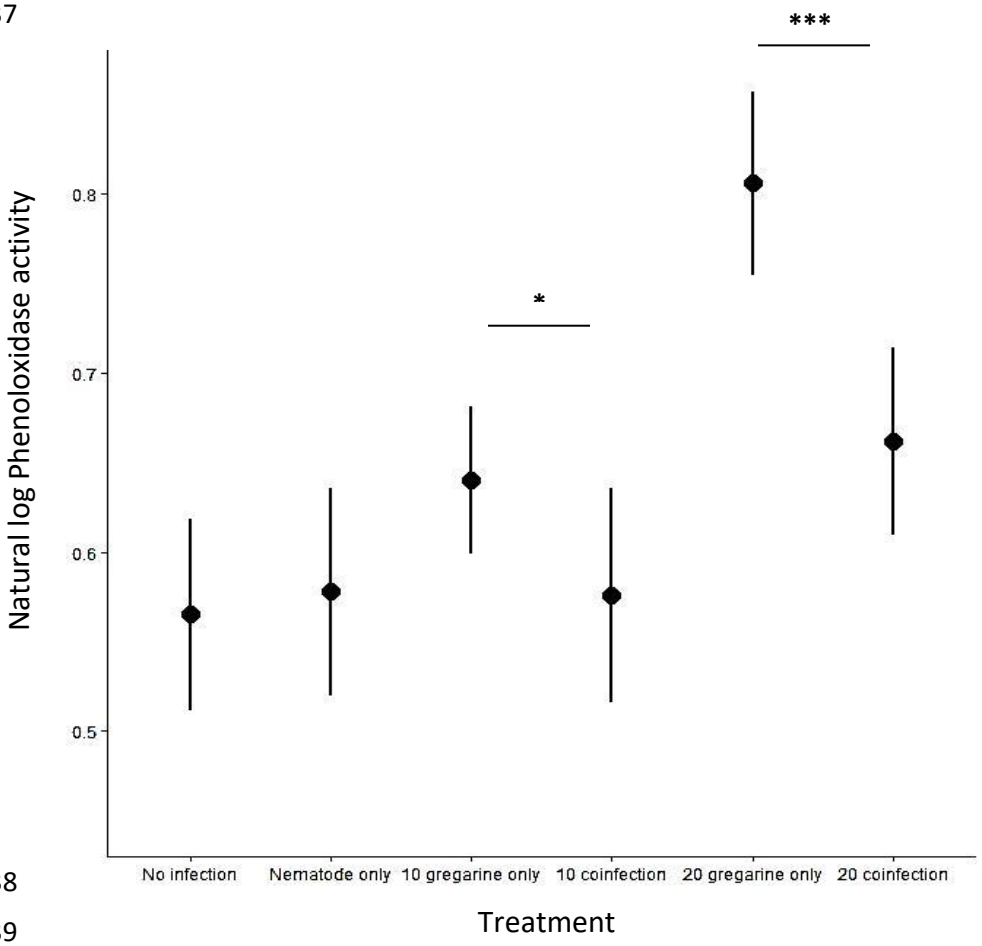


Figure 2.3: The effect of host treatment on the mean phenoloxidase activity in the haemolymph of German cockroaches post-exposure to *Steinernema carpocapsae*. Black lines denote the mean and the error bars represent the 95% confidence intervals. Asterisk (*) within the bar indicates the significant difference between the means (p<0.05).

Effect of host gregarine exposure on the lysozyme activity

Haemolymph lysozyme activity was associated with the host gregarine exposure ($F_{2,627}$=5.51, p<0.001). In particular, the lysozyme activity was significantly increased in highly infected gregarine hosts compared to the control, however, there was no significant difference between the control and low infection treatments (Fig. 2.4, Appendix 2.2). All cockroaches singly infected and coinfected with nematodes had suppressed levels of lysozyme activity compared with gregarine and control groups ($F_{2,312}$=12.461, p<0.001, Fig. 2.4).

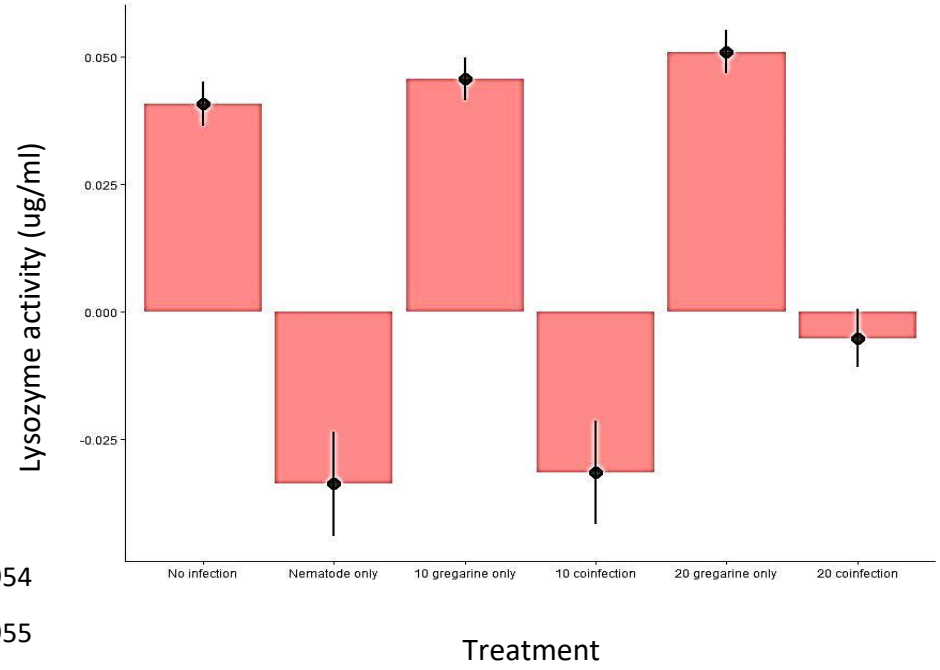


Figure 2.4: The effect of the host gregarine exposure (coinfected, single and controls) on the mean baseline lysozyme activity post-infection with *Steinernema carpocapsae.* The black circle denotes the mean of the generalised linear model while the error bar represents the 95% confidence intervals.

Effect of host gregarine exposure on the encapsulation response

There was no significant effect of host gregarine exposure on the encapsulation of an artificially inserted nylon filament ($F_{2,188}$=0.033, p>0.05). Neither, was there any trend that could indicate a potential difference between the gregarine infection treatments.

Effect of host gregarine exposure on the susceptibility to *Steinernema carpocapsae*

The percentage mortality of gregarine coinfected individuals were 23.3, 33.3, 30 and 30%, while singly infected hosts had a mortality rate of 43.3%, however, this difference was not significant. Neither, the presence of gregarine trophozoites in the gut of the cockroach nor host sex (Figs. 2.5 and 2.6), had a significant effect on host survivorship either when gametocyst exposure levels were considered independently or when they were pooled. Power analysis suggests that to differentiate between the coinfected individuals and the singly infected hosts, with a two-sided significance level of 0.05 and a power of 0.8, a total of 208 individuals per treatment was needed.

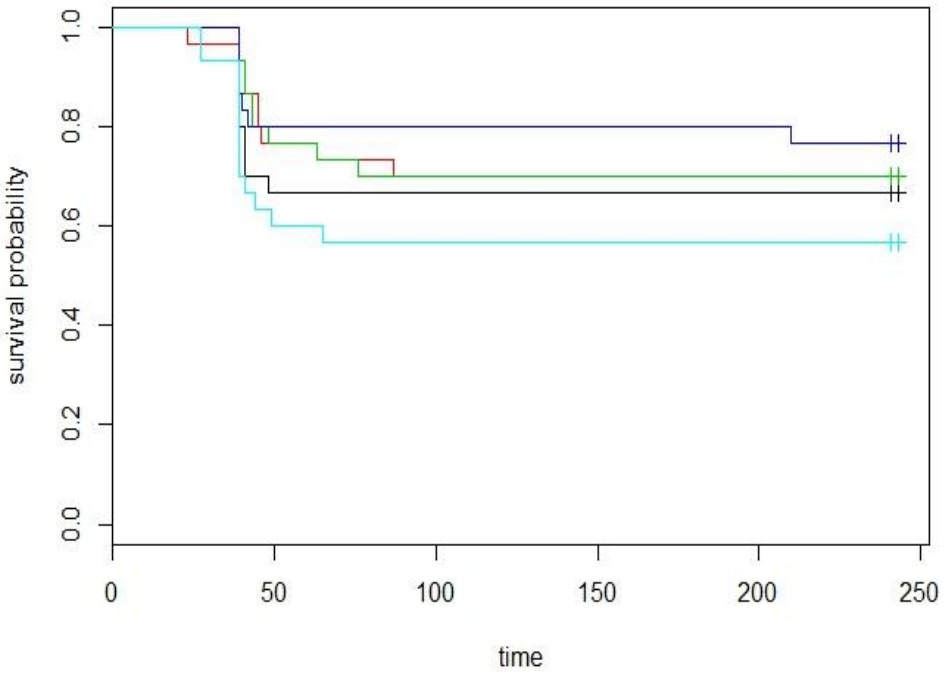
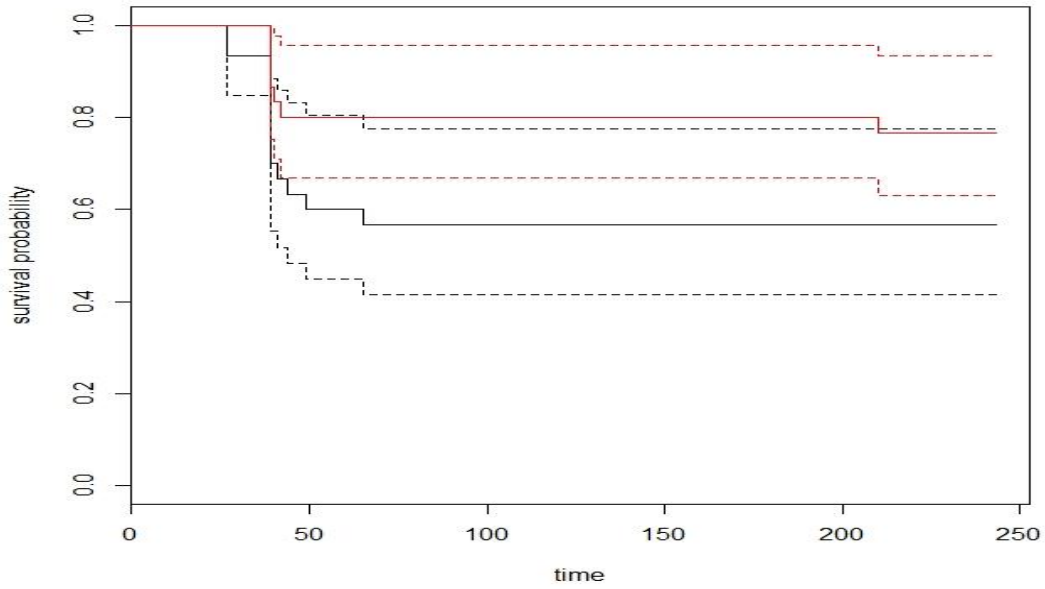


Figure 2.5: Kaplan-Meier probability of survival of cockroaches, excluding negative controls exposed to 5 (navy blue), 10 (red), 15 (green) and 20 (black) gametocysts and gregarine-free German cockroaches (light blue), 10 days post-exposure to *Steinernema carpocapsae*. The confidence interval was removed from the plot for visual clarity but interval for all groups overlapped.

A)



B)

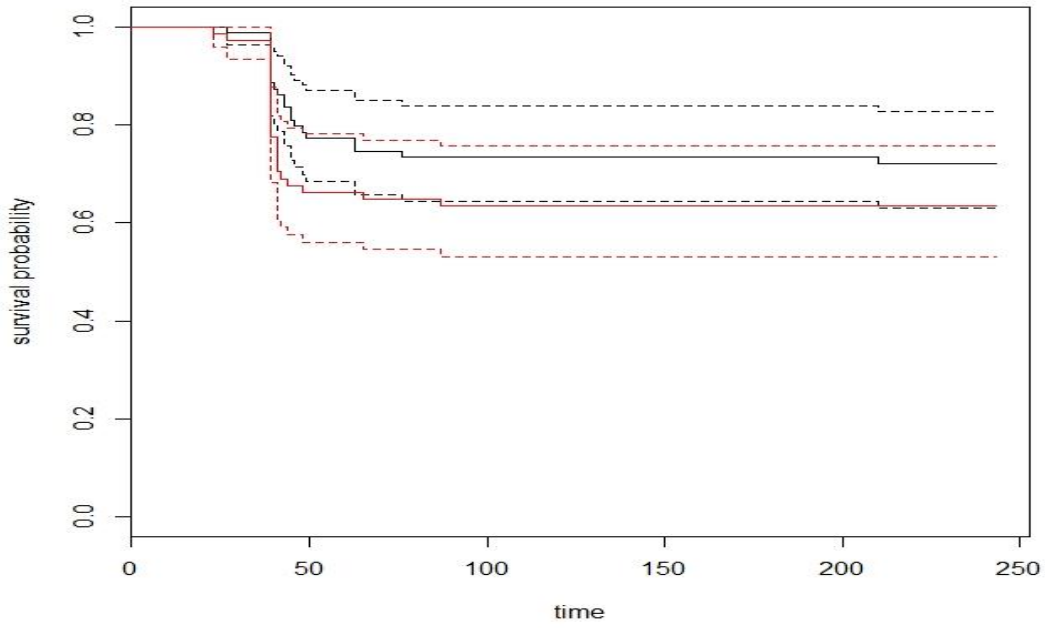


Figure 2.6: Kaplan-Meier probability of survival of (A) Coinfected treatment 5 (red line), singly infected German cockroaches (black), B) Female (black line) and male German cockroaches (red) 10 days post-exposure to *Steinernema carpocapsae* for a duration of 6 hours. Dotted lines represent 95% confidence interval.

2.5 Discussion

The host immune response (i.e. the production of phenoloxidase (PO) and the baseline lysozyme activity in the haemolymph) was found to be dependent on the level of endemic gregarine infection in the German cockroach host, thus partially supporting the hypothesis that gregarine density-dependent reduction in resources will deplete the amount of resources available to mount an adequate immune response. The finding from the current study also revealed suppressed levels of both PO and lysozyme activity in all hosts coinfected with nematodes compared to gregarine infection only. Despite the gregarine induced immune changes, and contrary to the hypothesis that immune modulation will increase susceptibility, hosts infected with different levels of gregarine infection did not vary significantly in their susceptibility to *Steinernema carpocapsae*. This contrasts with similar work on the effect of gregarines on host susceptibility to an entomopathogenic pathogen (fungus; *Metarhizium anisopliae*), where gregarine infected German cockroaches showed increased mortality (Lopes and Alves 2005). Whilst the result from the current study shows that the effect of the gregarine is not statistically significant, the trend is that cockroaches infected with low doses of gregarine had the highest survivorship. Power analysis suggests that the sample size (n=30) per treatment was below the number of cockroaches (n=208) that would be required to detect a difference between the survival curves. The increased immune response in gregarine infections might suggest that a small difference in survivorship between single and coinfected host might be detected with this larger sample size.

Gregarines attach to the cockroach host gut epithelium where they reduce host nutrient intake through direct utilisation of host food resources and can cause damage in the process of absorbing host nutrients (Takahashi et al. 2009; Landers 2010). Phenoloxidase, a general response to both micro- and macroparasitic infection, may have been activated for wound repair processes (Lai et al. 2002). The findings in this study revealed elevated levels of PO in hosts with just gregarine infection compared to

1014 the suppressed levels of PO in coinfected hosts. The endosymbiont *Xenorhabdus* used
1015 in the current study is known to prevent the activation of phenoloxidase cascade
1016 through the production of specific inhibitory compound Bromophenacyl bromide (Park
1017 and Kim 2003), and also the suppression of already activated PO activity through the
1018 production of a compound benzylideneacetone (Kim et al. 2011), which inhibits
1019 phospholipase (PLA2) needed for PO activation (Kim et al. 2011). A similar study
1020 identified that cosmid clones of a related endosymbiont (*Photorhabdus*) from the
1021 genomic DNA library suppressed previously activated PO levels *in vivo* and *in vitro*
1022 (Eleftherianos et al. 2009). The finding from the current study is consistent with other
1023 studies demonstrating a suppressed PO activity when hosts were infected with the
1024 entomopathogenic nematodes (Yokoo et al. 1992; Brivio et al. 2004; Kim et al. 2005;
1025 Eleftherianos et al. 2007; Eleftherianos et al. 2009; Seo et al. 2012; Eom et al. 2014;
1026 Abdolmaleki et al. 2017). Further, the results from this study suggest that the ability of
1027 the nematode to suppress the PO immune response is impaired when the host is
1028 infected with the endemic gregarine parasite.

1029 The aim of the current study was to explore the potential trade-off between host anti-
1030 macro and anti-micro response that may occur as a consequence of gregarine
1031 infection. The insect lysozyme specific response to a gregarine parasite has not been
1032 described prior to this study, however, some studies have highlighted the effect that
1033 gregarines may have on the host cellular response e.g. melanin pigmentation and
1034 encapsulation (Siva–Jothy 2000; Krams et al. 2017; Suhonen et al. 2018). An increase in
1035 the host cellular encapsulation response to gregarines was expected to downregulate
1036 the anti-micro response, as previously demonstrated in this system (Randall 2011). The
1037 current findings, however, suggest that the baseline anti-bacterial lysozyme activity in
1038 the haemolymph increased with increasing gregarine parasite intensity. The
1039 upregulation of lysozyme may reflect gregarine parasite stimulation of the Immune
1040 deficiency (IMD) and Toll pathways, which drive the transcription of antimicrobial
1041 peptide genes. In Anopheles mosquito, the IMD pathway triggered mostly by
1042 peptidoglycan bacterial cell wall was shown to possess antiparasitic properties which

were not only infection density-dependent but also effective against an apicomplexan protozoan parasite (*Plasmodium falciparum*) (Garver et al. 2012). In *Drosophila*, it has also been demonstrated that antimicrobial peptides can be induced when the host is infected by gut parasites (Boulanger et al. 2001). It is possible that lysozyme (an antimicrobial peptide) has antiparasitic capabilities as demonstrated by other insect antimicrobial peptides e.g defensin (Lowenberger et al. 1996; Shahabuddin et al. 1998; Boulanger et al. 2004; Hu and Aksoy 2005). There is evidence suggesting that a host antimicrobial response to midgut microbes confers protection against malaria, for example, by altering *Plasmodium* development in the host (Lowenberger et al. 1999; Aguilar et al. 2005; Dong et al. 2009; Meister et al. 2009; Cirimotich et al. 2011; Smith et al. 2014; Rodgers et al. 2017). In addition to the systemic activation of antimicrobial peptides, insects can activate antimicrobial peptides in response to a localized injury in the host gut (Ferrandon et al. 1998). Considering that gregarine trophozoites are attached to the host gut (via the epimerite embedded into the intestinal epithelium) where they absorb nutrients and in the process cause injury to the host gut tissue (Lacey 1997; Lucarotti 2000; Kuriyama et al. 2005). It is possible that the injury to the host gut may explain the upregulation of the antimicrobial peptide (lysozyme) demonstrated in the current study. The increased baseline lysozyme activity in the current study is consistent with results from another insect system, which demonstrated that the immune system was upregulated by an existing infection (non-pathogenic bacterium, *Escherichia coli*), such that the host immune response to the endosymbiont, *Photorhabdus* was enhanced (Eleftherianos et al. 2006a, b). The protective effect was associated with an upregulation of the microbial pattern recognition genes and the antibacterial effector genes (e.g cecropin, lysozyme) (Eleftherianos et al. 2006a, b). Conversely, lysozyme was suppressed in hosts infected with *S. carpocapsae* in the current study. Previous studies have also shown that *S. carpocapsae* possess inhibitory properties that impair immune cell function including downregulating insect antimicrobial peptide genes, which may explain the reduction in lysozyme activity in the single and coinfected hosts (Ji and Kim 2004; Duvic et al. 2012;

Bisch et al. 2015; Binda-Rossetti et al. 2016). To evade the host immune response, the cuticle of *Steinernema* produces cuticle-surface factors to inhibit the host antimicrobial response and prevent immune detection (Brivio et al. 2006; Castillo et al. 2011). It is, therefore, possible that *S. carpocapsae* interfered with the host antimicrobial enzyme cascade resulting in low levels of antibacterial activity in the host haemolymph. Similar to the suppressed PO immune response in coinfected hosts when compared to single infection, the current findings suggest that the presence of gregarine infection can potentially alter the ability of the nematodes to suppress the hosts' lysozyme activity.

Previous studies have demonstrated that the activation of the immune response is energetically costly for hosts (Norris and Evans 2000; Raberg et al. 2002; Siva-Jothy and Thompson 2002; Klasing et al. 2004; Rahnamaeian et al. 2015; Brace et al. 2017). The costs can be due to increased use of resources or trade-offs in life-history traits (Moret and Schmid-Hempel 2000; Bonneaud et al. 2003; Brace et al. 2015; Brace et al. 2017). The current study did not detect any difference in the host encapsulation response between the gregarine and uninfected groups, in contrast to previous work in our laboratory where gregarine infected hosts did show an increased encapsulation response compared to controls (Randall 2011). It is, however, possible that the immune response of the host was primed or upregulated in the previous work since hosts from a long term laboratory population were used, where longer-term infection and re-infection may have occurred. Another possible explanation could be that, the use of young adults in this study influenced the susceptibility of the host to a secondary infection. The cuticle pigment colouration of German cockroaches darkens with age, due to increased melanin production (Das and Gupta 1974), so the host may not be responding strongly (by depositing melanin pigments) when challenged with the artificially inserted nylon filament at the early adult stage used in the current study. Also, host resistance to a pathogen may be age-dependent where immunity is decreased due to the diversion of resources to other host requirements. Previous work in our laboratory has shown that gregarines cannot infect cockroach nymphs (Randall 2011), but it is unknown if immunity/resistance to infection decreases or increases

with an increase in age. For example, in the crayfish (*Cambarus bartonii*), younger and smaller hosts were less susceptible to infection, while older and larger adults showed increased susceptibility (Thomas et al. 2016). Conversely, in mosquitoes and bumblebees, the level of phenoloxidase was reduced with the host age (Jianyong et al. 1992; Chun et al. 1995; Whitehorn et al. 2010). It is also possible that the host lipid resources were not fully exploited by the gregarines in this relatively short experimental period of 10 days which may explain the non-significance in susceptibility and encapsulation response between the treatment groups.

In this study, a difference in susceptibility between the sexes might be expected because of the size differences between them, with female German cockroaches being generally larger than the males. Female German cockroaches tend to have higher lipid levels than males (Randall 2011), which may enhance their immune response compared to males (Rheins and Karp 1985; Kurtz and Sauer 1999; Sheridan et al. 2000; Rolff 2002). Variations in host immune response have been linked to sexual dimorphism in both invertebrate (Harris et al. 1986; Ahtiainen et al. 2004, 2005) and vertebrate hosts (Verhulst et al. 1999; Klein 2000; Klein et al. 2000; Moore and Wilson 2002; Klein 2004; Cousineau and Alizon 2014). Sex-biased immunity may be attributed to the difference in host metabolic functions. In mosquitoes, for example, males have a shorter life span and are less capable of mounting an adequate immune response (melanization) against foreign objects compared to females (Harris et al. 1986). Similarly, the female German cockroach is long-lived compared to the males, which could have explained the non-significant difference in survivorship between the sexes in the current data.

Although, unrelated to coinfection, one way for insect hosts to protect and prevent against the establishment of nematode infection is behavioural immunity, for example, grooming. Japanese beetles exposed to transmission stages of an entomopathogenic nematode, *Heterohabditis bacteriophora,* were able to brush off over 60% of nematodes that attached to their cuticle (Gaugler et al. 1994). The ability of the host to

groom, detect and avoid nematodes can reduce susceptibility to nematode infection within the host population (Gaugler et al. 1994; Koppenhöfer et al. 2000; Rosengaus et al. 2000; Půža and Mráček 2010). Some studies have also demonstrated that the gregarine gut parasite reduces the foraging ability of insects, and this effect can be gregarine density-dependent (Bouwma et al. 2005). Whilst the effect of gregarines on host susceptibility to nematodes was not statistically significant in the current study, it is possible that the changes to the host foraging behaviour may explain the survivorship trend in the data. In natural conditions, a reduced host movement could reduce the risk of exposure with entomopathogenic nematodes. Our understanding of the role of insect behavioural immunity in alleviating disease spread or reducing parasite growth is limited (Moore 2002; de Roode and Lefèvre 2012), and this study system provides an ideal model that can be manipulated to improve knowledge of host-parasite interactions.

By exploring the influence of underlying endemic infection, this study does not support a difference in the susceptibility of gregarine and non-gregarine hosts to secondary nematode infection in young adult cockroaches. Studies on other insect systems (e.g. house cricket, Colorado potatoe and Japanese beetle) have suggested that the differences in a host susceptibility to entomopathogenic nematodes can also be attributed to a variation in both the parasite traits (e.g. species, strain and endosymbiont) and host factors (e.g. Genotype, age and immune response) (Wang et al. 1994; Li et al. 2007; Ebrahimi et al. 2011). Given the apparent upregulation in the host immune response during high levels of coinfection, it is possible that there is a small protective response against other pathogens. This study does suggest that the effect of gregarines on the host immune response can be density-dependent. Since parasites are aggregated within their host populations, density-dependent differences are likely to have consequences for the host ability to defend against pathogens (Luong et al. 2011). Although unrelated to coinfection, the density-dependent variation of the host immune responses seen in the current study may explain the differences in the host time to death post-exposure to nematodes in another finding (Chapter 3), where

lower numbers of nematodes emerged from late death hosts. Considering that endemic infections are ubiquitous in natural systems, future work should explore the effect of a host-parasite burden on immune-mediated interactions, in particular where host resources are shared between pathogens. Resource availability can be a key factor determining the progression of epidemics since immunological responses to a foreign pathogen are costly and rely on host food intake (Ponton et al. 2013). Exploring the host immune capacity during coinfection in relation to the intensity of an existing infection is not only key for successful control strategies but also crucial for developing a better understanding of host immunity and its consequences for the dynamics of infectious diseases.

3.0 The effect of an endemic macroparasite on the quality and quantity of an epidemic parasite

3.1 Abstract

Endemic infections exist in almost all natural populations, therefore, epidemic infections will inevitably occur as a coinfection in hosts already infected by endemic parasites. Determining the impacts of these endemic parasites on the transmission dynamics and disease severity of epidemic parasites is, therefore, of paramount importance. Aggregation of endemic parasites within host populations is the norm and the depletion of host resources is normally parasite density-dependent. Parasite interspecific interactions (direct or indirect) can potentially impact both the quantity and quality (fitness) of transmission stages. To our knowledge, however, no study has investigated how different endemic parasite intensities within individual hosts, might drive epidemic disease emergence. Here, this study assessed the impact of different endemic parasite burdens on the quantity and quality of an epidemic parasite's transmission stages, where the parasite interspecific interaction was host resource mediated. It is hypothesized that the host's lipid resources would decrease in relation to gregarine burden and that this decrease, would lead to a reduced quantity and quality of infective juveniles emerging from the coinfected host. This hypothesis was explored using the previously established insect host model system, the German cockroach host, *Blatella germanica,* its endemic gut macroparasite (*Gregarina blattarum*) and an epidemic generalist entomopathogenic nematode (*Steinernema carpocapsae*). Specific parasite-free cockroaches were first infected with different intensities of gregarines and subsequently, these same hosts and uninfected controls were exposed to *S. carpocapsae.* Both the emergence of infective juveniles (i.e. transmission stage) over time and their individual lipid reserves, which are necessary for their infectivity and survival in the environment were monitored. Coinfection resulted in a reduced output of *S. carpocapsae* compared to singly infected hosts but

not in a parasite density-dependent manner. Host gregarine burden, however, had a non-linear effect on the lipid levels in the infective juveniles. Specifically, lipids were reduced at low burdens of the gregarine but increased at high burdens. This study demonstrates that where hosts lipid stores mediate the interaction between parasites, density-dependent depletion of the host resources due to endemic parasite burden can have fitness implication for the transmission stages emerging during coinfection. The same species of parasite can show different patterns of aggregation in different host populations or vary temporally in the same population, and these different patterns of aggregation may, in turn, lead to different effects on the of transmission of any emerging pathogen. Taking greater account of aggregation may, therefore, be essential in order to accurately predict disease outbreaks.

3.2 Introduction

Parasites are dependent on host energy reserves for survival and onward transmission into naive hosts (Tocque 1993). Between-host transmission is linked to the quality and fitness of the parasite transmission stages produced by infected hosts (Heffernan et al. 2005; Fraser et al. 2014; Stephenson et al. 2017). Parasites, in turn, may alter their host's energy reserves (Humiczewska and Rajski 2005; Hall et al. 2009). Simultaneous infection of a host by parasites of different taxa is common in natural populations (Petney and Andrews 1998; Cox 2001; Graham 2008; Choisy and de Roode 2010) and within-host competition between these parasites can directly impact resource availability of one or both parasite species (Fellous and Koella 2009, 2010; Randall et al. 2013; Griffiths et al. 2014). Such resource competition has been shown to alter the host immune response (Moret and Schmid-Hempel 2000; Koski and Scott 2001; Cornet et al. 2014), rate of parasite development (Dezfuli et al. 2001; Lagrue and Poulin 2008), survival (Fischer et al. 2009; McNamara et al. 2009) and the virulence of infection (Gower and Webster 2005; Choisy and de Roode 2010; Louhi et al. 2015; Bose et al. 2016; Kinnula et al. 2017). Further, interactions mediated through resource

competition have been highlighted as one of the most common forms of interspecific parasite interaction (Griffiths et al. 2014; Ramiro et al. 2016).

Endemic parasites, such as gastrointestinal protozoa and helminths, are found in most host populations (Wang et al. 2000; Viney and Cable 2011). Most endemic parasites show an aggregated distribution in host populations and this can lead to large variations in parasite intensity between hosts (Shaw and Dobson 1995; Hupalo et al. 2014). Host resource depletion is normally parasite density-dependent, yet, no study has investigated how different intensities of an endemic parasite might affect the transmission stages of a secondary infection, where host resources mediate the interspecific parasite competition. The interspecific interactions between parasites can alter parasite transmission, disease severity and dynamics within the host population (Ferrari et al. 2009; Knowles et al. 2013; Pedersen and Antonovics 2013; Viney and Graham 2013). Given the ubiquity and persistence of endemic infections in host populations, emerging or epidemic microparasitic infections will normally encounter hosts already infected by endemic parasites. Understanding the potential impact of a host's endemic parasite intensity on the output of an epidemic parasite's transmission stages during coinfection is important, as it may help to accurately predict the likelihood of epidemic spread and disease emergence in populations.

In this study, the model host system, the German cockroach (*Blattella germanica*), was used to investigate the role endemic parasites may have upon epidemic infection. This cockroach species is a generalist omnivore in which, the majority of its resources are stored as lipids in body fat (Schal et al. 1984; Cooper and Schal 1992; Shik et al. 2014). Cockroaches like many insect species are host to gregarine parasites, which are low virulence endemic protozoans. In German cockroaches, the endemic species is *Gregarina blattarum*, which reside in the host gut. Despite being unicellular, gregarines are actually macroparasites as they do not undergo replication inside the host, but deplete host resources in a density-dependent manner (Randall 2011) and display an aggregated distribution (Takahashi 2004) within the host population. Hosts become

infected with gregarines by oral ingestion of oocysts from the environment, which contain infective sporozoites (Clopton and Gold 1996; Kuriyama et al. 2005). These sporozoites then attach to the gut epithelium and further develop into trophozoites (reproductive stage). The trophozoites subsequently detach from the epithelium and pair to form gametocysts, which are shed into the environment via the faeces (Logan et al. 2012; Gigliolli et al. 2016). The released gametocysts sporulate to form oocysts which are ingested by an uninfected host completing the life-cycle. Given that endemic parasites are aggregated in their host, differences in the host's parasite intensity have the potential to differentially affect the resources available to both the host and the parasite (Booth et al. 1993; Tocque 1993; Seppala et al. 2008).

The emerging epidemic pathogen in this model system is the generalist entomopathogenic nematode, *Steinernema carpocapsae*. This highly virulent parasite is capable of infecting a broad spectrum of insect hosts (Kaya and Gaugler 1993; Georgis et al. 2006; Heve et al. 2017). These nematodes replicate inside their host and cause epidemics within host populations (Griffin 2012). The non-feeding, third larval (L3) infective juvenile (IJ) stage of this nematode relies solely on energy reserves (lipids, protein and carbohydrate) derived from the host for its infectivity within the host population (Patel et al. 1997; Qiu and Bedding 2000; Qiu and bedding 2000; Fitters and Griffin 2006). Glycogen and protein are provisioned as alternative energy reserves during IJ development (Selvan et al. 1993; Qiu and bedding 2000), and are used up significantly when lipid reserves are depleted. The majority of studies, however, have demonstrated that lipids are the main long term energy reserve of non-feeding IJ (Fitters et al. 1997; Patel et al. 1997; Patel and Wright 1997, Wright et al. 1997; Fitters et al. 1999; Menti et al. 2000; Hass et al. 2002; Menti et al. 2003; Fitters and Driffin 2004; Andalo et al. 2011). The stored energy reserves, in particular, neutral lipids can be an important indicator of the quality (fitness) of nematodes (Abu Hatab et al. 1998), and the amount within an IJ can range between 32 and 43% of the total body weight (Selvan et al. 1993; Hatab and Gaugler 1997). Given the importance of lipids in insect systems, and for practical reasons of resource limitation, this study focuses on the IJ

lipid content as a proxy for overall resource allocation. Further, previous work on this study system has already demonstrated that lipids are an important mediator of interspecific resource competition between gregarines and *S. carpocapsae* (Randall et al. 2013).

Following penetration of the host cuticle barrier, the IJs migrate to the host's haemocoel where they release their symbiotic bacterium *Xenorhabdus nematophilus,* which kills the host by causing septicaemia (Park and Kim 2000; Boemare 2002; Lewis et al. 2006). After a series of within-host reproductive and developmental cycles, the nematode switches to the production of IJs, usually between two to ten days post-infection (Koppenhöfer et al. 1997; ÜNLÜ and ÖZER 2003; Fujimoto et al. 2007), when available nutrients are close to depletion (Selvan et al. 1993; Nguyen et al. 2001; Pena et al. 2015). The presence of another parasite, such as *G. blattarum* during coinfection, can potentially accelerate the depletion of host resources, altering their availability for the nematodes. Previously, a reduction in the transmission potential of *S. carpocapsae* was demonstrated when the host was coinfected with naturally acquired *G. blattarum* infection (Randall et al. 2013). Density-dependent depletion of host resources could also have potential consequences for the quality (energy reserves) of emerging infective juveniles and hence their ability to successfully find and infect a suitable new host.

Here, experimental infections were conducted, using the model cockroach host-two parasite system, to assess the impact of different gregarine intensities on the output of an epidemic parasite's transmission stages. As the host's main energy stores (lipid) mediate the interaction between both parasites (Randall et al. 2013), it was predicted that the host lipid resource would reduce in relation to an increasing level of endemic gregarine infection, and that this would result in a negative relationship between gregarine intensity and the number of IJs. Further, it was hypothesized that there would be a negative relationship between the quality (energy provisioning) of emerging IJs and gregarine intensity.

3.3 Materials and Methods

3.3.1 Host and parasite cultures

Final stage nymphs were collected from laboratory colonies of specific parasite free (SPF) German cockroaches. These SPF colonies have been maintained in the laboratories at Cardiff University School of Biosciences since 2007. All experiments were conducted under the same environmental conditions (25±1˚C, 30% ±2% humidity and a 12 L: 12 D photoperiod), with cockroaches maintained as previously described in Chapter 2.

Freeze dried Infective Juveniles (IJs) of the entomopathogenic nematodes (EPNs) *Steinernema carpocapsae* were donated by BASF Agricultural Specialities Ltd but were passaged through cockroach hosts before use in the experiment. To passage the nematodes, an infection arena was created by pipetting distilled water containing nematodes onto a sterile sand substrate in a plastic pot (275 ml vol., 11.5 cm dia. x 7.5 cm height) coated with Fluon®. Cockroaches were then added to the infection arena and removed within 2 hours of death (which occurred between 48 and 72 hours post-exposure to the nematodes). Dead cockroaches were placed on White's traps (White 1927) to culture the IJs and new infection arenas were produced on a weekly basis using these recovered IJs, in order to maintain stock levels. For all experimental infection, freeze dried IJs were passaged once in SPF cockroaches and only IJs emerging from this single passage were used.

3.3.2 Coinfection

(i) *Experimental gregarine infection*

Using a stereo-dissecting microscope (GX Optical XLT-101) with fibre optic illumination, translucent un-sporulated gametocysts (ca. 40 μm dia.) were collected into a petri dish (4 cm dia. x 1.2 cm height) containing 10 ml distilled water (see Chapter 2). Adult

females cockroaches were collected (N=240) and grouped (N=10 per group) into plastic rearing pots (275 ml vol., 11.5 cm dia. x 7.5 cm height) with perforated lids. These pots were divided into six treatment groups (N=4 replicate pots, i.e. 40 cockroaches, per treatment). Two treatment groups were left unexposed to gregarines and were used as negative (uninfected) and positive (infected only with nematodes) controls. The remaining cockroaches were exposed to one of four gregarine gametocyst treatments, (i.e. 5, 10, 15 or 20 gametocysts, which were administered by placement on ground dog food (0.063g), held within the lid of a 150 µl Eppendorf tube. Cockroaches were then fed water and food *ad libitium* 2 hours post-exposure in the same rearing pot. Cockroaches in each group were exposed to oocysts within the rearing pots for 10 days in CT rooms to allow for trophozoite development in the host gut.

(ii) *Entomopathogenic nematode exposure*

Infection arenas consisted of small round plastic pots (275 ml vol., 11.5 cm dia. x 7.5 cm height) coated with Fluon and 75 g of dry sterile sand substrate with 8 ml water containing IJs (100 nematodes/ml) pipetted onto the sand (as for infection arenas created for the passage above). Control arenas were produced in the same way except that the nematode suspension was exchanged for 8 ml of distilled water. All treatments were placed in infection arenas containing IJs (N=10, one infection arena per treatment group), while negative controls (N=10) were placed in the control arena. Each treatment group had four replicates (i.e. N=40 cockroaches per treatment). The cockroaches in all the arenas were monitored for up to 120 hours (in which time all cockroaches in the infected treatment had died) and the time of death for each cockroach was recorded. The cadaver's from each arena were transferred individually to White's traps (i.e. one cockroach per White's trap), containing 20 ml distilled water to isolate IJs. The suspension containing emerging IJs was collected into a 30 ml Falcon tube every five days from each White's trap, the suspension was topped up to 30 ml with distilled water and a subsample was counted using a Sedgewick Rafter counting chamber under a compound microscope (x 40 magnification, Olympus UCC/BY 501). The White's trap was refilled with water after each collection.

3.3.3 Lipid assay

Ten individual cockroaches per treatment were placed on separate white's trap. The IJs emerging on day 10 for each treatment were pooled (into vials) and the lipid levels within the IJs were assessed. Each treatment had 4 replicates i.e 4 vials per treatment, each containing pooled nematodes from 10 cockroaches. The IJs were washed three times by sedimentation using tap water and allowed to migrate through a 34 µm sieve overnight (Fitters et al. 1999). The concentration of neutral lipids present in these live IJs was determined, for all treatments, using a colourimetric method based on oil red O staining (Published methods of Andalo et al. 2011). Briefly, the stain was prepared by dissolving 0.5g of the oil red O dye (Sigma) in 100 ml of absolute ethanol under constant agitation for 15 min. The solution was passed through a filter paper to remove particles and subsequently stored at 5°C in the dark (Andalo et al. 2011). Concentrated and washed IJs (0.5 ml) were transferred into a test tube containing 3 ml of oil red O stain solution and incubated at 60°C for 20 min. The mixture was left to cool at room temperature allowing the IJs to settle (Andalo et al. 2011). The oil red O supernatant (ca. 2.5 ml) was discarded and the concentrated stained IJ suspension was added to 3 ml of water:glycerine (1:1) solution. Forty randomly selected red-stained IJs from each treatment were examined from wet mount slides and photographed under the same low lighting environment using a Leica DMRB bright field photomicroscope. The images were cropped to remove the background stain by carefully drawing around the outer edge of the IJs using an apple pencil in Procreate® for iPad. The stain backgrounds were removed so that IJs could be superimposed onto a white background to aid colour recognition and avoid detection of non-specific background stain. All visual analyses were conducted in ImageJ for windows version 1.50i (Rasband 2016). IJ total body area was measured using the 8-bit image type and the same threshold (254). The ImageJ threshold method involves dividing an image into two or more classes of pixels by filtering the image from its background (Rosin and Ioannidis 2003). The total area stained red was measured using an RGB colour threshold of Red=244, Green=100 and Blue=110, to determine the percentage of neutral lipids

(triacylglycerol) within the emerging IJs. All images were analysed using the same set scale (9.37 pixels/inch).

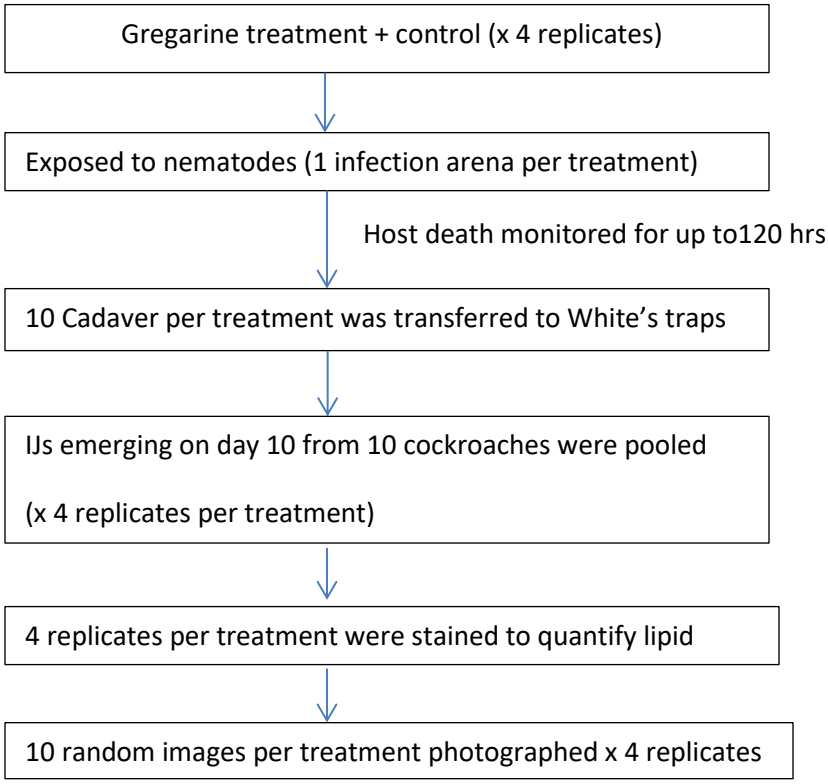


Figure 3.1: Representation of lipid assay experimental procedure. Specific parasite-free and gregarine infected cockroaches (5, 10, 15 and 20) were exposed to nematodes and only live emerging infective juveniles at day 10, were assessed for the concentration of lipid reserves.

3.3.4 Statistical Analysis

All statistical analyses were conducted using the R statistical programming software v.3.2.2 (R Core Team 2015).

Does infective juvenile emergence differ with gregarine burden?

A General Linear Model (GLM) with Gaussian error distribution and identity link function was used to determine the effect of gregarine burden on IJ output. The

dependent variable was transformed prior to analysis using natural log (Ln(x+1)) which resulted in a normal residual distribution. All second-order interactions between treatment, weight and sampling day were included in the starting model with host treatment, IJ count days and time to death included as categorical variables. The independent term, host gregarine treatment, was included as a categorical variable in the model. Insignificant terms were removed in a stepwise manner ($p>0.05$) and a *post-hoc* contrasts function using the R package "contrasts" (Max *et al.,* 2016) was used to assess differences between category levels where a categorical variable was found to be significant.

Does the host gregarine burden impact IJ quality?

The effect of host gregarine burden on the lipid reserves within the emerging infective juveniles was assessed using a generalized linear mixed model (GLMM), using a restricted maximum likelihood method in the ASReml-R package (v.4; VSN International Ltd.) The fixed model included the host gregarine burden and the replicates were added as a random term to account for pseudo-replication in the experimental design. The model residuals were checked for normality and the model was refined by stepwise deletion, using the log-likelihood ratio test for the random model and Wald test for the fixed model. The significant differences between groups were evaluated using the 95% limits.

3.4 Results

Effect of endemic gregarine infection on the output of *Steinernema carpocapsae*

Infective juvenile output was significantly associated with host treatment ($F_{4,789}=6.26$, $p<0.001$), time to death ($F_{3,789}=11.45$, $p<0.001$) and sampling day ($F_{3,789}=147.12$, $p<0.001$). There was no significant effect of host weight, nor the presence or absence of host oothecae on the emergence of IJs. All cockroaches infected with *G. blattarum* had significantly lower IJ output compared to singly infected hosts, however, there was

no significant difference in output between the different levels of gregarine infection (Fig. 3.2a). IJ emergence peaked at around 10 days and began to decline somewhere between 15 and 20 days (Fig. 3.3a) but this timing was not influenced by treatment. IJ output was affected by the hosts' time to death (Fig. 3.3b). Specifically, *S. carpocapsae* larval emergence from hosts that died at 48 and 72 h was higher than those that died at 96 and 120 h post-exposure.

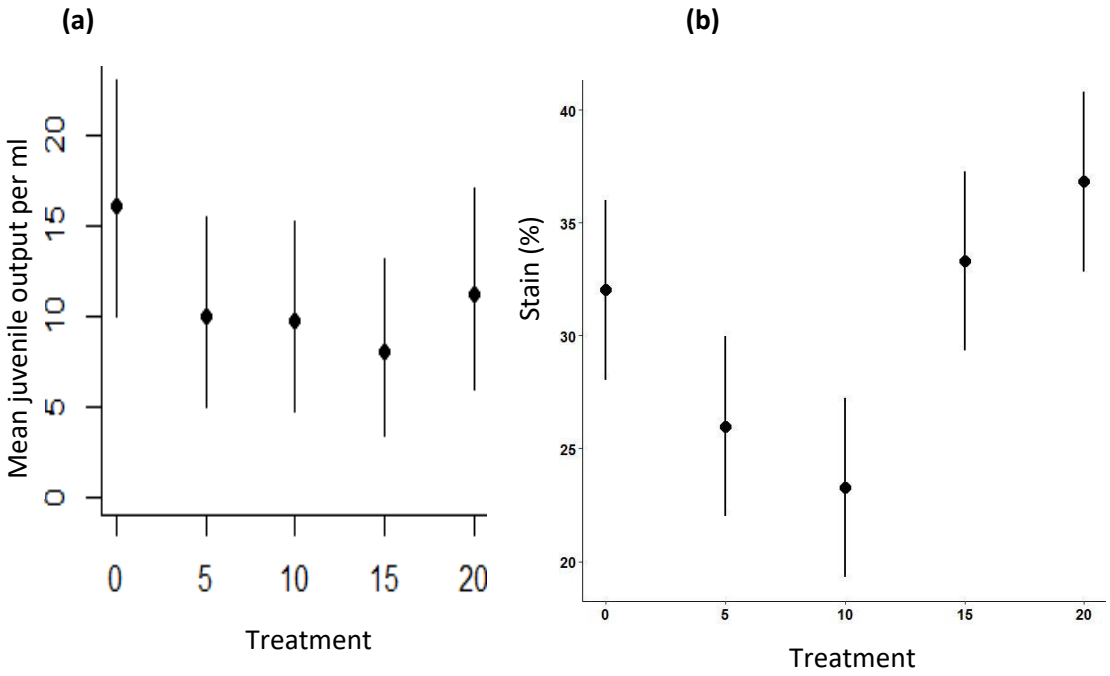


Figure 3.2: The effect of host treatment on the (a) mean juvenile output and (b) lipid levels of infective juveniles emerging from German cockroaches post-exposure to *Steinernema carpocapsae.* Black circles denote mean predictions of the model and bars represent the 95% confidence intervals.

Effect of endemic gregarine infection on lipid reserves

Energy reserves in emerging infective juveniles were significantly associated with host gregarine treatment ($F_{4,195}$=7.43, p<0.01). Specifically, IJs emerging from gregarine treatment of 5 and 10 gametocysts had lower lipid levels than singly infected hosts, equivalent levels at 15 and higher lipid levels at the 20 gametocysts (Fig. 3.2b, Table 3.1).

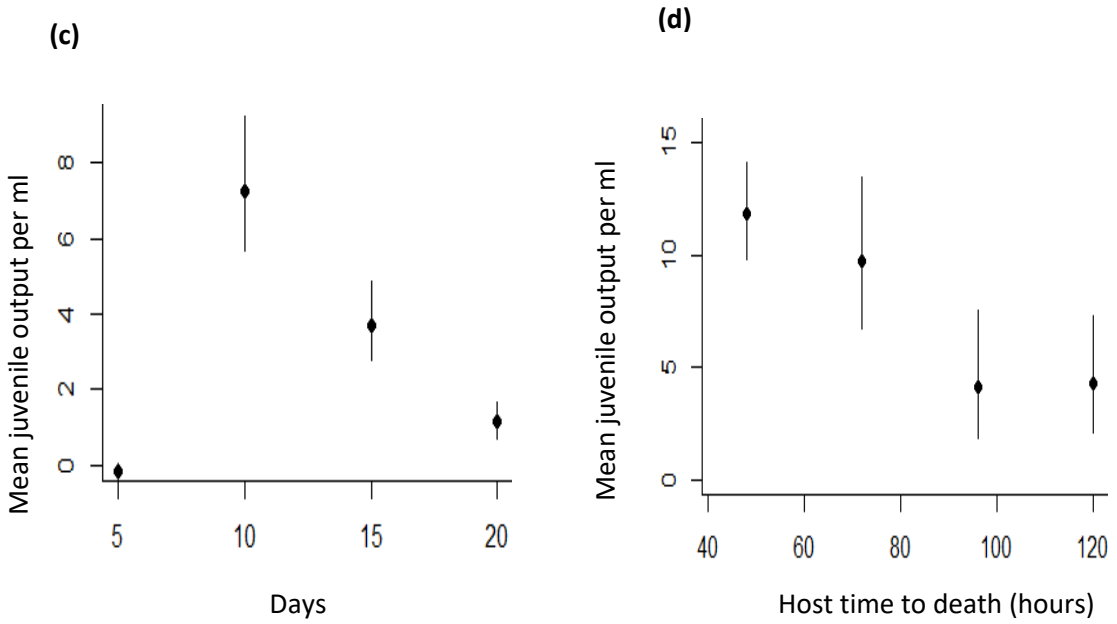


Figure 3.3: The effect of (a) sampling day and (b) hosts' time to death on the predicted mean infective juvenile output from German cockroaches post-exposure to *Steinernema carpocapsae*. Black circles denote mean predictions of the generalised linear model and bars represent the 95% confidence intervals.

Table 3.1: The mean difference in lipid reserves between the infective juveniles emerging during single and coinfection with *Gregarina blattarum*. Significance was assessed at the 95 % confidence level *p<0.01, **p<0.001, ***p<0.0001

Gametocyst exposure	**5**	**10**	**15**	**20**
0	-0.604	-8.75*	1.27	4.8
5		-2.70	7.31	10.84**
10			10.02**	13.54***
15				3.52

3.5 Discussion

Contrary to the hypothesis, the quantity of emerging epidemic parasite transmission stages (IJs) did not reduce with endemic parasite burden, although all levels of coinfection were associated with a significantly lower output of transmission stages than emerged from hosts with no endemic infection. In contrast, the quality of the emerging IJs did differ substantially with endemic parasite burden but in a non-linear fashion, which only partially supported the hypothesis that coinfection will negatively affect lipid provisioning in the emerging larvae.

Unexpectedly, IJs emerging from the highest level of gregarine treatment had higher lipid provisioning than the control cockroaches. Previous work has shown that gregarine infected female cockroaches ingested more food over time than uninfected controls, thus increasing their lipid intake (Randall 2011). The lipid levels in these hosts, however, remained lower than in uninfected controls, demonstrating that the cockroaches were unable to compensate for the loss due to infection (Randall 2011). Gregarines utilize host lipid for survival and the cytoplasm of gregarines does contain lipid deposits (Ciancio et al. 2001; Valigurova and Koudela 2005). Observations in our laboratory have also shown that, in highly infected hosts, gut trophozoites are numerous but small compared to gut trophozoites in low infection. It is possible, therefore, that the small trophozoites, in the heavily infected hosts, develop into gametocysts at a slower rate than larger trophozoites, and hence trophozoites may be retained for longer periods within hosts with high infection levels. As *S. carpocapsae* utilizes the entire cockroach resource, including its gut contents, it is possible that more lipids are available to *S. carpocapsae* in these heavily infected hosts, due to such retained gregarines.

Randall et al. (2013) demonstrated that the interaction between gregarines and *S. carpocapsae* is resource-mediated and that there was a negative correlation between the host's lipid resource and the parasite intensity within the host. It is perhaps surprising, therefore, that no difference was detected in IJ output between the

different levels of gregarine infection. One possible explanation could be that host resources were not fully exploited in the relatively short-term (10 days post-infection) standardized gregarine infection used in the current work. Randall et al. (2013), however, utilized a laboratory population of naturally infected gregarine individuals, where repeated infections were likely to have occurred over time. It has been shown in several systems that there is a strong correlation between the levels of infection at one point and repeated sampling from the same individual, even following clearance of the parasites by chemotherapeutics (Gryseels and Nkulikyinka 1989; Ahmed et al. 2012; Dunn et al. 2018; Ghosh et al. 2018). In simple terms, individuals tend to retain similar relative levels (low/high) of infection over time and hence those with high infections in the colony experiment may well have had chronically high infections for extended periods (and vice versa for low infections), meaning any resource depletion could be amplified through time.

An intriguing additional finding of this research, was that host time to death significantly influenced IJ quantity. One possible reason for the variation in output could be differences in the host immune response. It is possible that the immune response of these late death hosts is more effective against *S. carpocapsae,* which in turn, may have resulted in a reduced output of transmission stages. Alternatively, it is possible that early emerging *S. carpocapsae* IJs are more infective (i.e. shorter time to infect) (Therese and Bashey 2012). Early emerging IJs of the *Heterorhabditis megidis*, another entomopathogenic nematode species, have been shown to display better host finding ability compared to late-emerging IJs (O'Leary et al. 1998). It is, therefore, possible that well-provisioned IJs succeeded in infecting the hosts at 48 and 72 h, while poorer quality IJs infected the host at 96 and 120 h post-exposure.

The non-feeding emerging IJs adopt a ‘sit and wait’ ambush strategy in the environment, where they are dependent solely on the energy reserves provisioned during their larval development (L1 and L2) (Fitters et al. 1997; Medica and Sukhdeo 1997; Hass et al. 2002; Brivio et al. 2004). The current study suggests that the resource-

mediated interactions between parasites can affect not only the output but also the quality (fitness) of the IJs. Previous laboratory investigation has shown that about 66% of the hosts' sampled from the infected colonies were infected with the endemic gregarine parasites and that parasite aggregation (and hence the variation in parasite intensity) varied with time (Randall 2011). It is possible that the improved lipid provisioning in IJs emerging from the highly infected hosts, in the current study, could mitigate their reduced output. The IJs from the highly infected hosts may survive for long periods in the environment, or be more infective, which in turn may alter the spread of epidemics.

In summary, the current study suggests that resource mediated interactions can affect both the quantity and quality of epidemic parasite transmission stages and that this effect can be density-dependent. Endemic macroparasites show aggregated distributions and density-dependent variations would mean that hosts would carry the different burden of infection (high or low). Considering the combination of both infection burden and aggregation of endemic parasites may be necessary for accurate prediction of epidemic transmission and disease outbreaks. Future work should, therefore, focus on how different distributions of endemic parasites may alter the dynamics of between-host transmissions and disease emergence at the population level.

Chapter 4: Population-level effects of an endemic infection upon epidemic dynamics

4.1 Abstract

Despite the growing concern of emerging infectious diseases, very few studies have examined the effect of coinfection on disease dynamics and progression within host populations. Under natural conditions, coinfection of a host by multiple parasite species is commonplace. A critical challenge for epidemiological studies is linking how within-host interspecific interactions scale up to alter disease dynamics at the population level. Here, using a laboratory-reared population of German cockroaches that were experimentally infected with different levels of endemic gregarine infection, the fitness cost of coinfection on the between-hosts transmission of the epidemic parasite *Steinernema carpocapsae* were assessed. The transmission stages of this epidemic parasite are non-feeding, and are, therefore, dependent on the lipid energy reserves provisioned during development for both their survival in the environment and transmission into a new host. A curvilinear relationship between the host-parasite burden and lipid provisioning within the transmission stages has been demonstrated, such that low level endemic (gregarine) infection was detrimental to *S. carpocapsae* transmission stage lipid stores, while high gregarine levels had a positive effect. It is hypothesized that the transmission dynamics of the epidemic *S. carpocapsae* within the host population would be dependent on lipid provisioning of the transmission stages emerging at different levels of endemic gregarine infection. Groups of specific parasite free German cockroaches were infected with different intensities of endemic gregarines and then subsequently exposed to *S. carpocapsae* together with uninfected controls. Following *S. carpocapsae* host-induced death, populations of specific parasite free cockroaches were exposed to the donor (cadaver) from each of the coinfected and single infection groups, and subsequently, the time of host death was monitored post-

exposure to the donor. In support of the hypothesis, the transmission dynamics of *S. carpocapsae* within the host population increased in relation to lipid provisioning within the transmission stages. In particular, mortality was reduced when the hosts were exposed to donors coinfected with low endemic infection burden compared to high burden or single infection donors. This study demonstrates that where host lipid resources mediate the interaction between parasites, different patterns of parasite aggregation can alter the spread of epidemics within the host population. The study suggests that taking into account the impact of heterogeneity in parasite burden, together with coinfection could be crucial in order to achieve accurate prediction of epidemic outbreaks.

4.2 Introduction

Coinfection of a host by multiple parasites is the norm in natural systems (Cox 2001; Lello et al. 2004; Fenton et al. 2008). A significant number of studies suggests that the interactions (direct or indirect) between co-infecting parasites can impact the spread of infectious diseases (Bruce et al. 2000; Abu-Raddad et al. 2006; Wearing and Rohani 2006; Vasco et al. 2007; Lello et al. 2013; Ezenwa and Jolles 2015). In recent decades, disease ecologists have increasingly focused on understanding how these interactions between parasites may shape disease dynamics or infection outcome within a host population (Pedersen and Fenton 2007; Graham 2008; Telfer et al. 2010; Hoverman et al. 2013). In natural systems, the pattern and spread of diseases are observed at the host population level, while the potential impact of the mechanism of parasite interaction (and infection outcome) occur within-host (Viney and Graham 2013). Linking how the within-host/individual-level processes scale to alter the between-host transmission of a parasite lies at the forefront of disease ecology and poses a critical challenge for epidemiological studies (Mideo et al. 2008). Although, some studies have highlighted the potential impact of within-host processes on disease dynamics (Abu-Raddad et al. 2006; Jolles et al. 2008; Telfer et al. 2010; Ezenwa and Jolles 2011;

Gorsich et al. 2018), there is still a significant gap in understanding of how these processes alter between-host transmission. The output and quality of an epidemic parasite can be altered as a result of coinfection with an endemic parasite (i.e. reduced output of epidemic parasite transmission stages compared to singly infected hosts and non-linear effect of endemic gregarine burden on lipid levels in epidemic infective juveniles, see Chapter 3), therefore, a change in the transmission dynamics within the host population as a result of these within-host effects might be expected. Disentangling the complexities that arise from the non-linear individual-level processes (Abu-Raddad et al. 2006; Vasco et al. 2007; see Chapter 3), is not only essential for obtaining a predictive understanding of how individual-level processes scale up to modify between-host dynamics of diseases but also crucial for effective disease control strategies.

Endemic parasites (e.g. gastrointestinal protozoans) often survive in the host population for a protracted period of time and have the potential to alter infection dynamics during coinfection (Wuerthner et al. 2017; Lello et al. 2018). Most endemic parasites show an aggregated distribution within their host population where, a small proportion of highly infected hosts are responsible for the majority of parasite transmission (Woolhouse et al. 1997; Shaw et al. 1998; Hupalo et al. 2014). Within a host population, individuals will harbour different burdens of an endemic parasite as a result of parasite aggregation (Poulin 2013; Wilber et al. 2017). Such variations in parasite burden would also mean that the degree of resource exploitation will vary between hosts. Differences in the host-parasite burden can potentially influence within-host parasite interactions, which may alter the spread of an epidemic pathogen between-hosts (Susi et al. 2015). There is, therefore, a need for detailed experimental studies that are tailored to discern how these potentially non-linear within-host processes (occurring at the individual level; see Chapter 3) might culminate to influence population-level disease dynamics.

Parasite transmission is a fundamental process, critical for understanding host-parasite interactions and its effect on disease spread within the host population (McCallum et al. 2001). Transmission is a function of interactions between the host, parasite and environmental components (consisting of, the production of exiting/departing infective stages, parasite survival outside the host and entry into an uninfected host). Figure 4.1 highlights factors that can potentially lead to non-linearity in parasite transmission within the host population. For example, host factors such as age have been shown to affect the proliferation of endosymbionts in both *Manduca sexta* and *Cimex lectularius* (Eleftherianos et al. 2006; Fisher et al. 2018). Differences in parasite strain/genotype (Jensen et al. 2006; Salvaudon et al. 2007; Little et al. 2008; Pulkkinen et al. 2009) and changes to the environmental components have also been highlighted to shape parasite transmission (Patz et al. 2004; Aguirre and Tabor 2008; Lambin et al. 2010; McFarlane et al. 2013), also discussed extensively by Cable et al. (2017).

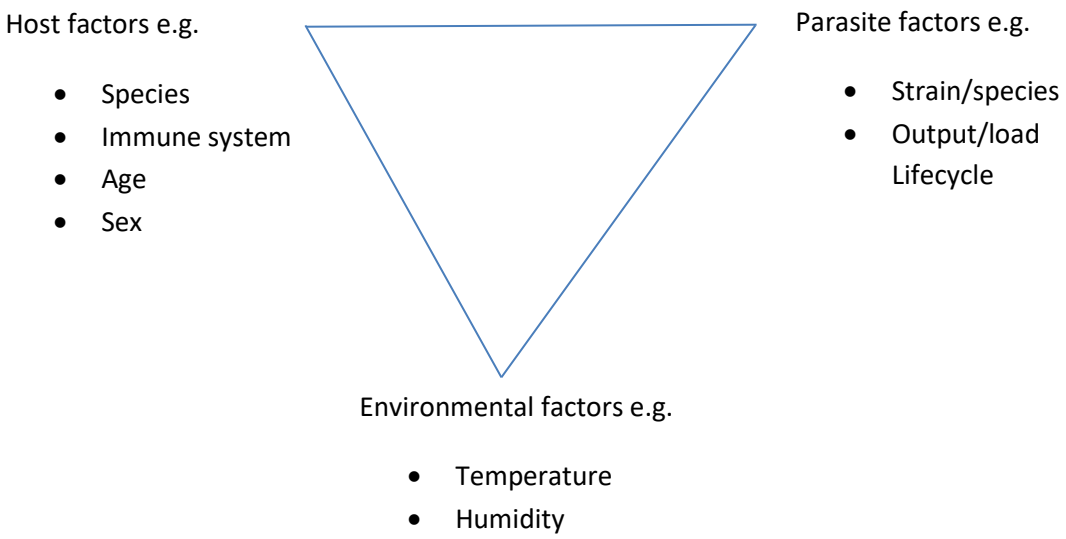


Figure 4.1: Key factors that contribute to non-linearity in parasite transmission.

The list above is not exhaustive but highlights key factors that may change transmission pattern under natural conditions. In the current model system, it has been previously demonstrated that the host characteristics, in this case, coinfection, can alter the output of transmission stages (Randall et al. 2013, Chapter 3). The interspecific

interactions between parasites have the potential to add further complexities to the biology of parasite transmission, and this highlights the need to develop a better understanding of how the altered characteristics (quality) of a parasite (during coinfection) may impact disease dynamics within the population.

In this study, controlled experimental infections were conducted to explore the fitness cost of the endemic protozoa, *Gregarina blattarum* on the transmission of a secondary epidemic parasite *Steinernema carpocapsae* in a laboratory population of the German cockroach (*Blattella germanica*). Host susceptibility to the epidemic parasite is not affected by the presence of an endemic infection (see Chapter 2). Here, this study explores whether individual-level effects (see Randall et al. 2013; Chapter 3) scale up to alter the between-host disease dynamics, with a specific focus on how differences in the lipid provisioning of transmission stages may alter transmission. Host lipid stores mediate the interaction between both parasites during coinfection (Randall et al. 2013). The transmission stages of the epidemic parasite are non-feeding, therefore, successful transmission into a new host and survival in the environment is dependent on the lipid energy reserves provisioned during their development (Qiu and Bedding 2000; Abu Hatab and Gaugler 2001; Menti et al. 2003). It is hypothesized that gregarines will reduce between-host transmission of *S. carpocapsae* but, this will be mitigated in populations exposed to the highly infected donor due to better parasite quality (Chapter 3).

4.3 Materials and methods

4.3.1 Endemic macroparasite infection protocols

Newly developed adult female cockroaches were collected (N=120) from specific parasite free (SPF) colonies and grouped (N=10) into plastic rearing pots (275 ml vol., 11.5 cm dia. x 7.5 cm height) with perforated lids (see Chapters 2 & 3). These pots were divided into six treatment groups (N=2 replicate pots i.e. 20 cockroaches per

treatment). Two replicate pots were exposed to one of the four gregarine gametocysts treatments (5, 10, 15 or 20 gametocysts). The remaining pots were left unexposed to gregarines and were used as negative and positive controls. Cockroaches were fed food and water *ad libitium* 2 hours post-exposure in the same rearing pots. All experiments were conducted under the same conditions as previously described in Chapters 2 & 3 (25±1˚C, 30% ±2% humidity and a 12 L : 12 D photoperiod).

4.3.2 Nematode infection protocols

Infection arenas were created using round plastic pots (Cater For You; 180 x 120 x 75 mm) coated with fluon (see Chapters 2 & 3), lined with 75 g of dry sterile sand substrate, and 8 ml of distilled water containing *S. carpocapsae* (100 nematodes/ml, BASF Agricultural Specialities Ltd) was pipetted onto the substrate. Each group of gregarine infected cockroaches and the positive control described above were placed in an infection arena, while the negative control was introduced to a similar arena but with 8 ml of distilled water only. Cockroaches in the nematode infected treatments died after 48 and 72 hours post-exposure. A cadaver from each group (coinfected and single infection) was used as a donor for the population-level study (naive SPF host).

4.3.3 Experimental set up for population-level effects of coinfection

Female SPF cockroaches (N=60) were collected and grouped into six (N=10, per treatment) small rectangular colony boxes (31 x 23 x 12 cm height) coated with fluon® and lined with dry sterile sand (260 g) of sufficient depth to fill an inverted petri dish lid (5 cm). The area of the sunken petri dish was pipetted with 8 ml of distilled water and a donor (cadaver) from the coinfected and single nematode infections was placed on the dampened, sand-filled petri dish within each colony box. The petri dish within the negative control group was treated in the same manner but without the introduction of a cadaver. The sand in the petri dish was kept damp to prevent desiccation of emerging infective juveniles. Each treatment group had 15 replicates (Table 4.1). All cockroaches in each colony box were provided with food and water *ad libitium* for the

duration of the study. Time to death of individual cockroaches within the populations was monitored every hour (24 hour/day) for 15 days.

Table 4.1: Representation of the experimental procedure. Recipient cockroaches (N=10, per replicate) were exposed to donor cockroaches (Gregarine infected, with 5, 10, 15 or 20 gametocysts, NC=negative control and PC= positive control, N=15 per treatment) placed within a dampened petri dish (5 cm) lid in the colony box, for the duration of 15 days.

Donor cockroach (cadaver) gametocyst treatment	**Donor nematode exposure**	**Recipient female cockroaches per colony box**	**Replicates**
Gregarine uninfected (SPF) - NC	Control- no cadaver	10 SPF	15
Gregarine uninfected (SPF) - PC	Yes	10 SPF	15
Gregarine infected-**5**	Yes	10 SPF	15
Gregarine infected-**10**	Yes	10 SPF	15
Gregarine infected-**15**	Yes	10 SPF	15
Gregarine infected-**20**	Yes	10 SPF	15

4.3.4 Statistical analysis

All statistical analyses were conducted using the R statistical package version 3.2.2 (R Core Team 2015). A subset of the data was created excluding the negative controls since no deaths occurred during the experimental period of 15 days. The effect of gregarine burden on between-host transmission was assessed by Kaplan-Meir survival analysis using the Survdiff function in the R "survival" package (Therneau 2015). A Cox proportional hazard model with a Gaussian distribution for random effects was created to incorporate the repeated sampling of each gregarine infection treatment, using the ''Coxme'' package (Therneau et al. 2003). The time taken for death of individual cockroaches within the population to occur and the censor (i.e. 0=unobserved death, 1=observed death during the study period) were included in the model as fixed effects while the treatment replicates were added as a random effect. Significance was assessed using the Chi-squared test statistic and associated p-value (with $p<0.05$ taken as significant).

4.4 Results

Host survivorship was significantly affected by treatment (X^2 = 13.8, df = 4, p<0.01, Table 4.2). In particular, hosts exposed to nematodes emerging from donors 5 and 10 had significantly higher survivorship rates compared to the donor from single infections, while survival rates equivalent to the positive controls were observed at 15 and 20 (Fig. 4.2). Host exposure to a positive control (nematode only) resulted in 86% mortality, compared to the coinfected groups that experienced 76, 77, 83 and 82% mortality following infection with, 5, 10, 15 and 20 gregarine gametocysts. When considering the overall risk of host death, i.e. percentage mortality by the end of the experiment, this was significantly affected by gregarine treatment (X^2 = 95.7, df = 4, p<0.001).

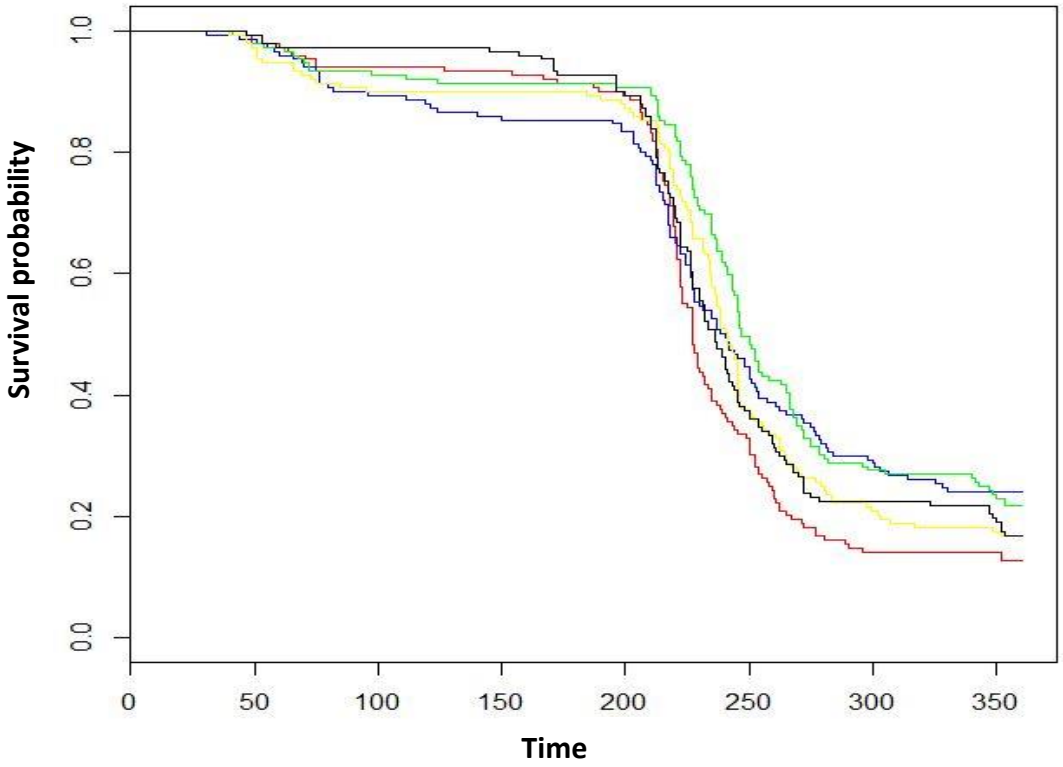


Figure 4.2: Kaplan-Meier survival probability of SPF cockroaches post-exposure to the donor from 0 (red), 5 (blue), 10 (green), 15 (yellow), and 20 (black) gametocysts for an experimental duration of 15 days, excluding negative controls. For visual clarity, the confidence intervals were removed.

Table 4.2: Cox mixed effect model fit by maximum likelihood. A decreased coefficient indicates increased survival probability when compared with gregarine free host. The hazard ratio is a comparison of survival probability between the gregarine treatments and the gregarine free host.

Fixed coefficients

Donor treatment	Coefficient	Hazard ratio	Standard error	Z value	P-value
5	-0.32	0.72	0.13	-2.44	0.01
10	-0.49	0.61	0.13	-3.71	0
15	-0.23	0.8	0.13	-1.77	0.08
20	-0.13	0.87	0.13	-1.04	0.3

Random effect

Group	variable	Standard deviation	variance
replicate	intercept	0.49	0.24

4.5 Discussion

In support of the hypothesis, the transmission dynamic of *Steinernema carpocapsae* within the host population were mitigated in populations exposed to donors with low gregarine burden. Using a host-two parasite system, the current study demonstrated that coinfection can alter the disease outcome of a naive host population. Specifically, the current finding revealed reduced mortality when the hosts were exposed to transmission stages emerging from donors coinfected with low endemic infection burdens compared to high burden or single infection donors. This finding does not agree with previous work by Susi et al. (2015), where more devastating epidemics were reported in populations with a higher level of coinfection when compared to a single infection. In natural conditions, endemic parasites vary in intensity (Poulin 2013) and the transmission potential of an epidemic parasite is reduced during coinfection with

an endemic parasite (Randall et al. 2013). In previous work (Chapter 3), a curvilinear relationship between coinfection load and parasite lipid content was demonstrated, where the lipid content within the epidemic parasite transmission stages was reduced at low gregarine burden but increased at high burdens. The current study builds on this finding by confirming that the donor response to different levels of gregarine infection in this model system impacts the disease dynamics of *S. carpocapsae* at the population level. The results indicate that intensity-driven changes in the host during coinfection carry key consequences for between-host transmission.

Randall et al. (2013) previously showed that the host lipid resource, which mediates the interaction between the endemic and epidemic parasites in this system, is depleted during coinfection. Since the output of transmission stages emerging from coinfected hosts are reduced compared to single infection (Randall et al. 2013, Chapter 3), a lower rate of transmission in populations exposed to the coinfected donor might be expected. Here, the effect is lost in populations exposed to donor infected with high levels of gregarines, probably because the reduced output is offset by an increased parasite quality (see Chapter 3). The result from this study does not agree with similar studies (Duncan et al. 2003; Campos-Herrera et al. 2012; Campos-Herrera et al. 2015), where resource competition between nematodes had no effect on the transmission of entomopathogenic nematodes. The current result suggests that competition for host resources may not solely mediate the interaction between both parasites used in this study system. Immune-mediated interactions between parasites have the potential to alter both the transmission and disease prevalence within the host populations (Eswarappa et al. 2012; Tollenaere et al. 2016). It is possible that the host immune response may have altered the fitness of the transmission stages emerging from the coinfected donor, which may explain survivorship in the current data (Fig. 4.2). Some studies have demonstrated that an altered immune response due to coinfection can mitigate the pathogenic effect of a coinfecting parasite, thus enhancing survivorship (Onah et al. 2004; Balmer et al. 2009; Gonzalez et al. 2018; Long et al. 2019).

An increasing number of studies have highlighted the significant role of resource availability in mediating the host-parasite interactions (Becker and Hall 2014; Cressler et al. 2014; Becker et al. 2015; Civitello et al. 2015; Knutie et al. 2017; Strandin et al. 2018), immune response (Chandra 1996; Sheldon and Verhulst 1996; Martin 2nd et al. 2007; Ezenwa and Jolles 2011; Budischak et al. 2015; Forbes et al. 2016) and the epidemiology of diseases (Civitello et al. 2018). Differences in host resource availability which are required for within-host processes and immunity can drive variation in transmission (and parasite virulence) at the host population level. A decrease in host infectivity has also been linked to the lipid reserves provisioned during larval development of the transmission stages (Fitters et al. 1997; Menti et al. 2000; Hass et al. 2002). It is possible that the non-feeding transmission stages, emerging from low-level endemic infection are less active and could not survive for long periods in the environment, thus impacting on the rate of transmission observed in the current study. Entomopathogenic nematodes are widely used as biological agents against an array of insect pest and are a proven replacement for chemical pesticides (Labaude and Griffin 2018). Empirical data from the current study highlights that in natural conditions where multiple infections of a host are the norm, differences in the quality and quantity of a parasite transmission stages have the potential to alter the efficacy of entomopathogenic nematodes used as a biocontrol agent.

The findings from this study demonstrate a pattern that mirrors the impact of non-linear within-host processes (parasite lipid provisioning, see Chapter 3) as a result of endemic infection burden, on the subsequent epidemic transmission between-hosts. The study suggests that the differences in lipid-parasite interactions could lead to different likelihoods of an epidemic outbreak. This calls for further investigation on the consequences of within-host fitness of a parasite on between-host transmission, in particular where host resources mediate the interaction. Taking into account the impact of coinfection on the within-host infection dynamics, may be central for effective disease control programs and predicting the outbreak of an epidemic pathogen.

Chapter 5: General Discussion

5.1 Summary

As most host populations are coinfected with macro- and microparasites, it is important to determine whether interspecific interactions between these parasite groups could shape within-host parasite dynamics and the spread of microparasitic infection. Macroparasites tend to be less well studied compared to microparasites, probably because macroparasite infections generally lead to morbidity rather than mortality, and their effects are usually density-dependent, meaning that low infection levels within a host tend to be less virulent. Conversely, the majority of high virulence pathogens are microparasites. Interactions between both macro- and microparasites, whether direct or indirect, have the potential to alter host immune response, parasite dynamics, disease severity and the effectiveness of control strategies. This study aimed to explore the potential role of macroparasites as a driver of microparasitic infection. Given that macroparasites typically show aggregated distributions, and therefore a wide range of intensities occur within a host population, this study also sought to determine the role of different macroparasite infection intensities upon infection dynamics. The key findings in this study demonstrate that macroparasites have a significant effect on the host immune response to infection, but this effect did not translate to altered susceptibility to infection with the model microparasite (Chapter 2). The quantity of microparasite transmission stages emerging from hosts was reduced due to macroparasite coinfection, but this was not macroparasite density-dependent. In contrast, the macroparasite coinfection load significantly altered the quality of microparasite transmission stages (Chapter 3). Ultimately, the transmission dynamics of the epidemic microparasite within the host population was dependent on the quality of transmission stages emerging from the coinfected host (Chapter 4).

5.2 Endemic infection and within-host interactions

Endemic macroparasites maintain a constant presence within the host population, and can negatively affect host health and significantly alter population dynamics (Stien et al. 2002; Lello et al. 2005; Randall 2011; Rose et al. 2014; Charlier et al. 2015; Weinstein and Lafferty 2015). In natural conditions, where coinfection of a host is the norm (Cox 2001; Griffiths et al. 2014; Ezenwa 2016), previous experience of an infection has the potential to alter the immune response against another parasite species (Resende Co et al. 2007; Cattadori et al. 2008; Graham 2008; Hoverman et al. 2013; Su et al. 2014; Tollenaere et al. 2016), and this can have consequences for disease susceptibility and severity (Su et al. 2005; Su et al. 2006; Chung et al. 2012; Budischak et al. 2015). Macroparasites are commonly over-dispersed within the host populations, with the majority of the parasite population concentrated in a minority of hosts (Poulin 2013). Heterogeneities in the host ability to transmit parasites can have implications for parasite dynamics and control measures (Keeling and Eames 2005; Lloyd-Smith et al. 2005; Tildesley et al. 2010). However, most studies do not take account of the heterogeneity in endemic parasite intensity between hosts within a population and how this might alter the strength of the host immune response. Given the differences in endemic parasite burden between-hosts, the interaction between coinfecting parasites may also vary.

This study revealed that there were gregarine (macroparasite) density-dependent effects on the host immune response (Chapter 2). In particular, the suppressed levels of phenoloxidase and lysozyme activity were found to be macroparasite density-dependent. However, these differences in immune response did not translate into a difference in susceptibility to the microparasite used in this study. As an increased immune response was seen in gregarine infected hosts, it is possible that the observed increase in phenoloxidase and lysozyme would be effective against a less virulent microparasite, but is insufficient to act against the highly virulent microparasite used in this study. The increased immune response in the current study is consistent with

other invertebrate studies demonstrating that the immune response can be upregulated by prior exposure to a pathogen and that this upregulation protects the host from secondary infection (Sadd and Schmid-Hempel 2006; Pham et al. 2007; Roth et al. 2008). For example, in the mealworm beetle, *Tenebrio molitor*, hosts immune responses were upregulated by Gram-positive bacteria and were highly protected against both Gram-positive and negative bacteria compared to those primed with Gram-negative bacteria, the protection, in this case, was attributed to increased haemocytes and antibacterial response (Dhinaut et al. 2018). The immune system can be biased by an upregulation towards a parasite such that the ability to respond effectively to another parasite is impaired (Salgame et al. 2013). In vertebrates, for example, the upregulation towards one arm of the immune response against macroparasites may negatively affect the efficacy of vaccines to emerging infections (Su et al. 2006; Iweala et al. 2007; Urban Jr et al. 2007; van Riet et al. 2007; Aira et al. 2017). There is scope for expansion of the current study, by exploring the molecular mechanisms that underlie the immune function, including the specificity of the immune response to endemic gut gregarines.

Despite a number of studies on gregarine abundance, host specificity and the effect of gregarines on host behaviour and life history (Clopton and Gold 1996; Clopton 2000; Marden and Cobb 2004; Takahashi 2004; Randall 2011; Kaunisto et al. 2017), there is a limited knowledge of how the host immune systems respond to the presence of endemic gregarine infections. An important general invertebrate strategy against the presence of a macroparasite (including gregarines), is the encapsulation response (Gillespie et al. 1997; Rolff and Siva-Jothy 2003; Ahtiainen et al. 2005; Strand 2008; Ilvonen and Suhonen 2016; Krams et al. 2017; Srygley and Jaronski 2018). The cellular encapsulation response is a common feature of insects against parasites that are too large to be phagocytosed by a single haemocyte (Strand 2008; Hillyer 2016). The encapsulation process involves the recognition of foreign pathogens as non-self, the activation of haemocytes (e.g. granulocytes) that migrate towards the pathogen, adhere and form a multi-layered capsule around it (Siddiqui and Al-Khalifa 2014).

The degree of encapsulation can vary with the host resource availability, age, sex, location of invading pathogen, and the presence of coinfecting parasites (Lie and Heyneman 1976; Giordanengo and Nenon 1990; Koella and Sorensen 2002; Bukovinszky et al. 2009). These factors could alter the quantity of circulating haemocytes, which in turn could impact the host encapsulation response (Ratcliffe 1985). Conversely, a prior pathogen infection could prime the immune system such that, the risk of future infections is reduced (Hamilton et al. 2008). Immune priming has the potential to generate non-linearities in parasite transmission and host susceptibility to infection (phenotypes with enhanced resistance), which may have important implications for disease dynamics within the host population (Moret 2006; Blagrove et al. 2012; Frentiu et al. 2014). Despite the lack of antibodies associated with vertebrates, the invertebrates are capable of mounting a primed immune response to secondary infection (Little et al. 2003; Schmid-Hempel 2005; Roth et al. 2008). Invertebrates achieve this by inducing major signalling pathways of the immune system that encode transmembrane proteins (Lemaitre et al. 1996; Rainey et al. 2014; Sim et al. 2014).

As stated in Chapter 2, there was no difference in encapsulation between the gregarine infected and uninfected groups. This finding is consistent with other studies that found no effect of gregarine burden on the host cellular encapsulation response (Siva-Jothy et al. 2001; Canales-Lazcano et al. 2005; Honkavaara et al. 2009). The discrepancy between the current finding and the previous work in this laboratory (Randall 2011), which showed that encapsulation response was greater in gregarine infected hosts, may be a consequence of trans-generational immune priming (Little et al. 2003; Moret 2006; Freitak et al. 2009; Roth et al. 2010; Tidbury et al. 2010; Hernández López et al. 2014). The previous work (Randall 2011) assessed encapsulation response from hosts collected from gregarine infected colonies, whereas the current study utilized hosts from gregarine free colonies that were then experimentally infected for a short period. An increasing number of studies have highlighted the impact of immune primed phenotypes on the spread of a secondary infection (Little and Kraaijeveld 2004;

Blagrove et al. 2012; Tate and Rudolf 2012; Tidbury et al. 2012; Frentiu et al. 2014; Dorigatti et al. 2018; Pereira et al. 2018; Nazni et al. 2019; Rahayu et al. 2019). Whilst, the current study did not aim to explore these effects, the model provides an ideal system that can be manipulated to explore immune primed phenotypes and its effect on coinfection both at the individual and population level. Further development of this study, could also explore the level and form of circulating haemocytes (e.g. granulocytes, plasmatocytes, lamellocytes) which are essential for encapsulation (Márkus et al. 2005; Cotter et al. 2008; Ebrahimi et al. 2011).

Contrary to the hypothesis in Chapter 2, macroparasite density-dependent effects on host immunity did not translate into a difference in susceptibility to microparasite infection. One limitation of the current study is that the effects of inbreeding and genetic variation of the host (cockroach lines established from initial purchase and bred in the laboratory since 2007) are unknown. A lack of genetic diversity as a result of genetic drift or inbreeding could mean that the ability of the host to resist new infections or adapt to new selection pressure is reduced (Frankham et al. 2002; Keller and Waller 2002; Whitehorn et al. 2010). The cockroaches in the current system are frequently subjected to bottlenecks as a result of periodic changes of the colony boxes (to avoid cockroach escape when the Fluon® needs replacing), which may result in genetic homogenization. However, the cockroach population are characterized by exponential growth (Ross 1976; Ross et al. 1984), which may reduce the potential impact of such genetic bottlenecks (Kimura 1983; Hague and Routman 2016). Future development could analyse the host genotype, compare responses of genetically distinct strains and link the immune-related genes to infection susceptibility.

5.3 Linking within-host infection dynamics with between-host disease dynamics

Parasites are one of the most abundant forms of life on earth (Windsor 1998), and understanding the changes in parasite fitness that may occur as a result of interspecific interactions, is important for predicting their effect on host populations (Gandon and

1991 Michalakis 2000; Antia et al. 2003; Dieckmann et al. 2005; de Roode et al. 2008).
1992 Parasites must undergo some within-host development, but this within-host process
1993 depletes host resources, induces tissue damage or provokes the immune response, all
1994 of which have the potential to indirectly shorten the window of parasite transmission
1995 (de Roode et al. 2008; Méthot 2012). Parasites, therefore, face a trade-off between
1996 prudent within-host replication and transmission (Anderson and May 1979, 1982;
1997 Galvani 2003; Dieckmann et al. 2005; de Roode et al. 2008; Alizon et al. 2009;
1998 Doumayrou et al. 2013). Whilst, the current study has focussed on the effect of a
1999 macroparasite on microparasite transmission, the macroparasite transmission might
2000 also be affected during coinfection. The presence of an epidemic parasite could
2001 potentially disrupt the balance between prudent within-host exploitation and
2002 transmission when the host is already infected with a macroparasite, and this can have
2003 implications for the macroparasite transmission (vanBaalen and Sabelis 1995; Frank
2004 1996), because a virulent epidemic parasite may not be well adapted to evolve such a
2005 balance but rather maximize transmission. Coinfection may further limit the
2006 opportunity for an epidemic parasite to evolve the best evolutionary strategy
2007 (Bremermann and Thieme 1989). When parasites co-occur within the host, the fitness
2008 of the transmission stages could depend upon the interspecific interactions between
2009 parasites (May and Nowak 1995; vanBaalen and Sabelis 1995; Martcheva and Pilyugin
2010 2006). For example, some studies have demonstrated that coinfection between
2011 microparasites can result in increased parasite virulence (Ebert and Mangin 1997;
2012 Escriu et al. 2000; Cooper and Heinemann 2005; De Roode et al. 2005) while other
2013 studies have documented reduced parasite virulence (Turner and Chao 1999; Read and
2014 Taylor 2001; Gower and Webster 2005; Harrison et al. 2006). The differences in
2015 virulence in these studies have been attributed to the exploitation rate of the parasite,
2016 selection pressure and host immune response. However, for the majority of studies
2017 that explore parasite fitness and disease transmission, the potential impact of a host-
2018 parasite burden on the fitness of transmission stages during coinfection has received
2019 very little attention.

The transmission of an epidemic parasite may be altered by the presence of another parasite (Chapter 3), but the results from this study do not agree with the majority of theoretical studies that have predicted increased parasite fitness as a result of increased host exploitation (Levin and Pimentel 1981; Frank 1992; May and Nowak 1994, 1995; vanBaalen and Sabelis 1995; Frank 1996; Mosquera and Adler 1998). The results, however, supports recent theoretical and empirical studies that have considered the impact of coinfection on parasite transmission and predicted decreased parasite fitness depending on the nature of interaction (Gandon et al. 2001; West and Buckling 2003; Massey et al. 2004; Gower and Webster 2005; Harrison et al. 2006). In Chapter 4, the results demonstrate that, where host resources mediate the interaction between parasites during coinfection, the burden of macroparasite infection can be a major determinant of the outcome on parasite transmission, i.e. low levels of macroparasite infection might result in a reduced microparasite transmission while an increased transmission may be expected from higher levels of macroparasite infection. The density-dependent effect identified in this study highlights that, by altering macroparasite infection burden, there may be a switch from negative to a positive relationship between the macroparasite and the quality of coinfecting parasite transmission stages, even if the underlying interaction is still resource mediated.

Entomopathogenic nematodes are an effective biocontrol agent for several insect pest populations due to their capacity to evade the host immune responses and kill the host (Ribeiro et al. 1999; Brivio et al. 2004; Ji and Kim 2004; Castillo et al. 2011; Bisch et al. 2015; Binda-Rossetti et al. 2016). For nematodes to remain an effective biocontrol agent, they must be highly infective to their hosts. A decrease in the lipid reserves provisioned during their development has been related to a decrease in the infectivity of nematodes (Fitters et al. 1997; Menti et al. 2000). A curvilinear effect of macroparasite intensity on the lipid reserves (quality) of nematodes emerging from the coinfected host was observed in this study (Chapter 3). As macroparasites infection load varies between hosts, taking account of different macroparasite distributions within the host population may be necessary for accurate prediction of microparasite

transmission between-host. Despite a number of promising laboratory results on the efficacy of entomopathogenic nematodes (Ramos-Rodríguez et al. 2006; Odendaal et al. 2016; Heve et al. 2017; Wagutu 2017), the field applications have yielded variable results (Schroeder et al. 1996; Mannion et al. 2001; Belair et al. 2003; Memari et al. 2016). It is possible that such variations are a consequence of different distributions of endemic infection in the different pest populations. In natural conditions where hosts harbour different burden of endemic infection, the current study suggests that combined therapies might actually prove useful. The curvilinear effect reveals that dosing with a low endemic burden of gregarines while simultaneously applying the entomopathogenic nematodes, might improve efficacy compared to dosing solely with nematodes treatment, although the natural endemic infection burden and diversity of parasites would also need to be taken into account.

In recent decades, disease ecologists have increasingly focussed on how interspecific interactions between parasites may scale to determine disease spread between-hosts (Abu-Raddad et al. 2006; Telfer et al. 2010; Ezenwa and Jolles 2011; Johnson and Hoverman 2012; Susi et al. 2015; Gorsich et al. 2018). In Chapter 4, the transmission dynamics of the epidemic parasite within the host population was dependent on the lipid provisioning within the transmission stages. It is possible that the non-feeding transmission stages that emerged from high levels of macroparasite infection were better suited (due to increased quality) to survive for longer in the environment or they were more infectious, which may explain the observed pattern of microparasite transmission. This study highlights the need to assess the level of endemic infection to accurately predict disease outbreak, failure to do this could result in unexpected variation in host-parasite dynamics.

In chapter 3, the results demonstrate a curvilinear relationship due to resource use but in other systems, curvilinear relationships could occur through the immune mechanism. The host immune response can be negatively correlated with parasite intensity (Biard et al. 2015), such that the immune response is upregulated at low

levels of infection but downregulated at a higher intensity, probably due to a depletion of host resources (Lochmiller and Deerenberg 2000). If the curvilinear relationship in the current study reflects other systems, then high burdens of macroparasite infection will result in increased microparasite fitness, which makes the transmission stages more competitive than those emerging from hosts with low levels of macroparasite. Increased microparasite fitness, on the other hand, may eventually lead to competitive exclusion of the macroparasite by reducing the pool of macroparasite susceptible hosts (Bremermann and Thieme 1989). There is still a gap in knowledge of the outcomes of macro- and microparasite coinfection despite several theoretical (Fenton 2008; Fenton and Perkins 2010; Ezenwa and Jolles 2011) and empirical studies (Lello et al. 2004; Jolles et al. 2008; Telfer et al. 2010) that have attempted to assess the implications of interspecific interactions for parasite dynamics. The differences in microparasite output, fitness and transmission described in this study reveal that, to accurately predict the effect of coinfection on between-host parasite transmission, there is a need to consider both the mechanism of interaction and the host-macroparasite burden. Resource competition and immune-mediated interactions are common during coinfection (Graham 2008; Randall et al. 2013; Griffiths et al. 2014), and this makes the current system an ideal model to further explore the individual-level impact of these interactions on parasite transmission.

The immune system of invertebrates shares close similarity with the vertebrate innate immune system (Vilmos and Kurucz 1998; Sheehan et al. 2018). The innate immune responses of insects and their vertebrate counterpart consist of both humoral and cellular components. Vertebrates have an adaptive immune system which enables specific immune responses based on previous experience to an infection (Cooper and Alder 2006; McFall-Ngai 2007). Although insects lack an adaptive immune system, studies have demonstrated that they possess a form of adaptive immune response (memory and long term upregulation of the innate response) (Hartman and Karp 1989; Kurtz and Franz 2003; Moret and Siva-Jothy 2003; Korner and Schmid-Hempel 2004; Kurtz and Armitage 2006; Schulenburg et al. 2007). Whilst, the immune system of

insects is less complex than the vertebrate system, the immune mechanisms are generic (Vilmos and Kurucz 1998; Kavanagh and Reeves 2007; Müller et al. 2008; Sheehan et al. 2018). The similarity between both systems enables insects to be used in some studies to overcome challenges associated with vertebrate systems (e.g. cost, ethics), whilst yielding comparable results. Furthermore, when host resources mediate the interaction between parasites (Antia et al. 2008; Graham 2008; Randall et al. 2013; Griffiths et al. 2014), the depletion of resources could, in turn, alter the strength of immune response against parasites (Koski and Scott 2001). Differences in a host's resource interaction can potentially lead to non-linearities in immune response (French et al. 2009) which may have consequences for host health.

The elimination of macroparasitic, soil-transmitted helminths as a public health problem by 2020 is one of the World Health Organization's strategic plan (WHO 2012). If the findings from Chapters 3 & 4 reflect other systems where host resources mediate the interaction between parasites (Griffiths et al. 2014; Budischak et al. 2015; Ramiro et al. 2016; Wale et al. 2017), then it suggests that integrated control programmes might prove useful, but caution should be taken with mass drug administration programmes, because the complete removal of an endemic pathogen may result in the outbreak of another pathogen. The differences in a host macroparasite burden can have important implications for microparasite fitness and between-host transmission and the relationship may be non-linear (Chapter 3 & 4). In natural conditions, hosts maintain relatively similar levels of macroparasite burden over time, even after drug treatment (Gryseels and Nkulikyinka 1989; Ahmed et al. 2012; Dunn et al. 2018; Ghosh et al. 2018). Therefore, for control programs to be effective, there is a need to consider the effect of different host-parasite burden and also the interspecific interactions that may occur between parasites when developing interventions.

An increasing number of studies have demonstrated that host resources play a significant role in mediating parasite interactions (Hall et al. 2007; Becker and Hall 2014; Cressler et al. 2014; Civitello et al. 2015). For example, increased resource

availability may support an increased immune response which reduces parasite output, transmission and parasite-induced damage (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000; Tschirren et al. 2007). Alternatively, since parasites are a sink of host resources, increased resource availability might promote parasite replication, development and fitness (Hall et al. 2007; de Roode et al. 2008; Seppala et al. 2008). In previous work, Randall *et al*. (2013) demonstrated that macroparasites deplete host resources, which affected the transmission potential of a co-infecting microparasite. The current study provides further evidence that where host resources mediate the interaction between coinfecting parasites, variations in resource availability have the potential to alter the spread of diseases at the population level (Chapter 3 & 4).

5.4 Future work

Differences in the effect of host age on the immune response to injury or parasitic infection are well documented in both vertebrates and invertebrates (Smith and Angus 1980; Jianyong et al. 1992; Linton and Dorshkind 2004; Rantala and Roff 2005; Whitehorn et al. 2010; Mackenzie et al. 2011; Kubiak and Tinsley 2017). In our laboratory, it remains unknown if the host immune response increases or decreases with age. In other insect systems, for example, the host encapsulation and lytic activity vary with host age but the relationship varies between different hosts and parasites (Brodeur and Vet 1995; Rantala and Roff 2005; Bukovinszky et al. 2009; League et al. 2017). However, due to the design of the current study, the effects that host age may have on the immune response and susceptibility to microparasite infection could not be determined. Future development of this study is to explore the effect of host age on the immune response to the macroparasite and then re-assess what effects this may have on microparasite dynamics and susceptibility to infection.

Malnutrition and poor host condition can influence the host susceptibility to parasitic infection (Welburn et al. 1989; Santos 1994; Anstead et al. 2001). It was hypothesized in the current study that the host lipid resource would decrease in response to higher levels of gregarine infection. Due to the short experimental infection period in this

study design, the resources may not have been fully exploited by the macroparasite to produce sufficient depletion of host resources. Further work on this study system is essential, for example, using hosts exposed to repeated doses of gametocysts and for an extended period of time. This will improve understanding of how extended depletion of resources, which mediates the interaction between both parasites, might alter infection and parasite dynamics.

The lipid reserves of microparasite transmission stages were unexpectedly lower in controls than the highest level of macroparasite infection (Chapter 3). This led to the hypothesis (following laboratory observation that macroparasite trophozoites are numerous and much smaller in highly infected hosts) that the unexpected difference was a consequence of more gregarine retention in heavily infected hosts. Future development of this study would be to assess the lipid levels of the macroparasite using colorimetric assays (Leyva et al. 2008; Cheng et al. 2011), to determine whether the gut macroparasites in this system are a sufficient supply of lipids for the microparasites. The microparasite in this system consumes the entirety of the host, undergoes within-host developmental cycles and produces infective juveniles only when resources are close to depletion.

Whilst the current system provided an interesting opportunity to explore microparasite dynamics and transmission, the consequences of the interaction on macroparasite dynamics could not be assessed because the microparasites used are obligate killers (i.e. no-host recovery post-infection). A development of this study would be to explore the consequence of interspecific interaction on the dynamics of the macroparasite using a less virulent microparasite. For example, the common soil bacterium *Bacillus subtilis* (Reavey et al. 2015), which could be used with the gregarine macroparasite as a model to further explore resource competition and the co-evolutionary consequences of coinfection.

5.5 *Conclusion*

The 20:80 rule broadly describes the aggregated distribution of macroparasites in which 80% of the parasite population is concentrated in 20% of the host population (Woolhouse et al. 1997; Shaw et al. 1998). What this means is that the distribution varies even for the same parasite species and therefore, hosts will harbour different burdens of macroparasites, as a result of extreme over-dispersion and consequently lose a different amount of resource to the parasite. In natural conditions where coinfection of a host is the norm (Cox 2001), resource competition may be the most common forms of interspecific interaction (Budischak et al. 2015; Ramiro et al. 2016; Wale et al. 2017). This study demonstrates that endemic macroparasite burden can have fitness costs for epidemic parasite transmission and that the within-host responses during different levels of macroparasite infection can potentially shape the dynamics of microparasite infection. Therefore, there is a need to assess the endemic macroparasite burden to effectively predict microparasite transmission. Whilst the current system reveals a specific example of the complex interaction and non-linear relationship between resource competition and host-parasite burden, it points to the importance of understanding such relationships in order to accurately predict epidemic spread. In order to provide a more detailed understanding of host-parasite interactions and its consequences for transmission, it is becoming clear that disease ecologists need to integrate experimental work (that aims to decipher precise mechanisms of interaction) with theoretical models to effectively predict epidemic outbreaks and the implications for control strategies. The use of insect systems can be useful in modelling host-parasite interactions and the testing of hypothesis that can be extrapolated into clinical studies (Mylonakis et al. 2007; Peterson et al. 2008; Lebeaux et al. 2013). Given the likelihood of macro-microparasite coinfections in natural conditions, this study reveals that macroparasite burden can drive non-linearity in microparasite transmission and highlights the need to consider macroparasite burden when assessing the risk of an epidemic outbreak.

References

Abbas, A. K. et al. 1996. Functional diversity of helper T lymphocytes. *Nature* 383(6603), 787.

Abdolmaleki, A. et al. 2017. Cellular and humoral responses of *Pieris brassicae* to infection by *Steinernema feltiae*, its symbiont bacteria, and their metabolites. *Nematology* 19(4), 477-487.

Åbro, A. 1971. Gregarines: their effects on damselflies (Odonata: Zygoptera). *Insect Systematics & Evolution* 2(4), 294-300.

Abu-Raddad, L. J. et al. 2006. Dual infection with HIV and malaria fuels the spread of both diseases in sub-Saharan Africa. *Science* 314(5805), 1603-1606.

Abu Hatab, M. et al. 1998. Influence of culture method on *Steinernema glaseri* lipids. *Journal of Parasitology* 84(2)

Abu Hatab, M. and Gaugler, R. 2001. Diet composition and lipids of in vitro-produced *Heterorhabditis bacteriophora*. *Biological Control* 20(1), 1-7.

Adamo, S. A. 2004. Estimating disease resistance in insects: phenoloxidase and lysozyme-like activity and disease resistance in the cricket *Gryllus texensis*. *Journal of Insect Physiology* 50(2-3), 209-216.

Agianian, B. et al. 2007. Preliminary characterization of hemolymph coagulation in *Anopheles gambiae* larvae. *Developmental and Comparative Immunology* 31(9), 879-888.

Aguilar, R. et al. 2005. Anopheles infection responses; laboratory models versus field malaria transmission systems. *Acta Tropica* 95(3)

Aguirre, A. A. and Tabor, G. M. 2008. Global Factors Driving Emerging Infectious Diseases Impact on Wildlife Populations. In: Sparagano, O.A.E. et al. eds. *Animal Biodiversity and Emerging Diseases: Prediction and Prevention*.

Agrawal, B. et al. 2019. Heterologous Immunity between Adenoviruses and Hepatitis C Virus (HCV): Recombinant Adenovirus Vaccine Vectors Containing Antigens from Unrelated Pathogens Induce Cross-Reactive Immunity Against HCV Antigens. *Cells* 8(5).

Ahmed, A. M. et al. 2012. High levels of *Schistosoma mansoni* infections among schoolchildren in central Sudan one year after treatment with praziquantel. *Journal of Helminthology* 86(2), 228-232.

Ahtiainen, J. J. et al. 2004. Sexual advertisement and immune function in an arachnid species (Lycosidae). *Behavioral Ecology* 15(4), 602-606.

Ahtiainen, J. J. et al. 2005. A trade-off between sexual signalling and immune function in a natural population of the drumming wolf spider *Hygrolycosa rubrofasciata*. *Journal of Evolutionary Biology* 18(4), 985-991.

Aira, N. et al. 2017. Species dependent impact of helminth-derived antigens on human macrophages infected with *Mycobacterium tuberculosis*: Direct effect on the innate anti-mycobacterial response. *PLoS Neglected Tropical Diseases* 11(2), e0005390.

Alizon, S. et al. 2009. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *Journal of Evolutionary Biology* 22(2), 245-259.

Andalo, V. et al. 2011. Influence of temperature and duration of storage on the lipid reserves of entomopathogenic nematodes. *Revista Colombiana de Entomologia* 37(2), 203-209.

Angelini, P. et al. 2008. Chikungunya epidemic outbreak in Emilia-Romagna (Italy) during summer 2007. *Parassitologia* 50(1/2)

Anderson, R. M. and May, R. M. 1979. Population biology of infectious diseases: Part I. *Nature* 280(5721), 361.

Anderson, R. M. and May, R. M. 1982. Coevolution of hosts and parasites. *Parasitology* 85(2), 411-426.

Anstead, G. M. et al. 2001. Malnutrition alters the innate immune response and increases early visceralization following *Leishmania donovani* infection. *Infection and Immunity* 69(8), 4709-4718.

Antia, R. et al. 2003. The role of evolution in the emergence of infectious diseases. *Nature* 426(6967), 658.

Antia, R. et al. 2008. The dynamics of acute malaria infections. I. Effect of the parasite's red blood cell preference. *Proceedings of the Royal Society B: Biological Sciences* 275(1641)

Arthurs, S. et al. 2004. An analysis of using entomopathogenic nematodes against above-ground pests. *Bulletin of Entomological Research* 94(4).

Athanassiou, C. G. et al. 2008. Insecticidal effect of *Steinernema feltiae* (Filipjev)(Nematoda: Steinernematidae) against *Tribolium confusum* (Coleoptera: Tenebrionidae) and *Ephestia kuehniella* (Zeller)(Lepidoptera: Pyralidae) in stored wheat. *Journal of stored products research* 44(1).

Athanassiou, C. G. et al. 2010. Mortality of four stored product pests in stored wheat when exposed to doses of three entomopathogenic nematodes. *Journal of economic entomology* 103(3).

Appel, A. G. et al. 1993. Laboratory and field evaluations of an entomogenous nematode (Nematoda, Steinernematidae) for German cockroach (Dictyoptera, Blattellidae) control. *Journal of Economic Entomology* 86(3), 777-784.

Bajgar, A. et al. 2015. Extracellular adenosine mediates a systemic metabolic switch during immune response. *PLoS biology* 13(4).

Balmer, O. et al. 2009. Intraspecific competition between co-infecting parasite strains enhances host survival in African trypanosomes. *Ecology* 90(12).

Bandilla, M. et al. 2006. A link between ectoparasite infection and susceptibility to bacterial disease in rainbow trout. *International Journal for Parasitology* 36(9), 987-991.

Barbercheck, M. 2015. *Insect-Parasitic Nematodes for the Management of Soil-Dwelling Insects* [Online]. The Pennsylvania State University Available at: http://ento.psu.edu/extension/factsheets/parasitic-nematodes

Bashey, F. et al. 2013. Alternative paths to success in a parasite community: Within-host competition can favor higher virulence or direct interference. *Evolution* 67(3), 900-907.

Bashir-Tanoli, S. and Tinsley, M. C. 2014. Immune response costs are associated with changes in resource acquisition and not resource reallocation. *Functional Ecology* 28(4).

Becker, D. J. and Hall, R. J. 2014. Too much of a good thing: resource provisioning alters infectious disease dynamics in wildlife. *Biology Letters* 10(7).

Becker, D. J. et al. 2015. Linking anthropogenic resources to wildlife-pathogen dynamics: a review and meta-analysis. *Ecology Letters* 18(5), 483-495.

Beechler, B. R. et al. 2015. Enemies and turncoats: bovine tuberculosis exposes pathogenic potential of Rift Valley fever virus in a common host, African buffalo (*Syncerus caffer*). *Proceedings of the Royal Society B-Biological Sciences* 282(1805).

Belair, G. et al. 2003. Efficacy of Steinernematid nematodes against three insect pests of crucifers in Quebec. *Journal of Nematology* 35(3), 259.

Beldomenico, P. M. et al. 2009. Host condition and individual risk of cowpox virus infection in natural animal populations: cause or effect? *Epidemiology and Infection* 137(9), 1295-1301.

Benesh, D. P. 2011. Intensity-dependent host mortality: what can it tell us about larval growth strategies in complex life cycle helminths? *Parasitology* 138(7), 913-925.

Bharti, N. et al. 2010. Measles hotspots and epidemiological connectivity. *Epidemiology & Infection* 138(9).

Bjørnstad, O. N. et al. 2002. Dynamics of measles epidemics: estimating scaling of transmission rates using a time series SIR model. *Ecological monographs* 72(2).

Biard, C. et al. 2015. Interpreting immunological indices: The importance of taking parasite community into account. An example in blackbirds *Turdus merula*. *Methods in Ecology and Evolution* 6(8), 960-972.

Billinis, C. 2013. Wildlife diseases that pose a risk to small ruminants and their farmers. *Small Ruminant Research* 110(2-3), 67-70.

Binda-Rossetti, S. et al. 2016. Effects of an entomopathogen nematode on the immune response of the insect pest red palm weevil: Focus on the host antimicrobial response. *Journal of Invertebrate Pathology* 133, 110-119.

Birger, R. B. et al. 2015. The potential impact of coinfection on antimicrobial chemotherapy and drug resistance (vol 23, pg 537, 2015). *Trends in Microbiology* 23(11), 742-742.

Bisch, G. et al. 2015. *Xenorhabdus bovienii* CS03, the bacterial symbiont of the entomopathogenic nematode *Steinernema weiseri*, is a non-virulent strain against lepidopteran insects. *Journal of Invertebrate Pathology* 124, 15-22.

Blagrove, M. S. et al. 2012. Wolbachia strain wMel induces cytoplasmic incompatibility and blocks dengue transmission in Aedes albopictus. *Proceedings of the National Academy of Sciences* 109(1).

Blandin, S. and Levashina, E. A. 2004. Thioester-containing proteins and insect immunity. *Molecular Immunology* 40(12), 903-908.

Boag, B. et al. 2001. Patterns of parasite aggregation in the wild European rabbit (*Oryctolagus cuniculus*). *International Journal for Parasitology* 31(13), 1421-1428.

Boemare, N. 2002. *Entomopathogenic nematology*. Biology, taxonomy and systematics of *Photorhabdus* and *Xenorhabdus.*

Bonneaud, C. et al. 2003. Assessing the cost of mounting an immune response. *The American Naturalist* 161(3), 367-379.

Booth, D. T. et al. 1993. Experimental demonstration of the energetic cost of parasitism in free-ranging hosts. *Proceedings of the Royal Society B-Biological Sciences* 253(1337), 125-129.

Boots, M. 2000. Density-independent resource limitation and the transmission of an insect pathogen. *Oecologia* 124(2), 172-175.

Bose, J. et al. 2016. Multiple-genotype infections and their complex effect on virulence. *Zoology* 119(4), 339-349.

Bottomley, c. et al. 2005. Population biology of multispecies helminth infection: interspecific interactions and parasite distribution. *Parasitology* 131(3), 417-433.

Boulanger, N. et al. 2001. Immune response of *Drosophila melanogaster* to infection with the flagellate parasite *Crithidia* spp. *Insect biochemistry and molecular biology* 31(2).

Boulanger, N. et al. 2004. Characterization of a defensin from the sand fly *Phlebotomus duboscqi* induced by challenge with bacteria or the protozoan parasite *Leishmania major*. *Infection and immunity* 72(12).

Bouwma, A. M. et al. 2005. Parasitism in a social wasp: effect of gregarines on foraging behavior, colony productivity, and adult mortality. *Behavioral Ecology and Sociobiology* 59(2).

Brace, A. J. et al. 2017. Costs of immune responses are related to host body size and lifespan. *Journal of Experimental Zoology Part a-Ecological and Integrative Physiology* 327(5), 254-261.

Brace, A. J. et al. 2015. Highway to the danger zone: exposure-dependent costs of immunity in a vertebrate ectotherm. *Functional Ecology* 29(7), 924-930.

Brady, M. T. et al. 1999. *Fasciola hepatica* suppresses a protective Th1 response against *Bordetella pertussis*. *Infection and Immunity* 67(10), 5372-5378.

Braun, A. et al. 1997. *Drosophila* immunity: Analysis of larval hemocytes by P-element-mediated enhancer trap. *Genetics* 147(2), 623-634.

Bremermann, H. J. and Thieme, H. 1989. A competitive exclusion principle for pathogen virulence. *Journal of Mathematical Biology* 27(2), 179-190.

Brivio, M. F. et al. 2004. The role of *Steinernema feltiae* body-surface lipids in host-parasite immunological interactions. *Molecular and Biochemical Parasitology* 135(1), 111-121.

Brivio, M. F. et al. 2006. Down-regulation of antibacterial peptide synthesis in an insect model induced by the body-surface of an entomoparasite (*Steinernema feltiae*). *Developmental & Comparative Immunology* 30(7), 627-638.

Brodeur, J. and Vet, L. E. 1995. Relationships between parasitoid host range and host defence: a comparative study of egg encapsulation in two related parasitoid species. *Physiological Entomology* 20(1), 7-12.

Brooker, S. et al. 2006. Contrasting patterns in the small-scale heterogeneity of human helminth infections in urban and rural environments in Brazil. *International Journal for Parasitology* 36(10-11), 1143-1151.

Bruce, M. C. et al. 2000. Cross-species interactions between malaria parasites in humans. *Science* 287(5454), 845-848.

Budischak, S. A. et al. 2012. Direct and indirect costs of co-infection in the wild: Linking gastrointestinal parasite communities, host hematology, and immune function. *International Journal for Parasitology Parasites and Wildlife* 1, 2-12.

Budischak, S. A. et al. 2015. Resource limitation alters the consequences of co-infection for both hosts and parasites. *International Journal for Parasitology* 45(7), 455-463.

Budischak, S. A. et al. 2018. Competing for blood: the ecology of parasite resource competition in human malaria–helminth co-infections. *Ecology Letters* 21(4), 536-545.

Bukovinszky, T. et al. 2009. Consequences of constitutive and induced variation in plant nutritional quality for immune defence of a herbivore against parasitism. *Oecologia* 160(2), 299-308.

Bulet, P. et al. 1999. Antimicrobial peptides in insects; structure and function. *Developmental & Comparative Immunology* 23(4-5), 329-344.

Busby, P. E. et al. 2016. Common foliar fungi of Populus trichocarpa modify Melampsora rust disease severity. *New Phytologist* 209(4).

Cable, J. et al. 2017. Global change, parasite transmission and disease control: lessons from ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372(1719).

Campos-Herrera, R. et al. 2012. Wide interguild relationships among entomopathogenic and free-living nematodes in soil as measured by real time qPCR. *Journal of Invertebrate Pathology* 111(2).

Campos-Herrera, R. et al. 2015. Unraveling the intraguild competition between Oscheius spp. nematodes and entomopathogenic nematodes: implications for their natural distribution in Swiss agricultural soils. *Journal of invertebrate pathology* 132.

Canales-Lazcano, J. et al. 2005. Fitness-related attributes and gregarine burden in a non-territorial damselfly *Enallagma praevarumhagen* (Zygoptera : Coenagrionidae). *Odonatologica* 34(2).

Cappello, M. 2004. Global health impact of soil-transmitted nematodes. *The Pediatric infectious disease journal* 23(7).

Castillo, J. C. et al. 2011. Insect immune responses to nematode parasites. *Trends in Parasitology* 27(12), 537-547.

Cattadori, I. M. et al. 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(16), 831-840.

Cattadori, I. M. et al. 2008. Parasite co-infection and interaction as drivers of host heterogeneity. *International Journal for Parasitology* 38(3-4), 371-380.

Cerenius, L. et al. 2010. Proteolytic cascades and their involvement in invertebrate immunity. *Trends in Biochemical Sciences* 35(10), 575-583.

Chandra, R. K. 1996. Nutrition, immunity and infection: from basic knowledge of dietary manipulation of immune responses to practical application of ameliorating suffering and improving survival. *Proceedings of the National Academy of Sciences* 93(25), 14304-14307.

Charlier, J. et al. 2015. Econohealth: Placing helminth infections of livestock in an economic and social context. *Veterinary Parasitology* 212(1-2), 62-67.

Chen, C. C. et al. 2005. Concurrent infection with an intestinal helminth parasite impairs host resistance to enteric *Citrobacter rodentium* and enhances Citrobacter-induced colitis in mice. *Infection and Immunity* 73(9), 5468-5481.

Chen, C. C. et al. 2006. Helminth-primed dendritic cells alter the host response to enteric bacterial infection. *Journal of Immunology* 176(1), 472-483.

Chen, Y. et al. 2013. Coinfection with *Clonorchis sinensis* modulates murine host response against *Trichinella spiralis* infection. *Parasitology research* 112(9), 3167-3179.

Cheng, Y. S. et al. 2011. Rapid quantitative analysis of lipids using a colorimetric method in a microplate format. *Lipids* 46(1), 95-103.

Cirimotich, C. M. et al. 2011. Natural Microbe-Mediated Refractoriness to Plasmodium Infection in *Anopheles gambiae*. *Science* 332(6031).

Choisy, M. and de Roode, J. C. 2010. Mixed Infections and the evolution of virulence: Effects of resource competition, parasite plasticity, and impaired host immunity. *American Naturalist* 175(5), E105-E118.

Chun, J. D. et al. 1995. Effect of mosquito age and reproductive status on melanization of Sephadex beads in *Plasmodium* refractory and *Plasmodium* susceptible strains of *Anopheles gambiae*. *Journal of Invertebrate Pathology* 66(1), 11-17.

Chung, E. et al. 2012. Variation in resistance to multiple pathogen species: anther smuts of *Silene uniflora*. *Ecology and Evolution* 2(9), 2304-2314.

Chyba, C. 2004. The global threat of new and reemerging infectious diseases: Reconciling US national security and public health policy. *Survival* 46(1), 163-164.

Ciancio, A. et al. 2001. Ultrastructure of trophozoites of the gregarine *Lankesteria ascidiae* (Apicomplexa : Eugregarinida) parasitic in the ascidian *Ciona intestinalis* (Protochordata). *European Journal of Protistology* 37(3), 327-336.

Civitello, D. J. et al. 2018. Assessing the direct and indirect effects of food provisioning and nutrient enrichment on wildlife infectious disease dynamics. *Philosophical Transactions of the Royal Society B-Biological Sciences* 373(1745).

Civitello, D. J. et al. 2015. Resources, key traits and the size of fungal epidemics in *Daphnia* populations. *Journal of Animal Ecology* 84(4), 1010-1017.

Clay, C. A. et al. 2009. Contact heterogeneity in deer mice: implications for Sin Nombre virus transmission. *Proceedings of the Royal Society B-Biological Sciences* 276(1660), 1305-1312.

Cleaveland, S. et al. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 356(1411), 991-999.

Clopton, R. E. 2000. *Stylocephalus occidentalis* n. sp (Apicomplexa : Eugregarinida : Stylocephalidae) from *Trimytis pruinosa* (Coleoptera : Tenebrionidae) in the Nebraska sandhills. *Journal of Parasitology* 86(3), 560-565.

Clopton, R. E. and Gold, R. E. 1996. Host specificity of *Gregarina blattarum* von Siebold, 1839 (Apicomplexa: Eugregarinida) among five species of domiciliary cockroaches. *Journal of Invertebrate Pathology* 67(3), 219-223.

Cooper, R. A. and Schal, C. 1992. Effects of protein type and concentration on development and reproduction of the German cockroach, *Blattella germanica. Entomologia Experimentalis et Applicata* 63(2), 123-134.

Cooper, T. and Heinemann, J. 2005. Selection for plasmid post–segregational killing depends on multiple infection: evidence for the selection of more virulent parasites through parasite–level competition. *Proceedings of the Royal Society B: Biological Sciences* 272(1561), 403-410.

Cooper, M. D. and Alder, M. N. 2006. The evolution of adaptive immune systems. *Cell* 124(4), pp. 815-822.

Cornet, S. et al. 2014. Impact of host nutritional status on infection dynamics and parasite virulence in a bird-malaria system. *Journal of Animal Ecology* 83(1), 256-265.

Cotter, S. et al. 2008. Selection for cuticular melanism reveals immune function and life-history trade-offs in *Spodoptera littoralis*. *Journal of Evolutionary Biology* 21(6), 1744-1754.

Cotter, S. C. et al. 2004. Costs of resistance: genetic correlations and potential trade-offs in an insect immune system. *Journal of Evolutionary Biology* 17(2), 421-429.

Cousineau, S. V. and Alizon, S. 2014. Parasite evolution in response to sex-based host heterogeneity in resistance and tolerance. *Journal of Evolutionary Biology* 27(12), 2753-2766.

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

Cressler, C. E. et al. 2014. Starvation reveals the cause of infection-induced castration and gigantism. *Proceedings of the Royal Society B-Biological Sciences* 281(1792).

Crompton, D. W. T. 2000. The public health importance of hookworm disease. *Parasitology* 121(S1), S39-S50.

Cunningham, A. A. et al. 2017. One Health, emerging infectious diseases and wildlife: two decades of progress? *Philosophical Transactions of the Royal Society B-Biological Sciences* 372(1725).

Cutler, J. et al. 2017. Susceptibility of cockroaches (*Gromphadorhina portentosa*, *Nauphoeta cinerea* and *Blaptica dubia*) exposed to entomopathogenic nematodes. *Biocontrol Science and Technology* 27(4), 556-564.

Das, Y. and Gupta, A. 1974. Effects of three juvenile hormone analogs on the female German cockroach, *Blattella germanica* (L.)(Dictyoptera: Blattellidae). *Cellular and Molecular Life Sciences* 30(9), 1093-1095.

de Lope, F. et al. 1998. Parasitism, immune response and reproductive success in the house martin *Delichon urbica*. *Oecologia* 114(2), 188-193.

De Roode, J. C. et al. 2005. Virulence and competitive ability in genetically diverse malaria infections. *Proceedings of the National Academy of Sciences* 102(21), 7624-7628.

de Roode, J. C. et al. 2008. Host plant species affects virulence in monarch butterfly parasites. *Journal of Animal Ecology* 77(1), 120-126.

de Roode, J. C. et al. 2008. Virulence-transmission trade-offs and population divergence in virulence in a naturally occurring butterfly parasite. *Proceedings of the National Academy of Sciences of the United States of America* 105(21), 7489-7494.

Demas, G. E. et al. 1997. Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 273(5).

Demas, G. E. et al. 2003. Reductions in total body fat decrease humoral immunity. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270(1518), 905-911.

Derting, T. L. and Virk, M. K. 2005. Positive effects of testosterone and immunochallenge on energy allocation to reproductive organs. *Journal of Comparative Physiology B* 175(8).

Desportes, I. and Schrével, J. 2013. *Treatise on Zoology-Anatomy, Taxonomy, Biology. The Gregarines (2 vols): The Early Branching Apicomplexa*. Brill.

Dezfuli, B. S. et al. 2001. Costs of intraspecific and interspecific host sharing in Acanthocephalan cystacanths. *Parasitology* 122, 483-489.

Dhinaut, J. et al. 2018. Immune priming specificity within and across generations reveals the range of pathogens affecting evolution of immunity in an insect. *Journal of Animal Ecology* 87(2), 448-463.

Dicker, R. C. et al. 2006. Principles of epidemiology in public health practice; an introduction to applied epidemiology and biostatistics.

Dieckmann, U. et al. 2005. *Adaptive dynamics of infectious diseases: in pursuit of virulence management*. Cambridge University Press.

Dimarcq, J.-L. et al. 1990. Insect immunity: expression of the two major inducible antibacterial peptides, defensin and diptericin, in *Phormia terranovae*. *The EMBO Journal* 9(8), 2507-2515.

Dobson, R. J. and Barnes, E. H. 1995. Interaction between *Ostertagia circumcincta* and *Haemonchus contortus* infection in young lambs. *International Journal for Parasitology* 25(4), 495-501.

Dong, Y. M. et al. 2009. Implication of the Mosquito Midgut Microbiota in the Defense against Malaria Parasites. *Plos Pathogens* 5(5).

Dorigatti, I. et al. 2018. Using *Wolbachia* for Dengue Control: Insights from Modelling. *Trends in Parasitology* 34(2).

Doumayrou, J. et al. 2013. An experimental test of the transmission-virulence trade-off hypothesis in a plant virus. *Evolution: International Journal of Organic Evolution* 67(2), 477-486.

Dowell, S. F. 2001. Seasonal variation in host susceptibility and cycles of certain infectious diseases. *Emerging infectious diseases* 7(3).

Drayton, J. M. and Jennions, M. D. 2011. Inbreeding and measures of immune function in the cricket *Teleogryllus commodus*. *Behavioral Ecology* 22(3), 486-492.

Duncan, L. et al. 2003. Competition between entomopathogenic and free-living bactivorous nematodes in larvae of the weevil *Diaprepes abbreviatus*. *Journal of Nematology* 35(2).

Dunn, J. C. et al. 2018. Longitudinal changes in the prevalence and intensity of soil-transmitted helminth infection following expanded community-wide mass drug administration in the delta region of Myanmar. *BioRxiv*, 341677.

Duvic, B. et al. 2012. Cecropins as a marker of *Spodoptera frugiperda* immunosuppression during entomopathogenic bacterial challenge. *Journal of Insect Physiology* 58(6), 881-888.

Duvic, B. and Söderhäll, K. 1992. Purification and partial characterization of a β-1, 3-glucan-binding-protein membrane receptor from blood cells of the crayfish *Pacifastacus leniusculus*. *European Journal of Biochemistry* 207(1), 223-228.

Dwyer, G. et al. 1997. Host heterogeneity in susceptibility and disease dynamics: Tests of a mathematical model. *American Naturalist* 150(6), 685-707.

Dzhivhuho, G. A. et al. 2018. Chronic schistosomiasis suppresses HIV-specific responses to DNA-MVA and MVA-gp140 Env vaccine regimens despite antihelminthic treatment and increases helminth-associated pathology in a mouse model. *Plos Pathogens* 14(7).

Ebert, D. and Mangin, K. L. 1997. The influence of host demography on the evolution of virulence of a microsporidian gut parasite. *Evolution* 51(6), 1828-1837.

Ebrahimi, L. et al. 2011. Hemocyte responses of the Colorado potato beetle, *Leptinotarsa decemlineata*, and the greater wax moth, *Galleria mellonella*, to the entomopathogenic nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora*. *Journal of Insect Science* 11.

Ebrahimi, L. et al. 2014. Effect of an entomopathogenic nematode, *Steinernema carpocapsae* on haemocyte profile and phenoloxidase activity of the Colorado potato beetle, *Leptinotarsa decemlineata*. *Biocontrol Science and Technology* 24(12), 1383-1393.

Ehlers, R. U. 2001. Mass production of entomopathogenic nematodes for plant protection. *Applied Microbiology and Biotechnology* 56(5-6), 623-633.

Eleftherianos, I. et al. 2006. RNAi suppression of recognition protein mediated immune responses in the tobacco hornworm *Manduca sexta* causes increased susceptibility to the insect pathogen Photorhabdus. *Developmental & Comparative Immunology* 30(12).

Eleftherianos, I. et al. 2007. An antibiotic produced by an insect-pathogenic bacterium suppresses host defenses through phenoloxidase inhibition. *Proceedings of the National Academy of Sciences* 104(7), 2419-2424.

Eleftherianos, I. et al. 2009. A single locus from the entomopathogenic bacterium *Photorhabdus luminescens* inhibits activated *Manduca sexta* phenoloxidase. *FEMS Microbiology Letters* 293(2), 170-176.

Eom, S. et al. 2014. Sequential immunosuppressive activities of bacterial secondary metabolites from the Entomopathogenic Bacterium *Xenorhabdus nematophila*. *Journal of Microbiology* 52(2), 161-168.

Epsky, N. D. and Capinera, J. L. 1994. Invasion efficiency as a measure of efficacy of the entomogenous nematode *Steinernema carpocapsae* (rhabditida, steinernematidae). *Journal of Economic Entomology* 87(2).

Eraud, C. et al. 2005. The energetic cost of humoral immunity in the Collared Dove, *Streptopelia decaocto*: is the magnitude sufficient to force energy-based trade-offs? *Functional Ecology* 19(1).

Escriu, F. et al. 2000. Evolution of virulence in natural populations of the satellite RNA of Cucumber mosaic virus. *Phytopathology* 90(5), 480-485.

Eslin, P. and Prévost, G. 1998. Hemocyte load and immune resistance to *Asobara tabida* are correlated in species of the *Drosophila melanogaster* subgroup. *Journal of Insect Physiology* 44(9), 807-816.

Eswarappa, S. M. et al. 2012. Within-host dynamics of multi-species infections: Facilitation, competition and virulence. *Plos One* 7(6).

Ezenwa, V. 2016. Helminth–microparasite co-infection in wildlife: lessons from ruminants, rodents and rabbits. *Parasite Immunology* 38(9), 527-534.

Ezenwa, V. O. and Jolles, A. E. 2011. From Host Immunity to Pathogen Invasion: The effects of helminth coinfection on the dynamics of microparasites. *Integrative and Comparative Biology* 51(4), 540-551.

Ezenwa, V. O. and Jolles, A. E. 2015. Opposite effects of anthelmintic treatment on microbial infection at individual versus population scales. *Science* 347(6218), 175-177.

Fellous, S. and Koella, J. C. 2009. Infectious dose affects the outcome of the within-host competition between parasites. *American Naturalist* 173(6), E177-E184.

Fellous, S. and Koella, J. C. 2010. Cost of co-infection controlled by infectious dose combinations and food availability. *Oecologia* 162(4), 935-940.

Fenton, A. 2008. Worms and germs: the population dynamic consequences of microparasite-macroparasite co-infection. *Parasitology* 135(13), 1545-1560.

Fenton, A. et al. 2008. Optimality analysis of Th1/Th2 immune responses during microparasite-macroparasite co-infection, with epidemiological feedbacks. *Parasitology* 135(7), 841-853.

Fenton, A. and Perkins, S. E. 2010. Applying predator-prey theory to modelling immune-mediated, within-host interspecific parasite interactions. *Parasitology* 137(6), 1027-1038.

Ferrandon, D. et al. 1998. A drosomycin–GFP reporter transgene reveals a local immune response in *Drosophila* that is not dependent on the Toll pathway. *The EMBO journal* 17(5).

Ferrari, N. et al. 2009. *Heligmosomoides polygyrus* reduces infestation of Ixodes ricinus in free-living yellow-necked mice, *Apodemus flavicollis*. *Parasitology* 136(3), 305-316.

Finney, C. A. M. et al. 2013. HIV infection deregulates innate immunity to malaria despite combination antiretroviral therapy. *Aids* 27(3), 325-335.

Fischer, B. et al. 2009. Unexpected patterns of plastic energy allocation in stochastic environments. *The American Naturalist* 173(3), E108-E120.

Fisher, M. L. et al. 2018. Growth kinetics of endosymbiont Wolbachia in the common bed bug, *Cimex lectularius. Scientific reports* 8(1).

Fitters, P. F. L. et al. 1997. Estimation of lipid reserves in unstained living and dead nematodes by image analysis. *Journal of Nematology* 29(2), 160-167.

Fitters, P. F. L. et al. 1999. Fatty acid composition of *Heterorhabditis* sp during storage. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 124(1), 81-88.

Fitters, P. F. L. and Griffin, C. T. 2004. Spontaneous and induced activity of *Heterorhabditis megidis* infective juveniles during storage. *Nematology* 6.

Fitters, P. F. L. and Griffin, C. T. 2006. Survival, starvation, and activity in *Heterorhabditis megidis* (Nematoda : Heterorhabditidae). *Biological Control* 37(1).

Forbes, K. M. et al. 2016. Food limitation constrains host immune responses to nematode infections. *Biology Letters* 12(9).

Forsthuber, T. et al. 1996. Induction of Th1 and Th2 immunity in neonatal mice. *Science* 271(5256), 1728-1730.

Frank, S. A. 1992. A kin selection model for the evolution of virulence. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 250(1329), 195-197.

Frank, S. A. 1996. Models of parasite virulence. *Quarterly Review of Biology* 71(1), 37-78.

Frankham, R. et al. 2002. *Introduction to conservation genetics*. Cambridge University Press.

Fraser, C. et al. 2014. Virulence and pathogenesis of HIV-1 infection: An evolutionary perspective. *Science* 343(6177), 1328.

Fredensborg, B. L. et al. 2004. Intensity-dependent mortality of *Paracalliope novizealandiae* (Amphipoda : Crustacea) infected by a trematode: experimental infections and field observations. *Journal of Experimental Marine Biology and Ecology* 311(2), 253-265.

Freitak, D. et al. 2003. Immune response is energetically costly in white cabbage butterfly pupae. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270(suppl_2).

Freitak, D. et al. 2009. Dietary-dependent trans-generational immune priming in an insect herbivore. *Proceedings of the Royal Society B: Biological Sciences* 276(1667), 2617-2624.

French, S. S. et al. 2009. Ecological immunology: The organism in context. *Integrative and Comparative Biology* 49(3), 246-253.

Frentiu, F. D. et al. 2014. Limited dengue virus replication in field-collected *Aedes aegypti* mosquitoes infected with *Wolbachia*. *PLoS neglected tropical diseases* 8(2).

Frost, P. C. et al. 2008. Bacterial infection changes the elemental composition of *Daphnia magna*. *Journal of Animal Ecology* 77(6), 1265-1272.

Fujimoto, A. et al. 2007. Dispersal, infectivity and sex ratio of early-or late-emerging infective juveniles of the entomopathogenic nematode *Steinernema carpocapsae*. *Journal of Nematology* 39(4), 333.

Galvani, A. P. 2003. Epidemiology meets evolutionary ecology. *Trends in Ecology & Evolution* 18(3), 132-139.

Gandon, S. et al. 2001. Host life history and the evolution of parasite virulence. *Evolution* 55(5), 1056-1062.

Gandon, S. and Michalakis, Y. 2000. Evolution of parasite virulence against qualitative or quantitative host resistance. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 267(1447), 985-990.

Garver, L. S. et al. 2012. Anopheles Imd pathway factors and effectors in infection intensity-dependent anti-Plasmodium action. *PLoS pathogens* 8(6).

Gaugler, R. et al. 1990. Fitness of a genetically improved entomopathogenic nematode. *Journal of Invertebrate Pathology* 56(1).

Gaugler, R. et al. 1994. Aggressive and evasive behaviors in *Popillia japonica* (Coleoptera: Scarabaeidae) larvae: defenses against entomopathogenic nematode attack. *Journal of Invertebrate Pathology* 64(3).

Georgis, R. et al. 2006. Successes and failures in the use of parasitic nematodes for pest control. *Biological Control* 38(1), 103-123.

Ghosh, S. et al. 2018. Changes in parasite traits, rather than intensity, affect the dynamics of infection under external perturbation. *Plos Computational Biology* 14(6).

Gigliolli, A. A. S. et al. 2016. The life cycle of *Gregarina cuneata* in the midgut of *Tribolium castaneum* and the effects of parasitism on the development of insects. *Bulletin of Entomological Research* 106(2), 258-267.

Gillespie, J. P. et al. 1997. Biological mediators of insect immunity. *Annual Review of Entomology* 42, 611-643.

Giordanengo, P. and Nenon, J. P. 1990. Melanization and encapsulation of eggs and larvae of *Epidinocarsis lopezi* by its host *Phenacoccus manihoti* - effects of superparasitism and egg laying patterns. *Entomologia Experimentalis et Applicata* 56(2), 155-163.

Godfray, H. 2001. Basis of the trade–off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster. Proceedings of the Royal Society of London. Series B: Biological Sciences* 268(1464), 259-261.

Gonzalez-Santoyo, I. and Cordoba-Aguilar, A. 2012. Phenoloxidase: a key component of the insect immune system. *Entomologia Experimentalis et Applicata* 142(1), 1-16.

Gonzalez, A. J. et al. 2018. Attenuation of Influenza a virus disease severity by viral coinfection in a mouse model. *Journal of Virology* 92(23).

Gorsich, E. E. et al. 2018. Opposite outcomes of coinfection at individual and population scales. *Proceedings of the National Academy of Sciences of the United States of America*.

Gouge, D. H. et al. 1999. Effect of temperature and lepidopteran host species on entomopathogenic nematode (Nematoda: Steinernematidae, Heterorhabditidae) infection. *Environmental entomology* 28(5).

Gower, C. M. and Webster, J. P. 2005. Intraspecific competition and the evolution of virulence in a parasitic trematode. *Evolution* 59(3), 544-553.

Graham, A. L. 2008. Ecological rules governing helminth-microparasite coinfection. *Proceedings of the National Academy of Sciences of the United States of America* 105(2), 566-570.

Graham, A. L. et al. 2007. Transmission consequences of coinfection: cytokines writ large? *Trends in Parasitology* 23(6), 284-291.

Graham, A. L. et al. 2005. Malaria-filaria coinfection in mice makes malarial disease more severe unless filarial infection achieves patency. *Journal of Infectious Diseases* 191(3), 410-421.

Green, M. S. et al. 2002. When is an epidemic an epidemic? *Israel Medical Association Journal* 4(1).

Grewal, P. S. and Richardson, P. N. 1993. Effects of application rates of *Steinernema feltiae* (nematoda, steinernematidae) on biological-control of the mushroom fly *Lycoriella auripila* (diptera, sciaridae). *Biocontrol Science and Technology* 3(1).

Griffin, C. T. 2012. Perspectives on the behaviour of entomopathogenic nematodes from dispersal to reproduction: Traits contributing to nematode fitness and biocontrol efficacy. *Journal of Nematology* 44(2), 177-184.

Griffiths, E. C. et al. 2015. Bottom-up regulation of malaria population dynamics in mice co-infected with lung-migratory nematodes. *Ecology Letters* 18(12), 1387-1396.

Griffiths, E. C. et al. 2014. Analysis of a summary network of co-infection in humans reveals that parasites interact most via shared resources. *Proceedings of the Royal Society B-Biological Sciences* 281(1782).

Gryseels, B. and Nkulikyinka, L. 1989. 2-Year follow-up of *Schistosoma mansoni* infection and morbidity after treatment with different regimens of oxamniquine and praziquantel. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 83(2), 219-228.

Hague, M. and Routman, E. 2016. Does population size affect genetic diversity? A test with sympatric lizard species. *Heredity* 116(1), 92.

Hall, S. R. et al. 2009. Resource ecology of virulence in a planktonic host-parasite system: an explanation using dynamic energy budgets. *The American Naturalist* 174(2), 149-162.

Hall, S. R. et al. 2007. Eating yourself sick: transmission of disease as a function of foraging ecology. *Ecology Letters* 10(3), 207-218.

Hamilton, R. et al. 2008. Two arms are better than one: parasite variation leads to combined inducible and constitutive innate immune responses. *Proceedings of the Royal Society B: Biological Sciences* 275(1637).

Han, R. C. and Ehlers, R. U. 2000. Pathogenicity, development, and reproduction of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* under axenic in vivo conditions. *Journal of Invertebrate Pathology* 75(1), 55-58.

Harris, K. L. et al. 1986. Comparative-studies on the melanization response of male and female mosquitos against microfilariae. *Developmental and Comparative Immunology* 10(3), 305-310.

Harrison, F. et al. 2006. Cooperation and virulence in acute *Pseudomonas aeruginosa* infections. *BMC Biology* 4(1), 21.

Hartgers, F. C. et al. 2008. Immune responses during helminth-malaria co-infection: a pilot study in Ghanaian school children. *Parasitology* 135(7), 855-860.

Hartgers, F. C. and Yazdanbakhsh, M. 2006. Co-infection of helminths and malaria: modulation of the immune responses to malaria. *Parasite Immunology* 28(10), 497-506.

Hartman, R. S. and Karp, R. D. 1989. Short-term immunologic memory in the allograft response of the American cockroach, *Periplaneta americana*. *Transplantation* 47(5), 920-921.

Hass, B. et al. 2002. Persistence of four *Heterorhabditis* spp. isolates in soil: Role of lipid reserves. *Journal of Nematology* 34(2), pp. 151-158.

Hatab, M. A. and Gaugler, R. 1997. Influence of growth temperature on fatty acids and phospholipids of *Steinernema riobravis* infective juveniles. *Journal of Thermal Biology* 22(4-5).

Heffernan, J. M. et al. 2005. Perspectives on the basic reproductive ratio. *Journal of the Royal Society Interface* 2(4), 281-293.

Helmby, H. et al. 1998. Altered immune responses in mice with concomitant *Schistosoma mansoni* and *Plasmodium chabaudi* infections. *Infection and Immunity* 66(11), 5167-5174.

Hernández López, J. et al. 2014. Trans-generational immune priming in honeybees. *Proceedings of the Royal Society B: Biological Sciences* 281(1785), 20140454.

Heve, W. K. et al. 2017. Biological control potential of entomopathogenic nematodes for management of Caribbean fruit fly, *Anastrepha suspensa Loew* (Tephritidae). *Pest Management Science* 73(6), 1220-1228.

Hillyer, J. F. 2016. Insect immunology and hematopoiesis. *Developmental & Comparative Immunology* 58, 102-118.

Hoeve, M. A. et al. 2009. *Plasmodium chabaudi* limits early *Nippostrongylus brasiliensis*-induced pulmonary immune activation and Th2 polarization in co-infected mice. *Bmc Immunology* 10.

Honkavaara, J. et al. 2009. Mating status, immune defence, and multi-parasite burden in the damselfly *Coenagrion armatum*. *Entomologia experimentalis et applicata* 132(2).

Hoffmann, J. A. 1995. Innate immunity of insects. *Current opinion in immunology* 7(1), 4-10.

Hotez, P. J. 2015. Correction: Blue Marble Health Redux: Neglected Tropical Diseases and Human Development in the Group of 20 (G20) Nations and Nigeria. *PLoS neglected tropical diseases* 9(8), e0004035-e0004035.

Hoverman, J. T. et al. 2013. Does timing matter? How priority effects influence the outcome of parasite interactions within hosts. *Oecologia* 173(4), 1471-1480.

Hu, Y. and Aksoy, S. 2005. An antimicrobial peptide with trypanocidal activity characterized from *Glossina morsitans* morsitans. *Insect biochemistry and molecular biology* 35(2).

Humiczewska, M. a. and Rajski, K. 2005. Lipids in the host-parasite system: digestive gland of *Lymnaea truncatula* infected with the developmental stages of *Fasciola hepatica*. *Acta Parasitol* 50(3), 235-239.

Hupalo, K. et al. 2014. Gregarine parasitism in two damselfly hosts: Comparison between species, sexes, and sites (Odonata: Calopterygidae). *Odonatologica* 43(3-4), 199-211.

Ilvonen, J. J. and Suhonen, J. 2016. Phylogeny affects host's weight, immune response and parasitism in damselflies and dragonflies. *Royal Society Open Science* 3(11).

Imler, J.-L. and Bulet, P. 2005. Antimicrobial peptides in *Drosophila*: structures, activities and gene regulation.*Mechanisms of Epithelial Defense*. Vol. 86. Karger Publishers, 1-21.

Iweala, O. I. et al. 2007. Th2 polarizing helminth infection alters the immune response to an oral *Salmonella*-OVA vaccine (41.5).

Jallow, S. and Mahdi, S. A. 2018. Possible implications of maternal HIV infection for increasing measles susceptibility in young infants. *Future Virology* 13(6), 371-373.

Jensen, K. H. et al. 2006. Empirical support for optimal virulence in a castrating parasite. *PLoS biology* 4(7).

Ji, D. and Kim, Y. 2004. An entomopathogenic bacterium, *Xenorhabdus nematophila*, inhibits the expression of an antibacterial peptide, cecropin, of the beet armyworm, *Spodoptera exigua*. *Journal of Insect Physiology* 50(6), 489-496.

Jianyong, L. et al. 1992. Relationship of hemolymph phenoloxidase and mosquito age in *Aedes aegypti*. *Journal of Invertebrate Pathology* 60(2), 188-191.

Johnson, P. T. J. and Hoverman, J. T. 2012. Parasite diversity and coinfection determine pathogen infection success and host fitness. *Proceedings of the National Academy of Sciences of the United States of America* 109(23), 9006-9011.

Jolles, A. E. et al. 2008. Interactions between macroparasites and microparasites drive infection patterns in free-ranging African buffalo. *Ecology* 89(8), 2239-2250.

Jomori, T. et al. 1990. Purification and characterization of lipopolysaccharide-binding protein from hemolymph of American cockroach *Periplaneta americana*. *European Journal of Biochemistry* 190(1), 201-206.

Jomori, T. and Natori, S. 1991. Molecular cloning of cDNA for lipopolysaccharide-binding protein from the hemolymph of the American cockroach, *Periplaneta americana*. Similarity of the protein with animal lectins and its acute phase expression. *Journal of Biological Chemistry* 266(20), 13318-13323.

Jones, K. E. et al. 2008. Global trends in emerging infectious diseases. *Nature* 451(7181), 990-U994.

Kajuga, J. et al. 2018. Management of white grubs (Coleoptera: Scarabeidae) with entomopathogenic nematodes in Rwanda. *Egyptian Journal of Biological Pest Control* 28(1).

Kamal, S. M. and El Sayed Khalifa, K. 2006. Immune modulation by helminthic infections: worms and viral infections. *Parasite Immunology* 28(10), 483-496.

Kamya, M. R. et al. 2006. Effect of HIV-1 infection on antimalarial treatment outcomes in Uganda: A population-based study. *Journal of Infectious Diseases* 193(1), 9-15.

Karp, R. D. et al. 1994. Evolution of adaptive immunity - inducible responses in the American cockroach. In: Beck, G. et al. eds. *Primordial Immunity: Foundations for the Vertebrate Immune System*. Vol. 712. 82-91.

Kaunisto, K. M. et al. 2017. Parasitism, immune response, and egg production of the spearhead bluet (*Coenagrion hastulatum*) damselfly. *Canadian Journal of Zoology* 95(5), 367-372.

Kavanagh, K. and Reeves, E. P. 2007. Insect and mammalian innate immune responses are much alike. *Microbe-American society for microbiology* 2(12).

Kaya, H. K. and Gaugler, R. 1993. Entomopathogenic nematodes. In: Mittler, T.E. et al. eds. *Annual Review of Entomology*. Vol. 38. 181-206.

Keeling, M. J. and Eames, K. T. 2005. Networks and epidemic models. *Journal of the Royal Society Interface* 2(4), 295-307.

Keller, L. F. and Waller, D. M. 2002. Inbreeding effects in wild populations. *Trends in Ecology & Evolution* 17(5), 230-241.

Kelly, T. R. et al. 2017. One Health proof of concept: Bringing a transdisciplinary approach to surveillance for zoonotic viruses at the human-wild animal interface. *Preventive Veterinary Medicine* 137, 112-118.

Kilpatrick, A. M. et al. 2006. Host heterogeneity dominates West Nile virus transmission. *Proceedings of the Royal Society B-Biological Sciences* 273(1599), 2327-2333.

Kim, Y. et al. 2005. Two groups of entomopathogenic bacteria, *Photorhabdus* and *Xenorhabdus*, share an inhibitory action against phospholipase A(2) to induce host immunodepression. *Journal of Invertebrate Pathology* 89(3), 258-264.

Kim, Y. G. et al. 2011. Bacterial metabolites of an entomopathogenic bacterium, *Xenorhabdus nematophila*, inhibit a catalytic activity of phenoloxidase of the diamondback moth, *Plutella xylostella*. *Journal of Microbiology and Biotechnology* 21(3), 317-322.

Kimura, M. 1983. *The neutral theory of molecular evolution*. Cambridge University Press.

Kinnula, H. et al. 2017. Coinfection outcome in an opportunistic pathogen depends on the inter-strain interactions. *Bmc Evolutionary Biology* 17.

Klasing, K. C. et al. 2004. What are the costs of immunity? *Poultry Science* 83, 444-444.

Klein, S. L. 2000. Hormones and mating system affect sex and species differences in immune function among vertebrates. *Behavioural Processes* 51(1-3), 149-166.

Klein, S. L. 2004. Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunology* 26(6-7), 247-264.

Klein, S. L. et al. 2000. Sex differences in Seoul virus infection are not related to adult sex steroid concentrations in Norway rats. *Journal of Virology* 74(17), 8213-8217.

Knowles, S. C. L. et al. 2013. Stability of within-host - parasite communities in a wild mammal system. *Proceedings of the Royal Society B-Biological Sciences* 280(1762).

Knutie, S. A. et al. 2017. Host resistance and tolerance of parasitic gut worms depend on resource availability. *Oecologia* 183(4), 1031-1040.

Koehler, P. G. et al. 1992. Susceptibility of cockroaches (Dictyoptera, Blattellidae, Blattidae) to infection by *Steinernema carpocapsae*. *Journal of Economic Entomology* 85(4), 1184-1187.

Koella, J. C. and Sorensen, F. L. 2002. Effect of adult nutrition on the melanization immune response of the malaria vector *Anopheles stephensi. Medical and Veterinary Entomology* 16(3), 316-320.

Koppenhöfer, A. M. et al. 1997. Survival of entomopathogenic nematodes within host cadavers in dry soil. *Applied Soil Ecology* 6(3), 231-240.

Koppenhöfer, A. M. et al. 2000. Synergism of imidacloprid and entomopathogenic nematodes against white grubs: the mechanism. *Entomologia Experimentalis et Applicata* 94(3).

Korner, P. and Schmid-Hempel, P. 2004. In vivo dynamics of an immune response in the bumblebee *Bombus terrestris*. *Journal of Invertebrate Pathology* 87(1), 59-66.

Koski, K. G. and Scott, M. E. 2001. Gastrointestinal nematodes, nutrition and immunity: Breaking the negative spiral. *Annual Review of Nutrition* 21, 297-321.

Krams, I. et al. 2017. Reproduction is costly in an infected aquatic insect. *Ethology Ecology & Evolution* 29(1).

Krist, A. et al. 2000. Spatial variation in susceptibility to infection in a snail–trematode interaction. *Parasitology* 121(4), 395-401.

Kubiak, M. and Tinsley, M. C. 2017. Sex-specific routes to immune senescence in *Drosophila melanogaster*. *Scientific Reports* 7(1), 10417.

Kuriyama, R. et al. 2005. Dynamic organization of microtubules and microtubule-organizing centers during the sexual phase of a parasitic protozoan, *Lecudina tuzetae* (Gregarine, Apicomplexa). *Cell Motility and the Cytoskeleton* 62(4), 195-209.

Kurtz, J. and Armitage, S. A. O. 2006. Alternative adaptive immunity in invertebrates. *Trends in Immunology* 27(11), 493-496.

Kurtz, J. and Franz, K. 2003. Innate defence: evidence for memory in invertebrate immunity. *Nature* 425(6953), 37.

Kurtz, J. and Sauer, K. P. 1999. The immunocompetence handicap hypothesis: testing the genetic predictions. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 266(1437), 2515-2522.

Labaude, S. and Griffin, C. 2018. Transmission success of entomopathogenic nematodes used in pest control. *Insects* 9(2), 72.

Lacey, L. A. 1997. *Manual of techniques in insect pathology*. Academic Press.

Lacey, L. A. and Georgis, R. 2012. Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. *Journal of Nematology* 44(2), 218.

Legaspi, J. C. et al. 2000. Evaluation of *Steinernema riobravis* (Nematoda: Steinernematidae) against the Mexican rice borer (Lepidoptera: Pyralidae). *Journal of Entomological Science* 35(2).

Lagrue, C. and Poulin, R. 2008. Intra- and interspecific competition among helminth parasites: Effects on *Coitocaecum parvum* life history strategy, size and fecundity. *International Journal for Parasitology* 38(12), 1435-1444.

Lancet, T. 2004. HIV/AIDS: not one epidemic but many. Elsevier.

Lai, S.-C. et al. 2002. Immunolocalization of prophenoloxidase in the process of wound healing in the mosquito *Armigeres subalbatus* (Diptera: Culicidae). *Journal of Medical Entomology* 39(2), 266-274.

Lamb, T. J. et al. 2005. Co-infected C57BL/6 mice mount appropriately polarized and compartmentalized cytokine responses to *Litomosoides sigmodontis* and *Leishmania major* but disease progression is altered. *Parasite Immunology* 27(9), 317-324.

Lambin, E. F. et al. 2010. Pathogenic landscapes: interactions between land, people, disease vectors, and their animal hosts. *International journal of health geographics* 9(1).

Landers, S. C. 2010. The fine structure of the developing oocyst of *Pterospora floridiensis* (Apicomplexa, Urosporidae). *European Journal of Protistology* 46(1), 1-9.

League, G. P. et al. 2017. *Anopheles gambiae* larvae mount stronger immune responses against bacterial infection than adults: evidence of adaptive decoupling in mosquitoes. *Parasites & Vectors* 10(1), 367.

Lebeaux, D. et al. 2013. From in vitro to in vivo Models of Bacterial Biofilm-Related Infections. *Pathogens (Basel, Switzerland)* 2(2), 288-356.

Lee, S. Y. et al. 2000. A Lipopolysaccharide-and β-1, 3-Glucan-binding Protein from Hemocytes of the Freshwater Crayfish *Pacifastacus leniusculus* purification, characterization, and cDNA cloning. *Journal of Biological Chemistry* 275(2), 1337-1343.

Lehmer, E. M. et al. 2018. Evaluating the impacts of coinfection on immune system function of the deer mouse (*Peromyscus maniculatus*) using sin nombre virus and *Bartonella* as model pathogen systems. *Journal of Wildlife Diseases* 54(1), 66-75.

Lemaitre, B. et al. 1996. The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. *Cell* 86(6).

Lello, J. et al. 2004. Competition and mutualism among the gut helminths of a mammalian host. *Nature* 428(6985), 840-844.

Lello, J. et al. 2005. The effect of single and concomitant pathogen infections on condition and fecundity of the wild rabbit (*Oryctolagus cuniculus*). *International Journal for Parasitology* 35(14), 1509-1515.

Lello, J. et al. 2013. The relative contribution of co-infection to focal infection risk in children. *Proceedings of the Royal Society B-Biological Sciences* 280(1754).

Lello, J. et al. 2018. Predicting the effects of parasite co-infection across species boundaries. *Proceedings of the Royal Society B-Biological Sciences* 285(1874).

Leonard, C. et al. 1985. The role of prophenoloxidase activation in non-self recognition and phagocytosis by insect blood cells. *Journal of Insect Physiology* 31(10), 789-799.

Leongson, K. et al. 2013. Altered immune response differentially enhances susceptibility to *Cryptococcus neoformans* and *Cryptococcus gattii* infection in mice expressing the HIV-1 transgene. *Infection and Immunity* 81(4), 1100-1113.

Levin, S. and Pimentel, D. 1981. Selection of intermediate rates of increase in parasite-host systems. *American Naturalist* 117(3), 308-315.

Levine, N. D. 1988. Progress in taxonomy of the Apicomplexan protozoa. *Journal of Protozoology* 35(4), 518-520.

Lewis, E. E. et al. 2006. Behavioral ecology of entomopathogenic nematodes. *Biological Control* 38(1), 66-79.

Leyva, A. et al. 2008. Rapid and sensitive anthrone–sulfuric acid assay in microplate format to quantify carbohydrate in biopharmaceutical products: method development and validation. *Biologicals* 36(2), 134-141.

Li, X.-Y. et al. 2007. Relationship between the successful infection by entomopathogenic nematodes and the host immune response. *International journal for parasitology* 37(3-4).

Lie, K. and Heyneman, D. 1976. Studies on resistance in snails. 6. Escape of *Echinostoma lindoense* sporocysts from encapsulation in the snail heart and subsequent loss of the host's ability to resist infection by the same parasite. *The Journal of Parasitology*, 298-302.

Liesenfeld, O. et al. 2004. Infection with *Toxoplasma gondii* reduces established and developing Th2 responses induced by *Nippostrongylus brasiliensis* infection. *Infection and Immunity* 72(7), 3812-3822.

Lifjeld, J. T. et al. 2002. Short-term fluctuations in cellular immunity of tree swallows feeding nestlings. *Oecologia* 130(2).

Linton, P. J. and Dorshkind, K. 2004. Age-related changes in lymphocyte development and function. *Nature Immunology* 5(2), 133.

Little, T. J. et al. 2003. Maternal transfer of strain-specific immunity in an invertebrate. *Current Biology* 13(6), 489-492.

Little, T. J. and Kraaijeveld, A. R. 2004. Ecological and evolutionary implications of immunological priming in invertebrates. *Trends in Ecology & Evolution* 19(2).

Little, T. et al. 2008. Parasite variation and the evolution of virulence in a *Daphnia*-microparasite system. *Parasitology* 135(3).

Lloyd-Smith, J. O. et al. 2005. Superspreading and the effect of individual variation on disease emergence. *Nature* 438(7066), 355-359.

Lochmiller, R. L. and Deerenberg, C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* 88(1), 87-98.

Logan, J. D. et al. 2012. The life cycle and fitness domain of gregarine (Apicomplexa) parasites. *Ecological Modelling* 233, 31-40.

Lokken, K. L. et al. 2018. Malaria parasite-mediated alteration of macrophage function and increased iron availability predispose to disseminated nontyphoidal *Salmonella* infection. *Infection and Immunity* 86(9), e00301-00318.

Long, S. R. et al. 2019. Intestinal helminth infection enhances bacteria-induced recruitment of neutrophils to the airspace. *Scientific Reports* 9.

Lopes, R. B. and Alves, S. B. 2005. Effect of *Gregarina* sp parasitism on the susceptibility of *Blattella germanica* to some control agents. *Journal of Invertebrate Pathology* 88(3), 261-264.

Louhi, K. R. et al. 2015. Interactions among bacterial strains and fluke genotypes shape virulence of co-infection. *Proceedings of the Royal Society B-Biological Sciences* 282(1821).

Lowenberger, C. A. et al. 1996. *Aedes aegypti*: Induced Antibacterial Proteins Reduce the Establishment and Development of *Brugia malayi*. *Experimental parasitology* 83(2).

Lowenberger, C. A. et al. 1999. Mosquito - Plasmodium interactions in response to immune activation of the vector. *Experimental Parasitology* 91(1).

Lucarotti, C. 2000. Cytology of *Leidyana canadensis* (Apicomplexa: Eugregarinida) in *Lambdina fiscellaria* fiscellaria larvae (Lepidoptera: Geometridae). *Journal of Invertebrate Pathology* 75(2).

Luong, L. T. et al. 2011. Strong density-dependent competition and acquired immunity constrain parasite establishment: Implications for parasite aggregation. *International Journal for Parasitology* 41(5), 505-511.

Mackenzie, D. K. et al. 2011. Senescence of the cellular immune response in *Drosophila melanogaster. Experimental Gerontology* 46(11), 853-859.

Macpherson, C. N. L. 2005. Human behaviour and the epidemiology of parasitic zoonoses. *International Journal for Parasitology* 35(11-12), 1319-1331.

Maizels, R. M. et al. 2012. Susceptibility and immunity to helminth parasites. *Current Opinion in Immunology* 24(4), 459-466.

Mannion, C. et al. 2001. Management of early-instar Japanese beetle (Coleoptera: Scarabaeidae) in field-grown nursery crops. *Journal of Economic Entomology* 94(5), 1151-1161.

Marden, J. H. and Cobb, J. R. 2004. Territorial and mating success of dragonflies that vary in muscle power output and presence of gregarine gut parasites. *Animal Behaviour* 68, 857-865.

Márkus, R. et al. 2005. Sterile wounding is a minimal and sufficient trigger for a cellular immune response in *Drosophila melanogaster*. *Immunology Letters* 101(1), 108-111.

Martcheva, M. and Pilyugin, S. S. 2006. The role of coinfection in multidisease dynamics. *SIAM Journal on Applied Mathematics* 66(3), 843-872.

Martin 2nd, L. B. et al. 2007. Immunological memory is compromised by food restriction in deer mice *Peromyscus maniculatus*. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 292(1), R316-R320.

Martin, L. B. et al. 2003. Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270(1511).

Masha, S. C. et al. 2019. *Trichomonas vaginalis* and HIV infection acquisition: a systematic review and meta-analysis. *Sex Transm Infect* 95(1), 36-42.

Massey, R. C. et al. 2004. Interference competition and parasite virulence. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 271(1541), 785-788.

Max Kuhn, S. W., Jed Wing, James Forester, Thorn Thaler. 2016. A collection of Contrasts Methods.

May, R. M. and Nowak, M. A. 1994. Superinfection, metapopulation dynamics, and the evolution of diversity. *Journal of Theoretical Biology* 170(1), 95-114.

May, R. M. and Nowak, M. A. 1995. Coinfection and the evolution of parasite virulence. *Proceedings of the Royal Society B-Biological Sciences* 261(1361), 209-215.

McCallum, H. et al. 2001. How should pathogen transmission be modelled? *Trends in ecology & evolution* 16(6).

McClelland, R. S. et al. 2007. Infection with *Trichomonas vaginalis* increases the risk of HIV-1 acquisition. *The Journal of Infectious Diseases* 195(5), 698-702.

McKean, K. A. et al. 2008. The evolutionary costs of immunological maintenance and deployment. *BMC Evolutionary Biology* 8(1), 76.

McFall-Ngai, M. 2007. Adaptive immunity: care for the community. *Nature* 445(7124), 153.

McFarlane, R. et al. 2013. Land-use change and emerging infectious disease on an island continent. *International journal of environmental research and public health* 10(7), 2699-2719.

McNamara, J. M. et al. 2009. Deterioration, death and the evolution of reproductive restraint in late life. *Proceedings of the Royal Society B: Biological Sciences* 276(1675), 4061-4066.

Medica, D. L. and Sukhdeo, M. V. K. 1997. Role of lipids in the transmission of the infective stage (L3) of *Strongylus vulgaris* (Nematoda: Strongylida). *Journal of Parasitology* 83(5), 775-779.

Meister, M. and Lagueux, M. 2003. *Drosophila* blood cells. *Cellular Microbiology* 5(9), 573-580.

Meister, S. et al. 2009. *Anopheles gambiae* PGRPLC-Mediated Defense against Bacteria Modulates Infections with Malaria Parasites. *Plos Pathogens* 5(8).

Memari, Z. et al. 2016. Are entomopathogenic nematodes effective biological control agents against the carob moth, *Ectomyelois ceratoniae*? *Journal of Nematology* 48(4), 261.

Menti, H. et al. 2003. Lipid utilisation during storage of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis megidis* from Greece and the UK. *Nematology* 5, 31-37.

Menti, H. et al. 2000. Infectivity of populations of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis megidis* in relation to temperature, age and lipid content. *Nematology* 2, 515-521.

Méthot, P.-O. 2012. Why do Parasites Harm Their Host? On the Origin and Legacy of Theobald Smith's" Law of Declining Virulence"—1900-1980. *History and Philosophy of the Life Sciences*, 561-601.

Mideo, N. et al. 2008. Linking within- and between-host dynamics in the evolutionary epidemiology of infectious diseases. *Trends in Ecology & Evolution* 23(9), 511-517.

Miller, C. V. L. and Cotter, S. C. 2018. Resistance and tolerance: The role of nutrients on pathogen dynamics and infection outcomes in an insect host. *Journal of Animal Ecology* 87(2), 500-510.

Moore, S. L. and Wilson, K. 2002. Parasites as a viability cost of sexual selection in natural populations of mammals. *Science* 297(5589), 2015-2018.

Moreira-Ludewig, R. and Healy, C. T. 1992. A rapid microtiter plate method for the detection of lysozyme release from human neutrophils. *Journal of Pharmacological and Toxicological Methods* 27(2), 95-100.

Morens, D. M. and Fauci, A. S. 2013. Emerging Infectious Diseases: Threats to Human Health and Global Stability. *Plos Pathogens* 9(7).

Moret, Y. 2006. 'Trans-generational immune priming': specific enhancement of the antimicrobial immune response in the mealworm beetle, *Tenebrio molitor*. *Proceedings of the Royal Society B: Biological Sciences* 273(1592), 1399-1405.

Moret, Y. and Schmid-Hempel, P. 2000. Survival for immunity: the price of immune system activation for bumblebee workers. *Science* 290(5494), 1166-1168.

Moret, Y. and Schmid-Hempel, P. 2001. Entomology: immune defence in bumblebee offspring. *Nature* 414(6863), 506.

Moret, Y. and Siva-Jothy, M. T. 2003. Adaptive innate immunity? Responsive-mode prophylaxis in the mealworm beetle, *Tenebrio molitor*. *Proceedings of the Royal Society B-Biological Sciences* 270(1532), 2475-2480.

Morse, S. S. 2012. Public health surveillance and infectious disease detection. *Biosecurity and Bioterrorism-Biodefense Strategy Practice and Science* 10(1), 6-16.

Morton, A. and Del Pino, F. G. 2007. Susceptibility of shore fly *Scatella stagnalis* to five entomopathogenic nematode strains in bioassays. *Biocontrol* 52(4), 533-545.

Mosquera, J. and Adler, F. R. 1998. Evolution of virulence: a unified framework for coinfection and superinfection. *Journal of Theoretical Biology* 195(3), 293-313.

Muller-Graf, C. D. M. et al. 2001. Population dynamics of host-parasite interactions in a cockroach-oxyuroid system. *Oikos* 95(3), 431-440.

Mutze, G. et al. 2002. Emerging epidemiological patterns in rabbit haemorrhagic disease, its interaction with myxomatosis, and their effects on rabbit populations in South Australia. *Wildlife Research* 29(6), 577-590.

Mylonakis, E. et al. 2007. Exploiting amoeboid and non-vertebrate animal model systems to study the virulence of human pathogenic fungi. *Plos Pathogens* 3(7), 859-865.

Nacher, M. et al. 2002. Intestinal helminth infections are associated with increased incidence of *Plasmodium falciparum* malaria in Thailand. *Journal of Parasitology* 88(1), 55-58.

Nasci, R. S. and Mitchell, C. J. 1994. Larval diet, adult size, and susceptibility of *Aedes aegypti* (Diptera: Culicidae) to infection with Ross River virus. *Journal of Medical Entomology* 31(1), 123-126.

Nazzi, F. et al. 2012. Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. *PLoS Pathogens* 8(6), e1002735.

Nazni, W. A. et al. 2019. Establishment of *Wolbachia* Strain wAlbB in Malaysian Populations of *Aedes aegypti* for Dengue Control. *Current Biology* 29(24), 424.

Nguyen, K. B. et al. 2001. Diagnostic and phylogenetic utility of the rDNA internal transcribed spacer sequences of *Steinernema*. *Journal of Nematology* 33(2-3), 73-82.

Nigam, Y. et al. 1997. Detection of Phenoloxidase Activity in the Hemolymph of Tsetse Flies, Refractory and Susceptible to Infection with *Trypanosoma brucei rhodesiense*. *Journal of Invertebrate Pathology* 69(3), 279-281.

Nkhoma, E. T. et al. 2012. The Effect of HIV Infection on the Risk, Frequency, and Intensity of *Plasmodium falciparum* Parasitemia in Primigravid and Multigravid Women in Malawi. *American Journal of Tropical Medicine and Hygiene* 87(6), 1022-1027.

Norris, K. and Evans, M. R. 2000. Ecological immunology: life history trade-offs and immune defense in birds. *Behavioral Ecology* 11(1), 19-26.

Nunn, C. L. et al. 2014. Interactions between Micro- and Macroparasites Predict Microparasite Species Richness across Primates. *American Naturalist* 183(4), 494-505.

O'Leary, S. A. et al. 1998. The effect of day of emergence from the insect cadaver on the behavior and environmental tolerances of infective juveniles of the entomopathogenic nematode *Heterorhabditis megidis* (strain UK211). *Journal of Parasitology* 84(4), 665-672.

O'Lorcain, P. and Holland, C. V. 2000. The public health importance of *Acaris lumbricoides*. *Parasitology* 121, S51-S71.

O'Neal, S. E. et al. 2007. Influence of helminth infections on the clinical course and immune response to *Leishmania braziliensis* cutaneous leishmaniasis. *Journal of Infectious Diseases* 195(1), 142-148.

Ochiai, M. et al. 1992. Immunocytochemical localization of β-1, 3-glucan recognition protein in the silkworm, *Bombyx mori. Cell and Tissue Research* 268(3), 431-437.

Odendaal, D. et al. 2016. Entomopathogenic nematodes for the control of the codling moth (*Cydia pomonella*) in field and laboratory trials. *Journal of Helminthology* 90(5), 615-623.

Onah, D. et al. 2004. Enhanced survival of rats concurrently infected with *Trypanosoma brucei* and *Strongyloides ratti*. *Veterinary parasitology* 119(2-3), 165-176.

Ots, I. et al. 2001. Immune challenge affects basal metabolic activity in wintering great tits. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 268(1472), 1175-1181.

Park, Y. and Kim, Y. 2000. Eicosanoids rescue *Spodoptera exigua* infected with *Xenorhabdus nematophilus*, the symbiotic bacteria to the entomopathogenic nematode *Steinernema carpocapsae*. *Journal of Insect Physiology* 46(11), 1469-1476.

Park, Y. and Kim, Y. 2003. *Xenorhabdus nematophilus* inhibits p-bromophenacyl bromide (BPB)-sensitive PLA2 of *Spodoptera exigua*. *Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America* 54(3), 134-142.

Patel, M. N. et al. 1997. Neutral lipids and the assessment of infectivity in entomopathogenic nematodes: Observations on four *Steinernema* species. *Parasitology* 114, 489-496.

Patel, M. N. and Wright, D. J. 1997. Fatty acid composition of neutral lipid energy reserves in infective juveniles of entomopathogenic nematodes. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 118(2), 341-348.

Patel, M. N. and Wright, D. J. 1997. Phospholipids and their fatty acids in infective juveniles of entomopathogenic steinernematid nematodes. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 118(3), 649-657.

Pathak, A. K. et al. 2012. Immuno-epidemiology of chronic bacterial and helminth co-infections: observations from the field and evidence from the laboratory. *International Journal for Parasitology* 42(7), 647-655.

Patz, J. A. et al. 2004. Unhealthy landscapes: policy recommendations on land use change and infectious disease emergence. *Environmental health perspectives* 112(10), 1092-1098.

Pedersen, A. B. and Antonovics, J. 2013. Anthelmintic treatment alters the parasite community in a wild mouse host. *Biology Letters* 9(4), 20130205.

Pedersen, A. B. and Fenton, A. 2007. Emphasizing the ecology in parasite community ecology. *Trends in Ecology & Evolution* 22(3), 133-139.

Pena, J. M. et al. 2015. Variation in the Susceptibility of *Drosophila* to Different Entomopathogenic Nematodes. *Infection and Immunity* 83(3), 1130-1138.

Pepin, M. et al. 2007. Emergence of animal and human infectious diseases. *Productions Animales* 20(3), 199-205.

Pereira, T. N. et al. 2018. *Wolbachia* significantly impacts the vector competence of *Aedes aegypti* for Mayaro virus. *Scientific Reports* 8.

Peterson, R. T. et al. 2008. Use of non-mammalian alternative models for neurotoxicological study. *Neurotoxicology* 29(3), 546-555.

Petney, T. N. and Andrews, R. H. 1998. Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. *International Journal for Parasitology* 28(3), 377-393.

Pham, L. N. et al. 2007. A specific primed immune response in *Drosophila* is dependent on phagocytes. *PLoS Pathogens* 3(3), e26.

Piot, P. et al. 2014. Ebola in west Africa: from disease outbreak to humanitarian crisis. *The Lancet Infectious Diseases* 14(11), 1034-1035.

Ponton, F. et al. 2013. Integrating nutrition and immunology: A new frontier. *Journal of Insect Physiology* 59(2), 130-137.

Poulin, R. 2013. Explaining variability in parasite aggregation levels among host samples. *Parasitology* 140(4), 541-546.

Poyet, M. et al. 2013. Resistance of *Drosophila suzukii* to the larval parasitoids *Leptopilina heterotoma* and *Asobara japonica* is related to haemocyte load. *Physiological Entomology* 38(1), 45-53.

Prokop, P. et al. 2010. Health and the avoidance of macroparasites: a preliminary cross-cultural study. *Journal of Ethology* 28(2), 345-351.

Pulkkinen, K. et al. 2009. Intensive fish farming and the evolution of pathogen virulence: the case of columnaris disease in Finland. *Proceedings of the Royal Society B: Biological Sciences* 277(1681), 593-600.

Půža, V. and Mráček, Z. 2010. Does scavenging extend the host range of entomopathogenic nematodes (Nematoda: Steinernematidae)? *Journal of invertebrate pathology* 104(1), 1-3.

Qiu, L. and Bedding, R. A. 2000. Energy metabolism and survival of the infective juveniles of *Steinernema carpocapsae* under oxygen-deficient conditions. *Journal of Nematology* 32(3), 271-280.

Qiu, L. H. and Bedding, R. 2000. Energy metabolism and its relation to survival and infectivity of infective juveniles of *Steinernema carpocapsae* under aerobic conditions. *Nematology* 2, 551-559.

Qiu, W. et al. 2012. powerSurvEpi: Power and sample size calculation for survival analysis of epidemiological studies. R package version 0.0. 6.

Rao, T. V. 2011. *Innate immunity* [Online]. Available at: https://www.slideshare.net/doctorrao/innate-immunity-nobel-prize-2011

R Core Team. 2015. R: A Language and Environment for Statistical Computing.

Raberg, L. et al. 2002. Basal metabolic rate and the evolution of the adaptive immune system. *Proceedings of the Royal Society B-Biological Sciences* 269(1493), 817-821.

Rahayu, A. et al. 2019. Prevalence and Distribution of Dengue Virus in *Aedes aegypti* in Yogyakarta City before Deployment of *Wolbachia* Infected *Aedes aegypti*. *International Journal of Environmental Research and Public Health* 16(10).

Rahnamaeian, M. et al. 2015. Insect antimicrobial peptides show potentiating functional interactions against Gram-negative bacteria. *Proc. R. Soc. B* 282(1806), 20150293.

Rainey, S. M. et al. 2014. Understanding the *Wolbachia*-mediated inhibition of arboviruses in mosquitoes: progress and challenges. *Journal of General Virology* 95(3), 517-530.

Ramiro, R. S. et al. 2016. Facilitation through altered resource availability in a mixed-species rodent malaria infection. *Ecology Letters* 19(9), 1041-1050.

Ramos-Rodríguez, O. et al. 2006. Pathogenicity of three species of entomopathogenic nematodes to some major stored-product insect pests. *Journal of Stored Products Research* 42(3), 241-252.

Randall, J. 2011. *Endemic infection in the German cockroach, Blattella germanica.* Cardiff University.

Randall, J. et al. 2013. Endemic infection reduces transmission potential of an epidemic parasite during co-infection. *Proceedings of the Royal Society B-Biological Sciences* 280(1769).

Rantala, M. and Roff, D. 2005. An analysis of trade-offs in immune function, body size and development time in the Mediterranean Field Cricket, *Gryllus bimaculatus*. *Functional Ecology* 19(2), 323-330.

Rasband, W. 2016. *Image J* [Online]. Available at: imagej.nih.gov/ij [Accessed.

Ratcliffe, N. 1985. Invertebrate immunity—a primer for the non-specialist. *Immunology Letters* 10(5), 253-270.

Read, A. F. and Taylor, L. H. 2001. The ecology of genetically diverse infections. *Science* 292(5519), 1099-1102.

Reavey, C. et al. 2015. Bacterial infection increases reproductive investment in burying beetles. *Insects* 6(4), 926-942.

Rera, M. et al. 2012. Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in *Drosophila*. *Proceedings of the National Academy of Sciences* 109(52), 21528-21533.

Resende Co, T. et al. 2007. Intestinal helminth co-infection has a negative impact on both anti-*Mycobacterium tuberculosis* immunity and clinical response to tuberculosis therapy. *Clinical and Experimental Immunology* 147(1), 45-52.

Rheins, L. A. and Karp, R. D. 1985. Effect of gender on the inducible humoral immune response to honeybee venom in the American cockroach (*Periplaneta americana*). *Developmental & Comparative Immunology* 9(1), 41-49.

Ribeiro, C. et al. 1999. Insect immunity—effects of factors produced by a nematobacterial complex on immunocompetent cells1. *Journal of Insect Physiology* 45(7), 677-685.

Rivero, A. et al. 2007. Resource depletion in *Aedes aegypti* mosquitoes infected by the microsporidia *Vavraia culicis*. *Parasitology* 134(10), 1355-1362.

Rodgers, F. H. et al. 2017. Microbiota-induced peritrophic matrix regulates midgut homeostasis and prevents systemic infection of malaria vector mosquitoes. *Plos Pathogens* 13(5).

Rodgers, M. A. et al. 2018. Preexisting Simian Immunodeficiency Virus Infection Increases Susceptibility to Tuberculosis in Mauritian Cynomolgus Macaques. *Infection and Immunity* 86(12).

Rodriguez, M. et al. 1999. Susceptibility to *Trypanosoma cruzi* is modified by a previous non-related infection. *Parasite Immunology* 21(4), 177-185.

Rohani, P. et al. 2003. Ecological interference between fatal diseases. *Nature* 422(6934), 885-888.

Rolff, J. 2002. Bateman's principle and immunity. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 269(1493), 867-872.

Rolff, J. and Reynolds, S. 2009. *Insect infection and immunity: evolution, ecology, and mechanisms*. Oxford university press.

Rolff, J. and Siva-Jothy, M. T. 2003. Invertebrate ecological immunology. *Science* 301(5632), 472-475.

Rose, H. et al. 2014. Exploiting parallels between livestock and wildlife: Predicting the impact of climate change on gastrointestinal nematodes in ruminants. *International Journal for Parasitology Parasites and Wildlife* 3(2), 209-219.

Rosengaus, R. et al. 2000. The social transmission of disease between adult male and female reproductives of the dampwood termite *Zootermopsis angusticollis*. *Ethology Ecology & Evolution* 12(4), 419-433.

Rosin, P. L. and Ioannidis, E. 2003. Evaluation of global image thresholding for change detection. *Pattern Recognition Letters* 24(14), 2345-2356.

Ross, M. H. 1976. Laboratory population studies of the German cockroach using a two-chromosome and a three-chromosome reciprocal translocation. *Annals of the Entomological Society of America* 69(6), 1073-1081.

Ross, M. H. et al. 1984. Population growth and behavior of *Blattella germanica* (L.)(Orthoptera: Blattellidae) in experimentally established shipboard infestations. *Annals of the Entomological Society of America* 77(6), 740-752.

Roth, O. et al. 2010. Paternally derived immune priming for offspring in the red flour beetle, *Tribolium castaneum*. *Journal of Animal Ecology* 79(2), 403-413.

Roth, O. et al. 2008. Strain-specific priming of resistance in the red flour beetle, *Tribolium castaneum*. *Proceedings of the Royal Society B: Biological Sciences* 276(1654), 145-151.

Rowley, A. F. and Powell, A. 2007. Invertebrate immune systems–specific, quasi-specific, or nonspecific? *The Journal of Immunology* 179(11), 7209-7214.

Rueckert, S. and Devetak, D. 2017. Gregarines (Apicomplexa, Gregarinasina) in psocids (Insecta, Psocoptera) including a new species description and their potential use as pest control agents. *European journal of protistology* 60, 60-67.

Ruiz-Daniels, R. et al. 2013. Do parasites adopt different strategies in different intermediate hosts? Host size, not host species, influences *Coitocaecum parvum* (Trematoda) life history strategy, size and egg production. *Parasitology* 140(2), 275-283.

Ryder, J. and Siva-Jothy, M. 2001. Quantitative genetics of immune function and body size in the house cricket, *Acheta domesticus*. *Journal of Evolutionary Biology* 14(4), 646-653.

Rynkiewicz, E. C. et al. 2015. An ecosystem approach to understanding and managing within-host parasite community dynamics. *Trends in Parasitology* 31(5), 212-221.

Sadd, B. M. and Schmid-Hempel, P. 2006. Insect immunity shows specificity in protection upon secondary pathogen exposure. *Current Biology* 16(12), 1206-1210.

Salazar-Castanon, V. H. et al. 2014. Helminth Parasites Alter Protection against Plasmodium Infection. *Biomed Research International*.

Salehzadeh, A. et al. 2007. Bacterial, fungal and parasitic contamination of cockroaches in public hospitals of Hamadan, Iran. *Journal of Vector Borne Diseases* 44(2), 105-110.

Salgame, P. et al. 2013. Effect of helminth-induced immunity on infections with microbial pathogens. *Nature Immunology* 14(11), 1118-1126.

Salvaudon, L. et al. 2007. Genotype-specific interactions and the trade-off between host and parasite fitness. *BMC Evolutionary Biology* 7(1), 189.

Santiago, H. d. C. et al. 1999. Coinfection with *Toxoplasma gondii* inhibits antigen-specific Th2 immune responses, tissue inflammation, and parasitism in BALB/c mice infected with *Leishmania major*. *Infection and Immunity* 67(9), 4939-4944.

Santos, J. I. 1994. Nutrition, infection, and immunocompetence. *Infectious Disease Clinics of North America* 8(1), 243-267.

Savioli, L. and Albonico, M. 2004. Focus: soil-transmitted helminthiasis. *Nature Reviews Microbiology* 2(8), 618-620.

Schal, C. et al. 1984. Behavioral ecology of cockroaches. *Biological Reviews of the Cambridge Philosophical Society* 59(2), 209-254.

Scheepmaker, J. W. A. et al. 1998. Susceptibility of larvae of the mushroom fly *Megaselia halterata* to the entomopathogenic nematode *Steinernema feltiae* in bioassays. *Biocontrol* 43(2), 201-214.

Schmid-Hempel, P. 2005. Evolutionary ecology of insect immune defenses. *Annual Review of Entomology*. Vol. 50. 529-551.

Schroeder, P. C. et al. 1996. Greenhouse and field evaluations of entomopathogenic nematodes (Nematoda: Heterorhabditidae and Steinernematidae) for control of cabbage maggot (Diptera: Anthomyiidae) on cabbage. *Journal of Economic Entomology* 89(5), 1109-1115.

Schulenburg, H. et al. 2007. How do invertebrates generate a highly specific innate immune response? *Molecular Immunology* 44(13), 3338-3344.

Seltmann, A. et al. 2017. Seasonal Fluctuations of Astrovirus, But Not Coronavirus Shedding in Bats Inhabiting Human-Modified Tropical Forests. *Ecohealth* 14(2), 272-284.

Selvan, S. et al. 1993. Biochemical energy reserves of entomopathogenic nematodes. *Journal of Parasitology* 79(2), 167-172.

Seo, S. et al. 2012. Phospholipase A2 inhibitors synthesized by two entomopathogenic bacteria, *Xenorhabdus nematophila* and *Photorhabdus temperata*. *Appl. Environ. Microbiol.* 78(11), 3816-3823.

Seppala, O. et al. 2008. Host condition as a constraint for parasite reproduction. *Oikos* 117(5), 749-753.

Shahabuddin, M. et al. 1998. *Plasmodium gallinaceum*: differential killing of some mosquito stages of the parasite by insect defensin. *Experimental parasitology* 89(1), 103-112.

Shapiro-Ilan, D. I. et al. 2012. Entomopathogenic Nematode Production and Application Technology. *Journal of Nematology* 44(2), 206-217.

Shaw, D. J. and Dobson, A. P. 1995. Patterns of macroparasite abundance and aggregation in wildlife populations: A quantitative review. *Parasitology* 111, S111-S133.

Shaw, D. J. et al. 1998. Patterns of macroparasite aggregation in wildlife host populations. *Parasitology* 117, 597-610.

Sheehan, G. et al. 2018. Innate humoral immune defences in mammals and insects: The same, with differences ? *Virulence* 9(1), 1625-1639.

Sheldon, B. C. and Verhulst, S. 1996. Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution* 11(8), 317-321.

Sheridan, L. A. et al. 2000. Sex differences in parasitic infections among arthropod hosts: is there a male bias? *Oikos* 88(2), 327-334.

Shik, J. Z. et al. 2014. Diet specialization in an extreme omnivore: nutritional regulation in glucose-averse German cockroaches. *Journal of Evolutionary Biology* 27(10), 2096-2105.

Siegel, J. et al. 2004. Use of steinernematid nematodes for post harvest control of navel orangeworm (Lepidoptera: Pyralidae, Amyelois transitella) in fallen pistachios. *Biological Control* 30(2), 410-417.

Siddiqui, M. and Al-Khalifa, M. 2014. Review of haemocyte count, response to chemicals, phagocytosis, encapsulation and metamorphosis in insects. *Italian Journal of Zoology* 81(1), 2-15.

Sim, S. et al. 2014. Mosquito immunity against arboviruses. *Viruses* 6(11), 4479-4504.

Siva–Jothy, M. T. 2000. A mechanistic link between parasite resistance and expression of a sexually selected trait in a damselfly. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 267(1461), 2523-2527.

Siva-Jothy, M. T. et al. 2001. Investment in immune function under chronic and acute immune challenge in an insect. *Physiological Entomology* 26(1), 1-5.

Siva-Jothy, M. T. et al. 2005. Insect immunity: An evolutionary ecology perspective. In: Simpson, S.J. ed. *Advances in Insect Physiology, Vol 32*. Vol. 32. 1-48.

Siva-Jothy, M. T. and Thompson, J. J. 2002. Short-term nutrient deprivation affects immune function. *Physiological Entomology* 27(3), 206-212.

Smith, W. and Angus, K. 1980. *Haemonchus contortus*: attempts to immunise lambs with irradiated larvae. *Research in Veterinary Science* 29(1), 45-50.

Smith, R. C. et al. 2014. The Plasmodium bottleneck: malaria parasite losses in the mosquito vector. *Memorias Do Instituto Oswaldo Cruz* 109(5), 644-661.

Soderhall, K. and Cerenius, L. 1998. Role of the prophenoloxidase-activating system in invertebrate immunity. *Current Opinion in Immunology* 10(1), 23-28.

Srygley, R. B. and Jaronski, S. T. 2018. Protein deficiency lowers resistance of Mormon crickets to the pathogenic fungus *Beauveria bassiana. Journal of Insect Physiology* 105, 40-45.

Stear, M. J. et al. 2000. The influence of age on the variation among sheep in susceptibility to natural nematode infection. *Veterinary Parasitology* 89(1-2), 31-36.

Steenhard, N. R. et al. 2009. *Ascaris suum* infection negatively affects the response to a *Mycoplasma hyopneumoniae* vaccination and subsequent challenge infection in pigs. *Vaccine* 27(37), 5161-5169.

Stephenson, J. F. et al. 2017. Host heterogeneity affects both parasite transmission to and fitness on subsequent hosts. *Philosophical Transactions of the Royal Society B-Biological Sciences* 372(1719).

Stewart, G. R. et al. 1999. Onchocerciasis modulates the immune response to mycobacterial antigens. *Clinical and Experimental Immunology* 117(3), 517-523.

Steyn, L. et al. 2019. Potential of South African entomopathogenic nematodes to control the leaf miner, *Holocacista capensis* (Lepidoptera: Heliozelidae). *South African Journal of Enology and Viticulture* 40(2), 1-1.

Stien, A. et al. 2002. The impact of gastrointestinal nematodes on wild reindeer: experimental and cross-sectional studies. *Journal of Animal Ecology* 71(6), 937-945.

Strand, M. R. 2008. The insect cellular immune response. *Insect Science* 15(1), 1-14.

Strandin, T. et al. 2018. Reviewing the effects of food provisioning on wildlife immunity. *Philosophical Transactions of the Royal Society B-Biological Sciences* 373(1745).

Stone, L. et al. 2007. Seasonal dynamics of recurrent epidemics. *Nature* 446(7135), 533.

Su, L. et al. 2014. Coinfection with an Intestinal Helminth Impairs Host Innate Immunity against *Salmonella enterica* Serovar Typhimurium and Exacerbates Intestinal Inflammation in Mice. *Infection and Immunity* 82(9), 3855-3866.

Su, Z. et al. 2005. Impairment of protective immunity to blood-stage malaria by concurrent nematode infection. *Infection and Immunity* 73(6), 3531-3539.

Su, Z. et al. 2006. Reduced protective efficacy of a blood-stage malaria vaccine by concurrent nematode infection. *Infection and Immunity* 74(4), 2138-2144.

Suhonen, J. et al. 2018. Parasitism affects variation in a male damselfly sexual ornament. *Ethology Ecology & Evolution* 30(3), 256-266.

Susi, H. et al. 2015. Co-infection alters population dynamics of infectious disease. *Nature Communications* 6.

Tachbele, E. et al. 2006. Cockroach-associated food-borne bacterial pathogens from some hospitals and restaurants in Addis Ababa, Ethiopia: Distribution and antibiograms. *Journal of Rural and Tropical Public Health* 5(1), 34-41.

Takahashi, K. T. et al. 2009. Observation by electron microscopy of a gregarine parasite of Antarctic krill: its histological aspects and ecological explanations. *Polar Biology* 32(4), 637-644.

Takahashi, K. T., Kawaguchi S, Kobayashi M, Toda T 2004. The variability in abundance of eugregarines living in the Antarctic krill. *National Institute of Polar Research* 17, 16-25.

Takem, E. N. et al. 2014. The association between malaria and non-typhoid *Salmonella* bacteraemia in children in sub-Saharan Africa: a literature review. *Malaria Journal* 13(1), 400.

Tate, A. T. and Rudolf, V. H. 2012. Impact of life stage specific immune priming on invertebrate disease dynamics. *Oikos* 121(7), 1083-1092.

Telfer, S. et al. 2008. Parasite interactions in natural populations: insights from longitudinal data. *Parasitology* 135(7), 767-781.

Telfer, S. et al. 2010. Species Interactions in a Parasite Community Drive Infection Risk in a Wildlife Population. *Science* 330(6001), 243-246.

Theopold, U. et al. 2004. Coagulation in arthropods: defence, wound closure and healing. *Trends in Immunology* 25(6), 289-294.

Therese, M. O. and Bashey, F. 2012. Natal-host environmental effects on juvenile size, transmission success, and operational sex ratio in the entomopathogenic nematode *Steinernema carpocapsae*. *Journal of Parasitology* 98(6), 1095-1100.

Therneau, T. M. et al. 2003. Penalized survival models and frailty. *Journal of computational and graphical statistics* 12(1), 156-175.

Therneau, T. M. 2015. *A Package for Survival Analysis in S.*

Thomas, M. J. et al. 2016. Ontogenetic shifts in a freshwater cleaning symbiosis: consequences for hosts and their symbionts. *Ecology* 97(6), 1507-1517.

Thompson, R. C. A. 2013. Parasite zoonoses and wildlife: One health, spillover and human activity. *International Journal for Parasitology* 43(12-13), 1079-1088.

Thompson, R. C. A. et al. 2010. Parasites, emerging disease and wildlife conservation. *International Journal for Parasitology* 40(10), 1163-1170.

Tidbury, H. J. et al. 2010. Within and transgenerational immune priming in an insect to a DNA virus. *Proceedings of the Royal Society B: Biological Sciences* 278(1707), 871-876.

Tidbury, H. J. et al. 2012. The epidemiological consequences of immune priming. *Proceedings of the Royal Society B: Biological Sciences* 279(1746), 4505-4512.

Tilahun, B. et al. 2012. High load of multi-drug resistant nosocomial neonatal pathogens carried by cockroaches in a neonatal intensive care unit at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia. *Antimicrobial Resistance and Infection Control* 1.

Tildesley, M. J. et al. 2010. Impact of spatial clustering on disease transmission and optimal control. *Proceedings of the National Academy of Sciences* 107(3), 1041-1046.

Tocque, K. 1993. The relationship between parasite burden and host resources in the desert toad (*Scaphiopus couchii*), under natural environmental-conditionS. *Journal of Animal Ecology* 62(4), 683-693.

Tocque, K. and Tinsley, R. C. 1994. The relationship between *Pseudodiplorchis americanus* (Monogenea) density and host resources under controlled environmental-conditions. *Parasitology* 108, 175-183.

Tollenaere, C. et al. 2016. Evolutionary and epidemiological implications of multiple infection in plants. *Trends in Plant Science* 21(1), 80-90.

Tomalak, M. 1994. Selective breeding of *Steinernema feltiae* (filipjev) (nematoda, steinernematidae) for improved efficacy in control of a mushroom fly, *Lycoriella solani* winnertz (diptera, sciaridae). *Biocontrol Science and Technology* 4(2), 187-198.

Toossi, Z. et al. 2001. Impact of tuberculosis (TB) on HIV-1 activity in dually infected patients. *Clinical & Experimental Immunology* 123(2), 233-238.

Tourtois, J. and Grieshop, M. 2015. Susceptibility of *Dalotia coriaria* (Kraatz)(Coleoptera: Staphylinidae) to Entomopathogenic Nematodes (Rhabditida: Heterorhabditidae and Steinernematidae). *Insects* 6(1), 224-235.

Tschirren, B. et al. 2007. Host condition and host immunity affect parasite fitness in a bird–ectoparasite system. *Functional Ecology* 21(2), 372-378.

Tuomilehto, J. 2013. The Emerging Global Epidemic of Type 1 Diabetes. *Current Diabetes Reports* 13(6), 795-804.

Turner, P. E. and Chao, L. 1999. Prisoner's dilemma in an RNA virus. *Nature* 398(6726), 441.

ÜNLÜ, I. O. and ÖZER, N. 2003. Evaluation of the reproductive potential and competition between two entomopathogenic nematodes, *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora*, Poinar 1976 (Rhabditida: Heterorhabditidae). *Turkish Journal of Biology* 27(3), 149-155.

Urban Jr, J. F. et al. 2007. Infection with parasitic nematodes confounds vaccination efficacy. *Veterinary Parasitology* 148(1), 14-20.

Valigurova, A. and Koudela, B. 2005. Fine structure of trophozoites of the gregarine *Leidyana ephestiae* (Apicomplexa : Eugregarinida) parasitic in *Ephestia kuehniella* larvae (Lepidoptera). *European Journal of Protistology* 41(3), 209-218.

Van den Hof, S. et al. 2002. Measles epidemic in the Netherlands, 1999–2000. *The Journal of infectious diseases* 186(10), 1483-1486.

Van Geertruyden, J. P. 2014. Interactions between malaria and human immunodeficiency virus 2014. *Clinical Microbiology and Infection* 20(4), 278-285.

van Riet, E. et al. 2007. Chronic helminth infections induce immunomodulation: consequences and mechanisms. *Immunobiology* 212(6), 475-490.

van Santen, S. et al. 2013. The iron link between malaria and invasive non-typhoid *Salmonella* infections. *Trends in Parasitology* 29(5), 220-227.

vanBaalen, M. and Sabelis, M. W. 1995. The dynamics of multiple infection and the evolution of virulence. *American Naturalist* 146(6), 881-910.

VanderWaal, K. and Deen, J. 2018. Global trends in infectious diseases of swine. *Proceedings of the National Academy of Sciences of the United States of America* 115(45), 11495-11500.

Vasco, D. A. et al. 2007. Tracking the dynamics of pathogen interactions: Modeling ecological and immune-mediated processes in a two-pathogen single-host system. *Journal of Theoretical Biology* 245(1), 9-25.

Verhulst, S. et al. 1999. A tradeoff between immunocompetence and sexual ornamentation in domestic fowl. *Proceedings of the National Academy of Sciences of the United States of America* 96(8), 4478-4481.

Vilmos, P. and Kurucz, E. 1998. Insect immunity: evolutionary roots of the mammalian innate immune system. *Immunology Letters* 62(2), 59-66.

Viney, M. and Cable, J. 2011. Macroparasite Life Histories. *Current Biology* 21(18), R767-R774.

Viney, M. E. and Graham, A. L. 2013. Patterns and Processes in Parasite Co-Infection. In: Rollinson, D. ed. *Advances in Parasitology, Vol 82*. Vol. 82. 321-369.

Wagutu, G. 2017. Efficacy of entomopathogenic nematode (*Steinernema karii*) in control of termites (*Coptotermes formosanus*). *Journal of Agriculture, Science and Technology* 18(1).

Wale, N. et al. 2017. Resource limitation prevents the emergence of drug resistance by intensifying within-host competition. *Proceedings of the National Academy of Sciences* 114(52), 13774-13779.

Wale, N. et al. 2017. A nutrient mediates intraspecific competition between rodent malaria parasites in vivo. *Proceedings of the Royal Society B-Biological Sciences* 284(1859).

Walker, J. W. et al. 2018. Transmissibility of emerging viral zoonoses. *Plos One* 13(11).

Wallinga, J. et al. 2005. A measles epidemic threshold in a highly vaccinated population. *PLoS Medicine* 2(11), e316.

Walzl, G. et al. 2000. Influenza virus lung infection protects from respiratory syncytial virus–induced immunopathology. *Journal of Experimental Medicine* 192(9), 1317-1326.

Wang, Y. et al. 1994. Variations in immune response of *Popillia japonica* and *Acheta domesticus* to *Heterorhabditis bacteriophora* and *Steinernema* species. *Journal of nematology* 26(1), 11.

Wang, E. et al. 2000. Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. *Journal of virology* 74(7), 3227-3234.

Wanji, S. et al. 2003. Heterogeneity in the prevalence and intensity of loiasis in five contrasting bioecological zones in Cameroon. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 97(2), 182-187.

Wearing, H. J. and Rohani, P. 2006. Ecological and immunological determinants of dengue epidemics. *Proceedings of the National Academy of Sciences of the United States of America* 103(31), 11802-11807.

Weinstein, S. B. and Lafferty, K. D. 2015. How do humans affect wildlife nematodes? *Trends in Parasitology* 31(5), 222-227.

Welburn, S. et al. 1989. Rate of trypanosome killing by lectins in midguts of different species and strains of *Glossina. Medical and Veterinary Entomology* 3(1), 77-82.

West, S. A. and Buckling, A. 2003. Cooperation, virulence and siderophore production in bacterial parasites. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270(1510), 37-44.

White. 1927. A method for obtaining infective nematode larvae from cultures. *Science*, 302-303.

Whitehorn, P. R. et al. 2010. Genetic diversity, parasite prevalence and immunity in wild bumblebees. *Proceedings of the Royal Society B: Biological Sciences* 278(1709), 1195-1202.

WHO. 2012. Soil-transmitted helminthiases: eliminating as public health problem soil-transmitted helminthiases in children: progress report 2001-2010 and strategic plan 2011-2020.

Wilber, M. Q. et al. 2017. When can we infer mechanism from parasite aggregation? A constraint-based approach to disease ecology. *Ecology* 98(3), 688-702.

Wilcox, J. J. S. et al. 2015. Comparative community-level associations of helminth infections and microparasite shedding in wild long-tailed macaques in Bali, Indonesia. *Parasitology* 142(3), 480-489.

Wilson, K. et al. 2001. Melanism and disease resistance in insects. *Ecology Letters* 4(6), 637-649.

Windsor, D. A. 1998. Controversies in parasitology, most of the species on earth are parasites. *International Journal for Parasitology* 28(12), 1939-1941.

Wiria, A. E. et al. 2012. Helminth infection in populations undergoing epidemiological transition: a friend or foe? *Seminars in Immunopathology* 34(6), 889-901.

Woolhouse, M. E. J. et al. 1997. Heterogeneities in the transmission of infectious agents: Implications for the design of control programs. *Proceedings of the National Academy of Sciences of the United States of America* 94(1), 338-342.

Wright, D. J. et al. 1997. Relative importance of neutral lipids and glycogen as energy stores in dauer larvae of two entomopathogenic nematodes, *Steinernema carpocapsae* and *Steinernema feltiae*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 118(2), 269-273.

Wuerthner, V. P. et al. 2017. The benefits of coinfection: trematodes alter disease outcomes associated with virus infection. *Journal of Animal Ecology* 86(4), 921-931.

Yamada, K. et al. 2018. Impact of sea spider parasitism on host clams: susceptibility and intensity-dependent mortality. *Journal of the Marine Biological Association of the United Kingdom* 98(4), 735-742.

Yang, S. et al. 2013. Cross-protective immunity against influenza A/H1N1 virus challenge in mice immunized with recombinant vaccine expressing HA gene of influenza A/H5N1 virus. *Virology Journal* 10.

Yokoo, S. et al. 1992. Suppression of the prophenoloxidase cascade in the larval haemolymph of the turnip moth, *Agrotis segetum* by an entomopathogenic nematode, *Steinernema carpocapsae* and its symbiotic bacterium. *Journal of Insect Physiology* 38(11), 915-924.