Patterns and consequences of coinfection in humans: implications for treatment and health

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[1]Figure and table legends have been compressed

Abstract

Coinfection by multiple species of parasite, including viruses, bacteria, protozoa, fungal pathogens, and helminths, affects hundreds of millions of people. Despite the potential for significant health effects, and important implications for treatment of infections, relatively little is know about the structure of coinfecting communities, the processes responsible for this structure, and the consequences for host health. This lack of knowledge limits the extent to which treatment of infection can account for coinfection.

The four original research chapters of this thesis include collation and analyses of large databases of coinfection information collected from published papers, analyses of coinfection data in a large database of death certificates, and analyses of the behaviour of a theoretical model of two coinfecting parasites.

Coinfection information in previously published literature indicates that coinfection tends to enhance parasite abundance and harm human health. The same literature shows that interactions among coinfecting parasites are likely to involve shared resources, as opposed to being mediated via the immune system.

Analysis of death certificates showed that the proportion of deaths attributed to coinfection was greatest in early adulthood, and that, positive associations between pairs of coinfections on death certificates were more common than negative associations.

The theoretical model of two coinfecting parasites revealed that indirect effects of treatment on untreated parasites may be predictable given information about the direction of interspecific interactions among parasites.

In sum, these findings indicate that coinfection in humans involves hundreds of different species combinations, that these communities are likely to be structured by bottom-up rather than top-down processes, and that coinfection can present a serious health risk. Furthermore, a better understanding of interspecific interactions among parasites could be used to improve treatment outcomes. Further research could show where specific treatments indirectly suppress more parasites than currently estimated.

Abstract

Acknowledgements

skills. In undergraduate tutorials, Richard Washington encouraged me to strive to understand statistics. Ali Rogers' essay comments showed me the importance of engaging one's readers with a positive, important, and well-considered argument. Phil Ineson taught me his two golden rules for research: to always know your research question, and to begin by sketching a graph. And Owen's rule is similarly good: visualise your data before analysing them. I hope this thesis does these words of wisdom justice.

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Emily C. Griffiths,
Sheffield,
April 2013

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Declaration

I wrote all chapters of this thesis. The research chapters are written as manuscripts and for each of them I reviewed the literature, retrieved and analysed the data, and wrote it up. The idea for Chapter 4 was my own, but inspiration for the other chapters arose from discussions with my supervisors. My supervisors made extensive and detailed contributions to all aspects of all the chapters. All the figures and tables are my own work, except that the human body in Fig. 3.8 was drawn by Mike Costelloe.

Related publications

Chapter 2 has been published, and has been lightly edited for this thesis. Chapter 3 is in review.

> Griffiths, E.C., A.B. Pedersen, A. Fenton, and O.L. Petchey. The nature and consequences of coinfection in humans. *Journal of Infection*, 63: 200-6, 2011.

> Griffiths, E.C., A.B. Pedersen, A. Fenton, and O.L. Petchey. Parasites interact most via shared resources in a summary human coinfection network. *Royal Society Interface*, in review.

I wrote an article about my PhD experience, which is not in this thesis.

> Griffiths, E.C.. Doing a PhD with multiple supervisors in multiple places. *BES Bulletin* 43: 31-32, 2012.

Chapter 1

Introduction

Over the last century, vaccines and antibiotics have improved the prevention and treatment of many infectious diseases, and, in the case of smallpox, even enabled eradication (Winslow, 1980). These interventions have significantly reduced deaths from infection so that by the 1970s human life expectancy in countries like the USA, UK, and Sweden was more limited by chronic, degenerative diseases like heart disease and cancer (Finch and Crimmins, 2004; Horiuchi, 2000; Omran, 1971; Rosenberg, 2009). In fact, between 1990 and 2010 the global health burden switched from mainly infectious to noninfectious causes (Murray et al., 2013).

However, in the 21st century infectious diseases remain a large challenge for individual health, in terms of morbidity and mortality, on a global scale. While many countries have relatively little infectious disease mortality, the economic burdens are substantial; in 2003 influenza cost the USA over \$87 billion in disease prevention, healthcare, and lost productivity (Molinari et al., 2007). In many poorer countries the majority of human deaths are still caused by infectious disease (Sanders et al., 2008). Many twentieth century efforts to locally eliminate or globally eradicate infections were very costly, and not always successful (Hopkins, 2013).

One of the difficulties in treating or preventing morbidity (illness) and mortality (death) from infectious disease is that existing interventions often ignore the potential for different infections to affect one another (Ameisen, 1999; Singer, 2009; Singer and Clair, 2003). Health services are faced by many infection-related challenges including sexually trans-

mitted infectious diseases (Eng and Butler, 1997; Forhan et al., 2009), hospital-acquired infections (Curtis, 2008; Meyer et al., 2011; Sandora and Goldmann, 2012), and children missing multiple vaccines (Duckworth et al., 2012), all of which increase the chances for infections to co-occur within individuals. To improve global health, health burdens from infectious diseases must be tackled, and to do this effectively interactions among infectious agents need to be considered (Wilson, 1995). This thesis aims to better understand how different infectious diseases interact within humans, and to determine what the consequences of these interactions are for effective treatment. To achieve this important goal, we need to understand the distribution and impact of coinfection in human populations.

What is known about coinfection in humans

Coinfection is the simultaneous infection of an individual by more than one type of parasite. Although the effects of coinfection on human health are rarely assessed (King, 2010; Pullan and Brooker, 2008), and there is no estimate of the number of coinfected individuals worldwide, studies of particular taxa and groups of individuals suggest that coinfection is extremely widespread. Over three billion individuals have parasitic worm (helminth) infections (Drake and Bundy, 2001), one billion of whom are coinfected with multiple helminth species (Crompton, 1999). One study in the Ivory Coast found that 75% of villagers were heavily coinfected with between three and ten intestinal parasite species (Raso et al., 2004). Beyond helminths, over 10 million individuals are coinfected with HIV and tuberculosis (World Health Organization, 2012). Bacterial coinfection has also been identified as a major cause of death among pandemic and seasonal influenza patients because the virus enables more bacteria to colonise and cause more severe infection than would occur in patients with just a bacterial infection (Chertow and Memoli, 2013; Dushoff et al., 2006; Finelli et al., 2008; Morens et al., 2008; World Health Organization, 2010). Marginalised groups within human populations are the most likely to be coinfected, due to high exposure, and inadequate prevention or treatment options (Boraschi et al., 2008). These groups include the global poor

(Bonds et al., 2010; Buck et al., 1978a; Lustigman et al., 2012; Steinmann et al., 2010), sex workers and injecting drug users (Singer, 2009; Singer and Clair, 2003), and men who have sex with men (Guo et al., 2009; Keystone et al., 1980).

Taken together, these studies suggest that coinfection is commonplace in humans and involves parasites of different taxa, transmission routes, and pathologies. How these patterns of coinfection arise and how they differ by parasite types is unknown because data on human coinfections have not yet been synthesised. Before attempting such a synthesis we must first consider the kinds of parasites that infect humans, and then consider what defines coinfection.

Parasites that infect humans

Across all taxa and ecosystems, more than half the species on earth, including the most abundant species (Zhao et al., 2013), are parasitic. These parasites live in and rely on the organism they infect (their host) for nutrition and survival during their life cycle (Begon et al., 2006; Brooks and Hoberg, 2000). "Parasite" is used broadly here to include viruses, bacteria, fungal parasites, protozoa, and helminths (i.e. micro-parasites - often called pathogens - and macroparasites *sensu* Anderson and May (1992)). Humans are infected by many different parasites (Anderson, 1990); a comprehensive search published in 2001 counted $1,415$ disease-causing (pathogenic) species reported as infecting humans (Taylor et al., 2001). The number of parasites of humans will likely increase as further sampling and better diagnostic tests reveal previously undiscovered parasites, and as new parasites invade the human population (Henderson and Morse, 1993), recent examples being influenza A (H1N1) in 2009, SARS in 2002, and Nipah virus in 1999 (Tabish, 2010).

Non-human hosts are also infected by various parasites, and coinfection is commonplace in domesticated animals including livestock and pets (Nieto and Foley, 2009), wild buffalo (Budischak et al., 2012; Ezenwa et al., 2010), rodents (Behnke et al., 2001; Telfer et al., 2010), and Soay sheep (Craig et al., 2008). There are growing concerns about how these parasite species may interact within animal hosts, as these interactions may affect

treatment and parasite dynamics (Lello et al., 2004; Telfer et al., 2010). This thesis focuses on coinfection in humans because much is known about the range of infectious diseases that are found to infect humans, as well as their pathologies. The concepts, techniques, and findings regarding coinfection in humans are likely to be relevant to other host systems.

Defining coinfection

This thesis defines coinfection as multiple pathogenic parasite species simultaneously infecting the same individual. In the literature, the term "coinfection" has various meanings, from parasites of different taxonomic kingdoms, to a mix of pathogenic and non-pathogenic parasites, to even multiple strains of the same parasite species. Some papers discuss coinfection of bacteriophages (Mills et al., 2013; Refardt, 2011), and prions (Leblanc et al., 2012). These groups are relatively little studied and are not considered in this thesis. By including viruses, bacteria, protozoa, fungi, and helminths in the definition of coinfection, the effects of a range of parasites on human health can be explored.

Commensal organisms are like parasites because they derive energy from their host, though they are not normally pathogenic. Commensals can be difficult to distinguish from mutualists or pathogens because their relationship with the host varies over time and under different conditions (Mims et al., 2000). While commensals are thought to affect the susceptibility, immunity to (Abt and Artis, 2013; Naik et al., 2012; Stecher and Hardt, 2008), and virulence (Lysenko et al., 2010) of parasites co-occurring in their host, these commensals also confer benefits to host physiology (Turnbaugh et al., 2008). While not addressed in this thesis, the role of commensals in coinfection is an important area for future study. For this thesis a pathogenic definition of coinfection seems reasonable since the aim is to understand the effects of coinfection on human health, and pathogenic organisms directly damage human health while commensals generally do not.

Some authors use coinfection to refer to multiple subspecies, like different malarial strains (De Roode et al., 2005), or genotypes of a trematode species (Karvonen et al., 2012). Across infection publications, 11% re-

port conspecific strains (Balmer and Tanner, 2011). This thesis does not consider co-occurring subspecies as coinfections because multi-genotype coinfections can involve different processes from multi-species infections (Louhi et al., 2013), such as viral recombination only occurring with superinfection of a conspecific strain (Blackard et al., 2002). This distinction is complicated by the fact that the differences between multi-genotype and multi-species infections often depends on the taxonomic group (Alizon et al., 2013). While further research is needed into how parasite species or subspecies differ and what the biological implications are (Alizon et al., 2013), before we can make such a comparison, we first need to establish the general patterns of coinfection when the line is drawn at each scales. Since differences within species are likely to be smaller, this thesis focuses on interactions between species to enable the breadth of interactions in coinfected humans to be studied more efficiently. Comparisons with multi-genotype research are an important subject for future research. Having defined coinfection as simultaneous infections of multiple pathogenic parasite species in the same host, the next step is to consider how coinfecting parasite species interact.

How do coinfecting parasites interact?

An interaction is the effect of an individual of one species on an individual of another species (Wootton and Emmerson, 2005). Parasites of different species do not always interact; the species might be independent, but the presence of multiple species in the same host can exert additive burdens on host health (Brogden et al., 2005; Brooker et al., 2007). Coinfection can also just be an association, for example two species may often coinfect humans if the same host characteristics are a risk factor for infection by both parasite species (Sousa, 1994). There are also debates over which statistical tools to use to detect parasite interactions (Cobey and Lipsitch, 2013; Fenton et al., 2010; Haukisalmi, 1994; Sousa, 1994), but they can be classified by the biological processes between the two parasites. Interactions between parasite species can be direct or indirect, analogous to the types of interspecific interactions found in free-living ecosystems. Accordingly, indirect interactions between parasites are top-down when

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mediated by predators (the immune system), or bottom-up when mediated by a shared host resource (Graham, 2008; Pedersen and Fenton, 2007).

Direct interactions include viruses infecting bacteria (Flores et al., 2011; Hanlon, 2007), helminths transmitting bacteria (Perkins and Fenton, 2006), and bacterial colonies releasing exoproducts that hinder other bacterial species (Hoffman et al., 2006). Indirect interactions that are mediated by the immune response (Kasprowicz et al., 2008) can be opportunistic coinfection by normally commensal bacteria when the immune system is suppressed by another infection (Chonmaitree et al., 2008; Cox, 2001), and trade-offs between different branches of host immunity (Bradley and Jackson, 2008; Graham, 2001; Maizels and Yazdanbakhsh, 2003; Page et al., 2006; Stewart et al., 1999). Lastly, examples of indirect interactions involving host resources are competition between malarial parasites for red blood cells (Antia et al., 2008; Read and Taylor, 2001), or influenza infection creating more pneumococcal binding sites (Peltola et al., 2005). There are many mechanisms by which parasites of different species interact in a host (Dobson, 1985), and these can have positive or negative effects (Brogden et al. (2005), also called synergistic or antagonistic) on the abundance of coinfecting parasites, and, in turn, on host health. Whether interactions are positive or negative, direct or indirect, they could affect parasite community dynamics, and treatment outcomes (Jackson et al., 2006; Lello et al., 2004).

Importance of coinfection for prevention and treatment of infectious disease

Finding parasite species that interact strongly is urgent given the many, and potentially large effects of parasite interactions on the outcomes of treatment and prevention efforts. For example, immune-mediated interactions can hinder preventative measures like vaccination. Generally, if one parasite affects the immune response, then vaccines and certain diagnostic tests are less effective (Buck et al., 1978c; De Bruyn, 2010). Vaccines that are less effective in individuals with other infections include helminths

(Cooper et al., 2001; Geiger et al., 2011; Harris et al., 2009), HIV (van den Berg et al., 2009), human papillomavirus (Pons-Salort et al., 2013), and enteric viruses (Armah et al., 2010; Guerrant et al., 1990; Madhi et al., 2010; Wang et al., 2012; Zaman et al., 2009, 2010). While these preventative measures are an important area for coinfection research, this thesis focuses on treatments because these are administered to coinfected individuals, whereas many of the aforementioned vaccine studies include individuals with single-species infections.

As with vaccines, treatments targeted toward particular parasite species can fail to improve the health of individuals who are infected by other species. Treatment of infection using drugs can be impeded or counterproductive because of interactions between coinfecting parasites (Behnke et al., 2001). One otherwise successful treatment had such serious side effects in coinfected patients that subsequent treatment programmes took extra precautions in coinfected populations (Diggle et al., 2007). In this case, treating onchocerciasis with a standard drug (ivermectin) triggered severe, and sometimes lethal, encephalitis within three days in patients with high burden *Loa loa* infections (Boussinesq et al., 1998, 2001; Chiodini, 2001; Gardon et al., 1997). This is thought to be because the drug caused a high rate of nematode death behind the eyes (Gardon et al., 1997; Twum-Danso, 2003). Thus, treatments can have reduced benefits for, and even damaging effects on, individuals coinfected with other parasites.

Knowledge of how parasite species and existing treatments affect the health of coinfected individuals is therefore needed to develop new, improved treatment options. Knowledge of parasite interactions is not always available and, where it is, its use in treatment programmes is just beginning. The only treatment guidelines for coinfection are for patients with Hepatitis B or C virus (HBV or HCV) or tuberculosis and HIV (Brook et al., 2010; Daftary et al., 2006; England et al., 2009; Pozniak et al., 2005), with prescription of existing drugs for both infections being the first treatment option (Firnhaber and Ive, 2009). The recommended drugs fail in two thirds of HCV-HIV coinfection cases, so new treatments are being developed that combine standard interferon with new HCV protease inhibitors (Chary and Holodniy, 2011) or microRNA blockers (Janssen

et al., 2013). Even for relatively well-studied hepatitis coinfection where treatment guidelines are being developed, there is much scope to better understand interactions between the viruses to improve treatment.

Treating coinfecting parasites simultaneously

Some treatment programmes are beginning to consider which parasites co-occur in human populations. One approach is to use integrated treatments that administer all available drugs together to tackle each infection (Molyneux et al., 2005; Zaman et al., 2009). Integrated treatment programmes have begun in communities with high helminth coinfection prevalence (Hotez, 2009; Hotez et al., 2007). Treating several helminth infections in communities with many coinfected individuals can be highly efficient and beneficial to health; the largest health improvements from deworming school children have been found in populations where 96% of children had hookworm and *Trichuris* coinfection (Stephenson et al., 1993; Taylor-Robinson et al., 2012). However, the potential for interactions between the drugs is poorly understood (Lammie et al., 2006), proper evaluation of these programmes is rare (Parker and Allen, 2011; Yamey, 2009), and effects on non-target parasites are rarely monitored (Basáñez et al., 2012; Eziefula and Brown, 2008; Righetti et al., 2012).

There is also integrated treatment in terms antiretroviral therapy for HIV during tuberculosis therapy. This reduced patient mortality in a randomised controlled trial (Abdool Karim et al., 2010), but the best time for initiating the treatments is unclear (Abdool Karim et al., 2011). Similarly for HIV-malaria coinfection it is unclear when integrated treatment should be preferred (Skinner-Adams et al., 2008).

Although current treatment guidelines recommend it, integrated treatment could be unnecessary if targeting a single infection indirectly reduces the burden of other infections. For example, malaria elimination efforts also bring reductions in cases of lymphatic filariasis (van den Berg et al., 2013). Research into optimal coinfection treatment is in its infancy, and other options besides integrated treatment are being considered.

New treatments based on the direction of parasite interactions

Rather than administering separate drugs for each infection, recent studies have suggested that treatments could be tailored to how parasites interact within coinfected hosts (Pasman, 2012; Shrestha, 2011; Siqueira Jr and Rocas, 2009). Traditionally, infection research studied each parasite independently (Belia, 2009), and often ignored how different infections affect one another (Kuramitsu et al., 2007). Understanding positive interactions between particular pairs of parasites has led to novel methods of infectious disease control, such as using antibiotics to kill the commensal bacteria *Wolbachia* in various host species. While the *Wolbachia* do not directly parasitise humans, antibiotics clear these commensals from their adult filarial nematode hosts, such as *Wuchereria bancrofti*, which are sterilised and die prematurely (Landmann et al., 2011; Slatko et al., 2010; Tamarozzi et al., 2011; Taylor et al., 2010). I know of no examples where one pathogenic species was targeted in order to also suppress another parasite species.

Treatments could also be improved by use of parasites that interact negatively with other parasite species. For example, parasites like symbiotic bacteria that compete with more pathogenic organisms are given to patients as probiotics after surgery or after antibiotics to reduce the risk of subsequent infection by more pathogenic species (Khodadad et al., 2013; Rayes et al., 2004). Viruses that delay progression of coinfection are also being considered as potential vaccines, as with GBvC or HIV-2 that can produce some protective immunity against HIV-1 (Bagasra et al., 2012; Esbjernsson et al., 2012). Introducing *Wolbachia* from *Aedes aegypti* mosquitoes can reduce transmission of dengue among humans (Hoffmann et al., 2011). Treatment of parasites that interact negatively with other parasites should also be avoided since treating parasites that confer crossprotective immunity to other parasites, as with *Plasmodium falciparum* malaria in some age groups (Bruce et al., 2000; Smith et al., 1999), might lessen the target infection and exacerbate non-target infections.

Appropriate manipulation of interactions among coinfecting parasites could improve treatment for millions of coinfected individuals worldwide

(Brogden et al., 2005; Costello et al., 2012; Cox, 2001). It is possible to make general statements about treatment of coinfection based on parasite interactions. For instance, if one parasite, like a helminth, induces anti-inflammatory immune responses then treatment could exacerbate coinfections (Jackson et al., 2004; Kamal and El Sayed Khalifa, 2006; Smith et al., 2011). However, altering treatments based on likely negative interactions does not necessarily improve patient health; randomised trials show that treating immunosuppressive parasites like helminths does not delay progression of other immunosuppressive parasites like HIV (Whitaker et al., 2012). A general theory of coinfection in humans would need to explain why non-target parasites respond to treatment in sometimes unexpected ways. This thesis aims to contribute to such a general perspective, and various approaches are available for this research.

Approaches to coinfection research

From pairs of parasites to whole communities

Most of the above studies only consider a few coinfecting species, but humans encounter a more diverse parasite community. There are also many concurrent within-host processes that may affect treatment success. Whether there are general rules governing how certain types of parasite species interact within the same host, and how these interactions can be manipulated to improve human health, is an open question, and one this thesis seeks to address.

Spanning multiple disciplines and scales

Parasite communities within individual hosts have been discussed previously, including a 1950s description of ecological understanding of parasitism and host immunology (Cameron, 1956), a 1986 chapter on helminth communities (Holmes and Price, 1986), and a 1992 symposium on ecological studies of immune responses to infection (Wassom, 1993). Nowadays coinfection is studied by many specialisms, including parasitologists, microbiologists, and public health practitioners. Just as knowledge of inter-

actions at the protein level requires a mix of biology, chemistry, and physics (Kastritis and Bonvin, 2013), so too is cross-disciplinary research of parasites needed to improve coinfection treatment (Fincham et al., 2003). In this thesis I therefore use a variety of relevant tools in an "antedisciplinary" endeavour (Eddy, 2005) in order to identify interspecific parasite interactions, and test their impact on health and treatment outcomes.

Not only are there multiple parasite species involved in coinfection, but processes occur at various biological scales to affect individual health. Parasite interactions occur at levels from the cellular level within coinfected individuals (Durmuş Tekir and Ülgen, 2013; Schneider and Klabunde, 2013; Zengler and Palsson, 2012), to migration through multiple organ systems, up to the human population level where complex, multi-stage diseases are currently a major medical challenge (Auffray et al., 2009; Tappenden, 2011; West, 2012). To help research this multi-scale problem, concepts and techniques normally applied to free-living communities can be useful for studying the community of parasites within coinfected individuals (Costello et al., 2012; Gonzalez et al., 2011; Meyer and Leveau, 2012; Pedersen and Fenton, 2007; Roche et al., 2012; Siqueira Jr and Rocas, 2009).

Making the most of existing data

Broad studies will most efficiently find how best to control complex diseases like coinfection (Barabási et al., 2011; Wolfram, 2002) and patterns of interaction among coinfecting parasites. Holistic understanding comes from combining knowledge of individual components (Pavlopoulos et al., 2011; Wilson, 1998), and published findings spanning cellular processes up to whole organ systems offer a good start for beginning to build up knowledge of interactions among the parasite community of humans. By bringing together specific insights from studies that each focused on a few parasite species, we can develop a systems level understanding of complex host organisms and their parasites (Moulin, 2005). This thesis will attempt to collate and describe these interactions using recently published reports of coinfected humans. Given the wealth of expert knowledge on the wide array of human parasites, it would be prudent to use existing

publications to gain an overview of the parasites and health of coinfected individuals. Data from these publications could reveal, for the first time, the types of parasites known to coinfect humans, and differences between the health status of coinfected individuals and those with single infections. In this thesis I present a systematic survey of a full year of publications on coinfection in humans to seek to fill these large gaps in current knowledge about coinfection.

Another way to collate existing data on coinfection is to construct a network from known parasite interactions. Finding out how parasites, resources, and immune system components interact in coinfected humans would improve mechanistic understanding of parasite community structure. Food webs are networks that have been used to study the structure of trophic (feeding) relationships among free-living organisms. Parasites are increasingly being included in these networks by describing the host species fed on by different parasite species, predominantly in marine or estuarine ecosystems (Hechinger et al., 2007; Kuris et al., 2008; Lafferty and Kuris, 2009; Lafferty et al., 2006, 2008; Lima Jr et al., 2012).

Applying these network ideas to coinfection, we can view parasitism as a trophic interaction within a host, where a parasite is the consumer and cells of the host are the resources, and where the host's immune system is the predator (Wassom, 1993). Were a network of trophic relationships of parasites within humans to be constructed, it would reveal the potential way for coinfecting parasites to interact (Pedersen and Fenton, 2007). This thesis will assemble such a network to understand the types of interaction that structure the parasite community of humans, the first network of its kind for any host species.

Further data on many infections in human populations are routinely collected by many organisations. Some datasets contain information on concurrent infections of individuals, but to my knowledge these have not yet been used to study coinfection. For example, in the constituent countries of the UK, every human death is recorded with its cause or causes. These causes of death can include infectious diseases. Accessing such data would provide unique insight into the kinds of parasites involved in human mortality. This thesis will analyse the relative proportions of single and

coinfection deaths in these data to infer how strongly one infection affects the odds of other infections being reported as a cause of death.

Testing the implications of parasite interactions using theoretical models

Developing a network to describe interactions in coinfected humans, analysing reports of the effects of coinfection from recent publications, and analysing a national dataset on causes of death to understand the odds of coinfection death, will provide suggestive evidence of how parasite interactions affect human health. Understanding how interactions affect each parasite species' abundance over time is also vital if treatment outcomes are to be improved. Coinfection has been recognised among many human communities for decades, but its effects are unclear and coinfecting parasites are rarely monitored by treatment programmes targeting particular infections (Buck et al., 1978d; Keusch and Migasena, 1982). One could do field experiments in humans to assess interactions, and do clinical trials of different treatments to infer how treatment affects the abundance of coinfecting parasites. Some such studies are underway, but besides the few examples highlighted above where treatments are known to be affected by interspecific parasite interactions, we have no general understanding of how treatments targeting particular species affect non-target parasites.

Theoretical models can test the effects of treatment on coinfecting parasites and host health in parasite communities across a range of various interaction types and strengths. Developing a general, theoretical model could also show effects of drug treatment over multi-year programmes using a range of scenarios more quickly, efficiently, and at lower cost than field trials (Westerhoff et al., 2009). Theoretical models also allow for greater experimental control than real human treatment programmes where ethical concerns make it difficult to conduct randomised, double-blind, placebo-controlled trials.

Several models have been used to study the dynamics of simultaneous infections for particular pairs of parasites, such as gonorrhoea and HIV coinfection (Mushayabasa et al., 2011), and malaria and trypanosomiasis

(Nannyonga et al., 2012). However, the findings from such models are relevant only to specific coinfections. More general models can show the range of possible outcomes, for instance by varying the efficacy of treatments or the types of coinfecting parasite. General theoretical models have been used to show the effect of treatments of single-species infections (Anderson and May, 1992). More recent general models relating to coinfection have explored the effects of species interactions on populations of coinfecting bacteria (Eswarappa et al., 2012), and of vaccination and immune interactions in a three-species helminth system (Lello et al., 2004). The findings from these models are more widely relevant as they were not created for specific parasites. However, I know of no models that measure treatment outcomes amidst within-host parasite interactions of varying type and strength in a heterogeneous host population. In this thesis, I build this type of model to better understand how a village-sized human population receiving treatment for one of two helminth infections will be affected by interactions between the parasites.

Objectives of this thesis

The goal of this thesis is to better understand the effects of coinfection on human health on a general level. I aim to study the effects of coinfection on morbidity and mortality, interactions among coinfecting parasites, and how these interactions affect treatment outcomes. To understand the type and strength of interactions among the parasites community of humans, I collected several novel sources of data on the parasite community of humans and used various tools to describe the structure of this community and study its implications for human health and treatment.

In Chapter 2 I systematically reviewed recent coinfection publications and analysed the data they reported to find the taxonomic groups of parasites that coinfect humans, and their health effects. I compared the reported coinfecting parasites with infectious diseases causing highest global morbidity and mortality. The results suggest that coinfection often involves micro-parasites, and is associated with higher parasite abundance and worse host health when compared with single species infections. This

is the first broad-scale test of whether coinfections have a higher health burden than single infections.

In Chapter 3 I collated specific information about the within-host interactions among coinfecting parasites reported in the publications reviewed for Chapter 2. I assembled these interactions into the first ever network of within-host interactions for any host species. This network suggests that there are strong parallels between the structure of within-host parasite communities and free-living communities of other organisms, and that interactions involving shared host resources are the most common type of interaction.

For Chapter 4 I compiled a dataset of reported causes of death from England and Wales to test whether coinfection affects mortality risk. This is another broad-scale analysis of the health effects of coinfection, but, unlike in Chapter 2, I focus on mortality using cross-sectional, national data. Results suggest that the odds of coinfection being reported on a death certificate is not randomly distributed and that, when compared with the occurrence of single infection deaths, the odds of coinfection were higher than expected for many pairs of parasites.

In Chapter 5 I take a different approach again and develop a theoretical model to test the effects of different parasite interactions, coinfection prevalences, and treatment regimes on host health. This model includes both immune- and resource-mediated interactions, and results show that the direction of the effect of species-specific treatment on non-target parasites could be predictable if the direction of parasite interactions and related within-host processes are understood.

Lastly, in Chapter 6, I conclude the thesis by discussing the results of each of the four research chapters, and drawing together these findings to address general patterns of coinfection in humans. I then discuss the broader implications of my work, specifically how treatments could be modified with knowledge of parasite interactions, and possible future directions for coinfection research.

Chapter 2

The nature and consequences of coinfection in humans

Abstract

The kinds of parasites that coinfect humans, how frequently they occur, and their human health impacts are poorly understood. One way to study these patterns is to collate data from publications on coinfection in humans. In this chapter I systematically reviewed a recent sample of such publications to find the parasites involved, their reported effects on host health, and their reported effects on parasite abundance. I also compared the proportion of coinfections involving these parasites with parasites causing the most global morbidity and mortality. Reported coinfections included all kinds of parasites, but were most likely to contain viruses and bacteria. Generally coinfected individuals had worse health (78% of publications, 40% of parasite pairs) and higher parasite abundance (57% of publications, 37% of parasite pairs) than individuals with only one infection. The most commonly reported coinfections differed from infections causing highest global mortality, with a lack of serious childhood infections. This suggests that coinfection tends to have deleterious effects on human health, and often involves different parasites from those of highest global health concern. The skew towards negative health effects, and unknown levels of sampling or reporting biases in coinfection research should prompt further collation and evaluation of human coinfection data.

Introduction

The many parasites that infect humans (e.g., viruses, bacteria, protozoa, fungal parasites, helminths) often co-occur within individuals (Brogden et al., 2005; Cox, 2001; Esch et al., 1990; Petney and Andrews, 1998; Rigaud et al., 2010). Helminth coinfections alone are thought to occur in over 800 million individuals (Hotez et al., 2007), and are especially prevalent among the global poor (Boraschi et al., 2008; Hotez, 2009; Steinmann et al., 2010). Other coinfections involve globally important diseases such as HIV (Lawn, 2004), tuberculosis (Resende et al., 2007), malaria (Muturi et al., 2006), hepatitis (Sagnelli et al., 2004), leishmaniasis (Alvar et al., 2008), and dengue fever (Pancharoen and Thisyakorn, 1998). It seems likely, therefore, that the true prevalence of coinfection exceeds one sixth of the global population and often involves infectious diseases of pressing human concern.

Improved understanding of coinfection prevalence is greatly needed (Brooker et al., 2010), partly because coinfecting parasites can interact either directly with one another or indirectly via the host's resources or immune system (Cox, 2001). Compared to infections of single parasite species, these interactions within coinfected hosts can alter the transmission, clinical progression and control of multiple infectious diseases (Chiodini, 2001; Pedersen and Fenton, 2007; Sternberg et al., 2011). Establishing the nature and consequences of coinfection requires data about the various infections individuals have (Esch et al., 1990), but such data are rare (Brooker and Utzinger, 2007; Pullan and Brooker, 2008; Steinmann et al., 2010).

Coinfection involves a range of parasites and can have various effects on host health (Cox, 2001). Studies in wild bats and Soay sheep suggest positive interactions are dominant (Craig et al., 2008; Lotz et al., 1991), though findings in wild mammals are highly variable (Marzal et al., 2008). Reviews of coinfection emphasise the need for further research, especially in humans (Cox, 2001; Holmes and Price, 1986; Petney and Andrews, 1998; Pullan and Brooker, 2008), where coinfection outnumbers single infection in many communities (Petney and Andrews, 1998; Raso et al., 2004), and

where helminth coinfections appear to worsen human health (Pullan and Brooker, 2008). There are many individual studies concerning coinfection, but these use different methods and are often narrowly focused. To gain a coherent picture of the nature and consequences of coinfection in humans the published literature was systematically reviewed for coinfecting parasites and their effects on other infecting organisms and human health. Coinfections involve a huge variety of parasites, and most studies report negative effects on host health. However, current coinfection research rarely focuses on parasites with highest global mortality.

Methods

Literature search

Published studies of coinfection in humans were found using the largest online citation database, Scopus (Elsevier Ltd.). Many disciplines study infectious diseases and various terms are used to describe coinfection. Search terms were: coinfection, co-infection, concomitant infection, concurrent infection, multiple infection, simultaneous infection, double infection bi-infection, bystander infection, polyparasitism, or multiple parasitism in the Title, Abstract, or Keywords of publications from 1995 up to 2010. An equivalent search on an alternative online citation database, Web of Science (Thomson Reuters), yielded similar trends in publications through time, but fewer results (grey vs. black dots, Fig. 2.1).

Due to the large number of publications matching the search terms, further study focused on publications from 2009. Publications about non-human hosts, non-infectious diseases or multiple genotypes of only one parasite species were excluded. A separate search for "infection" in the Title, Abstract, or Keywords of publications on Scopus was done to compare publication trends with the background trend in infection publications (triangles, Fig. 2.1).
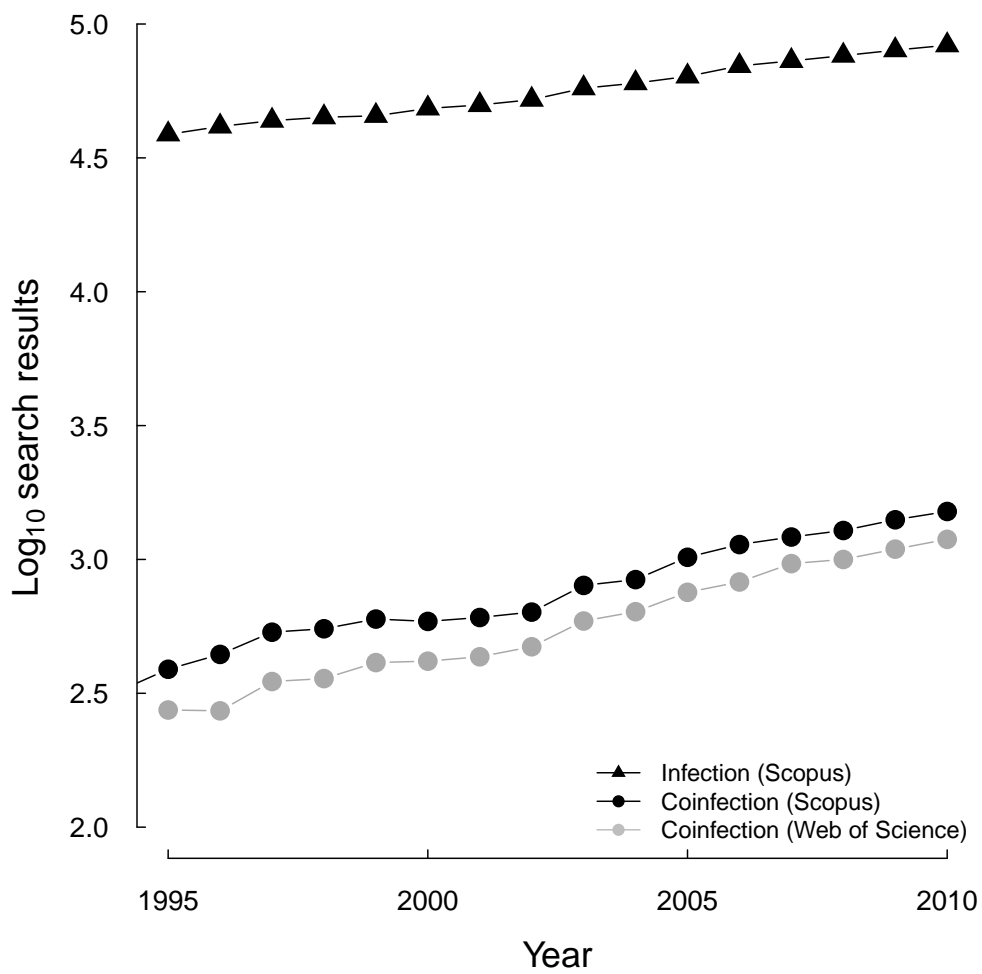
Fig. 2.1: Annual publications ($\log_{10}$) from a search for all coinfection terms in Scopus (black dots), and Web of Science (grey dots), and for infection in Scopus (black triangles).

Data collection

Data collected from each publication were: coinfecting parasites, journal, study type, and maximum number of parasite species per individual. Study types included experiments to find the effects of treating each infection, observational studies, and reviews or meta-analyses. Observational studies were either case notes on particular patients, studies of patient groups, or epidemiological surveys of human communities, most of which involved treatment.

Many publications reported the stated effect of one parasite on coinfecting parasite abundance (i.e. proxies for the intensity of infection, e.g. from measures of viral load, faecal egg counts, antibody response, bacterial cultures etc.) and/or host health (e.g. survival time, anaemia, liver fibrosis, immune cell counts). These effects of coinfection are relative to conditions observed under infections of single parasite species. Where these effects were reported the pair of coinfecting parasites, quality of measurement (rated as low e.g. anecdotal, adequate e.g. correlation, and high i.e. full reporting of appropriate statistical test supported by theoretical mechanisms), and other data (see below) were recorded. Data from review-type publications, case notes, and from publications not mentioning the effects of coinfection (93 publications for parasite abundance and 83 for host health) were excluded to avoid double counting, undue influence of individual cases, and the inclusion of irrelevant publications. Reported effects based on low quality evidence were also omitted (e.g. anecdotal or single patient data, 10 publications for parasite abundance, 24 for host health).

There was heterogeneity in the reporting of coinfection effects, both in the response variable (e.g. cell counts, biomarkers, survival rates), and the quantitative measure given (e.g. odds ratios, adjusted odds ratios, p-values, hazards ratios, raw comparisons). Many publications gave only qualitative statements of effect direction. Further study therefore focused on the direction of reported effects (positive, negative and no-effect) to maximise the data available. Reported directions of the effects on both parasite abundance and host health for each pair of coinfecting parasites

was coded $+1$ for positive effect, 0 for neutral, -1 for negative effects, and NA if no information about effect direction was given. The resulting dataset includes some repeated measures because some publications reported multiple pairs of coinfecting parasites and some coinfections were reported in multiple publications.

Analysis

I created two independent datasets containing the mean effect direction (i) per publication, and (ii) per coinfection to eliminate these sources of pseudoreplication. A negative mean implied a predominance of negative effects; a positive mean implied a dominance of positive effects. A mean close to 0 could result from either many neutral effects (parasite consistently had no discernible effect) or nearly equal numbers of positive and negative effects (parasite had different, possibly context-dependent effects). In either case, there is no clear indication of these parasites having a consistent effect on each other (or on host health), so conservatively I infer that there is no effect. These means were converted into three categories: negative (-1 to $-\frac{1}{3}$), neutral ($-\frac{1}{3}$ to $+\frac{1}{3}$) and positive ($+\frac{1}{3}$ to $+1$). Chi-squared tests (Bushman, 1994) based on double log-likelihood values (Crawley, 2007; Sokal and Rohlf, 1981) were done to establish whether totals in each category differed from those expected from two different null hypotheses (random and no-effect). The random null model had equal proportions of positive, neutral, and negative effects, while the no-effect null model was that coinfecting parasites do not interact, allowing for a 5% error rate (hence 2.5% negative, 2.5% positive, and 95% neutral reported effects). This follows a recommended vote-counting method using continuous response variables and 95% confidence intervals (Hedges and Olkin, 1985).

I explored the potential influence of missing data (NAs) on the analysis of coinfection effects (56 for parasite abundance, 47 for host health). These values represent reported coinfections where the effect on either parasite abundance or host health was not reported, despite the possibility that these coinfecting parasites did interact with each other or altered host health. I therefore assessed how potential interactions from these

unreported effects may alter the overall patterns of coinfection effects. To determine their potential impact, NAs were assigned one of three values with equal probability $(+1, 0, -1)$. The mean effect was then calculated per publication or coinfection pair as before, and a grand mean taken across all publications or coinfection-pairs. The grand mean represents an estimate of overall effect of coinfection on either host health or parasite abundance across either publications or coinfections, given a particular random assignment of $-1, 0, +1$ to NAs. Repeating this random assignment 1000 times produced a distribution of grand means.

Whether recent coinfection research focuses on the parasites causing the highest global mortality is considered in the discussion. Global totals for the number of deaths for each infection were reported by the World Health Organisation for the closest year available, 2008 (obtained from the Global Health Observatory website, World Health Organization (2009)). The ten categories causing the highest percentage of global deaths were compared with the percentage of reports of coinfection from 2009 involving these infections, and with their morbidity (years of life lost to disability, again for the closest year available, 2010, obtained from IHME (2012)).

Analyses were done in R version 2.15.1 (R Development Core Team, 2012).

Results

Hundreds of publications on coinfection are published annually and the numbers increased from 389 publications in the first year of search results to 1407 publications in 2009 (Fig. 2.1). This rate of increase has been almost double the rate of overall infection publications (linear regression $\log_{10}$(search results) year slope$= 0.041$ for coinfection, slope$= 0.023$ for infection, compare black dots with black triangles on the log-linear plot Fig. 2.1). These search results include studies of human and non-human hosts, and *in vitro* experiments. Of the 1407 publications retrieved for 2009, 253 reported multiple parasite species coinfecting humans. Publications came from 152 journals, but many journals $(46, 30.3\%)$ published a single coinfection article in 2009.

The majority of relevant publications from 2009 were observational studies (191 of 253, 75.5%), of which 126 (65.9%) involved patient groups, 46 (24.1%) were case notes and 17 (8.9%) surveyed a population. Two observational studies (1.0%) analysed death records. Fifty-seven publications (22.5%) were reviews or meta-analyses. Five publications (2.0%) were experimental, whereby treatment and controls were applied to both singly infected and coinfected groups. A majority of the relevant publications concerned coinfection by two parasite species (204 of 253, 80.6%), but more parasite species per individual were occasionally reported; a maximum of six parasites was reported once (Peng et al., 2009).

A total of 207 parasite taxa were reported in coinfection publications from 2009 across 677 reports of coinfections comprising 447 different pairs of coinfecting parasite taxa. All parasite types (viruses, bacteria, protozoa, fungal parasites, helminths) were reported in coinfections; the most common parasite group were viruses (796 viruses of 1354 coinfecting parasites [58.7%]), then bacteria (320, 23.6%), protozoa (107, 7.9%), helminths (78, 5.8%), fungal parasites (27, 2.0%), and undisclosed infections (26, 1.9%). In terms of specific parasites, HIV and hepatitis viruses featured highly in reported coinfections. The most frequently reported coinfections were: Hepatitis C (HCV) and HIV (82 reports of 677 total coinfections, 12.1%), Hepatitis B (HBV) and HIV (31, 4.6%), HBV and HCV (30, 4.4%), HIV and Human Papillomavirus (HPV, 27, 4.0%), and HIV and *Mycobacterium tuberculosis* (27, 4.0%).

Reported effects of coinfection

Effects of coinfection on parasite abundance and host health were sampled across 146 suitable publications according to parasite abundance and host health for 444 coinfections, involving 119 parasites. Among these coinfections, 191 (43.0%) measured the size or direction of effects on parasite abundance and 172 (38.7%) measured the size or direction of effects on host health. The remainder of coinfections had no effects reported.

Overall, positive effects of coinfection on parasite abundance were the most common reported across publications (6 negative, 14 neutral, 27

positive reports across 47 publications; Fig. 2.2A). Among specific pairs of coinfecting parasites neutral effects exceeded positive effects (7 negative, 91 neutral, 66 positive across 164 unique parasite pairs; Fig. 2.2C). In both cases these patterns were strongly significantly different from the random null model (grey line on Fig. 2.2, by publication [$X^2 = 14.7$, $d.f. = 2$, $p < 0.001$], by coinfection [$X^2 = 88.8$, $d.f. = 2$, $p < 0.001$]), and from the no-effect null model (black line on Fig. 2.2, by publication [$X^2 = 156.4$, $d.f. = 2$, $p < 0.001$], by coinfection [$X^2 = 276.4$, $d.f. = 2$, $p < 0.001$]).

Regarding the impact of coinfection on host health, there was a much greater number of negative effects reported in publications than either positive, neutral, or NA categories (46 negative, 10 neutral, 3 positive across 59 publications; Fig. 2.2B). When data were aggregated by specific parasite pairs the neutral effects exceed the negative effects (45 negative, 74 neutral, 4 positive across 123 unique parasite pairs; Fig. 2.2D). In both cases these patterns were significantly different from the random null model (grey line, by publication [$X^2 = 53.4$, $d.f. = 2$, $p < 0.001$, Fig. 2.2B], by coinfection [$X^2 = 77.2$, $d.f. = 2$, $p < 0.001$, Fig. 2.2D]), and from the no-effect null model (black line, by publication [$X^2 = 286.3$, $d.f. = 2$, $p < 0.001$, Fig. 2.2A], by coinfection [$X^2 = 176.0$, $d.f. = 2$, $p < 0.001$, Fig. 2.2C]).

It is unlikely that these patterns of the effects of coinfection would be changed by knowledge of the unreported effects (the NAs in Fig. 2.2). Even after NA values were assigned predominantly to the neutral category (i.e. under the no-effect null model), the distribution of the grand mean effect was positive for the effects on parasite abundance (Fig. 2.3A and C), and negative for effects on host health (Fig. 2.3B and D). None of the distributions of grand means overlapped zero (Fig. 2.3).

Reported coinfections compared with infections of global health importance

There were differences between the most commonly reported coinfecting parasites and the infections causing most global health burden (Fig. 2.4).
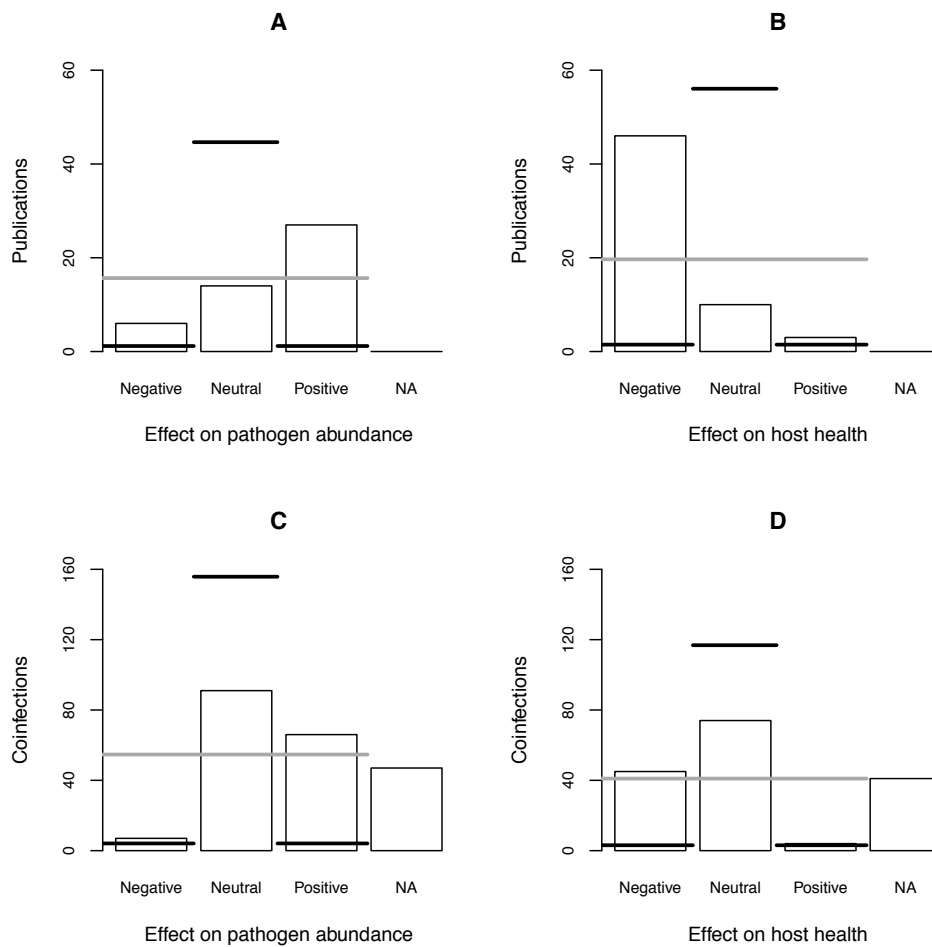
Fig. 2.2: Direction of reported effects of coinfection on the abundance of infecting parasites and host health averaged across publications and coinfections published in 2009. Horizontal lines indicate expected values of null hypotheses (black=no-effect, grey=random).
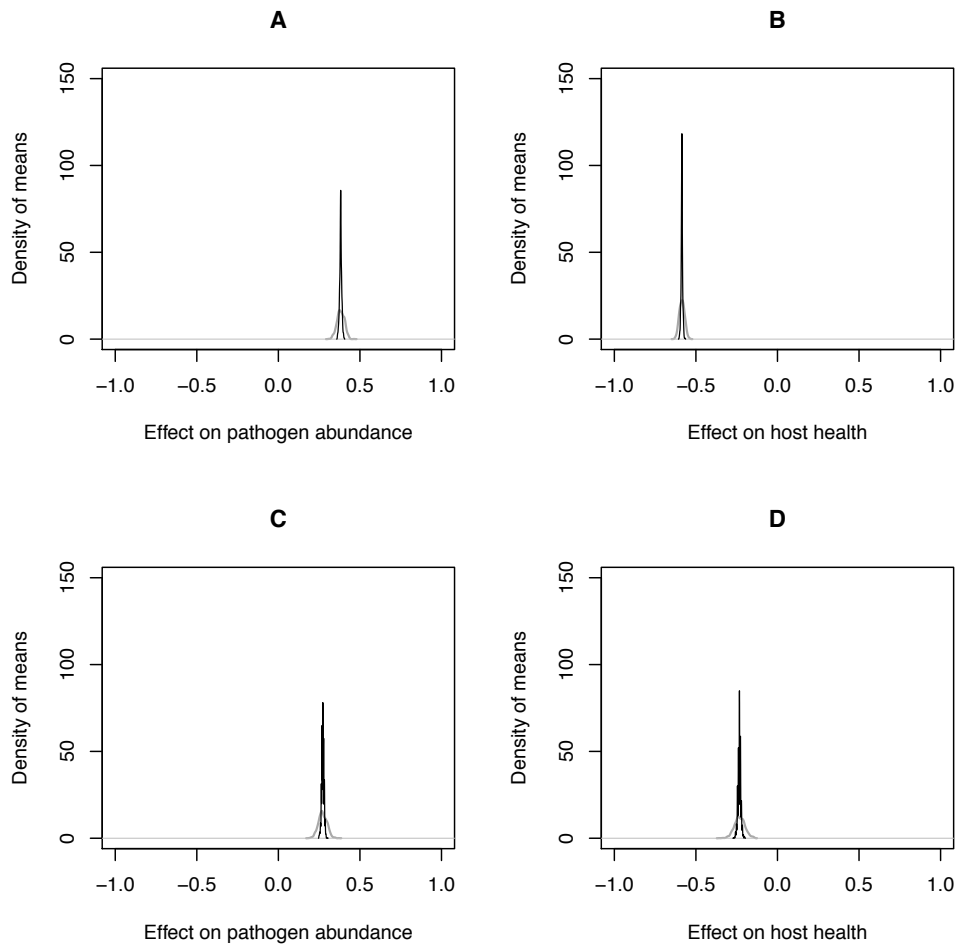
Fig. 2.3: Distribution of grand mean effects of coinfection including simulations of missing values according to the random (grey line) and no-effect (black line) null models. Lines generated by a Gaussian kernel estimator (smoothing bandwidths: random $= 5.1 \times 10^{-3}$, no-effect $= 1.2 \times 10^{-3}$).

Respiratory infections causing the most (40.5%) infection deaths, with the next greatest causes, diarrhoea and HIV/AIDS, causing half as many deaths. Other important infections by global mortality are tuberculosis, malaria, and predominantly childhood infections (measles, meningitis, pertussis, and tetanus).

Comparing the infections causing highest global mortality, global infectious disease morbidity, measured by years of disability caused by infection, are proportionately lower for respiratory infections and HIV/AIDS, but higher for diarrhoea and tuberculosis (compare grey and white bars on Fig. 2.4).

The tenth biggest infectious cause of mortality worldwide, HBV, is the only hepatitis virus featuring in the top ten infectious causes of mortality, causing 1.1% of infectious disease deaths, and 0.4% of years of disability from infection. In comparison, hepatitis viruses featured in one third of reported coinfections (220 of 677, 32.5%, fourth black bar from the left in Fig. 2.4)).

The top ten parasite species reported in coinfections were HIV (in 266 [39.3%] of 677 coinfections), HCV (20.4%), HBV (12.3%), *M. tuberculosis* (5.9%), Cytomegalovirus (CMV, 5.2%), Hepatitis D (HDV, 3.8%), unidentified bacterial infections (2.8%), Herpes Simplex virus (HSV, 2.7%), HPV (2.51%), and unidentified helminth infections (2.51%). While five of these viruses (CMV, HCV, HDV, HPV, and HSV) are among the most common reported coinfecting parasites, they contribute relatively little to global infection mortality. Four of the globally most common infectious causes of mortality received no, or very few, reports of coinfection in 2009 publications (all of them childhood infections: meningitis [0.15% of reports], tetanus [0.08% reports], pertussis [no reports], and measles [no reports]).

Discussion

Interest in coinfection has increased in recent years, with publications on human coinfection involving hundreds of parasite taxa across all major parasite groups. Recent publications show that negative effects of coinfec-

tion on human health are more frequent than no effect or positive effects. The most commonly reported coinfecting parasites are not the infections causing highest global mortality. These results raise questions concerning the occurrence and study of coinfection in humans and their implications for effective infectious disease management.

The overall consequence of reported coinfections was poorer host health and enhanced parasite abundance, compared with single infections. This is strongly supported by differences in the reported direction of effects ($p < 0.001$) from expectations of either no-effect or of random distributions, by the robustness of these trends to missing values, and by the diverse publications in which these coinfections were reported. The tendency for positive effects on parasite abundance also corroborates the negative effects on host health because larger infections are a mechanism by which disease can be exacerbated. The consistency of these detrimental coinfection effects across a wide range of parasites suggests a general tendency towards positive interspecific interactions. In publications about leprosy and tuberculosis coinfections there has been a recent turn away from antagonistic interactions toward more synergistic theories (Hohmann and Voss-Böhme, 2013). A majority of positive interactions is also seen in the animal coinfection literature (Manenti, 2011), and in Tanzanian children (Lello et al., 2013).

The reported coinfection effects could have at least two explanations. First, coinfection may be more likely to occur or be detected in individuals of poor health, which in turn leads to poorer prognosis among coinfected cases. The failure to always undertake a full-range of initial diagnostic tests and the relative paucity of experimental studies of coinfection in humans means sampling bias towards individuals of poorer health is possible, though there may be recall bias in the opposite direction because coinfected patients are less likely to take part in follow-up observations (Nansera et al., 2012). Such biases were difficult to account for in my analyses. The second explanation is that coinfecting parasites interact synergistically with each other, for example via the host's immune system, so that the presence of one enhances the abundance or virulence of the other. A clear example of this is HIV, which causes immunosuppres-

sion, increases the likelihood of additional infections, and occurred in two fifths of reported coinfections (Fig. 2.4).

Differences between reported coinfections and global mortality figures also suggest interactions between coinfecting parasites. Coinfections that were more commonly reported than their relative contribution to global mortality may involve particular synergistic parasite-parasite interactions, such as herpes viruses like CMV or HSV infection enhancing the risk of HPV coinfection (Baldauf et al., 1996). Conversely, infections that cause high mortality but had relatively few reports of coinfection could result from antagonistic interactions, reducing the likelihood of such coinfections occurring and being reported, like *Pseudomonas aeruginosa* exoproduct limiting *Staphylococcus aureus* colony formation (Hoffman et al., 2006). An alternative, and possibly more likely, explanation of the discrepancies between reported coinfections and global mortalities from infections could be greater funding availability (e.g. HIV/AIDS research), higher interests of virologists in coinfection and/or easier observations or more routine screening compared with other parasite types, for instance the greater difficulty of detecting intestinal helminths to the species level in a living patient. The lack of coinfection publications reporting on major infectious causes of childhood mortality remains unexplained. While some publications do consider childhood coinfection (Lello et al., 2013), and coinfection appears to be more common in childhood (Plata-Nazar et al., 2009), recently published coinfection research does not include the infections that kill the most infants globally. Fewer than 1 in 20 publications reported coinfections involving helminths, despite hundreds of millions of helminth coinfections globally (Hotez et al., 2007), which could arise from limited published research on helminthiases. To what extent disparities between global mortality data reflect real patterns, or biases in either research attention or reporting, remains to be seen. Proper evaluation of these potential biases is hindered by inadequate coinfection surveillance.

The disparity between infections that feature highly in global mortality statistics and those receiving most attention in published coinfection studies poses a challenge to infectious disease research. A general understanding of the effects of coinfection is important for appropriate control
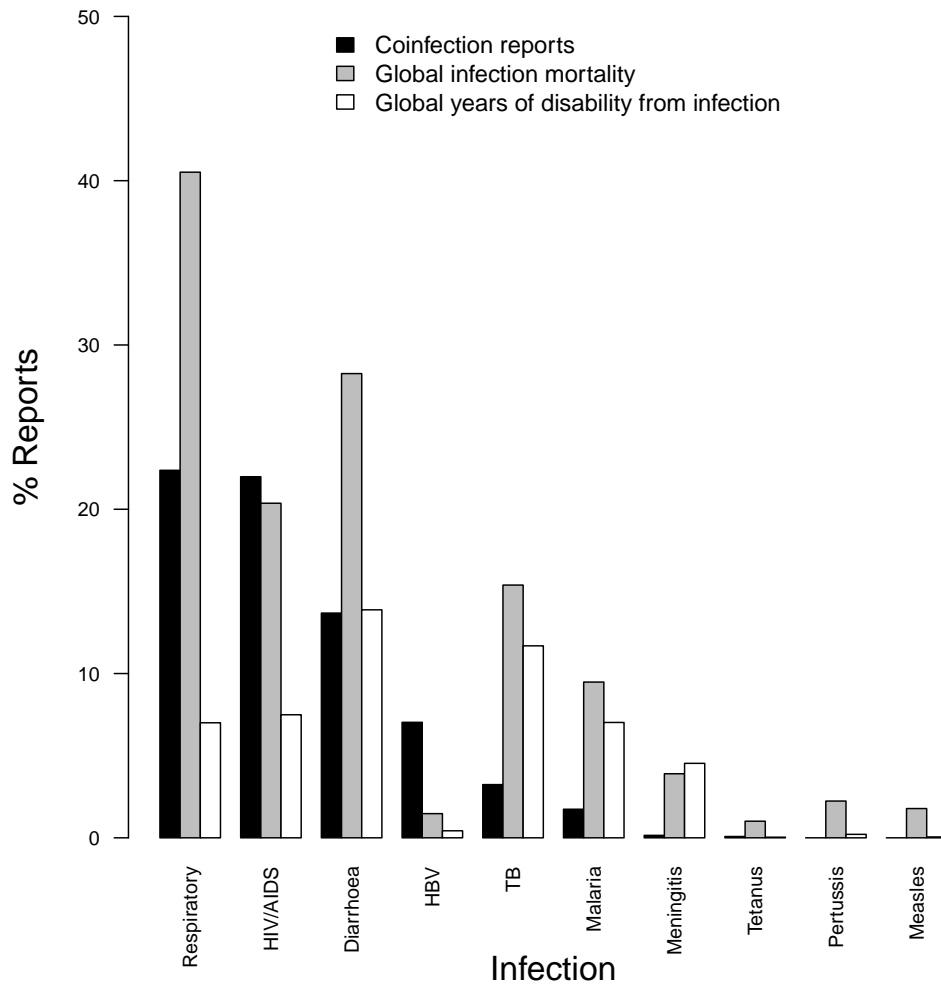
Fig. 2.4: Ten infections causing most deaths globally in 2008 (grey bars, World Health Organization (2009)), compared with percentage of times those infections were reported in coinfections in 2009 publications (black bars), and global estimates for the years of disability caused by those infections in 2010 (white bars, IHME (2012)).

of infectious diseases (Boraschi et al., 2008; Brogden et al., 2005; Hotez, 2009; Laserson and Wells, 2007). Poor or uncertain observational data regarding coinfection hinders efforts to improve health strategies for infectious disease in at-risk populations (Steinmann et al., 2010). For example, global infectious disease mortality data (World Health Organization, 2009) report only single causes of death, even if comorbidities were identified. If health statistics better represented coinfection, published coinfection research could be better evaluated. True patterns of coinfection remain unknown (Brooker and Utzinger, 2007), but my results suggest that it may differ from existing data on important infectious diseases.

Recently published reports of coinfection in humans show that coinfection tends to be detrimental to human health. Understanding the nature and consequences of coinfection is vital for accurate estimates of infectious disease burden. More holistic data on infectious diseases would help to quantify the size of the human health effects of coinfection. Improved knowledge of the factors controlling an individual's risk of coinfection, circumstances when coinfecting parasites interact, and the mechanisms behind these parasite-parasite interactions, especially from experimental studies, will also aid the design and evaluation of infectious disease management programmes. To date, most treatment programmes typically treat each parasite species as if it were in isolation. If coinfecting parasites tend to interact to worsen human health, as suggested here, treatments may need to be more integrated and specialist treatments developed for coinfection.

Chapter 3

Parasites interact most via shared resources in a summary human coinfection network

Abstract

Coinfection can have negative effects on human health (Chapter 2). How coinfecting parasites interact within a complex human host to produce these effects is currently unknown. Understanding the mechanisms that underly parasite interactions within a host could lead to improved coinfection treatments. In this chapter I use data from hundreds of published studies of coinfected humans to assemble a summary within-host coinfection network comprising direct and indirect interactions among parasites, resources, and host immune components. I then investigated the networks structure by quantifying parasite interaction types, and detecting modules of closely interacting components. Interactions between pairs of parasites were more often mediated indirectly through shared resources than through immune components or other parasites. Furthermore, the network comprised 10 groups of closely interacting parasites, resources and immune components, eight of which were associated with particular body parts, and seven of which were dominated by parasite-resource interactions. The summary network of reported coinfection in humans had a compartmentalised structure, with physical location and bottom-

up, resource-mediated processes most often influencing how, where, and which coinfecting parasites interact. This shows the utility of networks for understanding how coinfecting parasites interact, gives evidence for why parasite communities in human populations may be resistant to treatment, and provides hypotheses for how new treatments could modify the processes within coinfected humans.

Introduction

More than 1400 parasite species, including viruses, bacteria, helminths, protozoa, and fungi, infect humans (Taylor et al., 2001). Simultaneous infection of humans by multiple species (coinfection) is commonplace (Brogden et al., 2005; Cox, 2001; Petney and Andrews, 1998); helminth coinfection alone affects 800 million individuals (Hotez et al., 2007). Coinfection involves globally important diseases like HIV and tuberculosis (Fatkenheuer et al., 1999), is concentrated among the poor (Boraschi et al., 2008; Steinmann et al., 2010), and is often associated with worse host health and higher parasite abundance (Chapter 2). Coinfection can also reduce treatment efficacy (Chiodini, 2001; Cooper et al., 2001; Harris et al., 2009), and increase treatment costs (Rizzardini et al., 2011). These phenomena are likely driven by within-host interactions among coinfecting parasites.

Coinfecting parasites interact when individuals of one species affect individuals of another (an interspecific interaction, Wootton and Emmerson (2005)). Interactions may be direct (Hoffman et al., 2006), or indirect, mediated by other parasites, host immunity (Bruce et al., 2000; Christensen et al., 1987; Cox, 2001) or host resources (Antia et al. (2008), i.e. body parts consumed, damaged, or inhabited by parasites). These bottom-up resource-mediated and top-down immune-mediated interactions between coinfecting parasites have been likened to how species interact in free-living ecosystems (Pedersen and Fenton, 2007). Accordingly, treatment of one species could result in unexpected changes to another non-target parasite (Bruce et al., 2000; Druilhe et al., 2005; Lello et al., 2004). However, we currently know little about the frequency of these different interaction types, or how they are distributed in the human body (Franco et al.,

2003). Indeed, the potentially overwhelming diversity of coinfecting parasite types, and their many possible interactions, means that understanding the consequences of coinfection remains difficult.

Before the effects of treatment on coinfecting parasites can be predicted, we need to know how within-host parasite communities are structured. If parasite communities have consistent, non-random assembly processes, these could be used to develop general treatment guidelines. However, at present, we do not know the overall structure of the wider parasite community because most studies of coinfection are typically restricted to measuring interspecific interactions between pairs of parasites (80% of publications reviewed in Chapter 2 reported a single species pair). Here, I move beyond this pairwise view to study interspecific interactions among the parasite community of humans.

Network structure can reveal the biological function of complex systems (Albert and Barabasi, 2002; Strogatz, 2001), and networks have frequently been used to study free-living ecological communities. Summary networks, comprising aggregated samples across multiple places or times, are particularly useful for finding consistent forces influencing community composition or interaction pathways among groups of components, even when they are not directly measured from a single sample (Pimm, 2002). Researchers have begun to include parasites in ecosystem-wide networks (Lafferty et al., 2006), and in disease transmission networks (Danon et al., 2011; Poulin, 2010). Networks are also being applied to complex diseases (Cho et al., 2012), and within-host ecosystems, revealing relationships between the host and single parasite infections (e.g. *Mycobacterium tuberculosis*, Raman et al. (2010)), humans and their microbes (Thiele et al., 2012), and a summary network of many parasites across many fish species (Lima Jr et al., 2012). However, there has been no attempt to construct a comprehensive within-host interaction network for a single host species. Such a network could be constructed with three trophic levels of parasites, resources consumed by them, and host immune defences to show whether interactions among parasites are direct or indirect, or are predominantly resource-mediated or immune-mediated (Pedersen and Fenton, 2007). Understanding such community-wide patterns could in

the future help understand and predict treatment responses in individual coinfected patients.

Here I constructed the first summary network of within-host coinfection from published reports of coinfection in humans. The components (nodes) of the network are parasites (e.g. HIV, *Aspergillus*, hookworm), host immune system components (e.g. IgA, IL-10, macrophages), and host resources (including nutrients or cells consumed and cells, bodily fluids, tissues, organs, anatomic sites occupied or damaged by parasites). I then analyzed (i) the structure of the full network in terms of the distribution of links (interactions between nodes), (ii) the frequency of parasite interaction types (direct, immune-mediated, resource-mediated, or parasite-mediated), and (iii) how the network is arranged in modules of highly-connected nodes (see Table 3.1 and Fig. 3.1). I found the structure of interactions among parasites within coinfected humans to be similar to other ecological networks and, contrary to expectations from other research, to mainly be subject to bottom-up control.

Methods

Network development

A network of parasites, resources, and immune components (nodes) was assembled from 316 articles on human hosts with established coinfections published in 2009 (one year due to time constraints; see Chapter 2 for inclusion and exclusion criteria). Analyses later in this chapter show that results are unlikely to be affected by the restricted dataset (see accumulation curves and effects of publication sampling in Fig. 3.7).

Interactions in the network are denoted by links between two nodes, and all links in the network were binary (present or absent), since data for interaction strengths were unavailable from most publications. Quantified interaction strengths are useful when modelling network dynamics, and when some interactions may only occur under certain conditions (e.g. at particular points in the infection cycle, with plasticity of parasite phenotypes (Mideo and Reece, 2012; Viney, 2001). However, a binary network
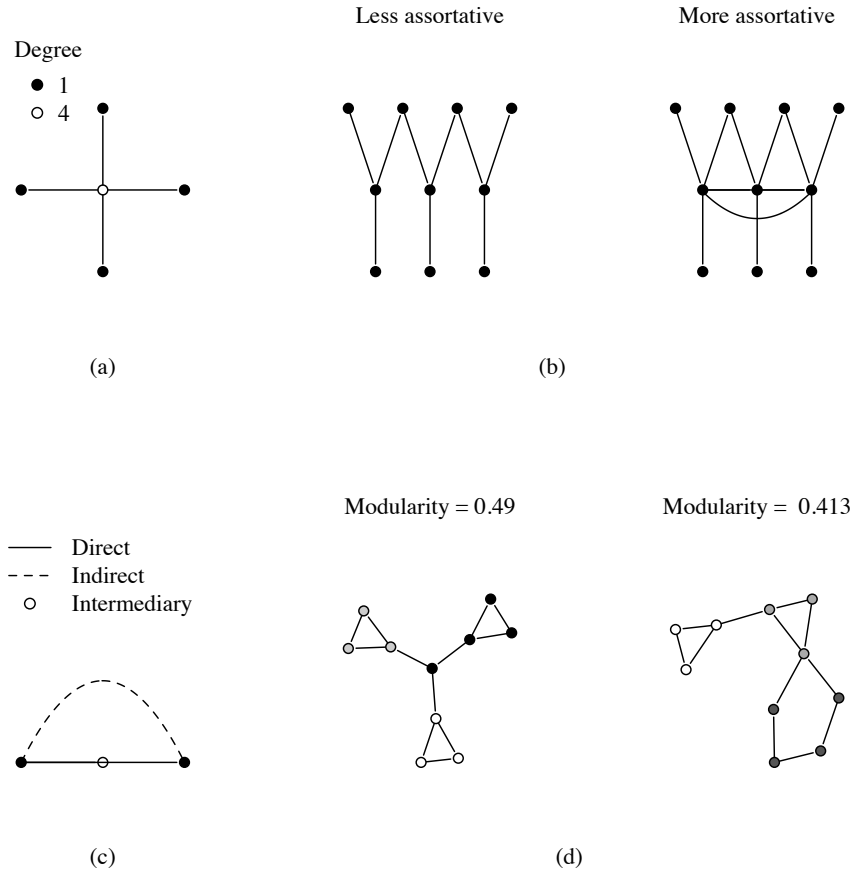
Fig. 3.1: Illustrative diagrams of network analyses undertaken: (a) node degree, (b) assortativity, (c) direct and indirect connections, and (d) modularity, where the modularity scores correspond to the maximum modularity obtained by sequentially collapsing nodes into modules. The left network in (d) was designed to have three modules and high modularity. The right network in (d) is a random network with the same number of nodes, links, and modules, but has lower peak modularity.

Table 3.1: Network metrics used herein and their biological relevance for understanding interactions among coinfecting parasites.

Measure	Meaning	Importance to coinfection	Outline
Degree	Number of nodes linked to a given node.	Reveals how interactive a node is.	Fig. 1a
Assortativity	Correlation of node degree across all pairs of linked nodes.	Strong positive correlation indicates polarisation between nodes with few and many interactions; cliques of highly interactive nodes may need special treatment.	Fig. 1b
Direct parasite interactions	Number of parasites linked to a given parasite.	Reveals coinfections where integrated treatment may be advisable.	Fig. 1c
Indirect parasite interactions	Number of parasites connected to each parasite by two links via an intermediary node.	Reveals interactions between coinfecting parasites mediated by another parasite or by host immunity or resources, where treatment choice may depend on host condition.	Fig. 1c
Modules	Groups in the network that have many links within them and fewer links to other groups.	Reveals areas of highly connected immune components, parasites & resources. Could enable typing of coinfection cases.	Fig. 1d

is sufficient for revealing the coarse topology of biotic interactions (see Chapter 2 for a fuller discussion of the difficulty of quantifying interaction strengths from this dataset).

Each individual publication reports only a subset of resource- and immune-interactions relevant to that specific study. To understand the wider potential for interactions among parasites I therefore combined interactions from many publications into a summary network (also called a community food web by Cohen (1978)), akin to many ecological networks of free-living systems that aggregate all the possible links between nodes in one ecosystem type (e.g. a freshwater stream, Woodward (2010)). Hence, the full network does not represent an individual coinfected host, but reflects all potential within-host interactions reported among the parasite community within humans.

Nodes

Nodes from reports collated across the sampled publications were named consistently by aggregating closely related nodes. This aggregation helps detect functionally similar interactions and is standard practice in network science (Dunne, 2006). Following common use in genetics (Bard and Rhee, 2004), I used an ontology (National Cancer Institute, 2011), the Universal Medical Language Service (UMLS) semantic hierarchy (US National Library of Medicine, 2010), and the following rules to ensure consistent node aggregation: (i) immune and resource nodes aggregated to cell type or above, except for components that interact directly with parasites, (ii) substance nodes designated by the UMLS type were aggregated by their biological function, and (iii) nodes relating to the human reproductive system were classified into gender-specific classes (e.g. female genitals, male genitals, and pregnancy), because differences between the sexes and reproductive status have been biologically important in other coinfection studies (Cattadori et al., 2008; Luong et al., 2010; Ned et al., 2005). Accordingly, some nodes above the cellular level were subsets of one another, such as knee and joint, gums and mouth, or colon and gastrointestinal. These nodes were not aggregated so as not to confound how particular interaction patterns were counted, for example, the number of

indirect links between parasites will increase as intermediary nodes are aggregated. Many links would be unrealistic if two parasites occupying very distinct parts of, for instance, the alimentary canal were linked to the same, aggregated resource node. Relations between nested nodes (like colon and gastrointestinal) are biologically important, and the module analysis allows these nodes to cluster together.

Since the amount of node aggregation (e.g. taxonomic resolution of nodes) can affect ecological network structure (Gilljam et al., 2011), I assessed the effect of three different node aggregation methods on the conclusions: (i) no aggregation, where node names matched those reported in publications, (ii) medium aggregation of cells into tissues, immune receptors into functional groups (Th1/Th2), and parasites to genus level, and (iii) high aggregation where tissues were aggregated into body parts, and parasites were aggregated to the family level.

Links

Links between nodes were first derived from the same publications that reported those nodes. Resource or immune links for some parasites were not mentioned in the publications, but in many of these cases the resources they consume and immune responses they triggered were known so were assigned using an encyclopedia of infections (Topley, 2006). Each link was classified in one of three ways according to the strength of evidence: (1) co-occurrences (two nodes observed in the same individual, (2) correlations (an association between two nodes is reported, without a known biological mechanism), or (3) mechanistic links (connected by a demonstrated biological process). While known mechanisms are a reliable basis for an interaction, there are potential causal processes that remain unknown, especially for poorly studied parasites. Establishing causality over mere association is particularly problematic in humans where experimentation is difficult. Two components found simultaneously in the same individual could potentially interact, even if the connecting mechanisms have not been identified or the interaction is so weak that it has not yet been detected. Therefore three versions of the network were analysed based on the link types described above: mechanistic links only, mecha-

nistic and correlative links, and all three link types together. The three versions cover a spectrum from a network with high degree of certainty (mechanistic only) to one where the mechanism of interaction has not been reported (all link types).

In most cases there was insufficient data to infer directionality between parasites and their various linked host (resource and immune) components. The presence of many undirected links, or ones where the direction of energy flow is ambiguous (e.g. non-mechanistic links between parasites, damage from migration through tissues, or immune interdependencies), and the inability to compute a network with a mixture of directed and undirected links means all three versions of the network were undirected. Most of the metrics I used (assortativity, direct/indirect interactions, and modularity) do not depend on link direction. Node degree can depend on direction by counting links to or from a node (in- and out-degree), but for this undirected network I used total degree.

A summary network

All the nodes and links across the time course of infections and across individuals form a summary network of coinfection in humans, akin to many ecological networks of free-living systems that aggregate all the possible links between nodes in one ecosystem type (Woodward, 2010). Hence, the full network does not represent an individual coinfected host, but reflects all potential within-host interactions reported among the parasite community within humans.

Network analysis

I analysed three structural features of each of the networks (Fig. 3.1 and Table 3.1): (i) how much the components interact with one another (distribution of node degree, Fig. 3.1a-b), (ii) the relative importance of different types of interaction between parasites (Fig. 3.1c), and (iii) whether the network contains modules of tightly interacting nodes (Fig. 3.1d). While many other network structural features exist, I chose these particular features because they reveal functionally important patterns

regarding interactions in coinfected humans (see Table 3.1). Many other analyses can be done to understand within-host networks of coinfection, but I present a selection of results to highlight what a summary within-host parasite network can reveal about coinfection. Analyses were done in R version 2.15.1 (R Development Core Team, 2012).

Degree distribution

I summarised the overall network structure by calculating node degree. A node's degree is the number of nodes that are one link away (i.e. the number of other nodes connected to each focal node). A networks degree distribution reveals how links are distributed among nodes, can indicate how resistant the network is to perturbation, and, being a commonly used network metric, enables us to directly compare the within-host coinfection network with other networks. Parameter(s) for exponential, power-law, Poisson, normal, and uniform degree distributions were estimated by maximum likelihood and the coefficient of determination (R^2) was calculated to find the model closest to the observed degree distribution (Dunne et al., 2002).

I also analysed the tendency for well-connected (high degree) nodes to be linked to other well-connected nodes (i.e. evidence for assortative mixing, or assortativity). If highly linked nodes are also connected to other highly linked nodes (high assortativity) there is greater potential for perturbations to spread across the network (Maslov and Sneppen, 2002). Assortativity was measured via Pearson's correlation coefficient (r) of the degree of each node either side of each link (Newman, 2003). Networks with high assortativity have high positive values of r (close to $+1$) because high degree nodes are also likely to be linked to other high degree nodes. Values of r close to 0 indicate a more even distribution of links, similar to a random network. Negative values of r (close to -1) indicate disassortativity whereby high degree nodes are dispersed across the network and are typically connected to low degree nodes.

Frequency of parasite interaction types

I counted the total number of direct and indirect links between every
pair of parasites to reveal the reported frequency of each type of parasite
interaction. Indirect links occur when two parasite nodes are linked via
an intermediate node (either a resource, parasite, or immune component).
The number of each type of parasite-parasite interaction was calculated
by isolating only the links relevant to a certain type of interaction, and
then counting the number of unique routes linking any parasite pair.

I compared the observed number of direct and indirect links with that
expected from chance using 1000 randomly rewired networks. I used a
constrained Poisson process to create random networks that had the same
number of nodes in each trophic level but each node had equal probability
of being linked to another node (Erdos-Renyi process of independent link
assignment, following Erdos and Rényi (1959); Strogatz (2001)). Many
networks deviate from such random distributions, but I used this null dis-
tribution to test whether the community of parasites coinfecting humans
was assembled by a neutral, independent process. Randomisations with
more biological detail could be explored in future, but as this is the first
summary network of parasites within humans I begin with a null model of
a Poisson distribution of links. I implemented the randomisation by reas-
signing the links from the upper triangle of the observed adjacency matrix
in random order. The total number of nodes and links was therefore equal
to the empirical network, though the redistribution of links meant that
individual node degrees differed (and hence the number of particular in-
teractions between certain nodes types in the network also differed). The
observed numbers of each type of interaction are significantly different
from the expected numbers of interactions if they lie beyond two stan-
dard deviations from the mean of the randomisation results. I calculated
p-values by comparing the observed number with the expected normal dis-
tribution using the mean and standard deviation from the randomisations
(at large means the Poisson tends toward a normal distribution). Ran-
domisations are an adequate significance test for frequency of interaction
types, but can fail to account for broader network structures like clusters

of nodes (Schwobbermeyer, 2007), which is why I also studied network modularity.

Modules

Modules were found using three search algorithms (Fig. 3.7a): (i) by sequentially removing the most peripheral link (Newman and Girvan, 2004); (ii) using statistical mechanics (the methodology of Reichardt and Bornholdt (2006), iterated 100 times); and (iii) using short random walks (Pons and Latapy, 2005). In brief, these algorithms search for classifications of nodes into groups (modules) that maximize the modularity. One measure of modularity, termed Q, ranges from 0 (no modular structure, many links between modules) to 1 (strong modular structure, few links between modules, Newman and Girvan (2004)). I analysed the set of modules with peak modularity (Q) for the mechanistic network, since this network makes a conservative assumption about the presence of interactions and reveals the strongest functional similarities (and differences) within the network. For each module I recorded the type (parasite, resource, immune) and identity of the node with highest within-module degree. These nodes contribute strongly to modularity and reveal the defining characteristics of each module.

I also looked within each module to test whether node types had more within-module links than would be expected from chance. I compared the observed number of within-module links to that expected from a binomial distribution where the number of trials was the total number of links to nodes of each type in the module. The number of observed links was considered significantly different from expected if the number was less than 5% probability in a two-tailed binomial distribution (i.e. $p < 0.025$ or $p > 0.975$). I repeated this test for two interaction types (immune-parasite and resource-parasite). Parasite-parasite links were omitted because this interaction type was very rare in the mechanistic network.

To test the robustness of the modularity analyses and results, specifically whether resource-dominated modules were also present in other possible module sets (identified by the same algorithms but having lower modularity), I compared the optimal module set with alternative module

sets. These alternatives had slightly lower Q values than the maximal value found (i.e. for four module sets with next-highest Q-values where $0.469 < Q < 0.4695$; see Results). Visually comparing the nodes in each module in these other high modularity sets with the 10 modules described above confirmed that all modules were consistently associated with bodily locations and the node with highest within-module degree was a resource node.

Results

The summary network of coinfected humans comprised 124 host resources, 305 parasite taxa, 98 immune system components, and 2922 links between these components. The network was compiled from 316 published papers. Most publications ($256/316$, 81%) reported data from multiple coinfected patients. The majority of links (1578) were based on mechanistic evidence, while 812 were from co-occurrence, and 532 from correlational evidence. I derived three versions of the summary network from these different link types: mechanistic links only, mechanistic and correlative links, and all links. I primarily describe results for the mechanistic-only version because these links have the greatest biological support. However, I also compare these results with those from the two other network versions.

Degree distribution

I calculated the degree of each node (the number of connections that each node has with other nodes; Fig. 3.1a, metrics defined in Table 3.1). The degree distribution for the network containing only mechanistic links most closely resembled an exponential distribution with the exponent 0.16 (s.d. 0.007, R^2=0.87, $p < 0.001$, Fig. 3.2a). This means that most nodes (i.e. parasites, resources or immune components) in the network were linked to few other nodes, in fact 89.7% of nodes ($456/508$) had < 15 unique links. Only 9 nodes (0.018%) had degree ≥ 35. These highly connected nodes were blood (70 unique links), respiratory tract (47), skin (40), lungs (39), HIV (37), IgG (37), macrophage (37), dental abscess (37), and liver (36).
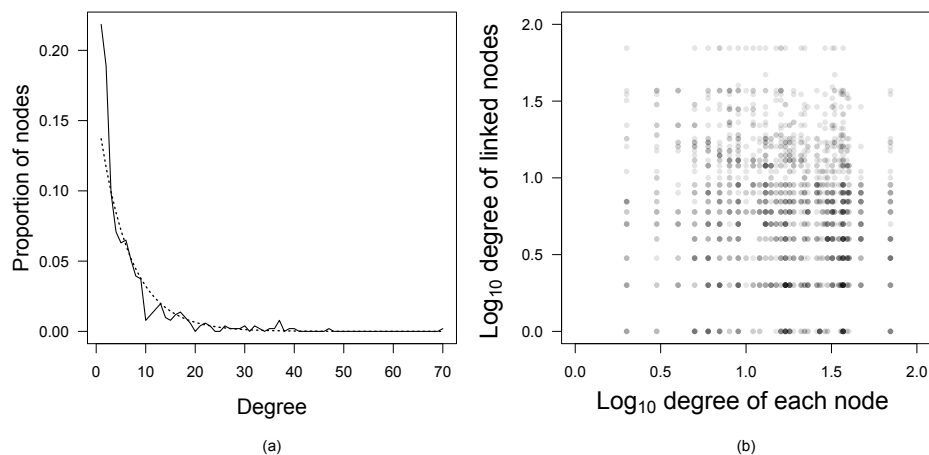
Fig. 3.2: (a) Degree distribution for the network containing only mechanistic links. Thick black line corresponds to the observed proportion of nodes with a degree greater than or equal to the value on the x-axis. Dashed line indicates the best-fitting statistical model, the exponential model (exponent 0.016, $p < 0.001$, $R^2 = 0.87$). (b) Assortativity: the degree of each node plotted against the degree of their linked nodes for all unique links for a network with only mechanistic links (Pearsons correlation $r = -0.12$, $p < 0.001$). Plotting symbols are transparent such that ten overlaid data points are black.

There was generally weak assortativity across all three versions of the summary within host network (r close to zero, ranging from -0.12 to 0.12, Fig. 3.3a, and Table 3.2), but significant disassortativity in the mechanistic network ($r = -0.12$, $p < 0.001$, Fig. 3.2b). Hence, most nodes were connected to a range of low, medium, and high degree nodes.

Frequency of parasite interaction types

Indirect interactions (i.e. paths of two links with an intermediate node, Fig. 3.1c) between parasites were more common than direct links. The ratio of indirect to direct links ranged from 1.09 times higher for parasite-mediated interactions within mechanistic and correlative link networks, to 829 times higher for resource-mediated interactions in the mechanistic-only network (Fig. 3.4, Table 3.2). Indirect parasite interactions were most often resource-mediated, and these were significantly more common than expected by chance ($p < 0.001$; rewiring randomisation test). Immune-mediated indirect interactions were about half as common as

(a)   (b)
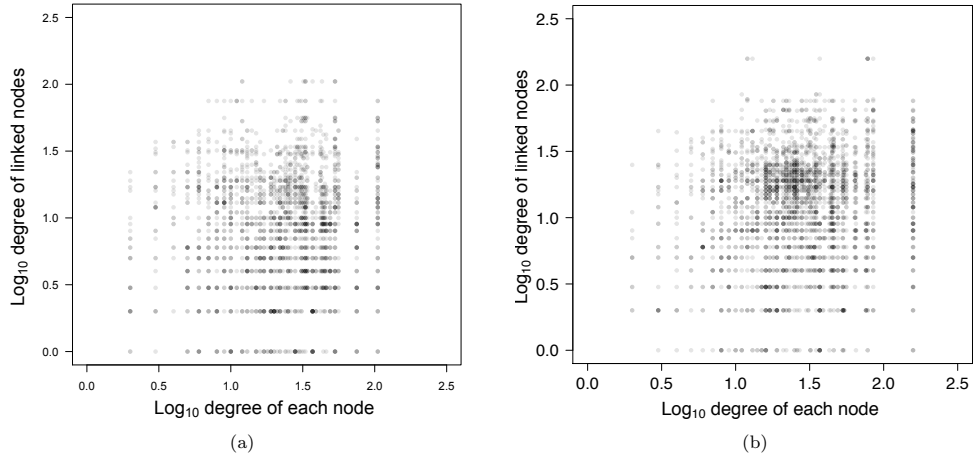
Fig. 3.3: Degree of nodes plotted against the degree of their linked nodes for all unique links. Plotting symbols are transparent such that 10 overlaid data points are black. (a) For mechanistic and correlative links and (b) all link types. The correlation for both networks was weak but significant, $p < 0.001$.

Table 3.2: Node degree, assortativity, number of each type of interaction between parasite species, and the number of modules for three versions of the summary within-host coinfection network containing different link types, with standard aggregation of node names. Indirect parasite interactions are represented as: I = Immune mediated, P = parasite mediated, and R = Resource mediated. *** denotes significantly different from randomised values ($p < 0.001$)

Measure	Mechanistic	Mechanistic & Correlative	All link types
Degree distribution	Exponential (λ=0.16, R^2 =0.866***)	Exponential (λ=0.12, R^2 =0.874***)	Exponential (λ=0.09, R^2 =0.895***)
Assortativity	-0.12***	$+0.07$***	$+0.12$***
Direct interactions	9***	128***	875***
Indirect interactions	P=1***, R=7464***, I=3652***	P=140***, R=9408***, I=5429***	P=4064***, R=11492***, I=5429***
Modules	10	9	8
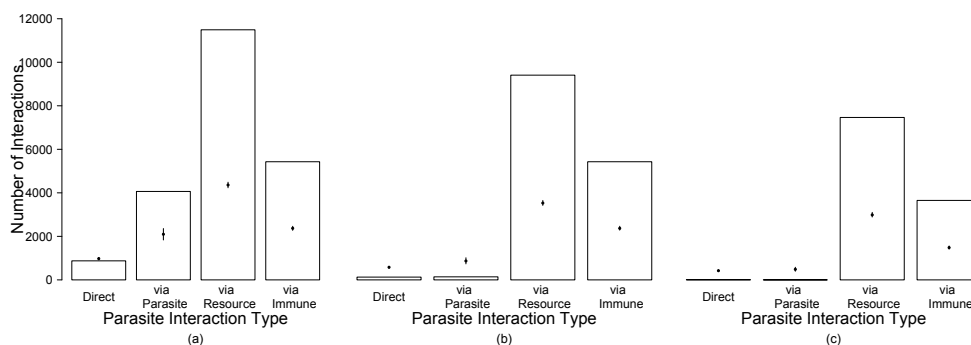
Fig. 3.4: The number of direct and indirect paths between parasites for (a) all link types, (b) mechanistic and correlative links, and (c) mechanistic links only. Vertical black lines represent expected distributions (2 sd, dot=mean) from 1000 simulations. All observed results deviated significantly from expected values (tested against normal distribution $p < 0.001$). Vertical axis scales for (a), (b), and (c) are identical.

resource-mediated interactions, though still significantly more common than expected by chance ($p < 0.001$). Furthermore, 167 publications ($167/316$, 53%) reported multiple parasite-resource interactions, but only 85 (27%) reported multiple parasite-immune interactions. The relative frequency of reported resource- and immune-mediated interactions were robust to the potential under-reporting of parasite-immune links (Fig. 3.5), and to the exclusion of publications relating to individual patients (Fig. 3.6).

Most parasite-parasite links were based on co-occurrence; networks excluding this type of evidence had relatively few direct or indirect interactions involving only parasites (and fewer than expected by chance; $p < 0.001$, Fig. 3.4b and c). The relative frequency of each type of parasite-parasite interaction was qualitatively similar for all three network versions (Fig. 3.4a-c, all $p < 0.001$; Table 3.2).
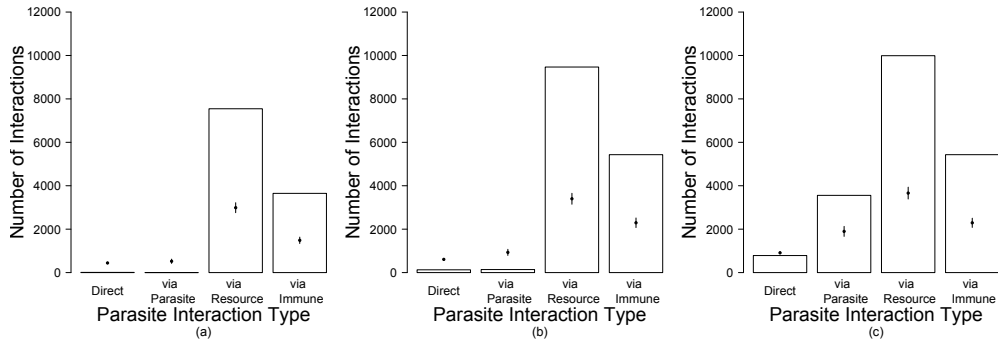
Fig. 3.5: Number of direct and indirect paths between parasites, with parasites lacking immune links removed from the network. Vertical black lines represent expected distributions from 1000 simulations (2 sd, point=mean, see Methods). (Right) All link types, (middle) mechanistic and correlative links, and (left) mechanistic links only. All observed results deviated significantly from expected values ($p < 0.001$).
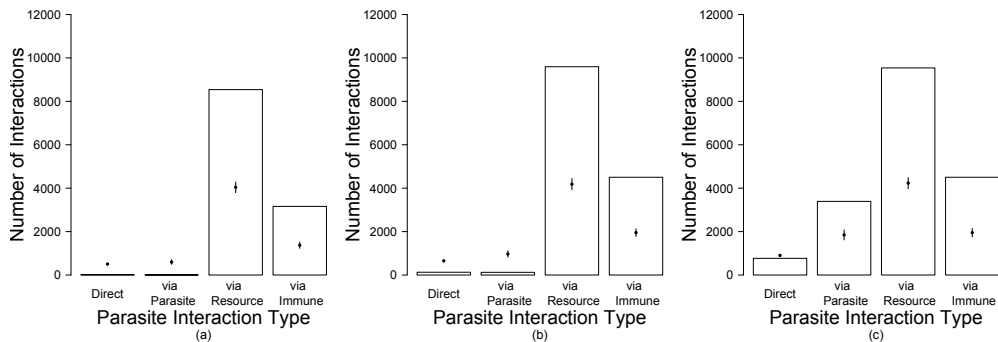
Fig. 3.6: Number of direct and indirect paths between parasites, with links from individual patients (case note publications) removed from the network. Vertical black lines represent expected distributions from 1000 simulations (2 sd, point=mean, see Methods). (Right) All link types, (middle) mechanistic and correlative links, and (left) mechanistic links only. All observed results deviated significantly from expected values ($p < 0.001$).

Modules

The mechanistic network contained a set of modules with highest modularity (peak Q value, illustrated in Fig. 3.1d) that best distinguished clusters of dense interactions, and included 10 modules ranging in size from 12 to 90 nodes ($Q = 0.4695$, Table 3.3, Fig. 3.7). Each of those modules contained a mix of parasites, immune components and resources. One module contained only bacterial parasites, however, all other modules contained multiple parasite types. Parasites were the most common node in 9 of the 10 modules (Table 3.3, except module 2 with 30 immune and 22 parasite nodes). All but two modules had more resource than immune nodes (except module 2 with 30 immune and 15 resource nodes, and module 4 with 25 immune and 9 resource nodes).

These 10 modules were associated with particular microhabitats within the human body (Fig. 3.8, Table 3.3), and this association was also found in other module sets with next highest modularity values. Visual inspection of these 10 modules showed associations with particular bodily systems (Modules 3, 4, 7, 9), body parts (Modules 1, 8, 10), and tissues (Module 6). Two modules were classified as mixed because they contained several sites of infection including the liver, oesophagus, genitals, and eyes (Module 2), and nose, skin, and urinary tract infections (Module 5). Resource nodes had the highest within-module degree for seven out of the 10 modules, and were more common than expected by chance in all modules (Fig. 3.9, $p < 0.001$). Parasite-immune interactions dominated the structure of the remaining three modules where they were also more common than expected by chance ($p < 0.001$). Of the three modules where non-resource nodes had the highest within-module degree, two were immune nodes (IgG and macrophages), and a parasite dominated the other (HIV).

(a)   (b)
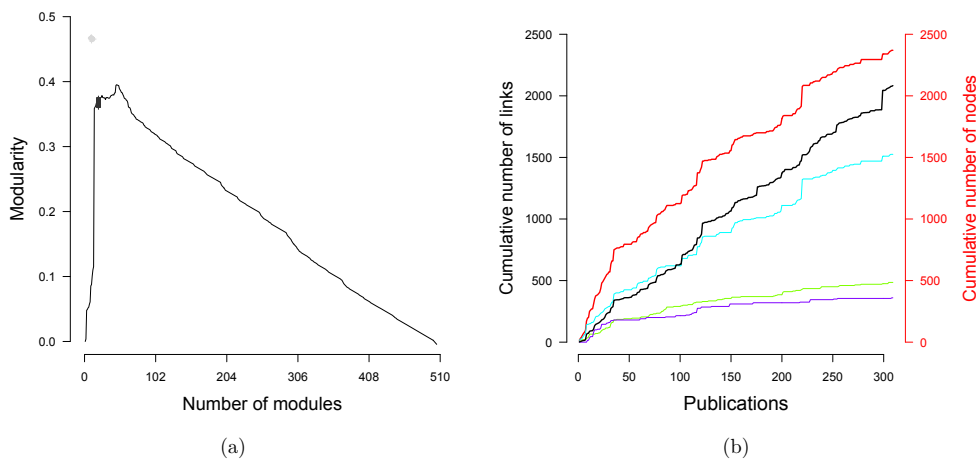
Fig. 3.7: (a) Number of modules and modularity score for each set of modules generated by different algorithms from the mechanistic network. Black line = sequential deletion of the weakest link from each individual node having its own module to one large module containing all nodes, light grey dots (indistinguishable from one another) = statistical mechanics, and black dots (indistinguishable) = random walks. Modularity (Q) is a measure of the strength of association within modules and division between modules. (b) Accumulation of nodes and links in the network with each sampled publication. All nodes (red line, right y-axis) resolved into immune (purple), resource (green), and parasite nodes (blue). Left y-axis for all links (black line).

Table 3.3: Classification of each module in the module set with peak modularity for the summary network containing only mechanistic links. I describe the number of nodes of each type in each module, and the identity and degree of the node with the highest number of links to other nodes in that module and proportion of within-module links these represent. I = immune, P = parasite, R = resource, GI = Gastrointestinal, UTI = Urinary Tract Infection, LRT=Lower Respiratory Tract.

Module	Name	I	P	R	Highest degree node	Degree	% of module links
1	Throat	1	11	3	Tonsil	9	69
2	Mixed	30	22	15	HIV	23	16
3	GI	12	39	17	IgG	20	17
4	Genitals	25	30	8	Macrophage	18	12
5	UTI, Skin	4	59	27	Urinary tract	24	11
6	Mucosa	11	30	11	Respiratory tract	19	18
7	Bowel	1	9	6	Colon	5	25
8	LRT	3	18	4	Lungs	15	45
9	Blood	1	29	10	Blood	24	42
10	Mouth	3	52	17	Dental abscess	27	23
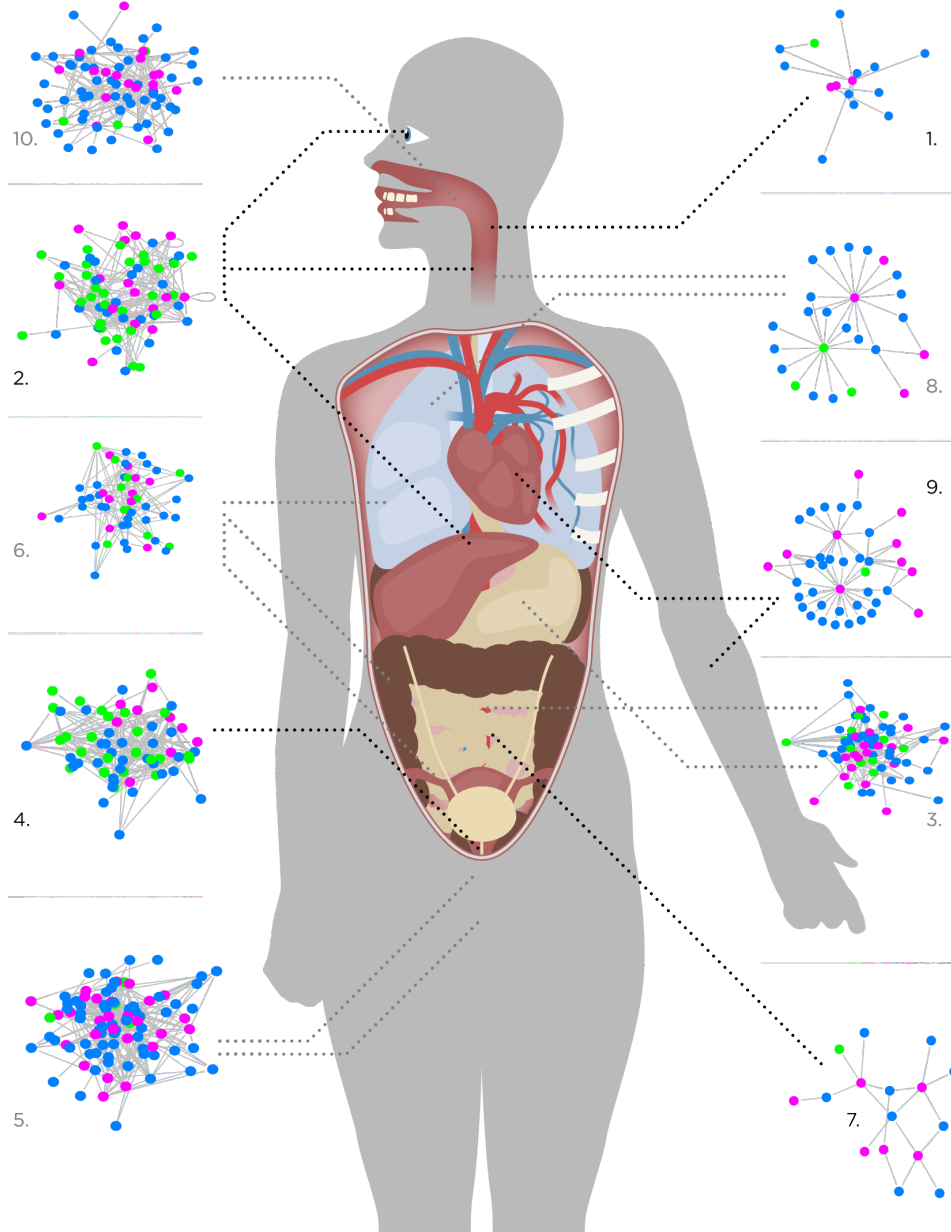
Fig. 3.8: The within-human coinfection network comprising parasite (blue), immune (green), and resource (pink) nodes connected by mechanistic links has ten modules, eight of which are associated with a particular bodily sites. Module numbers refer to Table 3.3. Grey and black numbers and dotted lines are stylistic only; the different shades are used to more clearly distinguish the lines corresponding to each module.
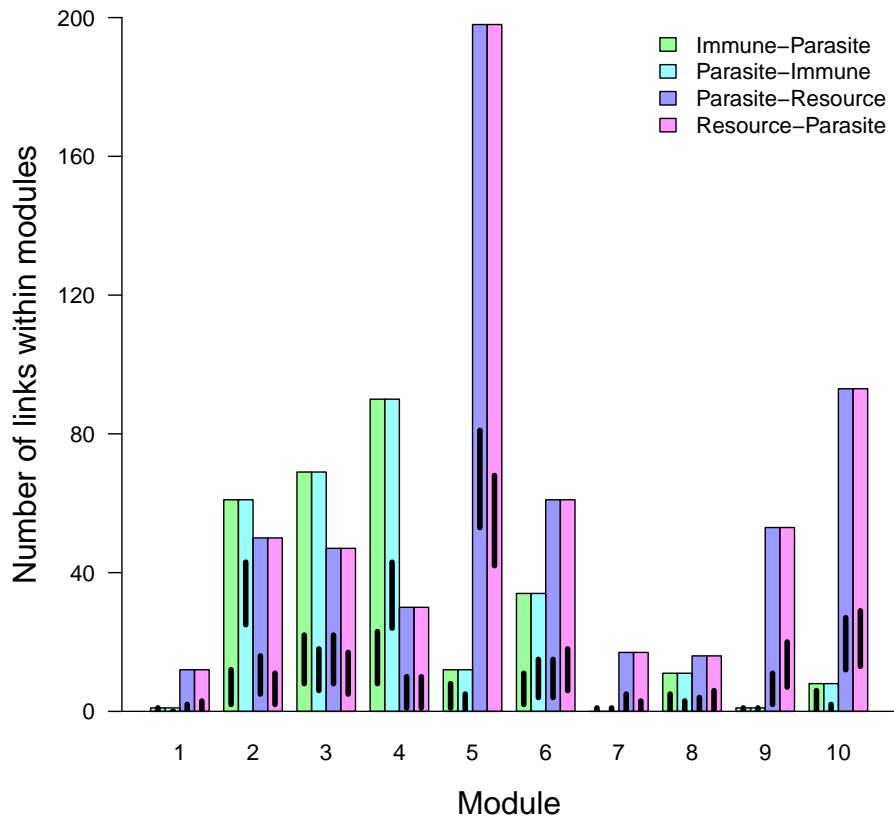
Fig. 3.9: Number of within-module interactions between host immune components and parasites, and between host resources and parasites in each of the ten modules of the mechanistic network. Thick black lines indicate 95% confidence intervals expected from the binomial test (see Methods). Bars that do that overlap with black lines (immune-parasite links for modules 1, 7, and 9) are not significantly different from random distributions ($p > 0.05$). The number of within-module links for all other modules and link types are significantly higher than expected by chance ($p < 0.001$)

Robustness of results

I tested whether measures of network structure were sensitive to the aggregation of nodes and the sample of publications (Tables 3.4-3.6, Fig. 3.10-3.14). The key findings of exponential degree distributions (Fig. 3.10), weak (dis)assortativity (Fig. 3.11), the relative frequency of parasite interaction types (Fig. 3.12), and resource-mediated outnumbering immune-mediated within-module interactions (Fig. 3.13) were all robust to node aggregation. While the number of nodes and links in the network increased linearly with each new publication (Fig. 3.7b), the ratio of resource- to immune-mediated links levelled off once 40 publications were sampled with resource-mediated interactions being dominant (Fig. 3.14a). The degree distribution exponent also reached an asymptote after 100 publications, but the R^2 value was unchanged even with only 5 papers sampled (Fig. 3.14b). Assortativity became weakly positive with a very low p-value, reaching an asymptote after 100 publications (Fig. 3.14c). The number of modules and the modularity score peaked once 50 publications were sampled, levelling off at lower values with fewer modules with more sampling (Fig. 3.14d).

Table 3.4: Summary of the results for number of nodes and links, node degree, assortativity, number of each type of interaction between parasite species, and the number of modules & associated Q value for different link types in a version of the network where node names were *not aggregated*. Indirect parasite interactions are represented as: I = Immune mediated, P = parasite mediated, and R = Resource mediated. *** denotes significantly different from randomised values with $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, no asterisk means NS.

Measure	Mechanistic	Mechanistic & Correlative	All link types
Nodes	612	644	664
Links	1651	2219	3073
Degree distribution	Exponential (λ=0.18, R^2=0.975***)	Exponential (λ=0.14, R^2=0.976***)	Exponential (λ=0.11, R^2=0.986***)
Assortativity	-0.02*	+0.05**	+0.11***
Direct interactions	16	163	854
Indirect interactions	P=5, R=9538***, I=3449***	P=177, R=10749***, I=5418***	P=934, R=10698***, I=5148***
Modules	10, $Q = 0.507$	12, $Q = 0.458$	13, $Q = 0.499$

Table 3.5: Summary of the results for the version of the network with medium aggregation, i.e. node names were *more aggregated* than with standard aggregation. Indirect parasite interactions are represented as: I = Immune mediated, P = parasite mediated, and R = Resource mediated. *** denotes significantly different from randomised values with $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, no asterisk means NS.

Measure	Mechanistic	Mechanistic & Correlative	All link types
Nodes	281	291	292
Links	1177	1606	2282
Degree distribution	Exponential (λ=0.12, R^2=0.887***)	Exponential (λ=0.09, R^2=0.849***)	Exponential (λ=0.06, R^2=0.887***)
Assortativity	-0.06*	+0.11***	+0.09***
Direct interactions	$10 **$	$124 **$	$648*$
Indirect interactions	P=3***, R=7316***, I=5009***	P=157***, R=8482***, I=6382***	P=6477, R=8415***, I=6382***
Modules	8, $Q = 0.358$	8, $Q = 0.335$	5, $Q = 0.374$
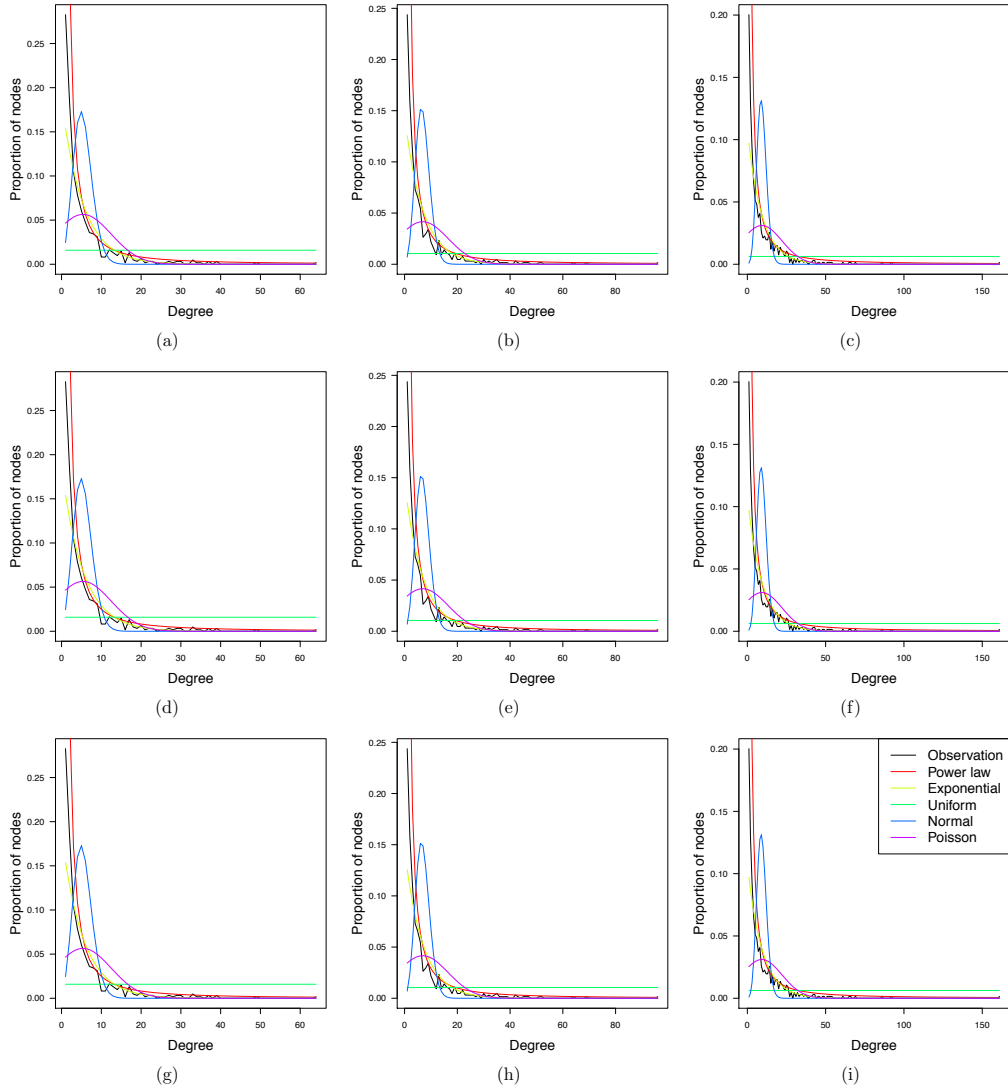
Fig. 3.10: Degree distribution for networks with (top row; a-c) no aggregation of node names, (middle row; d-f) medium aggregation, and (bottom row; g-i) high aggregation. Split by the link types in each network with (left column; a, d, g) mechanistic links only, (middle column; b, e, h) mechanistic and correlative links, and (right column; c, f, i) all link types. Thick black line is the observed proportion of nodes with a degree greater than or equal to the value on the x-axis. In all cases the best fitting model for the degree distribution was the exponential model (dashed line, see Tables 3.4-3.6- for statistics, all $p < 0.01$).
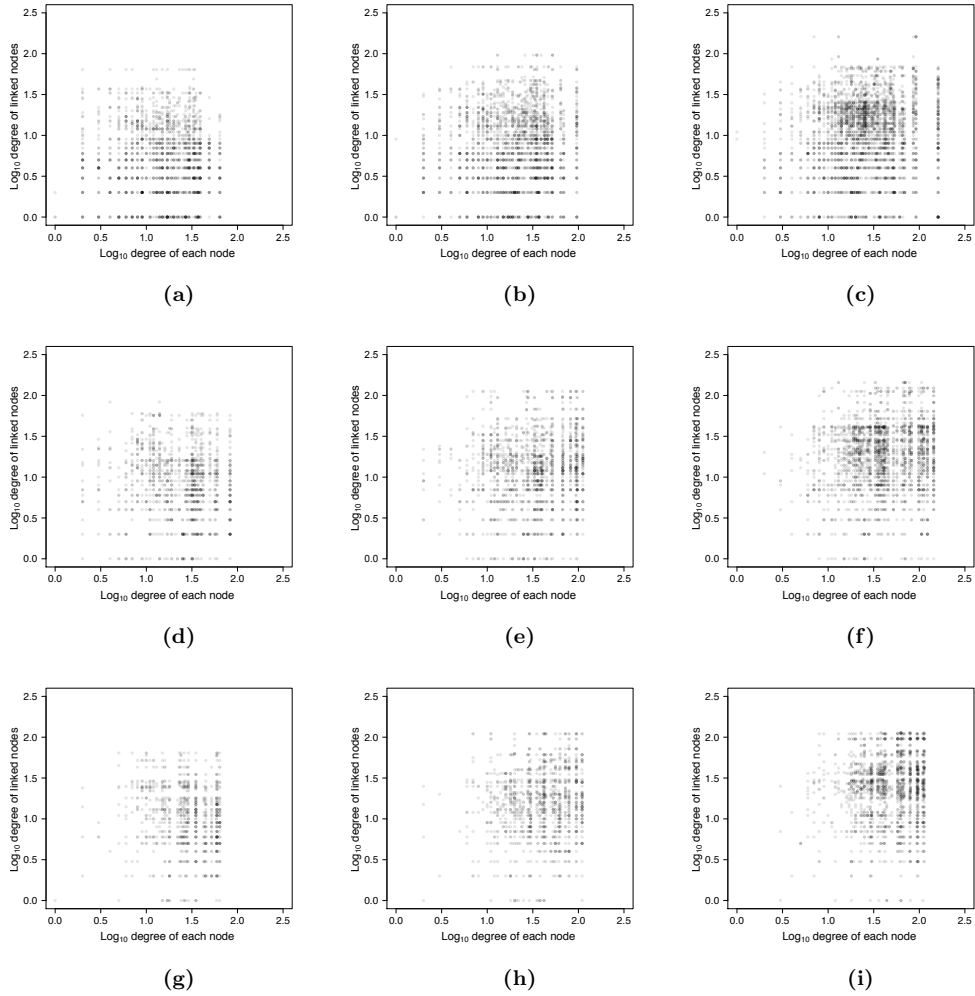
Fig. 3.11: Assortativity: the degree of each node plotted against the degree of their linked nodes for all unique links for networks with (top row; a-c) no aggregation of node names, (middle row; d-f) medium aggregation, and (bottom row; g-i) high aggregation. Split by the link types in each network with (left column; a, d, g) mechanistic links only, (middle column; b, e, h) mechanistic and correlative links, and (right column; c, f, i) all link types. Plotting symbols are transparent such that ten overlaid data points are black.
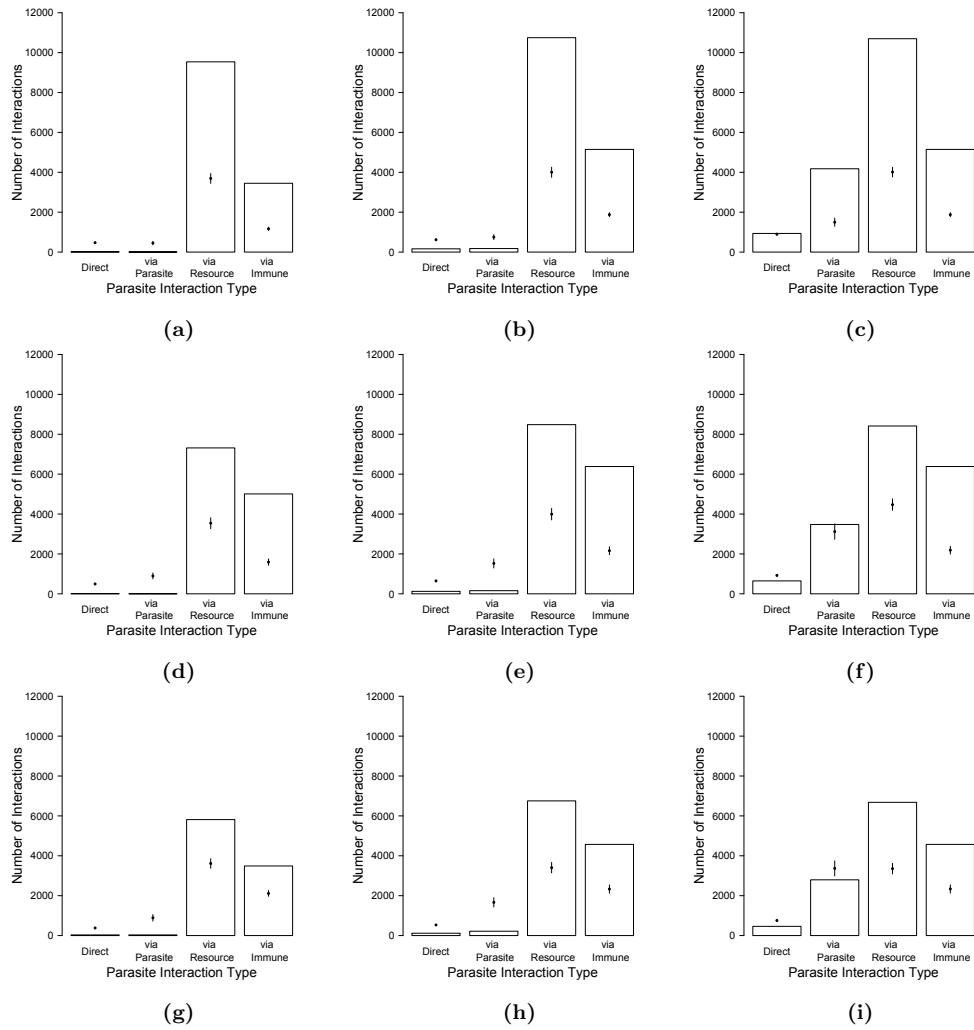
Fig. 3.12: Number of direct and indirect interactions between parasites for networks with (top row; a-c) no aggregation of node names, (middle row; d-f) medium aggregation, and (bottom row; g-i) high aggregation. Split by the link types in each network with (left column; a, d, g) mechanistic links only, (middle column; b, e, h) mechanistic and correlative links, and (right column; c, f, i) all link types. Vertical black lines represent expected distributions (2 sd, point=mean) from 1000 simulations (see Methods).
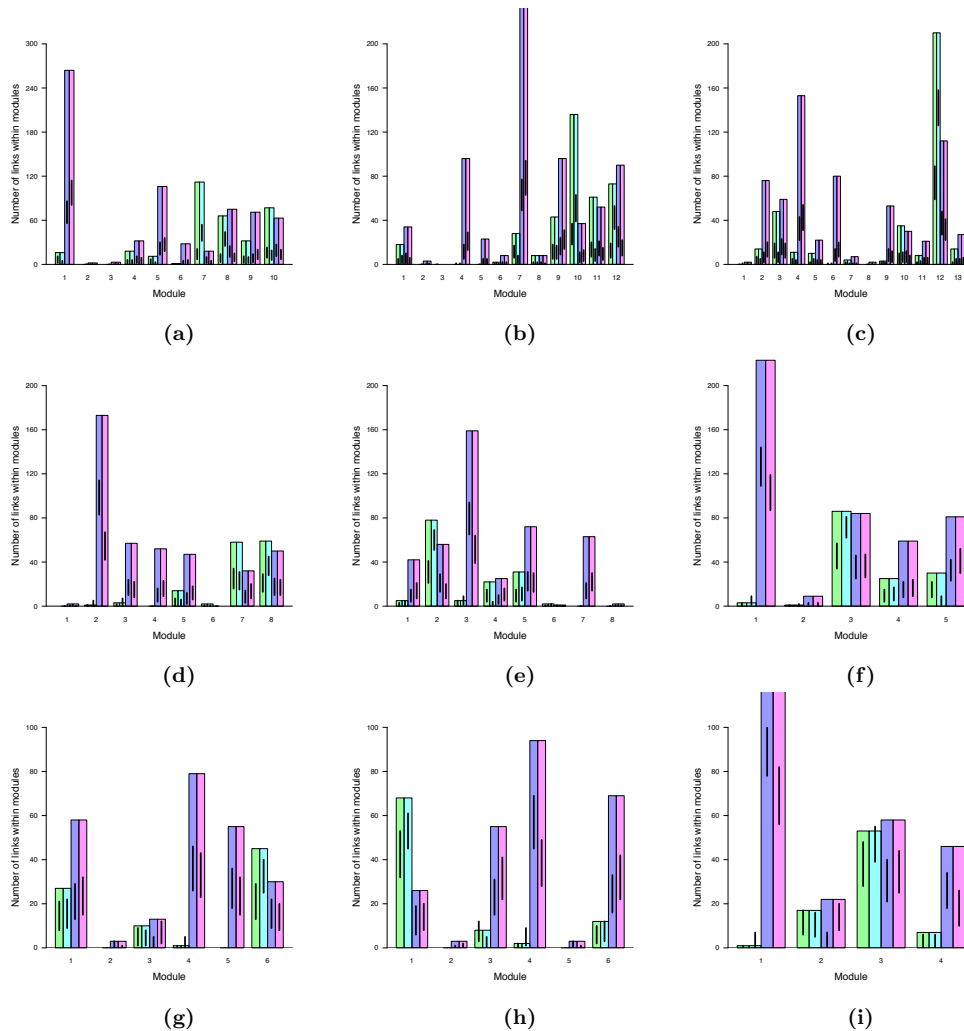
Fig. 3.13: Number of within-module interactions between parasites and immune components or resources in the module sets with peak modularity for networks with (top row; a-c) no aggregation of node names, (middle row; d-f) medium aggregation, and (bottom row; g-i) high aggregation. Split by the link types in each network with (left column; a, d, g) mechanistic links only, (middle column; b, e, h) mechanistic and correlative links, and (right column; c, f, i) all link types. Thick black lines indicate 95% confidence intervals from the binomial distribution (see Methods). Bars that overlap with CI lines are not significantly different from random distributions. Bar colours denote the type of nodes connected by a link: green = immune-parasite, blue = parasite-immune, purple = parasite-resource, pink bars = resource-parasite.
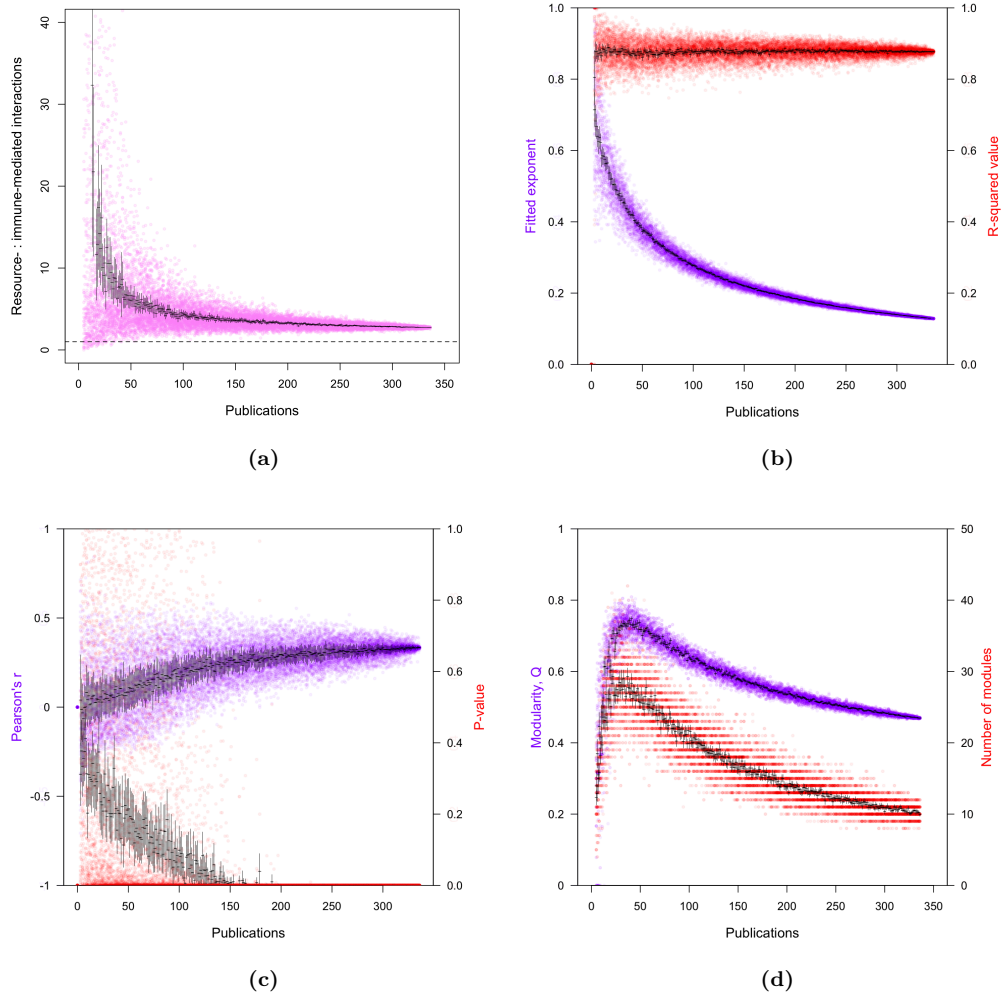
Fig. 3.14: Robustness of network structure to sampling within 2009 coinfection publications. (a) Ratio of resource- to immune-mediated parasite-parasite interaction frequency, (b) exponent and R^2 of model fit to degree distribution, (c) coefficient and p-value of Pearsons correlation between node degrees (assortativity), and (d) number of modules and Q modularity score for module set with peak modularity. Networks had only mechanistic links and standard aggregation of node names. Sampled from five to 336 publications. Links from Topley and Wilsons encyclopedia were broken down to reflect the average number of links reported in each 2009 paper, which increased the total number of publications to 337. Vertical black lines represent two standard errors around the mean (small horizontal black bar) from 50 simulations.

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Table 3.6: Summary of the results for the version of the network with high aggregation, i.e. node names were *much more aggregated* than standard aggregation. Indirect parasite interactions are represented as: I = Immune mediated, P = parasite mediated, and R = Resource mediated. *** denotes significantly different from randomised values with $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, no asterisk means NS.

Measure	Mechanistic	Mechanistic & Correlative	All link types
Nodes	163	167	167
Links	837	1187	1685
Degree distribution	Exponential (λ=0.09, R^2=0.877***)	Exponential (λ=0.07, R^2=0.856***)	Exponential (λ=0.06, R^2=0.887***)
Assortativity	-0.10**	$+0.09$***	$+0.08$***
Direct interactions	32 ***	118**	462***
Indirect interactions	P=32***, R=5815***, I=3490***	P=218***, R=6753***, I=4571***	P=2798***, R=6680***, I=4571***
Modules	6, $Q = 0.269$	6, $Q = 0.253$	4, $Q = 0.289$

Discussion

I developed a summary network of human coinfection from published reports of coinfecting parasites, the resources they consumed or inhabited, and immune components reacting with them. Although the summary within-host coinfection network is complex, it contains several clear structural patterns. First, most components interact with few other components, although some parasite species were highly interactive, e.g. HIV, *Staphylococcus aureus* and Hepatitis C virus each interacted with dozens of other nodes. Second, of all possible interactions between pairs of parasites, most were indirect. While many studies highlight immune-modulation by parasites (Bradley and Jackson, 2008; Lijek and Weiser, 2012; Maizels et al., 2004), I found twice as many interactions via shared resources than through shared immune responses. Finally, interactions were clustered around particular locations of the human body, suggesting that the parasite community of humans may be divided into microhabitat modules.

These findings indicate that the human summary coinfection network is similar to many free-living ecological communities, confirming prior

suggestions that coinfection can be understood using ecological concepts (Bruce et al., 2000; Graham, 2008; Pedersen and Fenton, 2007). In particular, assortative and disassortative processes were found in the summary coinfection network, similar to directed ecological networks (Foster et al., 2010; Hao and Li, 2011). This suggests that, while well-connected parasite species tend to interact with one another, other well-connected resource and immune nodes tend to interact with poorly-connected components. This feature may limit how far perturbations are likely to spread across the network (Maslov and Sneppen, 2002). Furthermore, the exponential degree distribution that I observed in the summary within-human network matches that of many food webs (Camacho et al., 2002; Dunne et al., 2002). The summary networks modularity ($Q = 0.469$) was within the range seen for many food webs (range 0.15 to 0.6) (Guimerà et al., 2010), suggesting that well-connected nodes are somewhat isolated and, again, restricting the wider effects of perturbations and maintaining network robustness (Alcántara and Rey, 2012; Krause et al., 2003; Maslov and Sneppen, 2002). Overall, therefore, many structural aspects of the summary coinfection network suggest it is robust to perturbations, such that treatment or vaccination of a particular parasite may have little impact on the remaining network. This finding is consistent with observations from treatment programmes in human communities where parasite populations rapidly return to pretreatment levels, and secondary effects on other parasites are rarely reported (Basáñez et al., 2012). Perturbation studies of parasite communities in other host species, more extensive monitoring of human treatment programmes, and dynamic coinfection networks are needed to more fully determine stability of parasite communities.

Resource- and immune-mediated indirect interactions between parasites were more common than expected by chance in the summary network. Coinfecting parasites were most likely to interact indirectly through shared resources than via the immune system, and network modules tended to be associated with microhabitats rather than immune phenotypes. The dominance of indirect effects matches other ecological systems and could be another reason why controlling parasites in coinfected populations is difficult (Borrett et al., 2010). While much coinfection research has fo-

cussed on immune-mediated interactions, and interactions between para- sites are often apparently presumed to be immunological (e.g. du Plessis et al. (2012)), the role of resource-mediated interactions has received less attention (Tompkins et al., 2011). However, host resources control the within-host dynamics of various taxa: red blood cell density affects malaria intensity in lab mice and in humans (Antia et al., 2008; Graham, 2008), associations among microbiota (Faust et al., 2012), exclusion of another hepatitis virus or *Trypanosoma* strain from certain tissues (Amaku et al., 2013; Franco et al., 2003), and microhabitat associations in parasite com- munities of nonhuman hosts (Lello et al., 2004; Stock and Holmes, 1988). My results indicate that resources may be more widely involved in struc- turing parasite interactions in humans than currently appreciated.

Such bottom-up control of the summary network could be produced by either facilitation or competition among parasites. In the case of facilita- tion, infection by one parasite encourages coinfection of the same resource, as with polymicrobial wound infection (Dalton et al., 2011). Conversely, ecological guilds of parasites may compete for particular resources (Lello and Hussell, 2008; Pedersen and Fenton, 2007). We need further stud- ies of the relative contributions of competition, facilitation, and how best to manipulate these interactions to improve treatment of coinfected pa- tients. If coinfecting parasites do interact more via resources, then new treatments could be developed that disrupt parasite colonisation, feed- ing, or reproduction using resources shared by coinfecting parasites. For instance, if certain dietary constituents like cranberry juice prevent ad- herence of bacteria to epithelial cells (Raz et al., 2004), do they prevent bacterial coinfection to the same extent? Within the recent specialism of nutritional and environmental medicine, research into how nutrients affect infection, let alone coinfection, has so far been relatively scant (Downing, 2009).

The apparent lesser influence of top-down immune control in the net- work suggests that either a strong immune response involving a few key components may prevent coinfection, or that the immune system tends to have weak interactions with specific parasites. Studies that consider both resource- and immune-mediated interactions are rare (see Chouvenc

et al. (2012) for an example in termites), so the relative contribution of immune and resource control on coinfecting parasite populations needs further study.

As with any analysis of literature-derived data, the results may be influenced by observational and reporting biases (as detailed in Chapter 2). In this analysis I attempted to address these issues where possible (see Fig. 3.10-3.14). Beyond this evaluation of the sensitivity of the results to possible biases, there may also be reporting biases in the sampled publications, for example toward describing infections in terms of the parasite's resource (Loscalzo, 2011). In addition, the publications may be subject to detection biases, for instance where establishing immune mechanisms may be relatively more difficult in humans than, say, in vivo experiments. Further research could identify whether individual networks assembled from particular coinfected patients are also resource dominated, could test for physiological or genomic shifts and biomarkers of coinfection, and compare networks from different patients and points in the infection cycle to measure the health consequences of particular topologies and dynamics. Such focused efforts would allow interaction strength and direction to be measured to enable probabilistic module detection (Tsuda and Georgii, 2013; Yang et al., 2013), and to help predict patient-specific treatment effects.

Reported interactions were most often indirect, and this result was robust to node aggregation and sampling of publications. It is therefore important to understand how treating one parasite species indirectly affects the community of coinfecting parasites. Given the growing interest in integrated control strategies where multiple species are treated simultaenously (Hotez et al., 2007; Lammie et al., 2006), we need to test whether knowledge of parasite interactions could improve treatment outcomes in human populations where coinfection is prevalent. Whilst the complexity of the parasite community of humans makes this process somewhat daunting, the summary network and analyses presented herein make this problem more tractable. In particular, the modules resolve interactions into potentially more manageable clusters of taxa. Some studies suggest that modules are a general biological pattern with evolutionary underpinnings

(Clune et al., 2012). Whether coevolution of host and parasite communities has resulted in resource-related modules because of increased stability or biological constraints like human physiology remains to be seen. With better understanding of the ecological interactions structuring parasite communities, the effects of treatment on the wider parasite community and on patient health could perhaps be predicted.

Chapter 4

Coinfection mortality in England and Wales from 2005 to 2008

Abstract

The parasites that tend to coinfect and cause death are unknown for any host species. Causes of human deaths, including infectious causes, are registered in many countries, and offer a unique but hitherto unexploited opportunity to study coinfection mortality.

I analysed a cross-sectional dataset of reported deaths from infectious causes in England and Wales. I tested whether the proportion of deaths from infectious causes involving coinfection differed across age and sex cohorts, and whether the number of infectious causes was randomly distributed across death certificates. I used two-way contingency tests to find the odds of each pair of infectious causes causing death from coinfection, and then analysed whether the strength of association between each pair was related to the similarity of the pair of infectious causes across four biological characteristics.

The proportion of deaths from certain infectious causes that involved coinfection peaked in adults in their 30s, and was higher in males. Death certificates contained between one and six infectious causes, and there were more death certificates with one or two infectious causes than expected

from a Poisson distribution. The reporting of most pairs of infectious causes were not associated with co-occurrence on death certificates (54%), but 39% of pairs were positively associated. The strength of these associations was not related to the similarity of the pair of infectious causes in the four characteristics tested.

These results indicate that coinfection causes many deaths in England and Wales, especially among younger adults, and that there is a tendency for infectious causes to co-occur on death certificates with higher odds than expected.

Introduction

Infectious diseases are the main cause of death of one in four individuals worldwide; for example, respiratory infectious disease caused 4.26 million deaths in 2008, diarrhoea caused 2.16 million, and HIV/AIDS caused 2 million (World Health Organization, 2009). Although HIV-tuberculosis caused 350, 000 deaths worldwide in 2008 (World Health Organization, 2012), the number of deaths involving other coinfections is unknown on a global or even national level.

Some coinfections increase the risk of death in humans, such as tuberculosis and HIV (Ditiu et al., 2011), HCV and HIV (Branch et al., 2012), and bacterial pneumonia with influenza (Chertow and Memoli, 2013; Dushoff et al., 2006; Finelli et al., 2008; Morens et al., 2008; Palacios et al., 2009; Rothberg et al., 2008; von Baum et al., 2011; World Health Organization, 2010). In contrast, some coinfections may decrease mortality rates, such as GBvC coinfection slowing HIV disease progression (Bagasra et al., 2012). Apart from these few examples, relatively little is know about which coinfections are associated with increased or decreased mortality risk, or indeed if there is a general pattern of coinfection in causes of death data.

One way to study the general associations between coinfection and death (or lack thereof) is to analyse a composite dataset of causes of death that were recorded on death certificates. Infectious diseases were the underlying cause of 1.2% of deaths in England and Wales between 2001 and

2011 (Office for National Statistics, 2011). While this level of infectious disease mortality may be relatively low compared with global levels, these data provide a national dataset of the distribution of infections recorded at death. These data enable tests of hypotheses about coinfection that are relevant to public health in England and Wales, and to understanding of coinfection in humans in general.

While coinfection could be an important determinant of human mortality, characteristics of coinfected individuals such as age and sex can also be important. These can be important risk factors for helminth coinfection status (Buck et al., 1978b), though not among the children from Zanzibar studied by Lello et al. (2013) where infection by a species of gastrointestinal helminth was the only consistent risk factor for coinfection. The role of age and sex in deaths from coinfecting helminths or other parasite types has, to my knowledge, never been studied. Older individuals are likely to be more susceptible to infectious disease (Weinberger et al., 2008), and less responsive to vaccines (Goodwin et al., 2006) as their immune system deteriorates. Health differences between the sexes are commonplace and are most likely attributable to biological and social factors (Arber and Ginn, 1993; Macintyre et al., 1996; Rieker and Bird, 2005; Verbrugge, 1989). These may also contribute to sex differences in coinfection deaths, but whether there is a consistent difference between males and females is unclear. For instance, hospitalised females have higher pneumonia mortality rates than males (Crabtree et al., 1999), and most fatal cases of measles are from viral or bacterial coinfection, especially in young females (Beckford et al., 1985; Dabbagh et al., 2009; Garenne, 1994; Griffin et al., 2012). In contrast, sepsis mortality rates are higher in males than females (Melamed and Sorvillo, 2009; Wehren et al., 2003).

To better understand how coinfections affect human mortality, it is also important to measure whether parasite species tend to co-occur, and what types of infectious disease are associated with this. The chance of a particular pair of infectious causes co-occurring could be higher if they are similar. For instance, it has been suggested that taxonomically similar parasite species may coinfect a host more often than expect by chance, due to similarities in life cycles, resource use, or immune responses (Pedersen

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and Fenton, 2007). The immune response to two coinfecting species of different taxonomic groups may stimulate divergent and weaker immune responses that limit damage to the host that would otherwise occur in single infected individuals (Graham, 2001). For example, anaemia was less severe in mice with malaria and helminth coinfection than in mice with just malaria (Fairlie-Clarke, 2011). Shared transmission routes may also increase the risk of coinfection, disease, and possibly death, e.g. blood-borne viral infections often coinfect injecting drug users (Chu and Lee, 2008; Singer, 2009). Similarly, having two infectious diseases that share a resource (i.e. have the same tropism) may exacerbate the health burden. For instance, hepatitis viruses A and C compete strongly in the livers of coinfected patients and this enhances pathology (Amaku et al., 2013). Coinfection by infections with the same timescale may also co-occur often. For instance, two chronic infections may have more of a chance for a serious interaction or complication to arise, and acute coinfection may enhance the risk of death because of severe stress exerted by two short-lived, virulent infections. Here, I test whether these characteristics are significant risk factors in reported coinfection death.

Other factors besides the age and sex of the individual, and the characteristics of infections reported on death certificates may well affect mortality rates. While further data were unavailable (see Methods), I consider other potential influences in the Discussion.

Infections often reported as causes of death may also be common but often mild infections, as opposed to rare but often deadly infections. It is therefore important to also test for a relationship between the number of individuals infected in the wider population, and the number of reported deaths caused by that infection.

Hypotheses

Using death certificate data on infectious causes of death from a recent four-year time period from England and Wales, and separate data on the numbers of reported infections in the same population and time period, I tested five hypotheses:

1. The number of deaths with a single infectious cause are positively correlated with the number of those infections reported in the population.

2. The proportion of deaths attributed to multiple infectious causes (as opposed to a single infectious cause) is related to age and sex.

3. The distribution of the number of different infections reported on death certificates is not random, indicating that infectious causes of death are not independent.

4. The occurrence on death certificates of a particular pair of infections is different from that expected given the occurrence of each infectious cause in isolation.

5. Associations between pairs of infections are positively related to similarity (or lack thereof) in their biological characteristics (i.e., taxonomic group, transmission route, tropism, or timescale).

Methods

Dataset

Death certificates in England and Wales report one underlying cause of death and up to 15 contributory factors; these are coded by the International Classification of Diseases (ICD, World Health Organization (1992)). The $139,459$ death certificates used here were registered from 2005 to 2008, contained at least one infectious disease and followed the coding system for causes of death in ICD-10 version 2005 (World Health Organization, 2005). Data were obtained via the Office for National Statistics.

In ICD-10 infectious diseases are coded in categories A and B, and numbers within these can indicate a parasite's genus (e.g. B68 *Taenia*) or species (e.g. B51 *Plasmodium falciparum*). Coinfection is indicated by multiple infectious causes reported on the death certificate, i.e. the doctor signing the death certificate attributed the death to more than one infectious cause. In addition, one specific code indicated coinfection

(B20, which denotes other infections arising from HIV infection). Any other code in isolation was assumed to be a single infection. This is a conservative assumption since other infections may have contributed to death but were not identified and so were not reported. The Discussion considers some possible implications of this assumption. Hereafter, the term "single infection death" indicates death certificates with one infectious cause reported in the underlying and contributing causes of death, and "coinfection death" indicates death certificates with more than one infectious cause reported.

Other data from the death certificates used here were sex and age at death (age categorised as $0-20$ then in decadal intervals up to $80+$). This type of categorisation of age is common in patient studies, and reflects the different care services accessed by different age cohorts (Freeman Jr, 1987), and ageing-associated diseases. All other information were removed by the Office for National Statistics to comply with data protection regulations.

In addition to the death certificate data, independent data on the number of notifiable infections in England and Wales from 2005 to 2008 (notifiable infections are those of interest to the government and reported by doctors) were obtained from the Health Protection Agency. This second dataset comprises the best data with national coverage on number of cases of certain infections, but only include a restricted number of infections: 10 notifiable infections did not appear on any death certificate, and 83 infectious causes of death were not notifiable infections. There were 96 infectious causes of death on death certificates, thus there were 13 infectious causes of death that were also notifiable infections.

Statistical analysis

All analyses were done in R version 2.15.1 (R Development Core Team, 2012).

Hypothesis 1: prevalence and mortality rate for certain infectious diseases

Spearman's rank correlation was used to test for a positive correlation between the number of reported cases of each notifiable infection and the number of single infection deaths. This analysis was done separately for males and females, because both datasets included information on sex and this is relevant for hypothesis 2.

Hypothesis 2: age, sex, and coinfection death

The relationship between age, sex, and coinfection death was modelled by logistic regression (generalised linear model (glm) with binomial error structure and a logit link function). The response variable in these models was the number of "successes" (coinfection death) and "failures" (single infection death). Age was fit as an eight level factor (described above) and sex was a two level factor. I tested for an interaction between age and sex in the glm. To aid interpretation, a binomial generalised additive model (gam) with a spline for age was also used. Splines with age have shown a good fit in other coinfection studies, e.g. Fenton et al. (2010). Analysis of deviance (χ^2) tests showed whether the interaction between age and sex should be dropped from the glm, or whether the age spline in the gam should be modified by sex or dropped from the model.

Hypothesis 3: distribution of infectious causes of death

The observed distribution of the number of infectious causes reported per death certificate was compared to a null expectation of independence following a Poisson distribution. I fit a Poisson distribution to the counts of each number of infectious causes per death certificate using the pois.exact

function in the R package epitools (Aragon, 2010). If the observed frequencies of number of infections per death certificate was beyond the values expected from this distribution (greater or lesser than 95% CI) then the chance of a death certificate having a given number of reported infections was non-randomly distributed.

Hypothesis 4: associations between pairs of infections

To test for associations between pairs of infectious causes of death, a two-way contingency test was performed for each pair of infections. A Fisher's exact contingency test was used because for many pairs of infections there were low numbers of occurrences (< 5). A negative association using this analysis (odds ratio< 1) indicates lower occurrence of coinfection than expected by chance, a positive association (odds ratio> 1) indicates a higher occurrence of coinfection than expected by chance. Here the critical value for determining a significant association was corrected for multiple comparisons using a Bonferroni correction of $\alpha = \frac{0.05}{k}$, where k is the number of pairs of infections, and hence the number of comparisons, that were tested.

Each pair of coinfections was categorised as having a positive association (significant OR> 1), negative association (significant OR< 1), or no association (OR not significantly different from 1). The observed proportions of these categories were compared to two null models using Chi-squared tests. The first null model had equal proportions of positive, no, and negative associations. The second null model was that coinfections do not interact, allowing for a 5% error rate (hence 2.5% negative, 2.5% positive, and 95% neutral reported effects). These tests use the same methods as those used and described in greater detail in Chapter 2.

Hypothesis 5: are similar pairs of infectious causes associated with coinfection death?

The above analyses for hypothesis 4 provided an estimate of the association between pairs of infections (i.e., the odds ratio). Linear models with $\log_{10}$ odds ratio as a response variable were used to test whether the asso-

ciation of a pair of infectious causes of death was related to the similarity of the biology of the pair.

Biological characteristics used were taxonomic group, transmission route, tropism, or timescale. Each infectious cause was assigned a category for each characteristic using a PubMedHealth (NCBI, 2012) search for its name in ICD-10 (World Health Organization, 2005), and two existing sources: a database of known human parasites (Taylor et al., 2001), and a database of characteristics of RNA viruses of humans (Brierly (in prep)). Taxonomic groups of infectious causes were: viruses, bacteria, fungal parasites, protozoa, helminths, or other (where the ICD code could refer to more than one of those five types, e.g. A09 diarrhoea or gastroenteritis of presumed infectious origin). Categories used for transmission routes were: contaminated food/water, inhalation, insect bites, via open wounds, contact with animals, skin contact, sexual contact, or environmental pathogens (following Taylor et al. (2001)). Tropisms were grouped into: neuronal, respiratory, circulatory, gastrointestinal, genital, skin, glandular, or multiorgan (following Brierly (in prep)). Lastly, timescales of infection were acute, chronic, or both (for diseases with both short- and long-term infections like *Chlamydia*, Q fever, or *Nocardia*).

Next the similarities between every pair of infections reported together on the death certificates was described in terms of four binary variables, one for each of the characteristics described above. In these four binary variables, a 1 indicates a match between the two infections, and a 0 no match. The lowest similarity was, therefore, four zeros, and highest four ones.

Parametric multiple regression was used with the four binary variables as explanatory variables, and the log odds ratio as the response variable. Starting with the full model (including the four way interaction), terms were removed in a stepwise manner using analysis of deviance (χ^2) tests. The final model showed large deviations from the assumptions of linear regression, even with other transformations of the response variable, removal of outliers, or removal of pairs of infections where no association was observed (OR= 1). For this reason, localised non-parametric regression was used, with one explanatory variable that was a single index of sim-

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ilarity combining all four binary explanatory variables (i.e. one variable ranging from 0 to 4).

Results

From the $2,028,734$ death certificates in England and Wales between 2005 and 2008, $130,758$ (6.4% out of $2,028,734$, $72,080$ female, $58,678$ male) death certificates attribute death to single infection, while $8,695$ (0.4%, $4,623$ female, $4,072$ male) listed multiple infections as causes of death. All other death certificates contained only non-infectious causes of death and were excluded from the analysis. In total there were 96 different infectious causes listed on the death certificates.

Hypothesis 1: prevalence and mortality rate for certain infectious diseases

There was no significant relationship between the number of death certificates with a particular infectious cause reported and the number of notified cases of that infectious disease for either sex (paired Spearman's Rank correlation for males $\rho = -0.14, P > 0.64, df = 12$ and for females $\rho = -0.02, P > 0.93, df = 12$, Fig. 4.1).

Hypothesis 2: age, sex, and coinfection death

The proportion of deaths that were coinfection deaths rose from < 20 years old to a peak in the $30 - 39$ age category, subsiding to lowest levels in the over 60s (Fig. 4.2, binomial gam with age spline by sex, deviance explained $= 99.2\%$). Sex modified the effect of age so that the rate of decline in the proportion of coinfection deaths from the peak in ages $30 - 39$ was greater in females than males (grey vs. black solid lines > 2 standard errors apart for ages $40 - 49$ and $50 - 59$ on Fig. 4.2). There was a significant increase in residual deviance when sex or age were removed from gam (deviance -46 and -978 respectively, both $P < 0.001$ using χ^2-test).
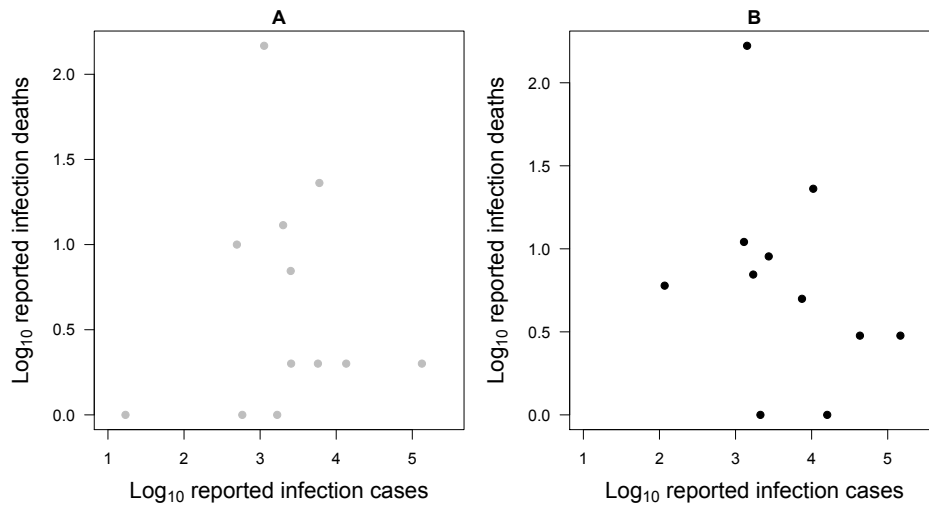
Fig. 4.1: Number of reported deaths from the 13 infectious causes of death for which there was data on the number of reported cases in England and Wales for (A) females, and (B) males.

Hypothesis 3: distribution of infectious causes of death

Out of $8,695$ reported coinfection deaths, $7,980$ (91.8%) had two infections, 609 (7.0%) had three, and 106 $(1.2\%])$ had four or more (Fig. 4.3). This observed distribution is broadly consistent with expectations based on independent and random distribution of infectious causes across the death certificates (bars for 3 to 6 reported infectious causes overlap with 95% CI from Poisson density function where λ was the observed mean number of reported infections per death certificate (1.07), Fig. 4.3). However, there were slightly more single and dual infection deaths than expected (bars for 1 to 2 reported infections higher than 95% CI, Fig. 4.3).

Hypothesis 4: associations between pairs of infections

The majority of pairs of infectious causes showed no association on death certificates $(197/366, 53.7\%,$ odds ratio (OR) not significantly different from zero using Bonferroni correction where $\alpha = 1.37 \times 10^{-4}$, neutral bar on Fig. 4.4). Next most common were positive associations $(144/366, 39.3\%,$ Fig. 4.4, OR significantly > 1). Only 25 infectious pairs were negatively
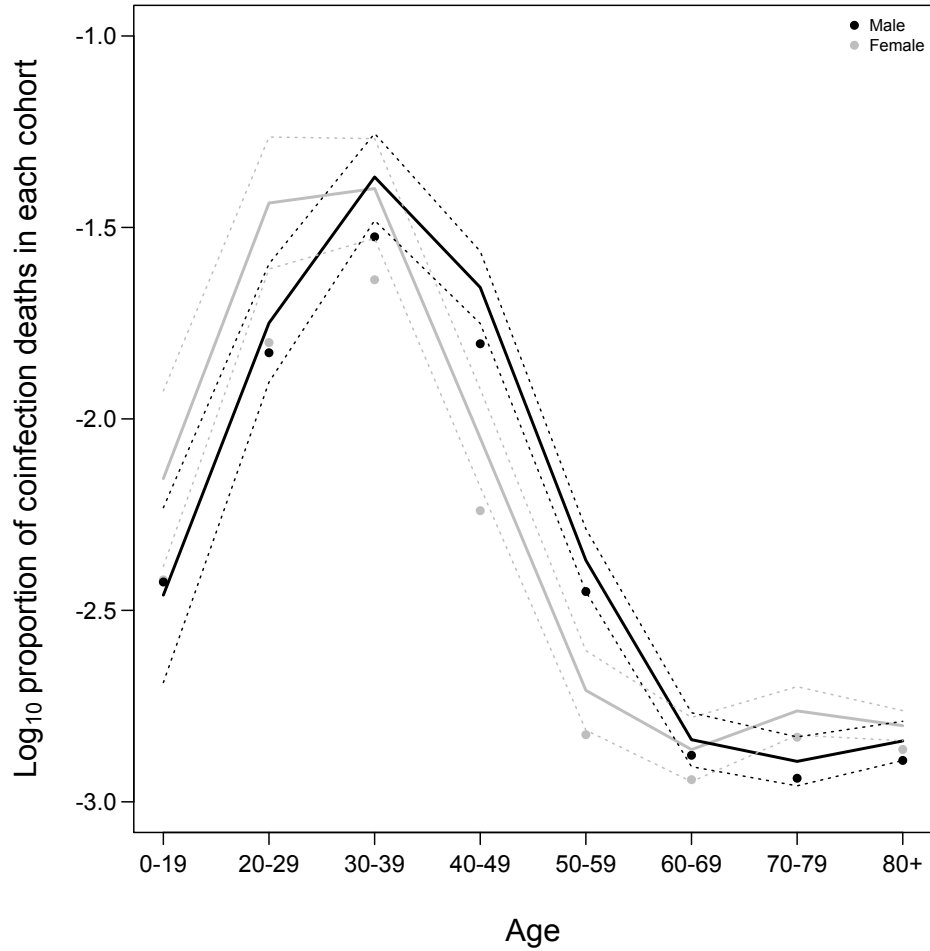
Fig. 4.2: Proportions of death certificates in the dataset that were coinfection deaths in decadal age categories and sex (female=grey, male=black). Points are the observed proportions, solid lines are the model predictions from a binomial gam P(multiple infection)=s(age):Sex, dotted lines are ±2s.e. around the model prediction.
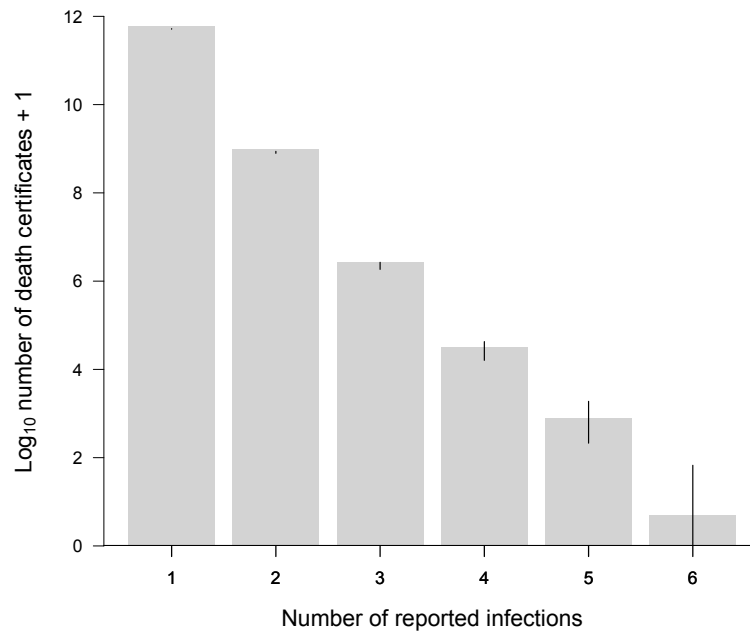
Fig. 4.3: Frequency distribution of reported infections on death certificates between 2005 and 2008 from England and Wales. Black lines are 95% confidence intervals from a Poisson distribution with $\lambda = 1.068371$.

associated (6.8%, Fig. 4.4, OR significantly < 1)

The distribution of these associations was significantly different from both the random and no-effect null hypotheses ($\chi^2 = 127, df = 2, P < 2.2 \times 10^{-16}$ and $\chi^2 = 2080, df = 2, P < 2.2 \times 10^{-16}$ respectively, bars significantly different heights from grey and black lines on Fig. 4.4).

Hypothesis 5: are similar pairs of infectious causes associated with coinfection death?

Among the 311 pairs of infectious causes, there was no relationship between the similarity in biology of the pair (in terms of taxonomy, transmission mode, tropism, and timescale of infection) and the odds of both infectious causes being reported on the same death certificate (horizontal regression line, Fig. 4.5). Other modelling approaches were attempted but flouted statistical assumptions (see Methods).
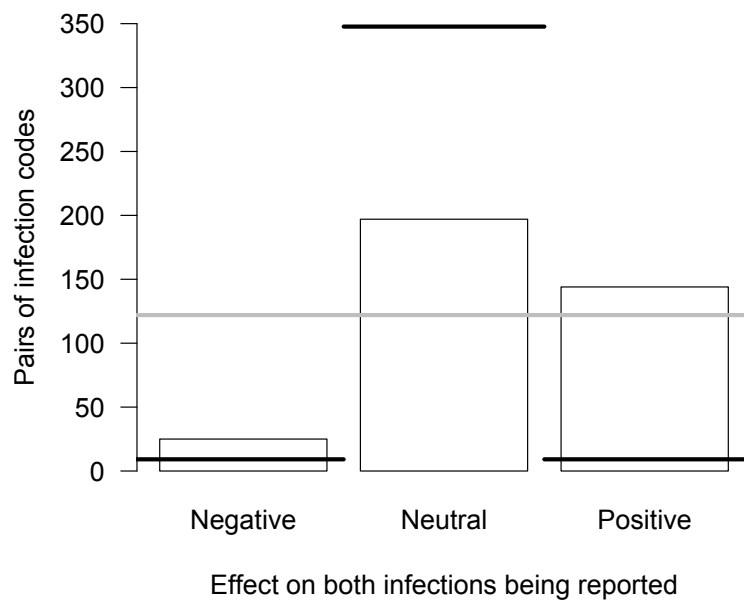
Fig. 4.4: Associations between every possible pair of infectious causes categorised into positive, negative, or neutral. Associations were derived from two-way contingency tests on the number of deaths reported with neither infection, each single infection, or both infections. Neutral indicates odds ratio not significantly different from one. Lines indicate null hypotheses (grey=random, black=no-effect).
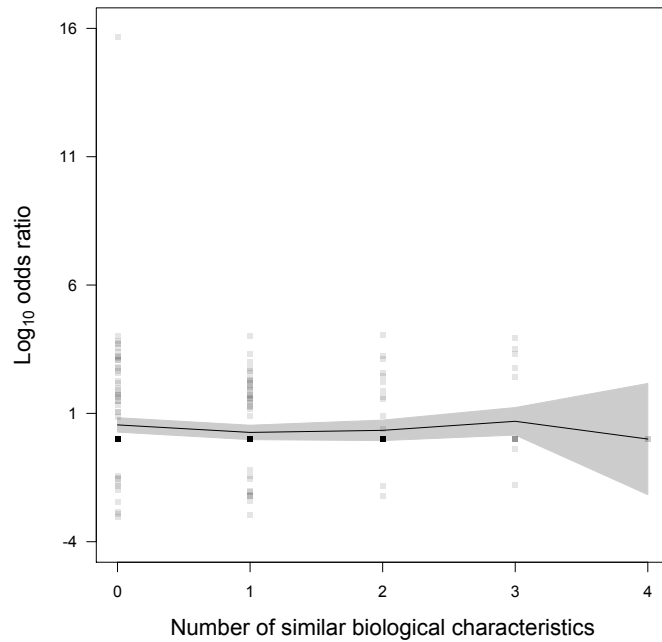
Fig. 4.5: Similarity in biology of pairs of infectious causes and the $\log_{10}$ odd ratio of their association on death certificates (from two-way contingency tests). The x-axis is the sum of four factors indicating whether the infectious causes shared the same tropism, transmission route, timescale of infection, or taxonomic group. For each factor, 1 denoted a shared characteristic (e.g. two chronic infectious causes would share the same timescale of infection), and 0 denoted different characteristics (e.g. a bacterial and a viral coinfection would have different taxonomic groups), so a pair of infectious causes with 4 on the x-axis had all four characteristics in common, while 0 means the infectious causes were different in all four characteristics. Points are transparent such that ten or more overlaid points are black. Black line is the locally smoothed non-parametric regression line with degree of smoothing $\alpha = 0.5$ Grey polygon is ± 2se.

Discussion

A four-year long dataset of human mortality in England and Wales, comprising almost $140,000$ deaths with reported infectious causes, yielded the first ever national cross-sectional analysis of coinfection as a cause of death. The infectious causes reported on these death certificates allowed estimation of whether pairs of infectious causes are more or less likely to be reported together. Adults between the ages of 20 and 40 had the highest probability of coinfections being reported on their death certificates. This probability of reported coinfection decreased into older age groups, but until age 60 was slightly higher in males than females. Positive associations of pairs of infectious causes co-occurring on death certificates were found for almost 40% of pairs. The strength of association between these pairs of infectious causes was not related to the number of biological characteristics shared by the pair. I now discuss the factors that may contribute to these patterns, before considering the limitations of the data.

Possible causes of observed patterns

There was a hump-shaped relationship between age and the proportion of infectious disease deaths that had multiple infectious causes reported. This early-to-mid adulthood peak in coinfection death contrasts with theories that the immune system's ability to respond to antigens declines in old age (Goodwin et al., 2006; Weinberger et al., 2008), and with noninfectious diseases where the risk of multiple simultaneous diseases (comorbidities) increases with age (Valderas et al., 2011). One possible explanation is that older adults are more frail from the inevitable decay of their complex bodily system (Topolski, 2009) and so have a higher risk of death from just a single infection, whereas younger adults are generally stronger so it takes multiple infectious diseases to kill them. Alternatively, those who survive to old age may have immune phenotypes that enable them to effectively respond to coinfections, but they eventually die from a single virulent infection, perhaps in conjunction with other diseases that are more common with age (Campbell et al., 1985), like cancer (Ferlay et al., 2007). There is evidence that young adults are more prone to severe immunopathologies

following infection. For example, critically ill hospitalised patients in the USA and Canada with influenza A(H1N1) tended to be $20 - 30$ years old (Jhung et al., 2011; Kumar et al., 2009) and had deficient adaptive immune responses (Bermejo-Martin et al., 2010). In coinfected individuals parasite abundance may also peak at intermediate age in rabbits and sheep (Fenton et al., 2010), which may translate to a higher risk of coinfection mortality in early adulthood in humans.

The number of death certificates with one or two reported infectious causes of death was higher than expected from random, but the number of deaths with three or more infectious causes was within expectations. This could be because infectious diseases with high virulence tend to competitively exclude other infections (as seen with higher virulence malaria strains outcompeting others Bell et al. (2006); De Roode et al. (2005)). In contrast, deaths from three or more infectious causes may be more of a random-draw process involving lower virulence infections. Reductions in the number of deaths reported from more than two infectious causes could also be explained by competition for resources suppressing parasite abundance (Dobson, 1985), coinfections of diverse taxa triggering immune responses that are less damaging to the host (Graham, 2001), or patients with many coinfections receiving closer medical attention.

While more than half of pairs of infectious causes of death were not associated with being reported together on the same death certificate, positive associations were four times more common than negative associations. These results are consistent with the skew toward positive interactions between parasites found in published reports of coinfection morbidity in humans (Fig. 2.2C). This skew could suggest that interspecific competition discussed above tends not to be strong enough to prevent coinfection of individuals before death, and that facilitation between pairs of infectious causes is more common. Such corroboration of findings suggests that medical care of coinfected patients is more problematic than those with single infections, being associated with higher parasite abundance, worse health outcomes, and often higher risk of multiple infectious causes of death.

More than four fifths of pairs of infectious causes were positively asso-

ciated, many with odds of reported coinfection death over three orders of magnitude greater than reported single infection death. Pairs with these higher odds ratios included: mycobacteria and gastrointestinal protozoa (A31 and A07, OR= 1795), mycobacteria and measles (A31 and B05, OR= 1570), streptococcal sepsis and malaria (A40 and B54, OR= 1212). Some of these same taxa also had significantly lower odds ratios when paired with other infectious causes, including respiratory tuberculosis and streptococcal sepsis (A40 and A16, OR= 0.244), and acute encephalitis and septicaemia (A41 and A86, OR=0.006). The direction of association for each taxon is therefore not consistent, but the strength of these associations means finding whether there are general causes behind these associations is important.

The odds of pairs of infectious causes being reported together was not associated with whether or not the pair shared the same transmission route, taxonomic group, timescale of infection, or tropism. This does not rule out the role of biological characteristics of parasites in modifying coinfection mortality risk. Further analyses could consider the particular categories within each factor rather than a binary variable of whether or not the pair shared the same category. Other biological characteristics could also be studied with continuous variables, for instance measuring generation time of the infecting organisms, time to transmission, average time infecting one host, etc. Further analysis could also compare the characteristics of the 20 out of 96 infectious causes that were never reported in coinfection deaths against the infectious causes that only occurred in coinfection deaths (e.g. $B20 - 23$ denoted HIV infections).

Quality of data available for studying coinfection mortality

As with any results based on reported disease, it is important to consider the influence of potential biases in reporting. There may be underreporting of coinfection death on death certificates if other infectious diseases were undetected, deemed not to have contributed to death, or reported using an ambiguous code. For example, some codes may conceal multiple species infecting the same individual (e.g. *Streptococcus* and *Staphylococcus* both in B95, *Klebsiella*, *Clostridium*, and others in B96). Using

multiple infectious causes (and code B20 as HIV coinfection) as indicators of coinfection therefore probably underestimates the number of coinfection deaths. Nevertheless death certificates offer novel insight into coinfection mortality, and there is no evidence that reporting of infectious causes of death is biased by age, sex, or infectious cause. Underreporting of multiple causes of death and wrong attribution of the cause of death was highlighted as a problem in the 1990s (James and Bull, 1996; Leadbeatter and Knight, 1993; Maudsley and Williams, 1996), and there have since been legal and education reforms for doctors completing the certificates (Aung et al., 2010; Swift and West, 2002; Tuffin et al., 2008). Inaccuracies on death certificates can be subject to legal challenge and lead to medical malpractice claims (Waldman and Spector, 2003). The Office for National Statistics also issues official guidance to doctors completing death certificates (Devis and Rooney, 1999; Tuffin et al., 2008). For all these reasons we can be confident that, in large causes of death datasets from recent years like the one used here, relationships among the number of reported causes of death, biological characteristics of infections, and host characteristics (age and sex) are detectable. Even if the extent of underreporting of infectious causes were known there is no reason to expect that this would detract from the patterns presented here.

There was no association between reporting of a subset of infectious causes on death certificates and the number of cases of those infectious diseases reported in England and Wales. This suggests that infections differ in their background risks of causing death. It is difficult to assess whether the number of reported coinfection deaths was proportional to the number of coinfection cases without data on coinfection prevalence in the wider population. To test for variation in the risk of coinfection death (coinfection mortality rate weighted by coinfection prevalence) between coinfections, we would need a coinfection dataset from a different cutoff in the infection cycle, such as recovery rates after hospitalisation, hospital diagnosis rates, symptomatic coinfection in doctors clinics, or the prevalence of asymptomatic coinfection surveyed in the population. Unfortunately none such data is currently available.

At a time when there are calls for greater reporting of causes of death

globally (Editorial, 2013), it should be noted that multiple causes of death must be reported in order to study interactions between different diseases (Chamblee and Evans, 1982; Goodman et al., 1982; Redelings et al., 2005, 2007). Adding to these calls for data on multiple causes of death to be more widely collected, this study has shown the utility of these data for studying coinfection mortality by inferring associations among infectious causes of death. Further such analyses are needed for accurate assessment of infectious disease mortality burden in countries with different parasite communities, human demographics, and health systems. Other opportunities for further research include estimating the sensitivity of results to different disease coding systems (Wilson and Bhopal, 1998) and death certificates from other years. Longitudinal studies can often reveal effect of ageing on disease better than cross-sectional data with chronological age categories (Hofer and Sliwinski, 2001). Tracking individuals with single infection or coinfection could also help control for parasite abundance, which is one of the key determinants of the type of immune response (Menon and Bretscher, 1998).

Causes of death can be associated with various factors including healthcare, socioeconomic status, family structure, geography, behaviour, host physiology, infectious dose, or virulence. Determining what factors control patterns of causes of death using national observational data alone is therefore difficult (Hansen et al., 2007). It is especially difficult as other health conditions, diagnostic tests, and treatment were not included in the dataset used here. The above discussion of causes of the observed patterns in single infection and coinfection deaths is therefore speculative, but raises many hypotheses to be tested in future. This study is the first of its kind to study coinfection mortality across the range of parasite types (from viruses to helminths). Several patterns have never been described before in humans or indeed any other host species, namely the distribution of coinfection deaths across age and sex cohorts, the distribution of number of infectious causes reported at death, and the odds of reported co-occurrence of hundreds of pairs of infectious causes. Understanding how coinfection modifies mortality risk will help target health resources to prevent deaths from multiple infectious causes.

Chapter 5

Indirect effects of treatment on non-target parasites under different one-way interactions

Abstract

There is potential for interactions among coinfecting parasites to undermine treatment efforts because treating one parasite species could indirectly exacerbate untreated parasites. To study how interactions among parasites modify treatment outcomes, an agent-based model of mass drug administration in a village-sized human population with two endemic macroparasites was developed.

The abundance of both parasite species was monitored in simulations with and without targeted drug treatment of one species, with three levels of coinfection. Simulations included one-way immune- or resource-mediated interactions of varying strengths and directions in coinfected hosts where target parasites affected the non-target parasite population. In each scenario the effects of either single or repeat treatments on non-target parasite abundance, and host morbidity (target plus non-target species abundance) were recorded. Simulations were repeated for each scenario using randomly generated initial conditions to test for variability in non-target parasite abundance. A sensitivity analysis of total treatment effects using half and double the original value of each parameter was also

done.

As may be expected, treating positively interacting parasites tended to also reduce non-target parasite abundance, whereas treating negatively interacting parasites generally increased non-target parasite abundance. However, the magnitude of this effect was sometimes negligibly or non-monotonically related to interaction strength, with the shape of this relationship depending on whether the interaction was resource- or immune-mediated. Indirect effects of treatment were amplified by higher coinfection prevalence and higher treatment frequency.

These results indicate that the direction of interspecific parasite interactions may be a good indicator of whether species-specific treatment programmes will have greater or lesser benefits for coinfected individuals. However, other within-host processes, such as resource- or immune-mediated intraspecific density dependence, can mask the effects of interaction direction such that the size of the indirect treatment effect may be less predictable. The model is theoretical, in that it represents a simplified and generalised one-host two-parasite system. Nevertheless, this model provides valuable insight into the potential indirect effects of targeted drug treatment under coinfection, and highlights areas where such future work may be directed, including quantifying the most influential parameters like rates of immune killing, and refining models of the relationships among parasites within coinfected humans.

Introduction

Parasite infections have been traditionally studied in isolation (Anderson and May, 1985; Cox, 2001), but coinfection by multiple parasite species is commonplace in humans (Buck et al., 1978a; Crompton, 1999; Petney and Andrews, 1998), and animals (Budischak et al., 2012; Ezenwa et al., 2010; Lello et al., 2004). Coinfected patients tend to have higher parasite abundance and worse health than those with one infection (Chapter 2). Thus we need to better understand the factors affecting the abundance of coinfecting parasites to help improve treatment of coinfected patients (Bruce et al., 2000; dos Santos et al., 2010; Hotez et al., 2010; Lello et al.,

2004; Lustigman et al., 2012).

Most treatment programmes are parasite-specific and therefore implicitly assume that parasite species can be managed independently (Cox, 2001; Gibson et al., 2010; Singer, 2009; Wenzel and Edmond, 2010). Here, I focus on drug treatments, but similar principles apply to other species-specific interventions like vaccination or prophylaxis. Successful parasite-specific drug treatments improve host health by suppressing target parasite abundance by using drugs to reduce or clear the target parasites. Drugs often vary in their efficacy against parasite species in the same or different phyla. For example, praziquantel is effective against schistosomiasis, but not directly on hookworm (Utzinger et al., 2002), and combined treatment of ivermectin and albendazole in cattle is effective against gastrointestinal nematodes except the resistant *Cooperia* genus (Entrocasso et al., 2008). Since many infections overlap in distribution (Brooker and Clements, 2009; Brooker and Utzinger, 2007; Brooker et al., 2000b, 2007, 2010; Molyneux et al., 2009; Soares Magalhães et al., 2011), there is high potential for drugs to indirectly affect other, non-target parasite species (Basáñez et al., 2012; Pasman, 2012). In such cases, the effect of drug treatment on non-target parasite abundance depends on how the target and non-target species interact within coinfected hosts. The health benefits of drug treatment will be reduced, or even overturned, if species not directly affected by the drug (non-target parasites) increase in abundance after treatment.

Interactions between parasite species can affect treatment outcomes, such as deworming treatments worsening HIV and tuberculosis epidemics (Bentwich et al., 1999, 2008; Elias et al., 2006; Nacher, 2002; Rafi et al., 2012; Sangaré et al., 2011; Wolday et al., 2002). There are new, integrated parasite treatment programmes for humans (Crompton, 2006; Fenwick, 2006; Hotez et al., 2008), but the effects of combining these drugs are only beginning to be explored (Gryseels, 2006; Lammie et al., 2006; van Genderen and van Hellemond, 2012), and the within-host interactions of most parasites are poorly understood (Alizon et al., 2013). Although rarely tested for, the occurrence, direction and magnitude of such interspecific parasite interactions could profoundly alter the net effects of targeted drug

treatment programmes.

Direction, magnitude, and mechanisms of parasite interaction

Within-host interactions between parasite species can be negative (antagonistic) from processes like resource competition, or positive (synergistic) from mutualisms or immunosuppression. Treating a positively interacting parasite will further reduce host morbidity because the non-target parasite population will also decline in coinfected hosts. Conversely, treating a negatively interacting parasite might bring reduced benefits to host health because the non-target parasite population will grow.

Parasites can interact directly with one another, for example by releasing an exoproduct that repels another species, or by consuming or infecting the other parasite. However, there appears to be greater capacity for indirect interactions mediated by resources available to the parasites, or the coinfected host's immune system (Chapter 3). Immune-mediated interactions include cross-reactivity, where one host antibody type or lymphocyte receptor binds to multiple parasite antigens, resulting in a negative interaction between parasites (Tanaka and Feldman, 1999), and trade-offs, where an immune reaction to one parasite reduces the reaction to a coinfecting parasite (a positive interaction, such as immunosuppression; see, for example, Austin et al. (1996)). Immune-mediated interactions are one cause of ineffective vaccines (De Bruyn, 2010), and has been observed in vaccines for *Vibrio cholerae* (Cooper et al., 2000, 2001; Harris et al., 2009), tetanus (Cooper et al., 1998, 1999), *Mycobacterium tuberculosis* (Elias et al., 2001; Wammes et al., 2010), and HBV (van den Berg et al., 2009), crossreactivity in influenza-HCV coinfection (Kasprowicz et al., 2008), and for gastrointestinal helminth-malaria coinfection in lab mice (Fairlie-Clarke et al., 2009, 2010).

Resource-mediated interactions involve parasites altering the space, energy, and nutrients available to coinfecting parasites. Where data on resource availability are lacking, resource-mediated interactions can be modeled by density dependent feedback loops. Negative density dependence means every additional parasite reduces the availability of resources (Anderson and May, 1978; Dietz, 1988), which can affect the stability of

the parasite population after chemotherapy (Churcher et al., 2006; Duerr et al., 2005; Nasell, 1976). Positive density dependence represents facilitation where population growth rates increase the more parasites there are (Basáñez et al., 1995).

Indirect effects of treatment on a non-target parasite population are likely to depend on the direction, strength, and mechanism of the interspecific parasite interaction (positive or negative, from weak to strong, and immune- or resource-mediated respectively). Furthermore, interactions within individual coinfected hosts can affect treatment outcomes at the population level, depending on how interactions affect parasite fecundity, how parasites transmit between hosts, and the proportion of hosts that are coinfected (coinfection prevalence). The indirect effects of treatment on coinfecting parasites will likely increase with coinfection prevalence because coinfected hosts, within which the parasites interact, are at higher density. Coinfection prevalence is enhanced by common risk factors including shared transmission routes, poor host hygiene or environment, and genetic susceptibility. Conversely, different risk factors for target and non-target parasites, such as environmental preferences, will lower coinfection prevalence.

Focus on coinfecting helminths

To explore the effect of treatment on non-target parasite abundance and host health (total parasite abundance) in different interaction scenarios, I focus on perhaps the most common coinfection globally. One billion individuals worldwide are estimated to be coinfected with multiple helminths (Crompton, 1999). Finding the most successful treatment options for helminths is important because they afflict the world's poorest (Bonds et al., 2010; King, 2010), and have a larger health burden than malaria or tuberculosis (Hotez et al., 2008). World Health Organisation guidelines recommend drug treatment for helminth infections (there are no vaccines, McSorley and Maizels (2012)). Treatment should be repeated at least annually depending on political will, budgetary constraints, attitudes, experience, and parasite prevalence in the population (Crompton, 2006).

Differences in treatment frequency could affect non-target parasite dy-
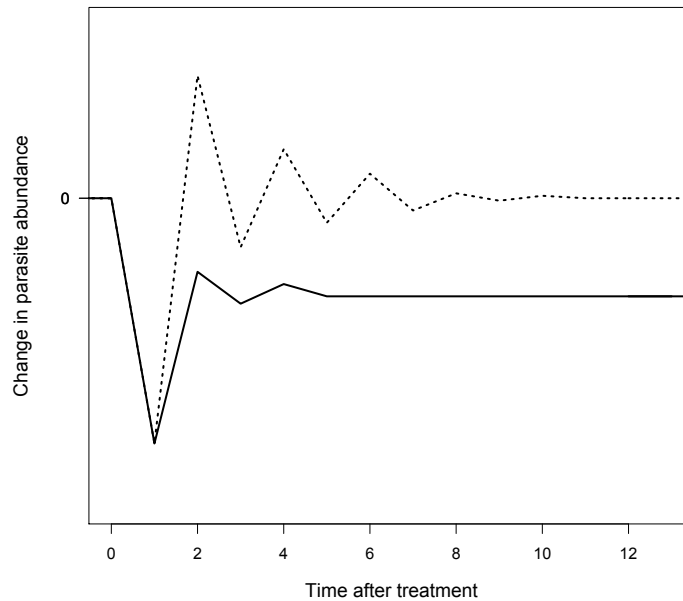
Fig. 5.1: Treatment at $t = 1$ perturbs the host-parasite system. Repeat treatment will move parasite abundance to a lower equilibrium (solid line), whereas after a pulse treatment parasite abundance will return to its former equilibrium (dotted line).

namics; a large or long-term indirect effect on the non-target parasite might only occur if the target parasite is treated repeatedly. One-off treatments can cause short-term, transient dynamics where the system reacts to treatment with an initial deviation that dampens over time (damping oscillation), before returning to its original equilibrium and closely matches the dynamics of an untreated system (Fig. 5.1 dashed line). Repeat treatments, if strong and long enough, also cause damping oscillations, but cause the system to move to a new equilibrium that is also dependent on species interactions (Fig. 5.1 solid line, see Bender et al. (1984)).

A mathematical modeling approach

Studies into how treatment frequency, coinfection prevalence, and inter-specific interactions affect treatment outcomes at the population level are rare, even though understanding indirect effects is crucial for effective

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treatment (Brooker et al., 2012; Lafferty, 2010). The complexity of coinfection in terms of the range of interactions described above, difficulties in ascertaining how parasites interact (Fenton et al., 2010; Hellard et al., 2012; Johnson and Buller, 2011), and the diversity of parasites that could simultaneously infect an individual means identifying particular coinfections and epidemiological contexts with significant indirect treatment effects is formidable. Meanwhile, projections from mathematical models can suggest optimal treatment strategies under different scenarios (e.g. Stolk et al. (2003); Winnen et al. (2002)), and offer useful opportunities for understanding within-host interactions and guiding policies for helminth treatment (Antia and Lipsitch, 1997; Michael et al., 2006, 2007; World Health Organization and TDR Disease Reference Group on Helminth Infections, 2012).

The effects of direct and indirect parasite interactions and are important in explaining patterns of host health (Bottomley et al., 2005), controlling energy flows in an estuarine ecosystem (Lafferty et al., 2006), and for increasing virulence and destabilising parasite dynamics (Eswarappa et al., 2012). There are models of treatment for specific coinfections including gonorrhea and HIV (Mushayabasa et al., 2011), and malaria and trypanosomiasis (Nannyonga et al., 2012), and of multiple untreated genotypes interacting via immunity or resources (Hellriegel, 1997). Lello et al. (2004) present an ordinary differential equation model with vaccination and immune-mediated interactions among three helminth species. However, the impact of immune- or resource-mediated interactions on drug treatment outcomes in a host population infected by two helminth species has not yet been modeled.

Here I develop a mathematical model to explore how different forms of interspecific parasite interaction mediate the effects of targeted antiparasite treatment. The model represents two helminth species in a human population, where the target parasite species can be treated, and the non-target parasite species is never treated. Although interactions between parasite species can be reciprocal, and can be among three or more species, for simplicity I assume only a one-way interaction where one species, the target species, interacts with a coinfecting, non-target parasite species. I

also assume that parasite abundance is positively related to host morbidity (following Eswarappa et al. (2012)).

Hypotheses

The following hypotheses relate to the mean abundance of the non-target parasite in the host population across ten years:

1. Non-target parasite abundance with and without treatment will depend on the sign of the interaction between target and non-target parasite species.

   a. With a positive interaction and no treatment of the target parasite, there will be higher non-target parasite abundance, and when there is a negative interaction abundance will be lower.

   b. After the target parasite is treated, when there is a positive interaction non-target parasite abundance will decrease, and when there is a negative interaction abundance will increase.

2. Stronger interactions will have greater effects on non-target parasite abundance both with and without treatment.

3. The effects of the interspecific interaction and of treatment will also depend on whether the interaction is mediated by resources or immunity.

4. The effects of treatment on non-target parasite abundance will be proportional to the coinfection prevalence.

5. Repeat treatments will have greater effects on non-target parasite abundance than single treatments.

I also hypothesise that:

6. Treatment effects on host morbidity (assumed to be proportional to combined abundance of target and non-target parasites) will depend on the type, sign, and strength of the parasite interaction. Assuming the two species have equal virulence, host morbidity will be lower

when a positively interacting parasite is treated because non-target parasite abundance will also decrease.

Modeling framework

These hypotheses are tested using an agent-based model of two co-endemic helminth species in a notional human village-sized population. Agent-based models are suited to modeling complex biological systems (Grimm and Railsback, 2005; Holcombe et al., 2012), and have three advantages for modeling parasite coinfection: (i) they can readily include variation among hosts, such as aggregated distributions of parasite abundance, which are fundamental to parasite dynamics and treatment success (Brogden et al., 2005; Grenfell et al., 1995; Johnson et al., 2012; King, 2010; Sabatelli et al., 2008; Schur et al., 2012; Soares Magalhães et al., 2011; Stein, 2011; Woolhouse et al., 1997), (ii) they simulate across scales, i.e. within and between-host infection dynamics (Bauer et al., 2009; Sabatelli et al., 2008), for example parasite abundance in each host, and transmission in the population (Plaisier et al., 1990), and (iii) they can have short-term, transient, nonequilibrium dynamics that often follow treatment.

Using this framework, different treatment regimes (single vs. repeated) are applied to the target parasite when target parasites have different effects on non-target parasites (immune- or resource-mediated, from strongly negative to strongly positive), in populations with different coinfection prevalences (low, moderate, or high). Some simulations had higher variability around mean non-target parasite abundance, prompting additional investigation of the contribution of stochastic processes.

Methods

Model overview

Each timestep of the discrete time model included an entire life cycle for each parasite species, including transmission, establishment, reproduction, and death of parasites, and possible death of a host. Target parasite abundance was reduced by treatment applied once or repeatedly,

and the indirect effect of treatment on non-target or total parasite abundance was measured. Simulations covered every unique combination of magnitude, direction (positive to negative), and mechanism (immune- or resource-mediated) of the interaction between the treated "target" parasite and the other "non-target" parasite, and repeat or single treatment. Individual hosts varied in their exposure to infection, and setting different correlations of exposure to the two parasites gave coinfection prevalence ranging from low ($< 20\%$), medium (35%) to high ($> 50\%$). Target and non-target parasites interacted either via resources (facilitation as a positive interaction, competition for resources as a negative interaction, modeled as a density dependent process based on contemporary parasite abundances), or via immune responses (crossimmunity as a negative interaction, an immune tradeoff as a positive interaction, modeled via an explicit immune component for each parasite species).

Each simulation recorded each parasite species' abundance and fecundity, and magnitude of the immune response specific to each parasite species in each host at each timestep for ten years after the first treatment. Parasite abundance was recorded at the end of each timestep, i.e. after establishment of new larvae, and after immune attack. Each timestep is roughly a year long, incorporating all parasite life stages. The mean and variability of non-target parasite abundance across the host population were calculated. Morbidity was the total abundance of both parasite species in each host, and the mean was calculated across hosts. There were 50 replicate simulations for each parasite interaction scenario. The agent-based model is described in more detail in the Appendix using an established and recommended protocol (Grimm et al., 2006).

Analysis

To show the effects of treatment, parasite abundance time series can be analysed in various ways (Chen and Cohen, 2001; Grimm and Wissel, 1997; Neubert and Caswell, 1997; Stott et al., 2011). Mean abundance across the ten timesteps after treatment was used as a proxy for how large a net effect treatment has. Ten timesteps was long enough to include the initial direct effects of treatment, rebound, and, in most simulations,
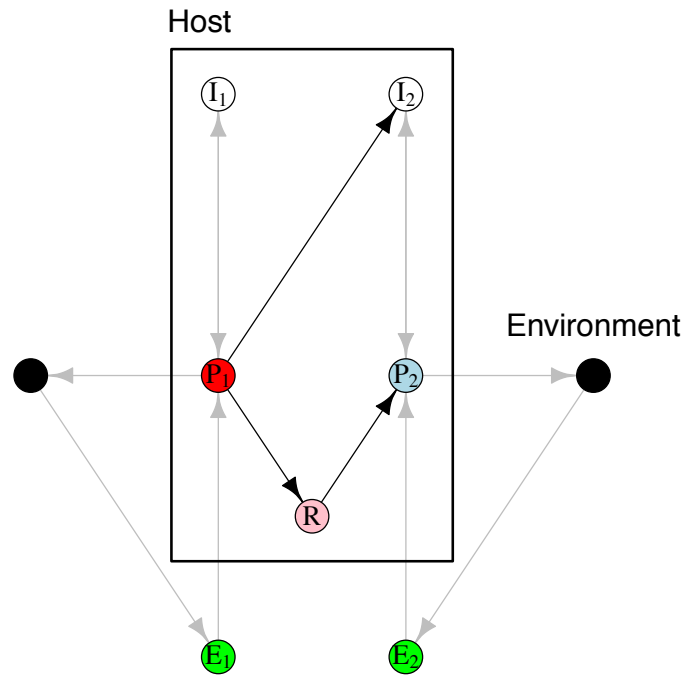
Fig. 5.2: Life stages of the target and non-target parasites (subscripted as species 1 and 2 respectively). Variables recorded within each host at each timestep are: specific immune response of host (I) that is stimulated by and kills adult parasites (P) infecting the host that reproduce and release eggs (black circles) that contribute to the environmental pool of larvae (E). The parasite species can interact within a coinfected host through immunity or resources (black lines). We model resources available for adult parasites (R) implicitly as interspecific density dependence. Eggs hatch into larvae that survive in the environmental pool and can be ingested by susceptible hosts next timestep.

equilibration. Ten years is also the timescale of many long-term helminth treatment programmes. The hypotheses focus on non-target and total parasite abundance because abundance was used in many original studies, and remains a good indicator, of interspecific interactions (Goldberg et al., 1999; Hutchinson, 1978; Poulin, 2001). Mean abundance in a human population is also used to evaluate helminth treatment programmes (Brooker et al., 2000a).

All simulations and analyses were done in R version 2.15.1 (R Development Core Team, 2012).

Sensitivity analyses

Results from model simulations can be sensitive to stochasticity, initial conditions, model structure, and the choice of parameter values. The 50 replicates using different sets of random numbers reveal uncertainty regarding stochastic dynamics and initial conditions assigned from probability distributions (i.e. host exposure). The model presents a basic macroparasite life cycle so there is little structural uncertainty. In terms of parameter values, global sensitivity analyses of other individual based ecological models of particular infections have sampled all variables within known confidence intervals to inform further experiments to refine parameter estimates of the most sensitive (Magori et al., 2009; Xu et al., 2010). The model presented here represents no specific coinfection, and consequently there are no empirical confidence intervals within which to sample parameter values for sensitivity tests. Nevertheless, even over arbitrary parameter ranges, sensitivity analyses are still useful to find the parameters relating to within-host interactions that most strongly control treatment effects on the non-target parasite.

Sensitivity tests were done for each parameter that related to the resource- or immune-mediated interactions. The metric used to assess sensitivity to changes in parameter values was total treatment effect on mean non-target abundance (the different in mean non-target parasite abundance between treatment and non-treatment runs), which was measured by taking the mean treatment effect across 50 simulations for each interaction value (from −1 to +1 in decimal intervals), and then calculat-

ing the absolute sum across those interaction values. This was repeated in turn for each parameter using half and double its original value (see Appendix table 6.1) under resource- and immune-mediated interactions. The only exception to this was the parameter for the proportion of the population treated where the upper parameter value was 1, since the original value of 0.7 could not be doubled (no more than the whole population can be treated). Press treatment and populations with medium coinfection prevalence were used for all sensitivity tests. By subjecting each parameter to the same treatment in turn allows the parameters to be ranked in terms of their relative sensitivities under each interaction type, and also provides insight into the impact of uncertainties in estimates of the effect of treatment in the model given particular within-host conditions.

Results

The model was simulated to test hypotheses that the (1) sign, (2) strength, and (3) type of interaction affected the mean abundance of the non-target parasite across ten timesteps with or without treatment of the other parasite. The effects of (4) coinfection prevalence and (5) treatment frequency on mean non-target parasite abundance were also tested, and how these interaction and treatment scenarios affected (6) host morbidity (total parasite abundance).

Hypothesis 1a: mean non-target parasite abundance in the absence of treatment depends on the sign of the interaction

Without treatment the target parasite generally led to lower non-target parasite abundance when there was a negative, immune-mediated interaction, and higher abundance when there was a positive interaction (treatment effect below and above $y = 0$ when interaction negative and positive respectively, Fig. 5.3A), supporting hypothesis 1a. However, the effects of resource-mediated interactions were sometimes the reverse of hypothesis 1a (Fig. 5.3B). Here, non-target abundance increased under weakly negative resource-mediated interactions before subsiding and going ex-
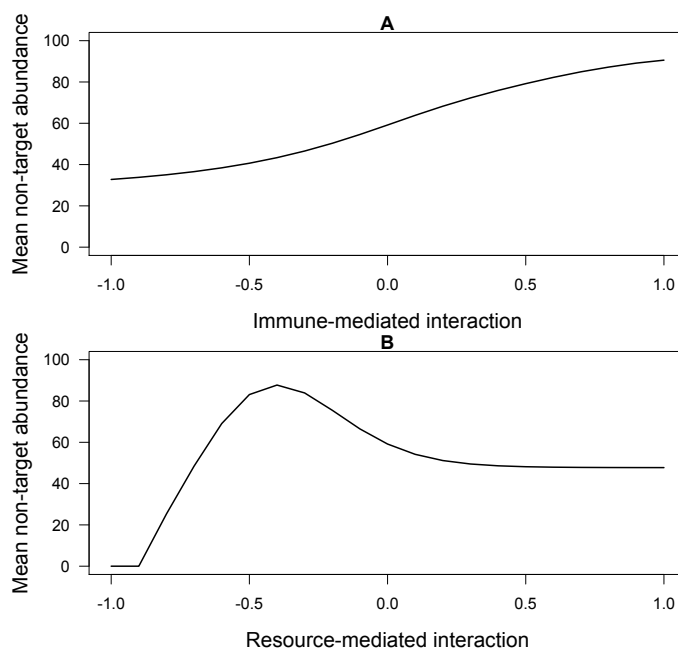
Fig. 5.3: Mean abundance of non-target parasite in the absence of treatment with either an interspecific immune- (A) or resource-mediated interaction (B).

tinct under strongly negative interactions (positive gradient for interaction strengths between 0.5 and 0, subsiding at interaction strengths around -0.5, and reaching zero for interaction strengths ≥ -0.9 Fig. 5.3B). Positive resource-mediated interactions did not alter parasite abundance (horizontal line for all interaction strengths > 0, Fig. 5.3B).

Hypothesis 1b: mean non-target parasite abundance in the presence of treatment depends on the sign of the interaction

Treatment of the target parasite generally increased non-target parasite abundance when there was a negative interaction, and decreased it when there was a positive interaction (treatment effect above and below $y = 0$ with negative and positive interaction respectively, Fig. 5.4), supporting hypothesis 1b. Treatment exacerbated the effect of resource-mediated interactions on non-target parasite abundance; the shape of the treatment response with a resource-mediated interaction (Fig. 5.4A, C, and E) is

similar to abundance without treatment (cf. Fig. 5.3B).

Treatment of a negative immune-mediated interaction consistently increased non-target parasite abundance (treatment effect on mean non-target abundance greater than 0 when interaction strength < 0, Fig. 5.4B, D, and F). Under positive immune-mediated interactions the treatment effect was negative, more stochastic, than seen under negative interactions, and non-linear (spoon-shaped), showing recovery of the non-target parasite under very strong positive interactions (Fig. 5.4B, D, and F).

Hypothesis 2: stronger interactions have greater effects on non-target parasite abundance

In untreated simulations the strength of the immune interaction resulted in a gentle S-shaped curve in non-target parasite abundance in untreated simulations (Fig. 5.3A), supporting the hypothesis that stronger interactions have greater effects on the non-target parasite. However, this is the only result consistent with hypothesis 2 across the interaction parameter space in either untreated or treated scenarios.

In treated scenarios under immune-mediated interactions (Fig. 5.4B, D, and F), the peak reduction in non-target parasite abundance was not at the strongest positive interaction, but at intermediate strength, with the effect diminishing at higher interaction strengths. Increasing the strength of negative immune-mediated interactions led to slightly higher non-target abundances after treatment.

For resource-mediated interactions, in both treated and untreated simulations, the highest levels of non-target parasite abundance occurred at intermediate negative interaction strength. Under strong negative interactions the non-target parasite went extinct before treatment was applied, so no treatment effect was observed here (abundance $= 0$ when interaction strength ≥ 0.9 in Fig. 5.3B corresponds with no change in abundance after treatment in Fig. 5.4A, C, and E). Increasing the strength of positive resource-mediated interactions caused little change in mean non-target parasite abundance either with or without treatment.
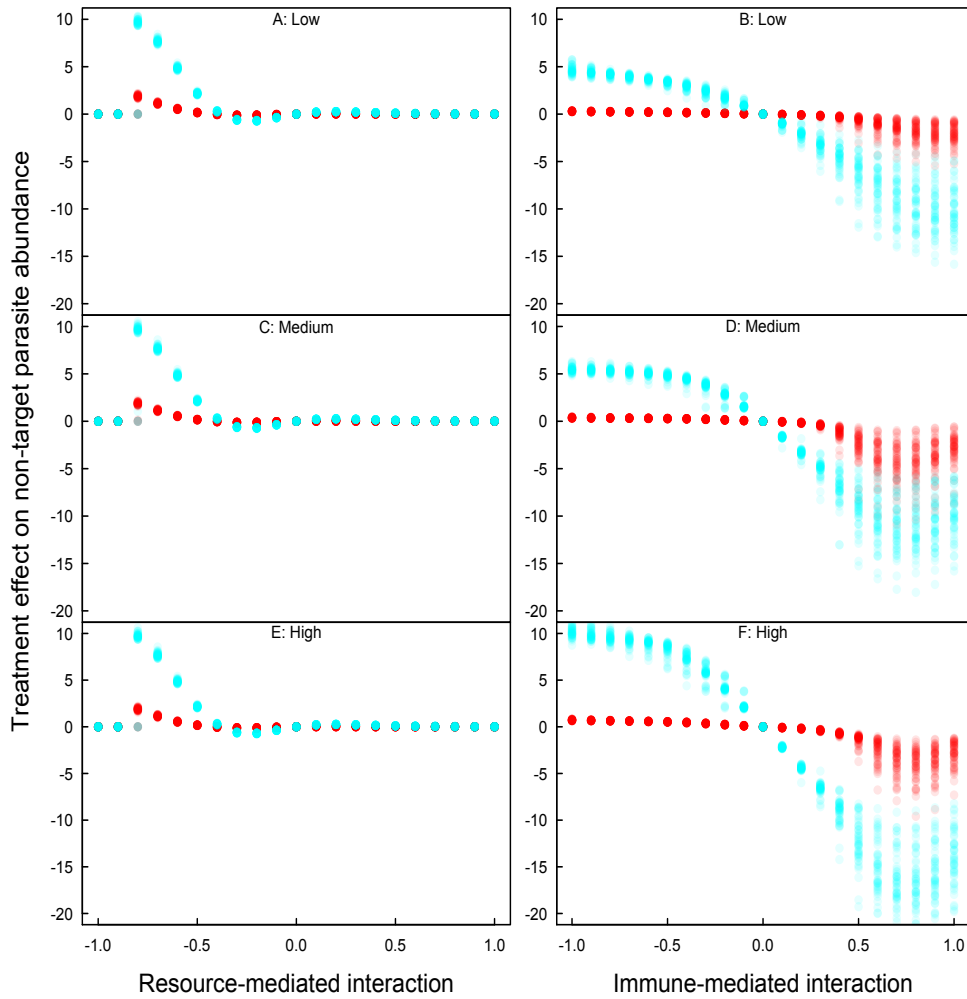
Fig. 5.4: Difference in mean non-target parasite abundance between treated and untreated simulations (red=single treatment, blue=repeat treatment) in low, medium, and high prevalence populations (top, middle, and bottom respectively) for resource- and immune-mediated interactions (left and right columns respectively).

Hypothesis 3: interaction and treatment effects depend on whether the interaction is mediated by resources or immunity

As seen in the contrasts made above, immune- and resource-mediated interactions produced different patterns of non-target abundance, both under untreated and treated scenarios (Fig 5.3 A vs. B, and Fig. 5.4 left vs. right column). Both mechanisms of interaction had some areas of negligible response of non-target parasite abundance to varying interaction strength (positive resource-mediated interactions, and negative immune-mediated interactions) or non-monotonicity (negative resource-mediated interactions, and positive immune-mediated interactions). Only immune-mediated interactions, particularly under strongly positive interactions, saw increased variability between simulations, with variability between simulations involving resource-mediated interactions remaining constant across the interaction parameter space (see Discussion).

Hypothesis 4: treatment effects on non-target parasite abundance are proportional to coinfection prevalence

As hypothesised, increasing the prevalence of coinfection in the host population accentuated the indirect effects of treatment on non-target parasite abundance for both interaction types (values stretch along y-axis from low to high prevalence from Fig. 5.4A to C to E, and from Fig. 5.4 B to D to F).

Hypothesis 5: repeat treatments have greater effects on non-target parasite abundance than single treatments

Again, as hypothesised, repeat treatments enhanced the effects on non-target parasite abundance compared with single treatments (blue points further from $y = 0$ than red points, Fig. 5.4).

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Hypothesis 6: treatment effects on host morbidity depend on the type, sign, and strength of the parasite interaction

Treatment of the target parasite always reduced host morbidity (assumed to be proportional to total parasite load, with equal virulence of the parasite species, $y < 0$ for all interaction strengths, Fig. 5.5). However, in accordance with hypothesis 6, the size of this reduction was moderated by the interaction between target and non-target parasite, and by treatment frequency: repeat treatments (Fig. 5.5, red points) brought greater reductions in morbidity than single treatments (Fig. 5.5, blue points), and host morbidity was further reduced where there was a positive interaction, and not reduced as greatly when there was a negative interaction. The greatest reductions in morbidity were seen under positive immune-mediated interactions, although there was substantial variation in treatment effects by this criterion between replicate simulations under immune-mediated interactions (Fig. 5.5B, D, and F show wide distance between blue and especially red points).

Sensitivity analysis results

The ranking of the variables from the sensitivity tests is shown in Fig. 5.6 where higher absolute treatment effects indicate a greater deviation between treated and untreated simulations across the relevant interaction. Treatment causes the biggest changes in non-target parasite abundance for parameters with lines higher up the y-axis on Fig. 5.6, and steeper lines indicate greater sensitivity to the value of that parameter. For both interactions, total absolute treatment effect was most sensitive to the proportion of the population treated (T), the effect of parasites on the host's specific immune response to them (γ_i), and the effect of immunity on parasite survival (SI). Total absolute treatment effect was relatively insensitive to changes in virulence (V), parasite survival (S), the effect of immunity on parasite fecundity (FI), and intraspecific density dependence (D_{half}). The ranking of the parameters depended on the interaction type (Fig. 5.6A vs. B), and on whether the parameter was half or double its original value.
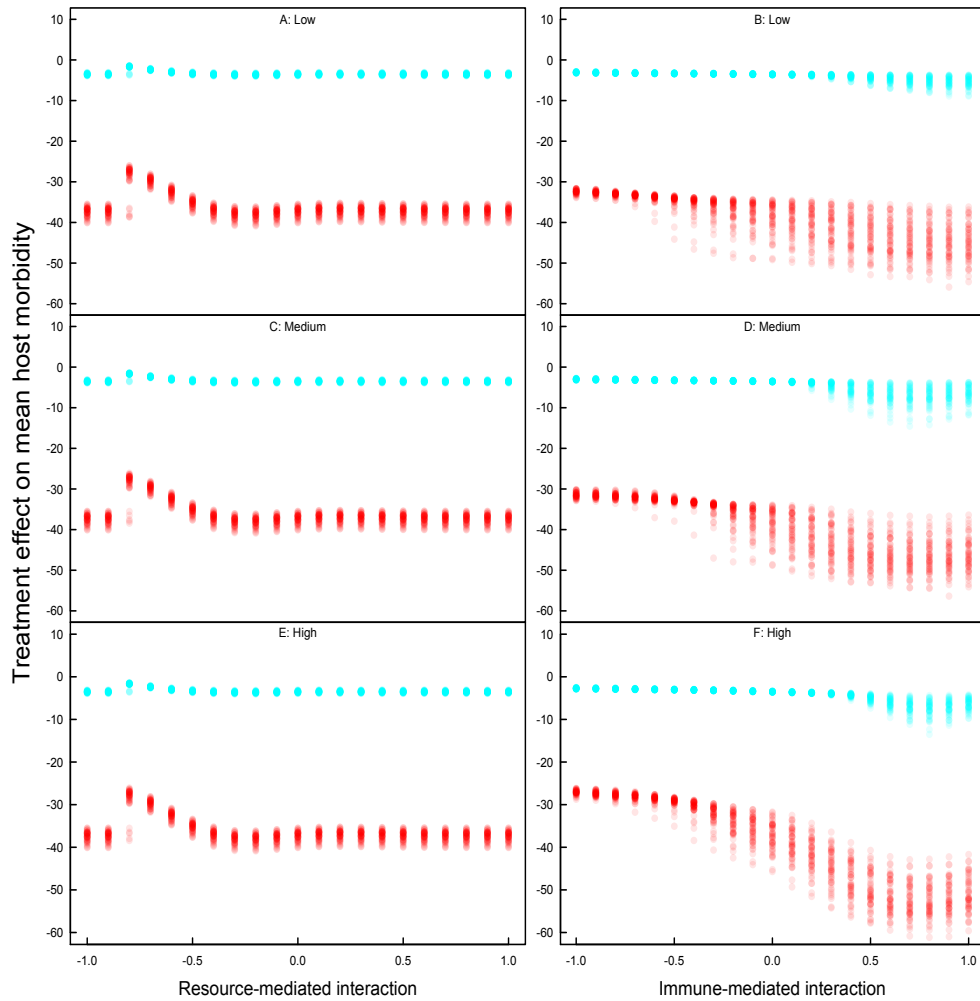
Fig. 5.5: Difference between mean total parasite abundance in treated and untreated simulations (red=single treatment, blue=repeat treatment) in low, medium, and high prevalence populations (top, middle, and bottom respectively) for resource- and immune-mediated interactions (left and right columns respectively).
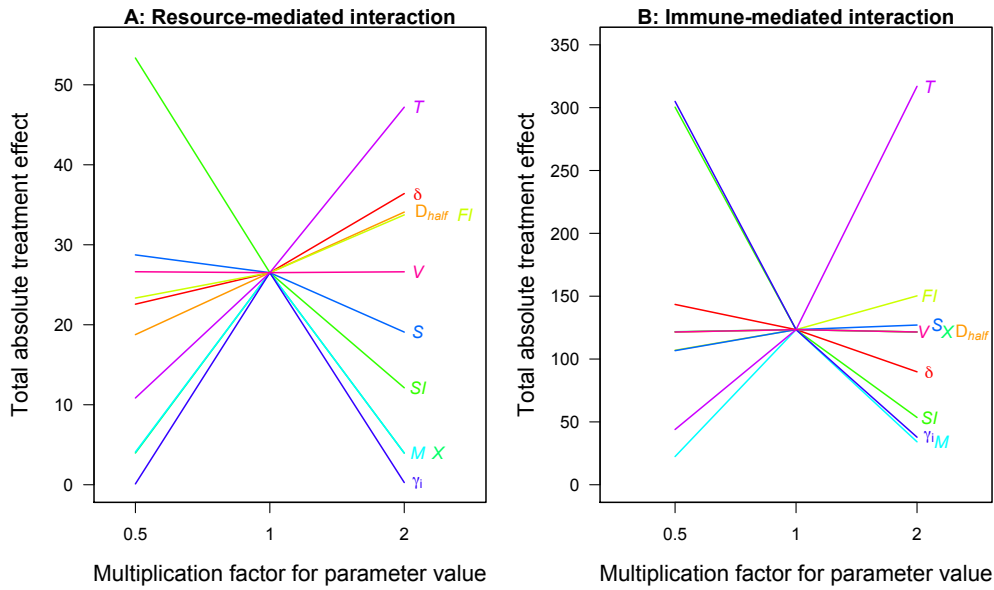
Fig. 5.6: Sum of the absolute difference between treated and untreated mean total parasite abundance across interaction parameter values for resource- (A) and immune-mediated (B) interactions. Each parameter is indicated by a label next to its line: δ = rate of decay of immune memory (red line), D_{half} = parasite density for half of larvae to establish (orange), FI = effect of immunity on adult parasite fecundity (olive), γ_i = species-specific immune stimulation (dark blue), M = maximum life expectancy (light blue), S = survival rate of adult parasites (medium blue), SI = effect of immunity on adult parasite survival (bright green), T = proportion of population treated (purple), V = virulence of parasites (magenta), and X = inflexion point of logistic virulence curve (teal). See Appendix table 6.1 for original parameter values corresponding with this notation.

Discussion

To test the indirect effects of treatment on coinfecting parasites, I simulated the population dynamics of two endemic helminth species in a village-sized population of humans with and without treatment of a target species, and with a range of interactions between the target and non-target species. Treatment either increased or decreased non-target parasite abundance, in a manner largely consistent with whether the underlying interaction between the species was negative or positive. However, this indirect effect of treatment was amplified by repeat treatments and higher coinfection prevalence, and modified by interaction type and strength in a complex manner, sometimes resulting in negligible and sometimes in non-monotonic relationships between interaction strength and the effect of treatment. As I will discuss, these responses have important implications for predicting or evaluating the outcomes of species-specific treatment programmes in coinfected populations. The results also prompt consideration of how parasite interactions can be inferred from perturbation studies, and highlight opportunities for future research.

Implications for treatment

In the model, the mean abundance of the non-target parasite was both raised and lowered when the target parasite interacted with the non-target parasite. Similarly, the change in morbidity in a treated population depends not just on how the targeted species is treated, but also on the presence of coinfecting species not directly affected by the drug. These results suggest that the net health benefits of the treatment programmes may be over- or under-estimated unless we account for the indirect effects of treatment on non-target parasites. Therefore surveys need to monitor for co-occurring species as well as target parasites before initiating treatment, and close attention paid to their responses following treatment. The implications of non-target parasites for the health of treated individuals should also be assessed.

To help predict or evaluate how non-target parasites are likely to modify treatment outcomes, the model highlights various factors that deter-

mine the indirect effects of treatment on non-target parasites, and conditions under which these effects do or do not arise. Key factors to consider are coinfection prevalence, treatment frequency and coverage, and the direction of the underlying one-way interspecific interaction. For example, in the model, when two species interacted, the longer treatment continued, or the higher the coinfection prevalence, the greater the deviation between treated and untreated simulations. The sensitivity tests also showed the proportion of the population receiving treatment to also be correlated with treatment effect. Another possible indicator is that the direction of a one-way interaction often corresponds to indirect effects of treatment of the opposite direction (e.g., a negative underlying interaction often results in a positive treatment effect, such that non-target parasite abundance increases post-treatment). Increases in non-target parasite abundance after treatment reliably reflected negative resource- or immune-mediated interactions, and decreases in non-target abundance reliably reflected positive resource- or immune-mediated interactions. However, some interactions, e.g. positive resource-mediated interactions, did not alter treatment outcomes. Thus, treatment may not necessarily affect non-target parasites despite the presence of an interaction. Such non-responsiveness happens when the effects of the interspecific interaction were obscured by intraspecific regulation (e.g. resource-mediated interspecific interactions were often obscured by intraspecific immune-mediated regulation). To understand the impact of parasite interactions on treatment success, interactions should therefore be understood in the context of other within-host processes. However, the nonlinear nature of the intraspecific processes produced non-monotonic relationships between post-treatment non-target abundance and interaction strength, so interaction strength may be an unreliable indicator of indirect effects of treatment programmes.

Studying parasite interactions

Intraspecific processes like species-specific density dependent immune responses affected the abundance of non-target parasites so strongly that they obscured the effects positive interspecific resource-mediated interactions. It is therefore important to know the form (direction, mechanism,

and magnitude) of all interactions among parasite species within coinfected individuals in order to predict treatment outcomes. In particular the sensitivity tests suggest that precise parameterisation of the stimulation of immunity by adult parasites and the effect of immunity on parasite eggs is particularly important. However, detection of interspecific interactions is known to be difficult from perturbed time series in general (Bender et al., 1984), and between coinfecting parasites in particular (Fenton et al., 2010; Hellard et al., 2012; Johnson and Buller, 2011). Appropriate models for many within-host processes are also unclear. How the rate of immune killing responds to parasite abundance is rarely discussed, but is crucial to indirect treatment effects when species interact via immunity in this and other coinfection models (e.g. Fenton and Perkins (2010)). The feeding relationships between parasites and host resources are perhaps discussed even less frequently, except in rare cases like the relationship between malaria and red blood cells (e.g. Savill et al. (2009)). The results from the sensitivity analysis suggest that estimating the inflexion point of any resource competition curve can have a large influence on the indirect of treatment on non-target parasites. Without quantified measurements of these relationships among parasites and host components, especially those parameters that treatment effects are most sensitive to, it will be difficult to predict the effects of treatment on communities of interacting parasites.

Further to these challenges to understanding the dynamics of coinfection within hosts, various model results suggest that inferences of interaction strength from mean treatment responses are uncertain because increases or decreases in treatment effects are not always correlated with interaction strength. The model results give at least three reasons for such uncertainty: wide regions of non-response, non-monotonic relationships, and high variability in treatment effects between replicate simulations.

First, negligible treatment effects were observed at various interaction strengths, including from very weak to very strong resource-mediated interactions. When effects of drug treatment were observed these tended to reliably reflect the direction of underlying interactions. However, the absence of a treatment response does not necessarily indicate the absence of an interspecific interaction. While certain interactions may be

occurring, as often occurred under resource-mediated interactions, other, stronger within-host processes like immune-killing can counteract these effects. Similar compensatory immune responses have also been shown to mask interspecific interactions in other multi-parasite systems (Bull et al., 2012).

Second, the largest treatment effect under positive immune interactions occurred at intermediate interaction strength; either side of this value treatment indirectly lowered mean non-target abundance, but to a lesser extent. When a negative treatment effect is smaller than this peak value there is therefore ambiguity in whether the immune interaction was weak or strong. Hence, a larger treatment effect is not necessarily indicative of a stronger interaction between the parasites.

Third, replicate host populations of identical size with identical processes, but with different randomly-selected values of host exposure, varied in the size of the treatment effect on mean non-target abundance. Indeed, the between-simulation variability under positive immune-mediated interactions (immunosuppression) was so high as to create a large area of ambiguity where the same treatment effect could have been caused by a wide range of interaction strengths.

These three possibilities need to be considered when interpreting the results of future studies of parasite communities. Indeterminacy of long term (equilibrial) effects of press perturbations in multi-species communities has long been known (Yodzis, 1988). In addition, the simulations presented herein show that there are areas of indeterminacy in the disequilibrium phase in the years after single or repeat treatment for a community of two parasite species.

Variability in non-target parasite abundance

Of particular interest in the model results were some immune-mediated interaction strengths that increased variability in mean non-target abundance between populations. To further understand what contributes to variation in mean parasite abundance, variability around the mean was measured in high prevalence simulations in two ways: (i) the amplitude of parasite dynamics (taken as the difference between the minimum and max-

imum mean parasite abundance per host in ten timesteps after treatment), which indicates temporal variability, and (ii) the distribution of non-target parasites across the host population (taken as the 95% confidence interval of the non-target abundance ten-year mean), which indicates between-host variability.

Post-treatment variability under resource-mediated interactions followed the same pattern as mean abundance for both temporal and inter-host variability (5.7A and C, cf. Fig. 5.4A, C, and E), with greater increases when treatment was repeated (red vs. blue points), and greatest increases at negative interactions up to -0.7, before parasite extinction reduced variability to zero.

Variability was larger for immune- rather than resource-mediated interactions (note same scales on Fig. 5.7A-B and C-D). For immune-mediated interactions, repeat treatments increased temporal variability more than single treatments when there was a positive interaction (blue points further from zero for positive interactions, Fig. 5.7B). Conversely, negative interactions increased temporal variability after single treatment more than repeat treatments (blue points closer to zero for negative interaction values, Fig. 5.7B). In terms of variability between hosts, negative immune-mediated interactions resulted in very little variability (Fig. 5.7D), whereas variability greatly increased under positive interactions, and these effects were magnified under repeat treatments.

These results (Fig. 5.7B and D) indicate that the observed increased stochasticity between replicates under positive immune-mediated interactions (Fig. 5.4 right column) is caused by increasing fluctuations in the parasite dynamics through time, and that inter-host variability contributes less to stochasticity because immunity tends to homogenise the host population by reducing the number of high burden hosts. It seems that positive immune interactions (immunosuppression) and the loss of immune memory as parasite populations decline contribute to delayed density dependence resulting in unstable population cycles. This contrasts with the more stable equilibrium observed even after treatment when immune interactions were weaker or negative. The potential for both stability and instability from delayed density dependence is known

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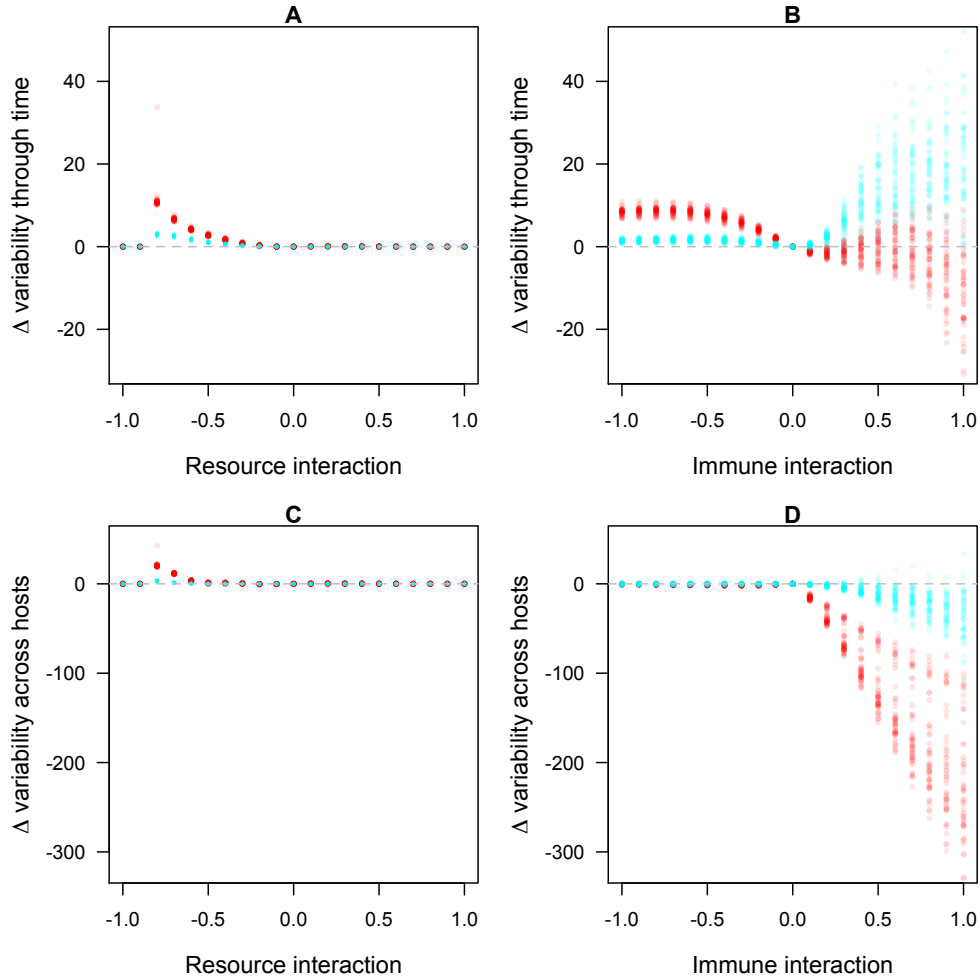
Fig. 5.7: Difference in variability in non-target parasite abundance between treated and untreated simulations (red=single treatment, blue=repeat treatment) in medium prevalence populations with a resource (A and C) or immune interspecific interaction (B and D). Variability is measured by the difference between minimum and maximum mean abundance in ten timesteps after treatment (temporal variability: A and B), and 95% CI in the abundance of non-target parasites among hosts (inter-host variability: C and D).

from lagged predator-prey systems (Sih, 1987; Turchin et al., 1999). The possibility of these dynamics being present within coinfected hosts is perhaps a new observation, though whether this is an artefact of the discrete model used here, or whether this is a real feature of immune interactions between parasites needs further testing.

Further research

In this chapter I have begun to explore how interactions among parasite species and variability between hosts affects population-level treatment outcomes. I developed an agent-based model with treatment of one parasite species that interacted via shared resources or immune responses. Further scenarios could be tested with small modifications to this model.

- How individual variation in interaction strengths affects ecosystems is a growing area of research (Wells and O'Hara, 2012), but how interactions between coinfecting parasites vary among hosts, and what impact it has on treatment programmes, is largely unknown. Components involved in immune- or resource-mediated interactions that vary between individuals, such as different parasite epitopes and immune receptors, nutritional status, or tissue repair rates, could easily be included in the model as another source of agent-based variation.

- Parasite communities are far more complex than presented in the model, and how these complexities alter the indirect effects of treatment need to be explored. For instance, greater parasite diversity exponentially increases the number of interactions and treatment options to be considered. Two-way interactions where both parasites affect one another, or where resource- and immune-mediated interactions occur simultaneously are also possible. And there are also evolutionary feedbacks, for instance drug treatments are likely to select for drug-resistant parasite strains, which adds to the parasites responding indirectly to treatment.

- The model should be parameterised for a specific pair of parasites,

and could be used to compare between real treatment options, for instance nutrition or environmental improvements as well as drug treatment.

Perhaps most importantly, to test whether these simulations are realistic, we need more monitoring of the effects of specific treatments on the wider parasite community, and studies of interactions among parasite species. More empirical studies of interactions in coinfected populations have been called for by other authors too (Basáñez et al., 2012; Righetti et al., 2012). Would a metanalysis of treatment effects on non-target parasites experiencing different interactions look like Fig. 5.4? The simulation presented here is just the beginning of research into how health in treated human populations is affected by interactions among the parasite community.

Chapter 6

Discussion

Infectious diseases pose a serious challenge to global health (Hopkins, 2013; Sanders et al., 2008), but the contribution of coinfection to this morbidity and mortality burden is only beginning to be investigated (Pullan and Brooker, 2008). This thesis has expanded the typical approaches used to study particular parasite coinfections to instead study the range of interactions among the diverse parasite community of humans using various cross-disciplinary methods. I have compiled three of the largest datasets of coinfection in humans, covering hundreds of species combinations and hundreds of thousands of individuals. I also developed a unique model representing treatment of a small human population infected by two interacting parasite species.

My research shows that coinfection is not rare, nor does it have random or negligible effects on human health. Rather, the published reports that I collated showed coinfection to be a diverse problem involving hundreds of parasite species. Coinfections reportedly have a tendency towards higher abundance infections, and towards worse host health than single infections. Most of the data contributing to these results comes from patients already receiving healthcare. Therefore, we can make, for the first time, a general conclusion about treatment of coinfected patients, namely that coinfection appears to have worse health outcomes than for those with only one infection, likely contributed to by the difficulties of tackling multiple species.

My research also revealed some other previously unknown general pat-

terns of reported interactions among coinfecting parasites, most notably that they are mostly indirect and often mediated by shared resources. My simulations of treatment effects on two parasite species in a heterogeneous host population suggested that the direction of a one-way interaction could inform decisions of the optimal treatment program in coinfected populations. Together these results suggest that, while treatment of coinfected patients appears to be more difficult than for those with one infection, treatments could be improved if parasite interactions were better understood. The findings from each research chapter are now discussed in turn, before considering shared themes and issues highlighted by the research chapters, and finally considering opportunities for advancing knowledge of this important global health issue.

Findings from research chapters

Patterns of human coinfection

Prior to this thesis many fundamental patterns of coinfection were not known, such as the different nutritional or pathological consequences for single- and co-infected hosts (Pullan and Brooker, 2008). The research in Chapter 2 collated data from recent publications on coinfecting parasites, and their reported effects on host health and parasite abundance. Reported coinfections included all kinds of parasites, but were most likely to involve viruses and bacteria. The most commonly reported coinfecting parasites differed from those causing highest global mortality, with a notable lack of serious childhood infections in reported coinfections. Coinfected individuals were generally reported to have worse health (78% publications) and higher parasite abundance (57% publications) than those with single infections.

Given the large and very significant effects observed, I am confident that these general findings are robust. It is still worthwhile to consider possible biases in and limitations of the data. The results do suggest biases in the coverage of coinfection research. For example helminth coinfection is estimated to affect between 800 million and one billion individuals

(Crompton, 1999; Hotez et al., 2007), but there were relatively few reports of this in the sampled publications. Also, most publications showed negative effects of coinfection on host health, but grouping the same data by the pair of coinfecting parasites led to most effects being negligible (though positive effects where parasite abundance was higher than single infections still exceeded negative ones, Fig. 2.2). Another caveat is the insufficient and inconsistent reporting of quantitative data, which precluded full meta-analysis. Nevertheless, having analysed the reported direction of associations and found a tendency toward reported negative effects of coinfection on human health (and positive effects on parasite abundance), the question emerges of how this arose.

The observational nature of the dataset from Chapter 2 means we can only speculate on the likely cause of this skewed distribution. Three explanations are plausible. (1) Coinfected individuals may have had worse health to begin with, such as a weakened immune system. Thus, the lower health status reported for coinfected individuals was the cause, rather than the consequence, of their coinfection. (2) The multiple infections could have had an additive effect where one infection causes a health burden, and a coinfection causes further health burden. Accordingly coinfection simply causes more damage to the host. (3) Coinfecting parasite populations might not have been independent and were interacting by some mechanism so that the presence of a parasite of another species alters the life history of a coinfecting parasite. To assess the interactions among parasites that may cause the statistical associations observed in published reports, the frequency of different interaction types was tested in Chapter 3.

Summary network of coinfecting parasites

The parasites infecting a host, the host's immune system, and the resources used by the parasites (e.g. host tissues) can be viewed as a network of interacting components, in the same way as one can characterise trophic relationships among species in a free-living community as a network (i.e. a food web). Just as in free-living communities, it is possible that communities of coinfecting parasites are controlled by top-down (immune control) or bottom up (resource control) processes (Pedersen and Fenton, 2007).

While some studies have collated all the host-parasite interactions for particular parasite species (e.g. Raman et al. (2010)), or included parasites in free-living food webs (Lafferty et al., 2006), in Chapter 3 I made the first attempt to construct a summary coinfection network for humans. Data on host-parasite interactions from the same set of recent publications used in Chapter 2 were assembled into a within-host network comprising direct and indirect interactions among parasites, resources, and immune components. Inclusion of resources consumed by coinfecting parasites as well as their immune interactions offered a novel comparison of the number of known routes for either top-down or bottom-up control.

Analyses of the structure of this network showed that interactions between pairs of parasites were more often mediated indirectly through shared resources than through immune components or other parasites. Interacting components of the network were also grouped into modules that were each associated with particular body parts. This structuring of the network by resource relationships offers the first indication that physical compartments within the body and bottom-up processes most often influence how, where, and which coinfecting parasites interact.

These findings underline the importance of studying resource-mediated interspecific interactions, and raises further questions. For example, these patterns originate from the same publications from Chapter 2 where coinfection was associated with worse host health, so does bottom-up control determine the health burden of coinfection? Although resource-mediated interactions were relatively common reported mechanisms for interactions between pairs of parasites, these interactions do not necessarily have the strongest effects on the parasite community. Measuring the strength of parasite interactions and their pathological consequences would enable further tests of the importance of resource-associated modules via new methods such as quantitative analyses of compartmentation via Bayesian clustering methods (Bogich et al., 2013; Tsuda and Georgii, 2013), and simulations to estimate the resilience and resistance of the parasite community of humans. There are various methodological difficulties in how to measure the strength of interactions between parasites and host components, and directly between parasites. For instance, how the rate of

consumption of host resources by parasites changes with parasite abundance or resource availability is rarely discussed. Even relationships between host immunity and parasites are difficult to model, especially as there is insufficient data for parameter estimation (Fenton and Perkins, 2010). The network I developed in Chapter 3 is an initial step towards studying all the interactions among parasites known to coinfect humans, and opens the door for future research.

Coinfection deaths in England and Wales

As well as not knowing how strongly coinfecting parasite species interact, the cohorts most at risk of coinfection were little studied. Risk factors for, and prevalence of, coinfection have so far been studied in narrow datasets, such as three gastrointestinal helminths and fever among 350 children in Tanzania (Lello et al., 2013), sexually transmitted infections among teenage girls in the USA (Forhan et al., 2009), and two tick-borne bacterial species in humans and other hosts (Nieto and Foley, 2009). Until now no study had considered the full range of parasites or the number of people coinfected across any national population. Moreover some studies implied coinfection was a problem of poorer countries (e.g. Lello et al. (2013)), though the mortality burden of coinfection in countries that invest heavily in universal access to healthcare was unknown.

Using data on reported causes of death from England and Wales, I showed in Chapter 4 that from 2005 to 2008 more than $135,000$ people died from infectious causes, and 6% of these were associated with coinfection. By analysing the first national-level distribution of coinfection deaths across age and sex cohorts, I showed that adults between their 20s and 40s were most likely to have coinfection reported on their death certificate, and relatively high proportions continued to slightly later ages in males. I found no consistent relationship between pairs of infections and the similarity of their biological characteristics. Most pairs of parasites co-occurred on death certificates at rates comparable with what was expected if the two infections caused death independently. However among the pairs of parasites where the odds of coinfection death were significantly different, the odds were most often higher than expected (Fig. 4.4). In

other words, the odds of coinfection death were, for more than half of pairs of infectious causes of death, based on the additive probability of having two infections. For two fifths of pairs, there were significantly higher odds of deaths reported as being from both causes. Referring back to the three potential drivers of association between coinfection and host health (page 123), for coinfection mortality in England and Wales we can conclude that pairs of infections mostly have additive effects on host mortality, but often the odds of coinfection were higher than the sum of their parts. Whether this association is because of host susceptibility, parasite interactions, or a mix of the two warrants further examination.

The network I assembled in Chapter 3 demonstrates that there is much evidence in publications that coinfecting parasite species have the potential to interact with one another within a human host. There is also evidence that host factors can affect the way individuals respond to their infections, and for those with weak, inappropriate, or pathological immune responses, worse health outcomes may be more likely (Ingram et al., 2011; Meyrelles et al., 2013). Generally speaking we would expect the proportion of coinfection deaths to be higher in older individuals because ageing of the immune system leads to higher susceptibility to infections (Weinberger et al., 2008) and lower immune responses (Goodwin et al., 2006). The results of Chapter 4 showed instead that the proportion of coinfection deaths was highest in early adulthood. People in this age bracket could be viewed as being in their prime, and that the higher proportion of coinfection deaths are indicative of highly pathological within-host interactions. This explanation is preferred among many infectious disease researchers nowadays, but wider anthropological factors should still be considered Singer (2009), especially as the dataset in Chapter 4 only revealed age, sex, and causes of death. Also in the analyses undertaken, the biological characteristics of co-occurring infections were not associated with the odds of coinfection death, so the nature of any within-host interactions is unclear. Whether those aged $20 - 40$ had characteristics that made them more susceptible to coinfection, or whether their immune response makes them more likely to die from coinfection remains to be seen.

While it is not possible, at least at this stage, to attribute the skew

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towards higher odds of coinfection being reported on death certificates to individual characteristics, parasite interactions, or both, these results corroborate the finding from Chapter 2 that coinfection is associated with worse host health. Moreover the lack of coinfections reported in the literature that involve childhood infections can be attributed to a lack of study; coinfections involving varicella zoster virus (chicken pox) and measles were reported on death certificates in England and Wales, and had a higher odds of coinfection death than deaths reported as being caused by either infection alone (infectious causes B01 and B05 had an odds ratio of 2096). Certain childhood infections are therefore associated with higher odds of coinfection death.

The lack of association between pairs of infections with similar biological characteristics, particularly shared tropisms, casts doubt on whether consuming the same host resource confers greater risk of coinfection and damage to health, as had been suggested when the results of Chapter 2 and 3 were taken together. However, more nuanced analyses of the biological characteristics of infections on death certificates and of relationships between coinfecting parasites and host resources are required. Nevertheless, my initial analysis of reported infectious causes of death shows that coinfection could have significant, detrimental health implications even in richer countries with universal healthcare.

Modelling indirect effects of treatment on coinfecting parasites

The fact that coinfection was associated with higher reported parasitemia (Chapter 2), worse health (Chapter 2), and higher odds of death (Chapter 4) despite most of the individuals in this dataset having access to healthcare suggests that current treatments developed to target single infections may be impeded by interactions among coinfecting parasites. This has been previously suggested in papers about coinfection in animals (Behnke et al., 2001; Lello et al., 2004). There were some models of vaccination and immune interactions (Lello et al., 2004), and of treatments for specific coinfections (Alemu et al., 2013; Mushayabasa et al., 2011). Yet there were no tests of how the indirect interactions among coinfecting parasites, that were so common in the network in Chapter 3, affect treatment of

parasite communities in humans. In Chapter 5 I developed a theoretical model of mass drug administration in a village-sized population to test how immune- or resource-mediated interactions between two endemic helminth species altered treatment outcomes.

I found that the indirect effect of treatment on non-target parasites was consistently related to the direction of the interaction between target and non-target parasite. Indirect treatment effects also increased in magnitude the higher the coinfection prevalence, and the more frequently treatment was applied. These results suggest that, for a two-parasite one-host species system, the direction of treatment outcomes on non-target parasites at the population level could be predicted using the direction of an interaction, the proportion of individuals coinfected, and treatment frequency. This indicates, at least theoretically for a one-way interaction, that understanding parasite interactions could help design better treatment programs for coinfected populations.

However, at certain interaction strengths I also found that the non-target parasite was unaffected by treatment, and where variability between simulations increased. These phenomena would hinder either precise prediction of treatment effects from interaction parameters, or inference of interaction parameters from observations of perturbed systems. This raises the question of how well we can understand the direction and strength of statistical associations and any underlying mechanistic interactions between coinfecting parasites.

Shared themes from the research chapters

Are observational patterns of coinfection meaningful?

For parasite interactions to be translated into clinical applications that could improve patient health, data about the presence and strength of interspecific interactions among parasites needs to be reliable. Using reports in the published literature and reports on death certificates, I showed that the overall effect of coinfection on human health appears to be negative. The extent to which such patterns are biologically meaningful rather than

artefacts of processes by which the data was produced and assembled, is crucial, but often unclear (Sutherland et al., 2013). Errors in the collection and presentation of empirical data can arise from random variation or structural biases at various stages: choice of system, sampling, measurement, statistical analysis, reporting of particular results, and biased publication of research (Levitt et al., 1993). Such errors may occur in the three datasets in this thesis (Chapters 2-4), though I have shown that results are robust to certain sources of error. The structure of the network that I collated from published papers in Chapter 3 is robust to the method used for sampling publications and the aggregation of node names, but it was not possible to assess the extent of publication or reporting bias in the source data. The key results in Chapter 2 were similarly robust to pairs of parasites where the presence (or absence) of an effect on host health or parasitemia was not reported in the reviewed publications. Tests for robustness were not done for the reported causes of death in Chapter 4. The sensitivity of all these Chapters' results to sampling by the original investigators is difficult to determine, but can be speculated upon. Such consideration is crucial if we are to have confidence in the main conclusion of this thesis that coinfection is commonplace and, in general, has negative effects on host health. While trends among biomedical publications (discussed below) might indicate biases that undermine this thesis' conclusions, this is counteracted by the diverse data sources presented herein corroborating one another.

Biomedical publications, like the infectious disease publications surveyed in Chapters 2 and 3, may report conclusions that are rarely supported by repeat studies or reanalysis (Easterbrook et al., 1991). Biomedical papers tend to report significant results, but between 14% and a majority contain false positives (Ioannidis, 2005; Jager and Leek, 2013). Accordingly, there could be consistent bias among infectious disease researchers to report data that show negative health effects, or to focus their research on more pathogenic parasites. There could also be a persistent bias towards, say, authors tending to describe the sites of infection rather than immune responses (Loscalzo, 2011).

Death certificates are very different sources of information compared

to published articles. Death certificates are legal documents signed by two doctors and collated by the state to study all causes of death, not just particular infectious diseases of research interest. The dataset in Chapter 4 includes a subset of all death certificates in England and Wales for which any infectious cause of death was reported. It is therefore unlikely that these data contain the same potential biases as in published articles, such as reporting biases toward worse coinfection outcomes, or biased documentation of sites of infection compared to immune responses.

Recently published studies using still other methods also suggest a general detrimental effect of coinfection on host health. For example, *Schistosoma spp.* coinfections (Abruzzi and Fried, 2011), polymicrobial *Staphylococcus aureus* infection (Park et al., 2012), and reported coinfections in non-human hosts (Manenti, 2011) have overall negative effects on host health. With increasing evidence, and a variety of data sources, we can reasonably conclude that coinfected hosts tend to suffer more from infectious disease than those with one infection.

How do parasites interact within a coinfected human?

Having established that coinfection often has general negative effects on human health, the interactions that could produce these effects need consideration. The network presented in Chapter 3 showed parasite feeding on shared resources to be the most commonly reported indirect interaction in coinfected humans. While the frequency of occurrence of this interaction type may be inflated by infection being often described in terms of host resources (Loscalzo, 2011), this finding should provoke greater consideration of non-immune interactions. Prior to this thesis immune-mediated interactions had been proposed as the major type of interaction in empirical and modelling studies (see citations and discussion in Graham (2008) and Tompkins et al. (2011), and Fenton and Perkins (2010) respectively), though the possibilities of bottom-up control had been discussed particularly in relation to coinfected mice (Fairlie-Clarke, 2011; Knowles, 2011; Pedersen and Fenton, 2007). The network shows that indirect interactions sharing a resource are relatively common in published reports; it is unclear whether publications fairly represent within-host interactions,

or how large an effect resource-mediated interactions have on coinfection dynamics relative to other interaction types. Nevertheless the network adds to existing calls for resource-mediated interactions to be considered alongside immune-mediated ones.

There are various options for how to develop methods to measure the strength of interactions among parasite species and their host. There are some computer and lab models of physiological changes caused by coinfection in host cells and parasites (Lutermann et al., 2012; Romano et al., 2013), but further work is needed. Where immune responses to parasites can be modeled using predator-prey models, there is uncertainty over how to model coinfection (Fenton and Perkins, 2010). How parasite populations respond to the availability of host resources is only discussed for certain parasites, like malaria (Savill et al., 2009), and no general model for within-host coinfection has been described. As an extension including theories of foraging in free-living systems (e.g. Petchey et al. (2008)), mechanistic functions of immune components attacking parasites, and parasites feeding on their resources (potentially being immune components) could be developed. Thus, foraging of parasites on host cells and tissues could explain rates of parasite growth within the host. While body size of consumers and their prey are important determinants of interactions in free-living ecological networks (Eklöf et al., 2013), they are yet to be applied to within-host parasite interactions. It is unclear how one would measure body size, handling time, or gape size of, say, cytotoxic T-cells fighting liver cells infected with hepatitis virus. Another complication is the wide range of within-host scales, with interactions involving components from molecules to tissues to populations of organisms. How descriptions of interactions among parasites should account for biological scale and different processes compared with free-living systems needs further work.

The typology I have presented in terms of direct, or resource- or immune-mediated also needs to be critically examined. The dichotomy this thesis has posed between resources and immunity as bottom-up and top-down respectively is blurred in at least five ways. First, many globally serious infections inhabit immune components, such as HIV infecting CD4

T-cells, or *Mycobacteria* infecting macrophages. Second, definitions of the immune system are broad, with some researchers considering anti-parasite behaviour relating to their offspring's food source to be immunological (Kacsoh et al., 2013). Third, both immune and resource components are limited by the host's energy intake so that there is a tradeoff between immune investment and tolerance of metabolic losses (McFall-Ngai, 2007; Rauw, 2012). Fourth, parasites' resource use can be determined by the host's immune phenotype, such as interferon type determining whether leprosy produces small, localised lesions or more disseminated disease (Teles et al., 2013), or *Salmonella* attracting neutrophils that lyse the red blood cells inhabited by malarial parasites (Cunnington et al., 2012). Fifth, immune components can be restricted to particular body parts, such as regulatory immune cells being specific to an organ (Malchow et al., 2013). Distinguishing between resources and immunity is therefore a non-trivial matter.

These ambiguities between immunity and resources affect the interpretation of Chapter 3's results in two ways. First, nodes like CD4 cells and macrophages are only classified as immune, even though they may be inhabited by many parasites. Consequently, the undirected links between immune components and parasites could reflect energy flows in either direction. Second, the module detection algorithms used in Chapter 3 mean that immune components like T-cells, macrophages, or antibodies are only assigned to a single module, even though they circulate around many parts of the body.

Future research could overcome some of these limitations by assigning direction(s) to indicate whether it is the immune component reacting to the parasite, or the parasite infecting the immune component (or both, in a bidirectional link). If the strength as well as the direction of each interaction were estimated, prediction of treatment effects would likely be more accurate (Novak et al., 2011), and the Bayesian clustering methods mentioned above could be used to allow nodes to appear in multiple modules. However, inferring interaction strength is particularly problematic for coinfecting parasites because, as mentioned above, there is no consensus on the functions and parameters to describe them. Even with

appropriate functions, gathering data to quantify the strength of hundreds of links in the network presented in Chapter 3 would be arduous. An initial option is to quantify interaction strength for a subset of the network. Even so, detecting interactions between parasite species is nontrivial.

How to detect parasite interactions

When publications report interactions between parasite species, this is often based on statistical associations (e.g. higher than expected rates of coinfection) rather than one species causing changes in the population size of coinfecting parasites (Poulin, 2001). However, establishing whether this association is caused by an interaction within a coinfected host is nontrivial. Much evidence is needed before concluding a particular interaction is occurring, as seen with debates over HIV-*Mycobacterium leprae* interactions (Hohmann and Voss-Böhme, 2013; Massone et al., 2011), interactions between sexually-transmitted viruses (Bollen et al., 2008; Cohen, 2006; Rotchford et al., 2000), and why differences in between single- and co-infected hosts appear at certain times post infection (Fairlie-Clarke, 2011). Furthermore, appropriate statistical methods for studying parasite interactions are strongly debated (Fenton et al., 2010; Hellard et al., 2012; Johnson and Buller, 2011).

My thesis work adds two further considerations for research into parasite interactions: potential non-response of perturbed systems, and variability through time and between hosts. I found in Chapter 5 that treatment effects on non-target parasites depended on the direction of an interaction, but that the absence or dampening of such a treatment effect did not necessarily indicate the absence or weakening of an interaction. Even when treatment effects were observed, the strength of the underlying interaction was sometimes obscured by temporal and demographic stochasticity. While binary questions of whether or not there is an association, or categorisation of coinfection effects into positive, negative, and neutral seems intuitive and is the focus of much debate, my research shows that we also need to consider the identity of the parasites involved, the mechanisms that link them, and how variable parasite populations are. Understandably coinfection in humans is often described as complex.

Coinfection is a complex system: how much do we understand?

Complex systems, such as the global climate system, brains, and human economies, have diverse and aggregated components, stable states, and locally intense, often nonlinear, interactions (Levin, 1998). Throughout the thesis I have shown that coinfection in humans, like communities of free-living species, has many if not all these characteristics of complex systems. Previously, the potentially overwhelming diversity of parasites and interactions among them had been spoken of, but evidence of complexity was lacking.

In terms of diversity, a survey of known human parasites numbered > 1400 species (Taylor et al., 2001). Further to this, the dataset collected in a single year of publications demonstrated that there are hundreds of different reported species co-occurrences, and in the human coinfection network built from these publications the number of nodes (infections) was still linearly increasing. In addition to the great diversity of parasites found, the network I analysed in Chapter 3 showed elements of robustness, such as an exponential distribution, weak, if any, assortativity, and compartments, which suggest that perturbations, such as drug treatments or vaccination, will affect few other nodes. Stability in terms of resistance was also found in the model of a two-species parasite community in Chapter 5, in that parasites were resilient to one-off treatment, and resistant to extinction even under repeat treatment. Lastly, the network had localised parasite interactions with 10 modules of interacting nodes largely centred around certain bodily sites. This thesis has advanced understanding of coinfection as a complex system, but treatments based on interactions among parasites are still rare.

Challenges to treating the complex system

Translating studies of complex systems into treatments is challenging for many reasons (Schadt and Björkegren, 2012). For coinfection in humans these include: (i) many components performing the same functions, (ii) multiple, nonlinear processes occurring simultaneously, and (iii) many in-

direct interactions confounding predictions of treatment effects. Studies of coinfection need to recognise that these three issues are inherent to complex systems, and find ways to deal with them.

In the immune system, different components perform the same function, such as multiple cytokines causing the same response in target host cells (Segel and Cohen, 2001). There is also the possibility that elimination of one parasite could simply open a niche for a different parasite to invade. Ideally treatments should be robust to this, but knowing that two or more nodes in a network share the same function is difficult a priori without experiments that knockout each node in turn. Such tests are often precluded in humans on ethical grounds, but the use of laboratory animals could be model systems.

I attempted to overcome these difficulties by representing coinfection within humans in silico using a network and a simulation. As mentioned above, there is no consensus over which functions should describe within-host interactions either in the model developed in Chapter 5, or for quantifying interaction strength in any future version of the network in Chapter 3. Where nonlinear functions for within-host processes are posited, as in the model in Chapter 5 following the models of Fenton et al. (2010) and Hauzy et al. (2010), the necessary data to parameterise models in humans are lacking. Thus I developed a theoretical model for a range of helminths species with different interspecific interactions. The results from this model showed that complex treatment responses are possible including non-monotonic or flat relationships with increases in the interaction parameter. When multiple nonlinear processes like density dependent establishment, fecundity, and immune attack occur simultaneously, perturbations like drug treatment might have counterintuitive effects. Improving our understanding of within-host biology, modelling it, and using the models to make predictions for treatment programs is therefore vital.

To translate model simulations into recommendations for real treatments the model presented in Chapter 5 is only the beginning. Future versions of the model could include more diverse parasite communities and species with different life histories, say varying in virulence, or having other hosts or vectors. Other treatment types could be modeled, such as

vaccination, nutritional improvements, or only treating high burden hosts. Two-way interactions can also be modeled where both species affect the another, as has been done for HIV and malaria coinfection (Alemu et al., 2013). However, the major caveat to such work is that, as I showed in Chapter 5, the simulated effects of treatment are sensitive to nonlinearities. Establishing the nonlinear functions of within-host processes, and collecting data to infer parameters are crucial.

Another potential caveat is that prediction might be difficult even when interspecific interactions are quantified. Perturbations of models of ecological communities of more than two species where the only process is population growth are predicted to often have effects of indeterminate direction and size (Yodzis, 1988), even with recent technical and computing advances (Novak et al., 2011). Nevertheless, this thesis indicates that whether non-target parasite populations in patients coinfected by a pair of parasites are higher or lower after treatment might be predictable when there is one-way interaction between the parasite species. While the impact of treatment on the wider parasite community of humans might be difficult to determine, decisions about target or integrated treatment that are informed by parasite interactions could be made for pairs of parasites and in individuals.

Personalised networks of coinfection

To test the potential for treating pairs of coinfecting parasites in terms of their interspecific interaction, first we would need to observe such coinfection systems in individuals, and then compare their outcomes with what was simulated by a computer model. Simulations of a realistic model could suggest the optimal treatment of coinfected patients that could then be tested by a medical trial. This could be part of a translational mathematical modelling branch alongside current efforts to incorporate new information for more personalised medical decisions (Auffray et al., 2009; Loscalzo, 2011; Ma et al., 2011; Milgrom and Tran, 2010). This could also extend the findings from the summary network in Chapter 3 to reveal the variability between individuals and their disease progression, which may require more flexibility in coinfection treatment strategies.

A move towards personalised networks would also incorporate estimates of interaction strength and various possible interventions, enabling evaluation of different treatment options. For example, many studies of hepatitis focus on interactions between HBV and HCV, but HDV coinfection and alcohol consumption have bigger effects on liver damage (Sagnelli et al., 2012). Hepatitis would be a good coinfection for developing networks to predict the outcomes of different treatment options, given that I found in Chapter 2 that almost two fifths of 2009 coinfection publications included hepatitis coinfection.

How could treatment of coinfection be improved?

Commensal parasites are being targeted by treatments to benefit host health (e.g. *Wolbachia*, Taylor et al. (2010)). Whether interactions between pathogenic parasites can be translated into treatments is still unclear. However, having reduced the complexity down to a theoretical model involving only two parasite species, I can make some of the first predictions of the general outcomes of species-specific treatment in coinfected populations. Results of simulations from Chapter 5 showed that reductions in non-target parasites can occur when a positively interacting parasite is treated. Coinfection, even at high prevalences, is therefore not sufficient justification for an integrated treatment program because targeting treatment at a positively interacting parasite can indirectly reduce the burden of coinfecting parasites.

Deciding on integrated treatment based solely on parasites co-occurring is therefore questionable; the presence of positive interspecific interactions should also be tested for. Treatments that target facilitatory parasites would be expected to indirectly reduce morbidity from non-target parasites. Extrapolating this finding from the model of helminth coinfection in Chapter 5 to other parasite types that are thought to interact positively, such as bacterial coinfection being fostered by malaria or influenza, this calls into question recommendations for integrated treatments. Such recent recommendations included giving antibiotics for suspected bacterial coinfections in malaria or influenza patients (Sandlund et al. (2013) and Davies (2011) respectively). Further tests for interactions and new

models of treatment of microparasite coinfection are needed. Ideally randomised trials comparing the outcomes of targeted and integrated treatments should be done before such medical guidance is given. At a minimum, data recording the multiple diseases that simultaneously affect patients should be collated (Sánchez et al., 2010; Schur et al., 2011). With more data on the disease trajectory of coinfected patients and their treatments, we could compare infected and coinfected patients, and coinfected patients receiving different treatments with fewer sampling, reporting, or publication biases.

Future research opportunities

The research in this thesis pioneered a broader approach to coinfection research, and has identified many avenues for future research.

Patterns of coinfection and related diseases

To further evaluate the general effects of coinfection on human health, more datasets are needed. The analytical approach of Chapter 4 could be extended by comparing coinfecting parasites in living patients with those reported on death certificates, analysing death certificates from other countries and years, or looking at other causes of death. For example, coinfection deaths could be associated with noncommunicable diseases because there are similarities between health services for infectious and noncommunicable diseases (Bygbjerg, 2012), the human immune system fights parasites, cancer, and autoimmune disease (Thompson, 1995; Vincent, 2006), and various infections can lead to cancer (Parkin, 2006). In addition, more systematic reviews of the human coinfection literature for years before and beyond 2009 would likely expand the database of parasites known to coinfect humans.

Evolution and treatment of complex systems

Studying complex systems often means a move toward big data and large networks, but these can be technically challenging, and, perhaps more im-

portantly, the biological understanding is often lacking (Anderson, 1994; Callebaut, 2012). Theory of coinfection as a complex system could be particularly advanced by study of evolution. This thesis has only considered the population dynamics arising from parasite interactions, but how the parasites, and, in turn, their interactions, evolve should also be studied. The evolution of resistance to drugs is a particular challenge for treatment of infectious diseases (Baquero, 1997; Hirsch et al., 1998; Koul et al., 2011). Pertinent to coinfection treatment, broad-spectrum drugs or combinations of drugs used in integrated control programs may select for parasites resistant to the most widely used drugs. For instance, applying antibiotics that target diverse coinfecting bacteria has been attributed to severe infections like necrotising fasciitis being increasingly dominated by single bacterial strains resistant to multiple antibiotics (Tsitsilonis et al., 2013). The double complication of coinfection with two differently resistant bacterial types is also increasingly common (Meyer et al., 2011). Whether administering multiple treatments to coinfected patients selects for resistance at a different rate from patients with single infections or coinfected patients receiving a single treatment needs further monitoring and modelling. While adding evolution to parasite interactions often makes the problem less tractable (Woolhouse et al., 2002), evolutionary feedbacks such as the evolution of resistance to drugs could easily be added to the model I developed in Chapter 5. This research is urgently needed to inform prudent drug treatments in coinfected patients.

Coinfection diagnosis and treatment in practice

The possibility that current treatments might be unnecessary or counter-productive must be tempered, given the dearth of data on how coinfected patients are being treated. The datasets used in Chapters 2 to 4 included treated individuals, but how their parasite communities were changed by treatment is unclear. Studies of how coinfected patients are diagnosed and treated in clinical practice would enable assessment of how parasite communities are being perturbed. Diagnosis of coinfection is often reported as occurring solely by symptoms, or once a patient's condition did not improve after initial treatment (Alavi et al., 2012; Baba et al., 2013;

Xuefei et al., 2013). How often does this occur? Which diagnostic tests are done first? Once diagnosed, how are coinfection treatments decided? What is the relative importance of patient condition, patient characteristics like age and sex, and guidelines for treatment of the infections? What are the strategies used by physicians treating patients with multiple diseases besides infection (Fortin et al., 2007), and can they be applied to coinfection?

As well as understanding parasite interactions within coinfected patients, we also need to find how social and clinical environments affect parasite communities parasites (Utzinger et al., 2011; Waldman et al., 2013). The model presented in Chapter 5 could be made more relevant to public health by considering societal inequalities, risky behaviours, and poor environments. Coinfected individuals are often from marginalised groups like criminals or drug abusers, and these social factors contribute to the particular vulnerabilities of these individuals to coinfection (Freudenberg and Galea, 2006; La Fleur et al., 2012; Ruan et al., 2004; Singer, 2009; Tshikuka Mulumba et al., 2012; Zhou et al., 2012). Reducing social inequalities and risk factors is one of the most promising avenues for reducing HIV-HCV coinfection (Klein et al., 2012). Models could thus be expanded to consider the role of altered social structure, sanitation, or needle exchange in coinfection dynamics. These models often rely on biomedical indicators (e.g. parasite abundance) to indicate health, but social attitudes to health will affect the behaviour of coinfected patients and their clinicians and the use of model findings in treatment programmes.

Research into biological systems like coinfection often dehumanises and objectifies coinfection, but social factors like emotion, culture, and stigma around diagnoses are important for patients (Daftary, 2012; Farrell and Comiskey, 2013; Hansen et al., 2007; Rödlach et al., 2012; Singer, 2009; Singer and Clair, 2003), and can affect compliance with treatment (Allen and Parker, 2011; Parker and Allen, 2011; Parker et al., 2008; Soriano et al., 2004). How can compliance with multiple treatments for multiple infections be maximised? What is the effect of patients' noncompliance on health outcomes? While health is defined in this thesis as the presence of disease causing morbidity or mortality, in other discourses "health" in-

cludes more social aspects to mean either complete wellbeing, or, increasingly, how resilient individuals are to changes in their lives (Jadad and O'Grady, 2008). There is considerable disagreement within and among patients and professionals how to define health (Tikkinen et al., 2012). How do coinfected individuals view their health? Do other measures of health also differentiate between coinfected and single infected individuals, and for which kinds of parasites? Exploring social aspects of coinfection is an important next step because human behaviour affects individual health, treatment compliance, and the parasite community.

Final conclusions

Through the varied datasets, methods, and investigations presented in this thesis, I have shown that coinfection, while diverse and complex, tends to have detrimental effects on the morbidity and mortality of human hosts. By building a network of parasites, host resources, and host immune components, I was able to show that most interactions among coinfecting parasites appear to be indirect, and are clustered around shared sites of infection. Simulations of an agent-based model showed that, despite the indirect nature of resource- and immune-mediated interactions, if we knew the direction of an interaction, we could often predict treatment outcomes of non-target parasites. This opens the way for recommendation of optimal treatment regimes based on interactions between pairs of parasite species. If there is a positive interaction between the parasites then specific treatment of the facilitatory species may be sufficient. If there is a negative interaction, co-treatment may be advisable. Most pairs of parasites appear to not be associated, but given the severe harm to host health in terms of morbidity and mortality we need to find the types of parasites that do co-occur more frequently than expected and that interact to damage host health. Taking a broader view of interspecific interactions in coinfected humans has opened up many more avenues for study of this complex system, including how treatment can be improved to maximise host health.

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Appendix: Agent-based model from Chapter 5

Purpose

The purpose of this model was to explore how treating one parasite affects non-target parasite abundance, and overall host morbidity, under a range of interaction scenarios. Specifically the model explores the effects of antihelminthic drug treatment applied singly or repeatedly across a range of parasite interaction strengths, mechanisms, and directions in populations with low, medium, and high coinfection prevalence.

State variables

State variables were: each host's exposure to two parasite species, the number of adults of each species (parasite abundance), size of the specific immune response to each species, number of eggs released by each parasite population into the environment (Fig. 5.2). The size of the environmental pool of each parasite species, and each host's age and mortality risk were also calculated each timestep.

Processes

The model represents a village-sized human population living in a common environment with two direct-transmitted endemic helminth species. Each timestep includes a full parasite life cycle comprising transmission from environment to hosts, establishment in hosts, immune response of hosts

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to parasites contributing to parasite mortality and reduced fecundity, and reproduction of surviving parasites releasing eggs from hosts into the environment. This model structure reflects a typical macroparasite life history where host exposure, establishment of parasites, and density dependence regulate helminth infections in sheep (Grenfell et al., 1987) and humans (Basáñez and Boussinesq, 1999).

The host population is assumed to be at equilibrium, with every host having the chance of dying each timestep, calculated from a background age-dependent mortality rate and the combined burden of both parasite species. Dead hosts were immediately replaced by identical, but uninfected new hosts (age 0). 70% of randomly selected hosts received treatment during treatment timestep(s), reflecting treatment coverage similar to other repeated mass drug administration programs (Mathieu et al., 2006; Wanji et al., 2009). The model was iterated for 500 hosts until state variables and host age distribution reached equilibrium (timestep 45).

Permanent residence in dense populations has been an important driver of infectious disease dynamics in human history (Dobson and Carper, 1996), but I chose a village-sized population because mass drug administration to a majority of the host population is more feasible here, and such settlements represent nearly half of the world population who live outside large urban areas. Modeling a population of 500 hosts is large enough to include a range of parasite susceptibilities, while also being computationally tractable. Submodels (see below) of parasite life stages were iterated in the same order every timestep for each host (e.g. infection, establishment within host, immune stimulation, death and immune attack on worms, parasite reproduction, immune attack on eggs, then possible host death). The order of hosts to which this sequence of submodels was applied was randomized in each iteration. Once at equilibrium the hosts were treated with antihelminthic drug for either one timestep (one-off treatment) or each timestep (repeat treatment) until the model run ended 10 timesteps later. Each parameter set was simulated 50 times, after which point the mean of all state variables was at equilibrium.

Design concepts

The model is a double-programmed, updated R version of the Fenton et al. (2010) Mathematica model of two macroparasites interacting within a mammalian host population. Such independent rewriting of code improves confidence in model features and results (e.g. Magori et al. (2009)). Parasite life history stages and their order are unchanged between the two models. Modifications include adapting the method for correlating host exposure rates for both parasites to allow for negative correlation, adding resource-mediated interactions, increased host longevity and increasing risk of host death with age (to represent typical human survivorship rather than the constant higher mortality rates of the original shorter-lived rabbits or sheep), and including treatment in the model.

Correlated exposure to infection

Each host has a lifelong exposure score to each parasite species that is a probability of that host encountering parasite larvae in the environment, reflecting fixed genetic, environmental, or behavioural risk factors to infection (Bensted-Smith et al., 1987; Bundy et al., 1987; Hall et al., 1992; Jong-Wook, 2003; Quinnell et al., 2001; Schad and Anderson, 1985). Host exposure to the two parasite species could be correlated (positively or negatively) to represent hosts with certain tendencies or environs that foster coinfection of similar parasite species like shared transmission routes (positive correlations in exposure) or differential exposure to the parasites like asynchronous parasite phenology (negative correlations). The values of the correlated exposure parameter were -0.9 (strong negative correlation, for low coinfection prevalence), 0 (no correlation, medium prevalence), and $+0.9$ (strong positive correlation, high prevalence). Correlated exposure is an association based on between-host parasite ecology, and is not a within-host interaction between parasite species. In other words although infection rates can be correlated in the host population, they behave independently within individual hosts unless an interaction is added.

Host immunity and immune-mediated parasite interactions

Immune responses associated with helminth infection are complex and some may be defective (Schweitzer et al., 1992). Following other models of immunity to parasites, details like particular cell types are ignored, and the size of an overall immune response is modeled. This immunity increases with parasite density (Kepler et al., 2009), and with cumulative experience of infection, albeit with some decay of immune memory with time post infection (Anderson and May, 1992). Immunity to each parasite accumulated over each host's lifespan at a rate determined by that parasites cumulative population size within that host (Haswell-Elkins et al., 1989), but immune memory decayed slowly over time so that immunity diminished without parasite stimulation (see Bleay et al. (2009)). Host immunity had a large negative effect on parasite numbers by increasing adult parasite mortality and reducing per capita fecundity, concuring with macroparasite infections in mammalian models (Bleay et al., 2007; Paterson and Viney, 2003; Roberts, 1999; Stear et al., 2007).

In simulations where the parasite species interacted via immunity, the crossimmunity parameter ranged from −1 (immunosuppression proportional to entire abundance of target parasite) to +1 (enhancement of immune response proportional to entire abundance of target parasite), reflecting differences in immune affinity caused by one parasite species (following Fenton et al. (2010)).

Parasite density and resource-mediated interactions

The model also included the possibility of a resource-mediated interaction. Progression of parasite life stages can change with parasite abundance (density), affecting establishment, growth, survival, and reproduction of each parasite species (Medley and Anderson, 1985; Paterson and Viney, 2003; Shostak and Scott, 1993). In the model non-target parasite species' density could also affect target species' density. This mimics one-way resource availability without tracking resources explicitly (Krebs, 1995). A density-dependent function was used where the rate of parasite establishment or fecundity of parasite species i at timestep T ($E_{i,T}$) varied with

total parasite density either positively or negatively using equation 2 from Hauzy et al. (2010):

$$E_{i,T} = E_{max} \cdot \frac{D_T{}^{x_i}}{D_{half}{}^{x_i} + D_T{}^{x_i}} \tag{6.1}$$

where E_{max} is the highest proportion of larvae that can establish or worms that can reproduce, D_{half} is the density of total parasites when half the maximum establishment or reproductive rate is reached, D_T is the total parasite density at time T, and x_i is the shape parameter, that is the effect of parasite density either positive or negative on parasite i. The shape of the function can represent competitive $(-1 < x_i < 0)$, facilitative $(0 < x_i < 1)$, or no interaction between the parasites $(0$, Fig. 6.1). As well as allowing the density of the target species to affect the non-target species, intraspecific density dependence is modeled so that mortality rates increase and fecundity decreases at higher conspecific densities (following studies of hookworm, Kotze and Kopp (2008); Norozian-Amiri et al. (1994)).

Treatment

Single ("pulse") and repeated ("press") treatments (*sensu* Bender et al. (Bender et al., 1984)) were simulated. Sometimes treatments are slightly effective at killing coinfecting parasites (e.g. Waikagul et al. (2005)), but for simplicity I assumed that a drug only directly affects the target parasite and has no effect on non-targets. Treatment was applied at specified time(s) to a random sample of 70% of hosts after parasite dynamics had equilibrated. Treated hosts were assumed to clear their adult worms for that timestep, representing a totally effective dose of antihelminthic drugs, like praziquantel for schistosomiasis, ivermectin for onchocerciasis, or albendazole for hookworm. The effect of treatment was instantaneous (i.e. same timestep, not persisting to the next timestep); consequently susceptible hosts can be reinfected. Mass drug administration was modeled because this is WHO-recommended practice (Crompton, 2006) and is generally more effective than selective or targeted treatments at reaching infected individuals and reducing mean worm abundance (Richardson
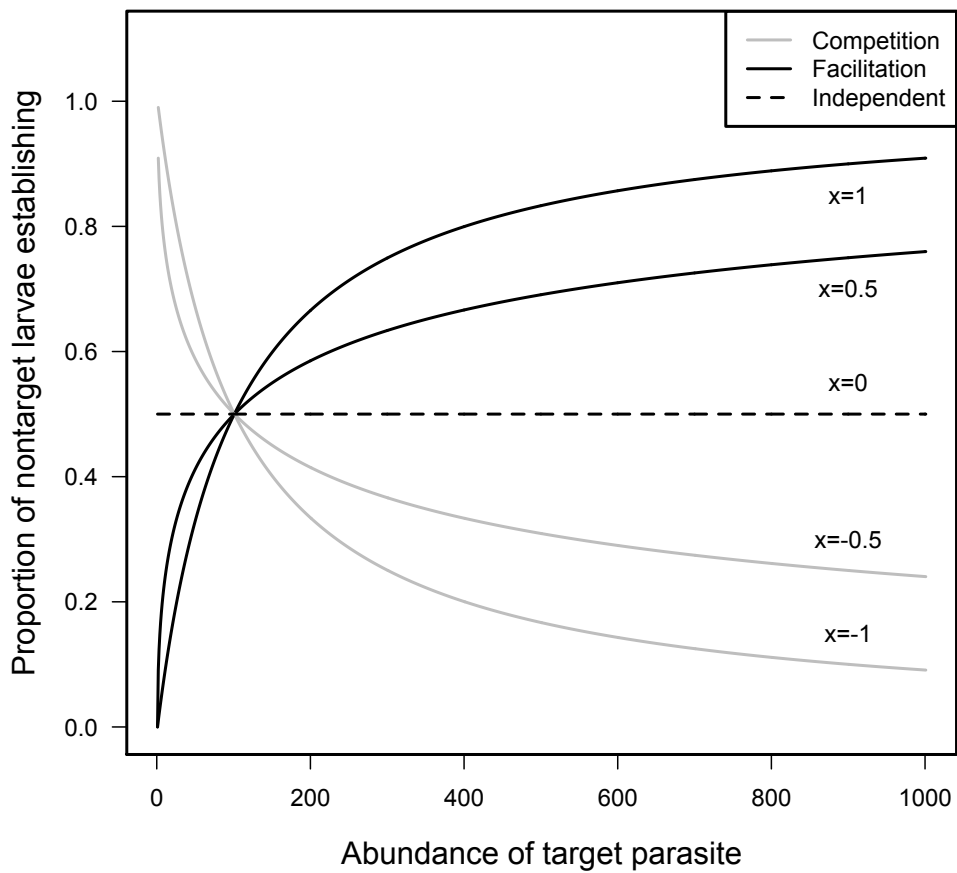
Fig. 6.1: Response of the establishment rate (E) of the non-target larvae to the abundance of adult target parasites depending on the shaping coefficient, x. Follows equation 6.1. This density dependent function is also applied to fecundity.

et al., 2011), even when subtotal enrollment is accounted for (Asaolu et al., 1991; Olsen, 1998).

Stochasticity

There were four stochastic aspects to the model that enable tests of treatment in varying host populations: (i) the initial assignment of host susceptibilities, (ii) the order of hosts at each timestep, (iii) demographic stochasticity by rounding population size of parasite adults and eggs to integer values, and (iv) host death. The demographic stochasticity from discretising numbers of adult parasites and their progeny is especially important for modeling infections at the individual host scale when parasite numbers within each host are small ($n < 100$), such as gastrointestinal helminths of humans. All parasite dynamics were deterministic, i.e. transmission, reproduction, parasite mortality, and interspecific interactions.

Initialisation

The model was first parameterised with the values of Fenton et al.'s theoretical model (Fenton et al., 2010). New parameters, such as for host mortality, were set at biologically realistic values (Table 6.1). Host exposure was assigned from the negative binomial distribution, which is a good statistical model for worm burden (Crofton, 1971; Richardson et al., 2011), of which host genetic susceptibility is a major driver (Holland, 2009; Wilson et al., 2002).

Input

The model was simulated for each parasite interaction term in intervals of 0.05 between -1 and $+1$. All functions were applied at every timestep to every host, except for treatment, which was applied at specified time(s). Each set of parameters was simulated 50 times.

Table 6.1: Values of parameters used in the Individual Based Model. Notation corresponds with the equations in the Supplementary Methods, with - denoting that there is no relevant equation presented.

Parameter	Notation	Value
Number of hosts	-	500
Number of parasite species	-	2
Number of timesteps	-	100
Replicate simulations	-	50
Host exposure	P	
Mean for negative binomial distribution	-	1
Dispersion for negative binomial distribution	-	6000
Coexposure to both species	-	$-0.9, 0, 0.9$
General parasite dynamics	-	
Initial environmental egg pool		1000
Density dependent shaping parameter	x	-1 to $+1$
Maximum fecundity and establishment rate	E_{max}	1
Parasite density for half density dependent rate	D_{half}	40
Adult parasite survival rate	S	0.8
Immune decay rate	δ	0.5
Adult parasite fecundity	F	200
Immune effect on adult parasite survival	SI	0.001
Immune effect on adult parasite fecundity	FI	0.001
Environmental egg pool decay rate	-	0.75
Parasite-specific immune responses		
Impact of parasite 1 on immune response to parasite 1	$\gamma_{1,1}$	$+1$
Impact of parasite 2 on immune response to parasite 2	$\gamma_{2,2}$	$+1$
Impact of parasite 1 on immune response to parasite 2	$\gamma_{1,2}$	-1 to $+1$
Impact of parasite 2 on immune response to parasite 1	$\gamma_{2,1}$	0
Host mortality		
Maximum host life expectancy	M	70
Virulence inflexion point	X	2000
Treatment		
Effect of drug on adult parasite number	-	1
Proportion of hosts treated	T	0.7
Timestep when treatment started	-	50

Submodels

Parasite life history stages and host mortality were calculated for each host at each timestep in the same order as the following submodels. These submodels correspond to those of Fenton et al. (2010), with some modifications detailed below.

Host exposure to parasites

Individual host exposure to parasites is modeled by a probability of infection sampled from a negative binomial distribution ($X \sim \text{NB}(\mu, k)$, with mean μ and dispersion parameter k). I converted each integer into a probability by dividing by the total. These randomly assigned probabilities were shuffled to create coexposure using the Iman Conover method until exposure was rank correlated as close as possible to a target correlation (Iman, 1982) (using the R package mc2d version 0.1-8 (Pouillot and Delignette-Muller, 2010; Pouillot et al., 2010)). This alteration to the method of Fenton et al. enables negative and positive correlation.

Larval parasite transmission

The number of parasite larvae of parasites species i ingested by a host (N^U) at timestep T is given by:

$$N_{i,T}^U = P_i \cdot L_{i,T} \tag{6.2}$$

where P_i is host exposure to species i, and L_i is the number of parasite larvae of species i remaining in the environmental pool from the previous timestep. In this function larvae remaining in the environment do not die between timesteps. Larvae of nematodes infecting sheep like *Ostertagia spp.* are known to live on pasture into the following year (Boag and Thomas, 1970; Gibson and Everett, 1972; Gibson et al., 1967; Gulland and Fox, 1992), and, depending on climate, larvae can survive round the year (Gupta et al., 1987; O'Connor et al., 2006).

Establishment of parasite larvae

The number of ingested larvae of species i establishing and reaching maturity within a host at timestep T $(N_{i,T}^{A})$ is given by:

$$N_{i,T}^{A} = N_{i,T}^{U} \cdot E_i \qquad (6.3)$$

where E is the larval establishment rate for species i.

Specific host immune responses

The magnitude of a host's specific immune response (I) of species i at time T is given by:

$$I_{i,T} = I_{i,T-1}(1 - \delta) + \gamma_{i,i} \cdot N_{i,T}^{A} + \gamma_{j,i} \cdot N_{j,T}^{A} \qquad (6.4)$$

where δ is the decay rate of immunity per unit time, j is the other parasite species (i.e. not species i), γ is the effect of the first subscripted species on the immune response to the second subscripted species, and N^{A} is the number of adult parasites infecting the host.

Adult parasite survival

The number of adult parasites of species i in a host at the end of timestep T $(N_{i,T})$ is given by:

$$N_{i,T} = (N_{i,T}^{A} + N_{i,T-1}) \cdot S_i.e^{-S_i \cdot N_{i,T-1} - SI \cdot I_{i,T}} \qquad (6.5)$$

where S_i is parasite survival rate of species i, SI is the effect of immunity on parasite survival.

Adult parasite fecundity

The number of parasite eggs (faecal egg count, FEC of species i released from a host in timestep T is given by:

$$FEC_{i,T} = N_{i,T} \cdot F_i . e^{-F_, \cdot N_{i,T} - FI \cdot I_{i,T}} \qquad (6.6)$$

where F is the reproductive rate (per capita fecundity) of an adult parasite of species i and FI is the effect of immunity on fecundity. In each timestep the model predicts that the more adult worms in an individual, the more eggs produced, although the relationship is nonlinear and variable (Fig. 6.2). Hosts die after parasite reproduction so some infected hosts release eggs, but subsequently die and lose all their parasites (eggs > 0 when worms $= 0$ on Fig. 6.2). Many publications report wide variation in individual fecundity and worm abundance relationships, with hosts with few worms releasing many eggs, and hosts releasing few eggs having many worms. The model does not exhibit such wide variation (few points along the axes on Fig. 6.2) because it represents a year, rather than the shorter daily to weekly timescales of most empirical data. Studies of nematodes in sheep show egg counts close to animal death are positively correlated and that eggs and adult parasites follow similar distributions across hosts (Grenfell et al., 1995).

Host mortality risk

The risk of a host's death $P(d_T^Y)$ related to its age Y at timestep T is given by:

$$P(d_T^Y) = 1 - 0.5^{\frac{1}{(M-Y_T)/2}} \qquad (6.7)$$

where M is the maximum host life expectancy. This is the Gompertz-Makeham model and replaces the constant background host mortality risk in the original model (Fenton et al., 2010) with one where mortality risk increases exponentially with host age (Milne, 2008).

Mortality risk was also associated with parasite burden (reflecting immunopathology like inflammation (Finch and Crimmins, 2004), severe
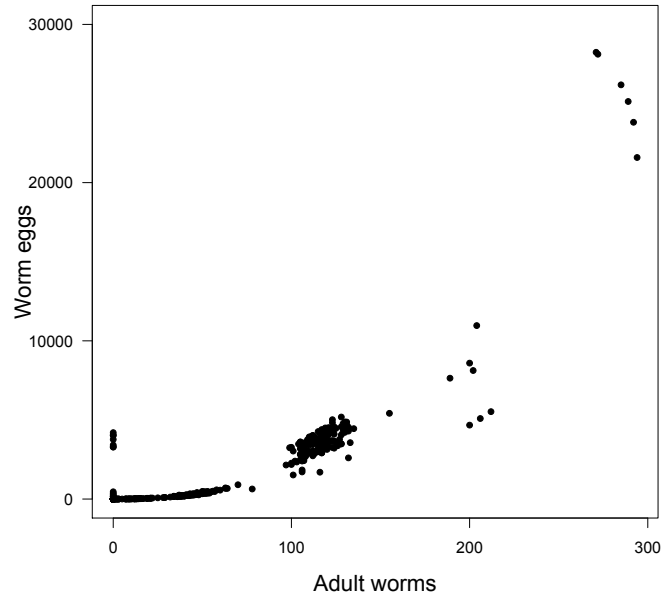
Fig. 6.2: Number of eggs produced in one timestep by adult parasites of the target species for 500 hosts.

malnutrition, organ failure etc.) by a sigmoidal relationship (Equation 6.8). The shape of this sigmoidal curve was equal for both parasite species so that the parasites had equal virulence. The probability of a host's death related to total parasite burden N at timestep T ($P(d_T^N)$) is given by:

$$P(d_T^N) = \frac{1}{1 + e^{(-V \cdot (\sum_i^n (N_{i,T}) - X)}}$$ (6.8)

where n is the number of the parasite species, V is the virulence of adult parasites, and X is the inflexion parameter for the sigmoid curve.

Mortality risks associated with age (Equation 6.7) and parasite burden (Equation 6.8) each contributed to two separate failure functions with a single-draw process determining host death $P(\text{death}) = $ mortality risk.