11 · *Host–Parasite Interactions in African Buffalo: A Community-Level Perspective*

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Introduction

Parasites, spanning viruses, bacteria, helminths, protozoa and arthropods, live within or on a host, often affecting individual host health, survival and reproduction. Furthermore, these individual-level effects of parasites can have consequences that cascade to the population, community and ecosystem levels (Wilson et al., 2019). Historically, host–parasite interactions were studied from a one host–one parasite perspective. However, given that most hosts are infected with more than one type of parasite simultaneously (Cox, 2001), the study of concurrent infection (i.e. coinfection) has gained increasing attention from ecologists, epidemiologists, veterinarians and biomedical scientists (Hoarau et al., 2020; Mabbott, 2018; Salgame et al., 2013). Crucially, wildlife studies occupy a unique niche in this research area because they can help uncover the real-world contexts in which coinfection, and the interactions occurring between coinfecting parasites, are most important (Ezenwa, 2016).

Just like free-living species in ecological communities, parasite species live in communities within their hosts where they interact by competing against or facilitating one another, with consequences for parasite community structure, host health and host fitness (Beechler et al., 2019; Graham, 2008; Pedersen and Fenton, 2007; Telfer et al., 2010). Many of the initial efforts to study parasite interactions in wildlife focused on co-occurrence patterns, revealing that coinfection is common, and that parasites and pathogens interact within hosts both directly and indirectly (e.g. Bush and Holmes, 1986; Lello et al., 2004). For instance, parasites may compete for space or resources (Budischak et al., 2018a; Clerc et al., 2019), such that the presence of one parasite decreases the likelihood

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another succeeds at growth and replication. Alternatively, one parasite may increase the success of another by providing resources or space (Dutt et al., 2021; Zélé et al., 2018). Parasite community interactions are further governed by the host immune response, where cross-immunity may cause one parasite to negatively affect the establishment and growth of another (Raberg et al., 2006), or where one parasite may suppress the host immune response in a way that is beneficial to other parasites (Graham, 2008). Recently, advances in molecular, immunological and statistical methods have enabled an increasingly mechanistic and/or predictive understanding of these types of parasite interactions in wild species (e.g. Clerc et al., 2019; Fountain-Jones et al., 2019; McDonald et al., 2020).

Studies of African buffalo have played a key role in advancing research on wildlife coinfection. Multiple facets of African buffalo ecology and life history make them an excellent system for understanding parasite interactions in free-living animals (Ezenwa et al., 2019). Buffalo are relatively long-lived, large-bodied, gregarious animals that are common throughout sub-Saharan Africa. Furthermore, buffalo are host to a broad diversity of parasites, including bacteria, viruses, protozoa and helminths (Ezenwa et al., 2019). These attributes allow parasite studies to be conducted on relatively large numbers of individuals across multiple spatiotemporal scales (Garabed et al., 2020). Physiological similarity between domestic cattle and African buffalo further enables the use of readily available physiological (Couch et al., 2017), immunological (Beechler et al., 2012) and diagnostic tools (Glidden et al., 2018), as well as therapeutics to measure animal responses to infection, describe parasite community composition (Beechler et al., 2019), and manipulate host–parasite interactions (Ezenwa and Jolles, 2015). In this chapter, we describe insights on parasite interactions derived from the study of African buffalo. Focusing on results drawn from two large studies performed in Kruger National Park (KNP), South Africa (see Box 11.1), we discuss how pairwise and multi-parasite perspectives have been used to understand which parasite taxa interact most strongly, the mechanisms accounting for these interactions and the implications for both hosts and parasites. We also highlight general patterns that have emerged across parasite systems. We outline key technical tools, both computational and laboratory, that facilitate the ability to draw strong inferences and link phenomena across scales. We conclude by identifying future research directions that will help advance scientists' understanding of the causes and consequences of parasite interactions.

Box 11.1 *Studying Parasite Interactions in the Wild*

Experimental and longitudinal approaches are important ingredients for studying parasite interactions in natural systems. By directly manipulating parasites in situ, researchers can identify how co-occurring parasites, as well as hosts, respond to changes in the parasite community and simultaneously investigate factors both internal and external to the host that govern variation in observed responses. Likewise, longitudinal approaches allow for parasite and host characteristics to be tracked over time, providing insight about the order in which events occur and helping to distinguish cause from effect. Either approach is valuable on its own, but in combination, these two methods represent a powerful tool for unravelling the causes and consequences of parasite interactions in free-ranging wildlife. Studies on wild African buffalo in KNP used these approaches to address a range of questions about parasite interactions.

Study 1 followed ~200 free-ranging young female buffalo captured from two herds in southern KNP over a four-year period. The animals were fitted with VHF collars (see Figure 11.1a) with recaptures occurring every 6 months to monitor changes in parasite communities, host physiology, health and performance (see Table 11.1). These animals were captured in the south-eastern portion of the park and animals were allowed to move and disperse as normal (Spaan et al., 2019). A goal of the study was to understand how gastrointestinal worms and bovine tuberculosis (bTB) interact, so half of the study animals received a long-acting anthelmintic drug applied every 6 months to reduce their worm burdens, while the other half were used as controls. Study animals were bTB-free at the onset of the experiment so that effects of anthelmintic treatment on bTB infection incidence and severity could be quantified.

Study 2 followed one herd of ~80 mixed age and sex buffalo, housed in a 900 ha semi-natural, predator-free enclosure in central KNP (see Figure 11.1b) that had been in place since the early 2000s and managed by KNP veterinary wildlife services. In this 'mesocosm' setting, study animals were captured every 2–3 months to collect finer-scale information on parasite communities and host traits. This short capture interval allowed for a better understanding of transmission patterns of microparasites like viruses and bacteria that are quick to spread throughout a population.

(a)

(b)

Figure 11.1 (a) African buffalo fitted with a VHF collar. (b) Double fence surrounding the 900 ha semi-natural enclosure containing the buffalo herd of Study 2.

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Table 11.1 *Measures of health and immunity used in African buffalo.*

Measure	Method	Citation
Physiology and health		
Body condition	Manual palpation of buffalo	Ezenwa et al. (2009)
Pregnancy status	Rectal palpation	Beechler et al. (2015)
Lactation status	Manual milking of teats	NA
Cortisol as a measure of stress	Radioimmunoassay of faecal samples	Spaan et al. (2017)
Haematocrit and red blood cell measurements	Haematological assessment of whole blood	Beechler et al. (2009)
Total protein, albumin, kidney and liver enzymes	Chemistry profile on plasma	Couch et al. (2017)
Immunity		
White blood cell counts	Blood smear on whole blood	Beechler et al. (2009)
Bacteriacidal ability	Bacterial killing assay on plasma and whole blood	Beechler et al. (2012)
Lymphocyte proliferation ability (LPA)	Whole blood LPA	Beechler (2013)
Cytokines (IFNy, IL4, TNFa, IL12)	Enzyme-linked immunosorbent assay (ELISA) of plasma	Beechler et al. (2015); Ezenwa and Jolles (2015); Glidden et al. (2018)
Acute phase proteins (SAA, Hapto)	ELISA of plasma	Glidden et al. (2018)
Total globulins	Chemistry profile on plasma	Couch et al. (2017)

For both studies, samples (e.g. blood, faeces) collected at capture were used to quantify and describe the parasite community. These samples were also used to perform a suite of assays to assess host physiology, immunity and overall health (see Table 11.1). In combination with information about external environmental conditions (e.g. seasonality), the data on host traits and parasites were used to test a range of hypotheses about the nature and implications of parasite interactions. For both studies, animal handling and scientific permits were acquired from appropriate institutions (see Ezenwa and Jolles, 2015 for Study 1 and Jolles et al., 2021 for Study 2 permit information).

Parasite Interactions: Combining Pairwise and Multi-Species Perspectives

Studies of parasite interactions in African buffalo have ranged in scale from studies focused on pairwise parasite interactions to studies examining interactions among multiple co-occurring parasites using taxonomic and trait-based approaches (Table 11.2). In most cases, a key goal of the work has been to uncover how the presence of more than one parasite modifies host and parasite responses to infection. Below, we review these studies to identify notable commonalities across them as well as methods used to develop a multi-parasite perspective. Findings highlight the value of pairwise and multi-parasite perspectives: integrating both perspectives identified immunological and ecological mechanisms underlying pairwise interactions and assessed the relative importance of those mechanisms in more complex parasite communities.

The studies of pairwise parasite interactions cover a broad taxonomic scope, including bacteria, viruses, protozoa and helminths (Table 11.2, Theme 1). They investigate parasite interactions and the consequences for hosts and parasites in real-world settings. For example, in laboratory studies, immunological mechanisms of interaction between parasites are well described, but if and how these interactions manifest in wild populations has been unclear. Early work in African buffalo provided seminal evidence that cross-regulated immune responses can shape parasite population dynamics (Jolles et al., 2008), and that as in laboratory rodents, infection with parasites like gastrointestinal helminths can induce immune cross-regulation (Ezenwa et al., 2010). Studies in the KNP buffalo population (Box 11.1) expanded on this foundation using manipulative experiments and longitudinal tracking of individuals to confirm that in a wild setting, clearance of one type of parasite (gastrointestinal helminths) has ramifications for host immunity and the severity of infection with a second parasite, in this case *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB) (Ezenwa and Jolles, 2015). Another key result from the pairwise studies was the broad importance of bTB on host immunity, health and survival. *M. bovis* infection was associated with lower innate immunity and higher inflammatory cytokine secretion, measured as *Escherichia coli* killing capacity and interleukin-12 concentration, respectively (Beechler et al. 2012, 2015). Accordingly, prior infection with bTB was associated with an increased likelihood of acquiring both *Brucella abortus* (the causative agent of brucellosis) and Rift Valley fever virus (RVF) (Beechler

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Table 11.2 *Summary of research on parasite interactions in African buffalo. The studies span a range of designs, including experimental (E), longitudinal (L) and case control (CC) studies. The design column indicates the study design type and whether the data were derived from Study 1 or 2 as described in Box 11.1. The scale column indicates whether the study focused on two parasites (pairwise), a group of parasites (e.g. helminths or* Theileria*), or all of the parasites that were screened (WC, for whole community). It also describes whether the interaction was between microparasites (micro–micro), macroparasites (macro–macro) or both (macro–micro). The interaction type and effects columns define whether the parasites positively (facilitation) or negatively (competition) influenced each other during a subsequent observation or across multiple observations (succession).*

Parasites (diseases)	Design	Scale	Interaction type	Effects on other parasite/parasite communities/host	Citation
Theme 1: Pairwise studies					
Mycobacterium bovis (bTB) vs. *Brucella abortus* (brucellosis)	L – 1	Pairwise. Micro–micro	Facilitation and competition	Effects on other parasite: Brucellosis infection was twice as likely in buffalo with bTB compared to uninfected buffalo, but brucellosis infection was not correlated with risk of bTB. Mathematical modelling suggests the net effect of bTB on transmission and mortality results in competition at the population-level: R_0 and endemic prevalence predictions for bTB were lower in populations in which both pathogens co-occur. Effects on host: Mortality rates were higher after individuals became infected with either bTB or brucellosis, with the highest risk occurring in co-infected buffalo. Neither infection reduced fecundity, measured by calf observations.	Gorsich et al. (2018),
bTB vs. helminths	E – 1	Pairwise. Micro–macro	Competition	Effects on other parasite: Experimental anthelmintic treatment did not influence risk of bTB. The predicted R_0 of bTB is 8-fold higher in treated populations due to decreases in mortality when worm burdens decline. Effects on host: Mortality rates were lower in buffalo that received the treatment compared to controls.	Ezenwa and Jolles (2015),

bTB vs. helminth resistance	L/E – 1	Pairwise, Micro–macro	Competition	Effects on other parasite: There was no effect of natural host resistance to worms on bTB infection risk. Effects on host: Worm-resistant individuals were more likely to die of bTB than were non-resistant individuals despite having lower worm burdens, and bTB progressed more quickly in the lungs of non-resistant individuals. Anthelmintic treatment moderated but did not eliminate this pattern, implicating 'resistance' to worms and not simply current worm infection as a driver of the interaction.	Ezenwa et al. (2021)
bTB vs. Rift Valley fever virus	L – 1	Pairwise. Micro–micro	Facilitation	Effects on other parasite: RVF infection was twice as likely in buffalo with bTB compared to uninfected buffalo. Mathematical modelling suggests this results in larger and faster spreading RVF outbreaks. Effects on host: Foetal abortion rates were 6.6 times higher in coinfected buffalo compared to infection with RVF alone.	Beechler et al. (2015)
Helminths, namely *Haemonchus* spp. vs. *Cooperia fuelleborni*	E – 1	Pairwise. Macro–macro		Effects on host: Host body condition was lower in buffalo that underwent experimental anthelmintic treatment compared to controls. In controls, increases in *Haemonchus* egg counts were negatively associated with changes in condition, while increases in *Cooperia* egg counts were associated with increases in condition. Neither parasite directly influenced survival or fecundity (likelihood of being pregnant or lactating). However, treated buffalo had higher survival and both treated and untreated buffalo in good condition had both higher survival and fecundity.	Budischak et al. (2018b)
Schistosoma matthei vs. *Cooperia fuelleborni*	–L – 1	Pairwise. Macro–macro	Facilitation	Effects on other parasite: Schistosome burdens varied seasonally. Wet season gains in burden were not correlated with helminth coinfection, but coinfection did influence the magnitude of dry season reductions in burden. Buffalo infected with *Cooperia* maintained higher schistosome burdens throughout the dry season.	Beechler et al. (2017)

(cont.)

Table 11.2 (*cont.*)

Parasites (diseases)	Design	Scale	Interaction type	Effects on other parasite/parasite communities/host	Citation
Theme 2: Multi-parasite studies applying taxonomic approaches					
6 gastrointestinal helminths[a]	–E – 1	Helminths	Succession	Effects on parasite community: After anthelmintic treatment, helminth communities in treated buffalo had a lower total abundance and higher diversity compared to communities in undisturbed control buffalo. With increasing time since treatment, treated helminth communities resembled those found in undisturbed control buffalo.	Budischak et al. (2016)
6 respiratory pathogens associated with bovine respiratory disease[b]	L – 1	WC	Facilitation	Effects on parasite community: Five of the respiratory pathogens were continuously circulating. Viral coinfection was the best predictor of viral infection; host physiology and season had little effect on odds of viral infection. Coinfection with bTB was positively associated with risk of BRSV but none of the other pathogens. Anti-helminthic treatment was not associated with any of the respiratory pathogens.	Glidden et al. (2021)
10 *Theileria* phylotypes[c]	–L – 1	*Theileria*	Facilitation, competition, succession	Effects on parasite community: Interaction networks change over time; young animals are infected with *Theileria* interaction networks composed of many negative and positive interactions, while adult interaction networks are composed of three positive interactions. 7/10 phylotypes exist with 80–90% prevalence in adult animals, this coexistence is likely the result of phylotype-specific immunity and facilitation. Two phylotypes infect young animals early (within 1 month), but are later displaced in adult animals and facilitation.	Glidden et al. (2021),

Theme 3: Multi-parasite studies applying trait-based perspectives					
bTB, brucellosis, 3 haemoparasites,[d] 6 respiratory pathogens[b] coccidia, strongyle nematodes, *Schistosoma matthei*	C–C – 1	WC	–	Effects on parasite community: Animals that acquired bTB experienced a greater increase in parasite assemblage richness and functional richness compared to age-matched buffalo that did not acquire bTB. The traits of parasite communities after bTB were less variable (measured as multivariate dispersion) and dominated by contact-transmitted parasites with simple life cycles and fast replication times.	Beechler et al. (2019),
11 haemoparasites[d] vs. BTB, brucellosis, helminths, coccidia, ticks (*Ambylomma hebraeum*, *Rhipicephalus* spp.)	L – 1	WC	Facilitation and competition	Effects on parasite community: Parasites infecting the same tissue type were associated with the probability of haemoparasite infection (e.g. other haemoparasites). For pairs of haemoparasites, the direction of the association can be predicted based on shared resources, cross immunity, and having a shared vector. In contrast, associations between haemoparasites and parasites infecting other tissue types were weak or non-existent.	Henrichs et al. (2016)
Tick-borne parasites Gastrointestinal parasites Respiratory pathogens	–L – 2	WC	–	Effects on parasite community: Parasite communities follow patterns of succession similar to free-living communities. The median age of first infection differs between parasite taxa, with tick-borne and helminths parasites first occurring in animals less than 1 year old and directly transmitted infections first occurring after 2 years.	Combrink et al. (2020)

[a] *Cooperia fulleborni*, *Haemonchus* sp., *Parabronema* sp., *Trichostrongylus* sp., *Africanastrongylus giganticus*, *Africanastrongylus buceros*.

[b] Bovine adenovirus-3 (AD-3), bovine herpes virus (BHV), bovine parainfluenza-3 (Pi-3), bovine respiratory syncytial virus (BRSV), *Mycoplasma bovis* (MB), *Mannheimia haemolytica* (MH).

[c] *T. parva*, *T.* sp (buffalo), *T.* sp (bougasvlei), *T. velifera*, *T. velifera* B, *T. mutans*-like 1, *T. mutans*-like 2, *T. mutans*-like 3, *T. mutans*, *T. mutans* MSD.

[d] *Anaplasma centrale*, *A. marginale*, *A.* sp. Omatjenne, *A. phagocytophilum*, *Babesia* sp., *Ehrlichia ruminantum*, *Theileria parva*, *T. mutans*, *T.* sp (buffalo), *T.* sp (sable), *T. velifera*.

et al., 2015; Gorsich et al., 2018). These studies provide a starting platform for investigating the consequences of bTB for a wider range of co-occurring parasites. Given the broad effects of bTB on African buffalo survival, health and susceptibility to other parasites, mechanistic models are useful to predict the consequences of bTB for the population-level dynamics of a second parasite. For example, Gorsich et al. (2018) parameterized a mechanistic model of bTB–brucellosis dynamics that represented a host's increased likelihood of acquiring brucellosis and increased mortality rates if the host was also infected with bTB. The model predicted the net consequences of these effects for prevalence and R_0, thereby linking the within-host mechanisms explored in previous papers (Beechler et al., 2012, 2015) to population-level patterns of disease spread.

Further studies investigated multi-parasite interactions by applying a multi-parasite approach (Table 11.2, Themes 2 and 3). These studies can be conceptually divided into those that quantify higher-order association patterns that emerge from complex multi-parasite interactions (Theme 2) and those that simplify complex multi-parasite interactions into generalizable patterns (Theme 3). The former relies on taxonomic approaches where parasites are classified according to their taxonomy, while the latter applies a trait-based perspective where parasites are classified by their biological features, such as how they differ in physiological, morphological or life-history traits. The two approaches reveal how different dimensions of the parasite community respond to change. However, because traits or trait distributions can be directly linked to host or environmental conditions, trait-based approaches allow for more mechanistic predictions about how a community may change in response to coinfection (see McGill et al., 2006 for a review of traits-based approaches in ecology).

Importantly, the multi-parasite approaches (Table 11.2, Themes 2 and 3) and the pairwise approaches (Table 11.2, Theme 1) have proven highly complementary. For example, Beechler et al. (2019) applied a trait-based approach to evaluate how parasite community composition – including 14 parasites ranging from viruses, bacteria, protozoa and helminths – differed before versus after buffalo were infected with bTB. Interestingly, parasite communities tended to have higher taxonomic and functional richness (e.g. unique parasite traits) after hosts acquired bTB infection (Figure 11.4a). Furthermore, while the number of unique parasites tended to increase after bTB, the traits of these parasites were functionally similar to each other, as quantified by

multivariate dispersion. Multivariate dispersion measures the amount of trait space occupied by a given community; thus, after bTB infection, parasite communities became more homogenous in terms of their traits even though the communities contained more species. This pattern was associated with communities becoming dominated by certain traits (e.g. contact transmission, fast replication rate), with less representation of other traits (slow transmission rate, environmental transmission).

Taxonomic studies occurring in the same system corroborate this result. The dominance of contact-transmitted, fast-replicating parasite taxa is supported by a longitudinal, multi-parasite study investigating associations among five contact-transmitted, fast-replicating respiratory pathogens (Theme 2). This analysis showed that after accounting for bTB infection, pathogen co-occurrence explained the largest proportion of variation in three focal viruses (bovine adenovirus-3, bovine herpes virus-1, bovine parainfluenza-3), with all three positively influenced by coinfection (Glidden et al., 2021). Additionally, the lack of slowly transmitted or environmentally transmitted pathogens is supported by pairwise-longitudinal and experimental studies investigating associations between bTB, brucellosis and gastrointestinal nematodes (Theme 1). Coinfection with both gastrointestinal nematodes and brucellosis was associated with higher mortality in bTB-positive individuals (Ezenwa and Jolles, 2015; Gorsich et al., 2018). These examples illustrate the value of combining approaches to investigate parasite communities.

Notable Commonalties Across Studies

Studying diverse parasite interactions can reveal commonalities across combinations of parasites that provide new insight into how the consequences of coinfection manifest in nature. At least one such common thread has emerged from studies of parasite interactions in wild African buffalo – the presence of conflicting outcomes across scales. Two studies of pairwise parasite interactions, one focused on interactions between gastrointestinal helminths and bTB (Ezenwa and Jolles, 2015), the other focused on interactions between brucellosis and bTB (Gorsich et al., 2018), both found evidence that from a host perspective, whether the outcome of coinfection is negative or positive differs at the individual host versus population scale.

Understanding the mechanisms that cause interactions between parasites is a cornerstone of coinfection research, in large part because uncovering these mechanisms should facilitate the development of effective

disease intervention and control strategies. Immunological mechanisms are widely implicated as a driver of interactions between helminth parasites and many microbial pathogens (e.g. viruses, bacteria). For example, in mammals, helminth infections typically trigger a T-helper cell 2 (Th2)-type immune response, but the upregulation of this response can suppress T-helper cell 1 (Th1)-type immune responses directed against microbial pathogens (Mosmann and Sad, 1996). The individual-level repercussions of this immune cross-regulation can include any or all the following: increased susceptibility to microbial infection, faster disease progression and more severe disease (Ezenwa and Jolles, 2011). Moreover, these individual-level effects may scale up to influence the spread of microbes at the population-level if their combinatorial effects are sufficient to alter microbial population growth rates (Fenton, 2008). Consequently, in populations where hosts are faced with concurrent helminth and microbe infection, treating individuals for their worms (e.g. via anthelmintic drug therapy) may be an effective strategy for mitigating the negative health impacts of certain microbial infections and reducing the population-level spread of these microbes (Hotez et al., 2006). However, how eliminating helminths will affect the population dynamics of a coinfecting microbe depends on the net effect of helminths on the different parameters relevant to microbial transmission.

The anthelmintic treatment study of wild buffalo in KNP (Box 11.1, Study 1) tracked the effect of experimental deworming on two key parameters that influence bTB dynamics in buffalo: (i) the probability that an individual becomes infected with the disease, and (ii) the disease-associated mortality rate. Results showed that although treatment boosted buffalo anti-bTB immunity, treated animals were equally likely as untreated controls to acquire bTB (Ezenwa and Jolles, 2015). In contrast, treatment drastically reduced bTB-associated mortality, with treated animals almost nine times less likely to die of their bTB infections compared to controls (Ezenwa and Jolles, 2015). The population-level consequences of these effects were estimated by considering the impact of treatment on the basic reproductive number (R_0) of bTB, a metric that generally reflects how fast a pathogen can spread in a host population. Theoretically, anthelmintic treatment can either decrease or increase the R_0 of bTB, but in this case, treatment was associated with a nearly eightfold increase in bTB's R_0 (Ezenwa and Jolles, 2015). As R_0 is defined as the number of secondary cases produced by a single infected individual in a fully susceptible population, this means that, on average, an anthelmintic-treated buffalo infects eight conspecifics with bTB for every one infected

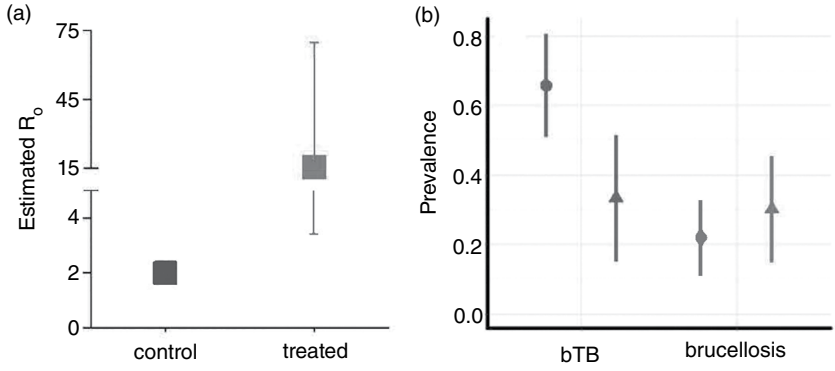

Figure 11.2 (a) The estimated reproductive number (R_0) of bTB in buffalo subpopulations that did (treated) versus did not (control) receive anthelmintic drug treatment. R_0 was approximately eight times higher for treated individuals (2 vs. 15.5), with upper and lower estimates of 3.4 and 69.8, respectively. (b) The estimated prevalence of bTB in buffalo populations with single bTB (left panel, line with circle) or concurrent bTB and brucellosis (left panel, line with triangle) infections. bTB prevalence declined in the presence of brucellosis, but there was no reciprocal effect of bTB on *Brucella* prevalence (right panel, line with circle vs. line with triangle).

by an untreated buffalo. Thus, although anthelmintic treatment has a positive outcome for individual health (i.e. bTB infected buffalo that receive treatment survive better), there appears to be a population-level cost of this strategy in terms of faster bTB spread (Figure 11.2a). This conflicting outcome likely arises because of the asymmetrical effects of worm treatment on bTB infection probability and mortality – as anthelmintic-treated, bTB-infected buffalo live longer (positive individual-level effect), they have more time to spread the disease to others (negative population-level effect). In the real world, therefore, broad-scale anthelmintic treatment and elimination or eradication of helminth parasitism may have unintended effects on the dynamics of certain microbial infections like bTB, despite vastly improving individual health outcomes.

In many cases, the within-host mechanisms underlying interactions between parasites are unknown, and *a priori* hypotheses about potential modes of intervention or control are lacking. Nevertheless, longitudinal studies can be used to understand the impacts of coinfection on both individuals and populations and extract insight about potential consequences of control strategies. Interactions between bTB and brucellosis in buffalo were studied in this way, with results pointing to another intriguing case of conflicting outcomes for individuals and populations. Taking

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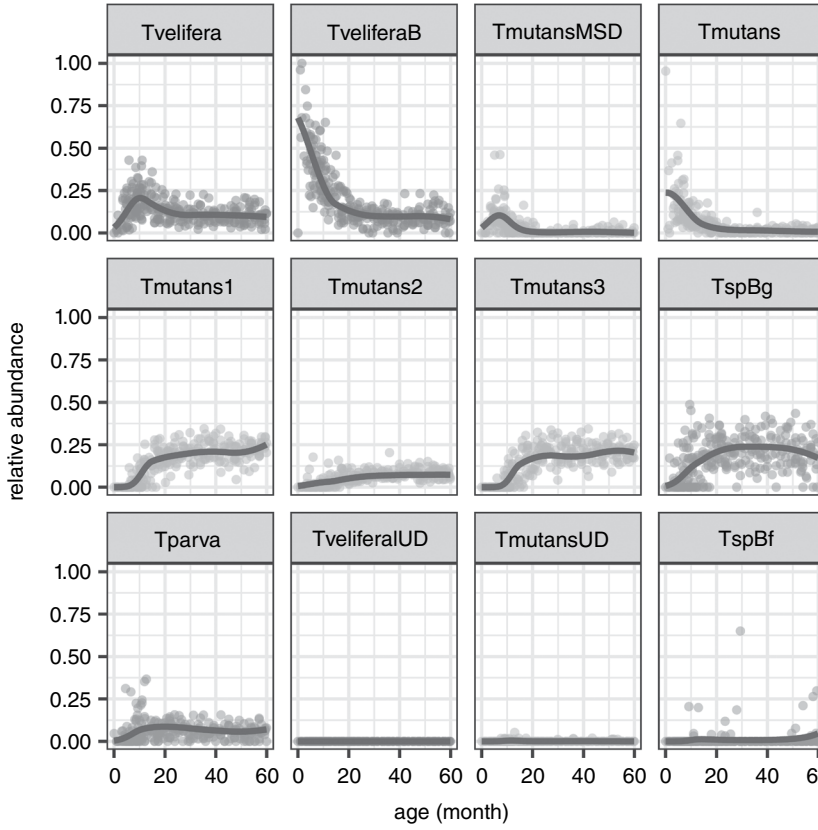

Figure 11.3 The succession of *Theileria* subtypes in African buffalo demonstrates the unique applicability of combining longitudinal study designs with high-throughput sequencing to identify how pathogen communities change over time. By combining infection time series with information on host traits (Table 11.1) we can determine the assembly processes that shape African buffalo parasite communities. Here, the bTB axis represents within-host parasite relative abundance, the *x*-axis represents animal age. The regression line is the output of a general additive mixed model with a Dirichlet-multinomial distribution, allowing for modelling composition and clustered data.

advantage of the coupled longitudinal design of Study 1 (Box 11.1), the interactions between these two chronic bacterial infections on the risk of infection of buffalo to each pathogen and mortality were examined. While bTB infection increased brucellosis risk, there was no consistent effect of brucellosis on bTB infection risk, revealing an asymmetry in the effects of these pathogens on one another (Gorsich et al., 2018). In terms of mortality risk, buffalo infected with both bTB and brucellosis

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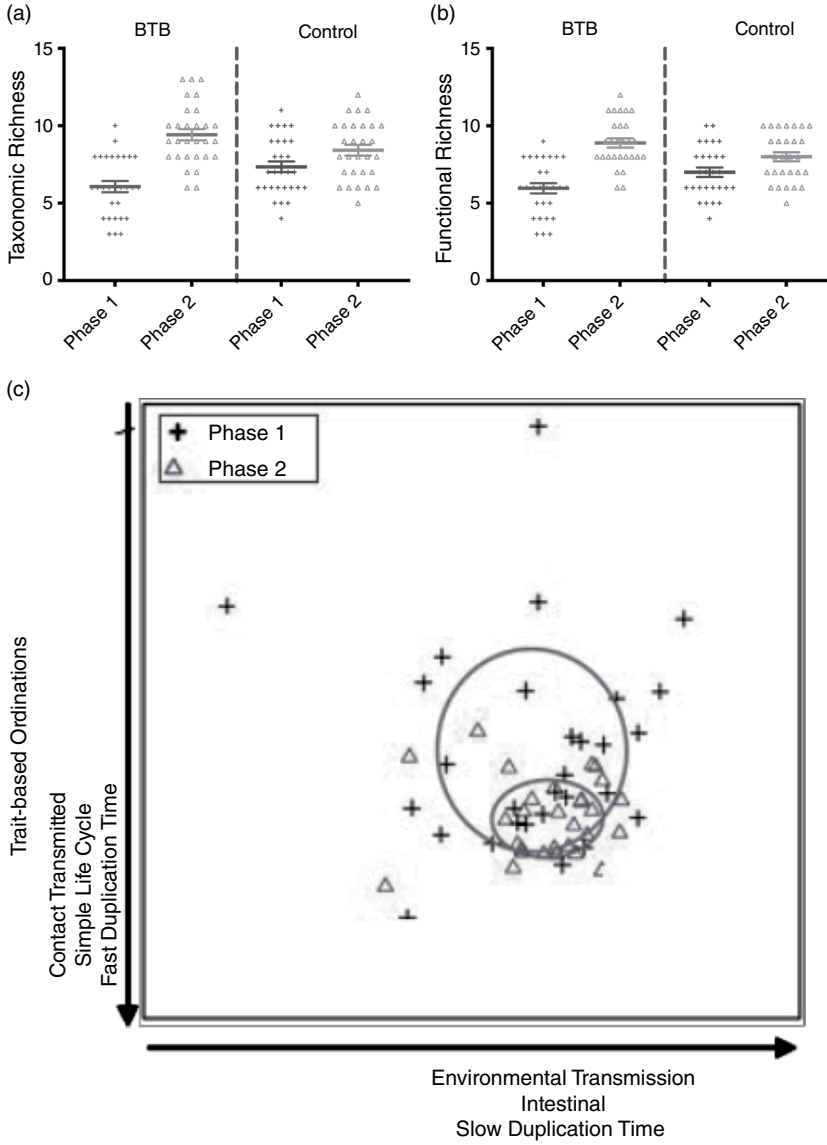

Figure 11.4 Bovine tuberculosis (bTB) infection altered the parasite communities in buffalo. By assessing changes in parasite communities both taxonomically (by species and genus) as well as functionally (e.g. using parasite traits such as speed and site of replication), Beechler et al. (2019) showed that animals that acquired bTB had higher parasite richness after bTB (phase 2) than before (phase 1) both taxonomically (panel a) and functionally (panel b). Furthermore, the magnitude of this increase was greater than that experienced in non-bTB infected control animals. Additional analysis suggested that becoming infected with bTB shifted the parasite community to be dominated by parasites with three key traits (panel c): direct contact transmission, fast replication time and simple life cycle (rather than complex with intermediate hosts). These results suggest that bTB altered the parasite community in buffalo in particular ways, lending the ability to predict how the invasion of bTB in other host populations may affect parasite communities.

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experienced a more than eightfold increase in mortality compared to uninfected buffalo (Gorsich et al., 2018). A mathematical model exploring how these changes in infection risk and mortality affected the R_0 and prevalence of both pathogens showed that the presence of brucellosis reduced both the R_0 of bTB and population-level prevalence, whereas the presence of bTB had no consistent effect on brucellosis (Gorsich et al., 2018). Thus, even though bTB infection renders individual buffalo more susceptible to brucellosis, the presence of brucellosis moderates the prevalence of bTB at the population level (Figure 11.2b), highlighting yet another conflicting pattern across scales. In this case, the conflict arises because buffalo infected with bTB are more likely to acquire brucellosis and die (a negative individual-level outcome); however, this decreases the timeframe over which they can spread bTB to others, thereby reducing bTB prevalence at the population level (a positive population-level outcome). This result means that coinfection can help moderate the population-level spread of pathogens in certain circumstances, even if the individual-level outcomes of coinfection are undesirable.

These two case studies reveal an intriguing contrast between individual- and population-level consequences of infectious diseases in multi-parasite systems. Both examples suggest that the presence of one parasite or pathogen (helminths, brucellosis) can moderate the spread of another (bTB). In both contexts, cross-scale contrasts arise because the relative effects of helminths and brucellosis on individual host susceptibility to bTB are negligible compared to effects on mortality during coinfection (Gorsich et al., 2018). If this imbalance between mortality and susceptibility/transmission effects is general, the implications for designing disease control and intervention strategies may be applicable to multiple host–pathogen systems. In wildlife, single-pathogen focused disease control and management programmes may inadvertently increase the prevalence of non-target infections or facilitate the invasion of novel pathogens. More generally, the presence of conflicting cross-scale outcomes raises intriguing new questions about parasite interactions. Among these questions, identifying the circumstances in which such conflicting outcomes are most likely, including the host and parasite attributes that contribute to this pattern, represent new frontiers in research on coinfection.

Tools for Studying Parasite Interactions

The growing understanding of the community ecology of buffalo parasites is in part attributable to the variety of empirical approaches (e.g. experiments, longitudinal tracking, case control designs) that have been

used to draw inference as well as the parallel development of new computational and laboratory tools. Here, we highlight these tools as they apply to the studies described in Table 11.2.

Pairwise Interactions: Causes and Consequences

Expanding our knowledge of host- versus parasite-level outcomes of coinfection is one of the largest contributions the buffalo study system has made to parasite community ecology. The ability to track large numbers of individual buffalo make them well suited for revealing these processes. Specifically, following animals through time allowed for parameterization of dynamical compartmental models as the number of susceptible, infected, coinfected and recovered (in some cases) individuals could be accurately assessed in real-time, and fecundity and/or survival rates could be parameterized using time-to-event analyses (Beechler et al., 2015; Ezenwa and Jolles, 2015; Gorsich et al., 2018). As dynamic compartmental models integrate multi-scale information to describe mechanistic processes, this methodological framework paints a clear and robust picture of multi-scale outcomes of parasite coinfection. Notably, in Ezenwa and Jolles (2015), the application of an anthelmintic drug typically used for cattle supported causal inference as well as guaranteed a relatively even sampling design of helminth infection status across animals.

A longitudinal study design and application of an anthelmintic drug also aided Budischak et al. (2016) in revealing patterns of parasite succession and in characterizing the outcome of a *gain* in infection on host health. Likewise, Beechler et al. (2017) benefited by uncovering seasonally dependent effects of helminth infection on schistosome *loss*. In both of these studies, mixed effects models were able to parse out the effect of explicit covariates and animal-level random variation. As such, not only do these methods account for repeated measures and non-independence among samples, but they also identify the presence of latent animal traits not included in the model.

Community-Wide Analyses: Characterizing Complexity and Emergent Patterns

Advancing statistics and diagnostic approaches have been crucial in understanding communities beyond pairwise interactions. The ubiquity of concomitant infections in African buffalo has made it an ideal system for application of these techniques. For example, Glidden et al. (2021) used

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diagnostic tools initially developed for cattle to identify five respiratory pathogens infecting buffalo. Conditional Markov-random field models were then used to estimate the relative effect of the associations among all pathogens versus animal traits related to animal exposure and susceptibility on odds of infection. Notably, these methods can also be used to estimate how association strength among pathogens varies with host traits. In the study described here, the authors found that association strength differed with herd membership and animal lactation status (Glidden et al., 2021). If examining interaction networks at multiple scales (individual, population, meta-population) joint-species distribution models can yield similar results (Fountain-Jones et al., 2019; Tikhonov et al., 2017).

Advanced time series analyses can also be used to detect causal associations (i.e. true interactions) between parasites in longitudinal observational data, without an experimental component, thereby making it possible to quantify multidimensional parasite communities. In Glidden (2020), empirical dynamical modelling, a technique that uses time series to detect information transfer among variables (Clark et al., 2015), was paired with high throughput sequencing of an 18S genus-specific marker specific to describe non-linear and time-varying interactions among 12 subtypes of *Theileria*. This empirical dynamical modelling revealed that parasite interaction complexity decreases as animals age, with adult animals' interaction networks containing only four facilitative interactions and no competitive interactions, whereas the *Theileria* community was connected via a dense web of both facilitative and competitive interactions in juvenile animals. General additive mixed models were then used to estimate the non-linear relationships among interaction strengths and host immune response, detecting a correlation between antibody concentration and mean interaction strength, suggesting that change in interaction networks may be related to shifts in immune dynamics. In this context, panel regression models are also a powerful tool as they use time series of multiple units (e.g. individuals) to detect causal relationships (Dudney et al., 2021). If considering linear interactions, autoregressive models (Solvang and Subbey, 2019) can similarly leverage time series data to identify causal interactions and characterize true interaction networks (Clark et al., 2015). Overall, increasingly accessible computational tools have expedited insight on the complex and interacting factors shaping high-dimensional pathogen community assemblages, with particularly novel tools allowing for causal inference from observational studies.

Trait-Based Analyses: Bridging Complexity to Prediction and General Rules

In recent years, the community ecology of free-living organisms has moved to expand characterization of biodiversity to include functional and trait-based descriptions. The sharing of pathogens of veterinary importance between livestock and buffalo has resulted in a fairly detailed knowledge of pathogen traits in African buffalo (e.g. infected tissue, transmission route), enabling classification of functional diversity. Unsupervised machine learning methods were used by Beechler et al. (2019) to cluster pathogen communities by functional traits pre- and post-bTB infection. Classification by traits has also eased interpretation of grouped analyses such as in Combrink et al. (2020), where time-to-event analyses were used to measure age of first infection across a range of parasite and pathogen taxa, and clear patterns emerged based upon pathogen transmission route and taxonomic group where animals were typically first infected by tick-borne protozoa and last infected with directly transmitted respiratory viruses and bacteria. Combrink et al.'s (2020) investigation was made possible by the ability to track African buffalo from birth, allowing observation of natural parasite succession. The same study system (Study 2, Box 11.1) was used to identify patterns of succession of *Theileria* subtypes, while the application of non-linear regression and high-throughput sequencing allowed for a fine-scale identification of groups of *Theileria* subtypes with unique life-histories (colonization, relative abundance in adults; Figure 11.3).

Advances Making Work More Feasible

Advances in genomic techniques have accelerated the ability to describe pathogen communities in African buffalo across a multitude of taxa from the genera to strain level (Glidden et al., 2020) and explore relationships between pathogens and microbiomes (Couch et al., 2021; Sabey et al., 2021). Pairing genomic tools with non-invasive sampling, such as 18S sequencing of faecal samples to exhaustively characterize gastrointestinal parasite communities (e.g. Gogarten et al., 2020), will continue to further our understanding of pathogen community assembly, the effect of coinfection on host fitness, and variation in pathogen communities across scales. Improvement in contact and GPS collars will support a better integration of pathogen exposure and host movement data into our understanding of pathogen community dynamics (Owen-Smith et al.,

2020). The development and reduction in cost of transcriptomics will further help to characterize complex immune responses to infection and coinfection (Sallé et al., 2020).

At the forefront of parasite community ecology, these genomic tools are starting to be used to uncover evolutionary drivers of observed coinfection patterns, helping to explain how trade-offs among evolved pathogen defences may drive responses to coinfection (Ezenwa et al., 2021). These tools have the capacity to answer long-standing ecological questions, in a range of wildlife host–parasite systems.

Conclusions and Future Directions

Our past studies on parasite communities in African buffalo have contributed novel insights into the mechanisms by which parasites interact within their hosts, and how these interactions scale up to affect individual hosts, population-level disease dynamics, and parasite community structure. We have employed experimental and longitudinal approaches to infer causal links between infection patterns by different parasites, and have viewed coinfections both through the lens of pairwise species interactions, and at a broad community-wide scale. Along the way, we have developed and refined methods for diagnosing a range of infections in African buffalo and quantifying buffalo immune responses, other aspects of host physiology, and fitness. We have also taken advantage of new methods for analysing multivariate longitudinal data sets including interacting networks of dozens of parasite taxa. This work has set the stage for African buffalo to serve as a tractable model system for the study of disease processes in natural populations. However, our studies have raised more questions than they have answered. With new technologies and tools becoming available for data collection and analysis, there is broad scope for future disease ecological investigations in African buffalo. In particular, this model system is poised to: (i) help advance our understanding of ecological and evolutionary disease dynamics in the context of environmental change, and (ii) provide an empirical basis for evaluating whole-system impacts of disease interventions.

Current environmental changes are presenting wild animals with novel physiological challenges and assemblages of infectious organisms. In this context, mechanistic disease models are essential to providing robust predictions of disease dynamics and impacts of disease control interventions. Statistical extrapolation relies on previously observed variation to predict future conditions; however, when environments

shift outside the boundaries of previously observed state space, non-linearities in host and pathogen functional responses may lead to novel infection patterns and outcomes (Kock et al., 2018). Improvements in animal tracking technologies and metabolic loggers can provide continuous, fine-scale data on animal movement, interactions, activity levels and metabolic rate. Coupled with high-resolution environmental data streams, and non-invasive sampling for infectious diseases, these technological advances set the stage for studies connecting environmental variation with host physiological and behavioural responses (Williams et al., 2021), contact patterns (Hamilton et al., 2020) and, ultimately, disease dynamics (Devan-Song, 2021). Building on this, assessing the metabolic and fitness costs of infections in natural populations becomes tractable, yielding insights on selection gradients imposed on hosts by parasites and pathogens. Complementary to this, quantitative molecular diagnostics provide detailed information on life-history variation among parasite strains and across different hosts. Faster, cheaper, deeper genetic sequencing techniques can elucidate host immunogenetic variation and parasite population genetics (Galen et al., 2020; Jax et al., 2021). Taken together, these data streams promise to provide an unprecedented empirical foundation for coevolutionary studies in model natural host–pathogen systems, such as African buffalo and their parasite community.

Our previous work has uncovered the ubiquity of interactions among coinfecting parasites, and their importance in shaping disease dynamics and host fitness. However, host–microbe interactions include the full spectrum of mutualistic to parasitic interactions. Elucidating the involvement of the microbiome in host health and disease in natural populations confronted with the full gamut of environmental variability and infectious challenges is an exciting frontier in disease ecology (Leung et al., 2018; Williams et al., 2018). Importantly, disease control interventions are likely to affect not only the specific target organisms, but also – directly or indirectly – the host's extended infracommunity of parasites and microbiota. Previous work on successional processes in parasite communities of African buffalo has used novel analytical techniques for disentangling host and microbial factors that shape microbial infracommunity dynamics (Budischak et al., 2016; Combrink et al., 2020; Glidden, 2020). However, the glimpse into parasite life-history variation and succession that we have provided is far from comprehensive. The intersection of quantitative molecular diagnostics (e.g. Glidden et al., 2020; Sisson et al., 2017) and analytical techniques for resolving community dynamics in complex, interacting species networks (e.g.

Sugihara et al., 2012) as applied in Glidden (2020) allows causal inferences to be drawn from observational parasite community data sets. This places a much broader understanding of parasite community responses to perturbations – such as disease control interventions, pathogen invasions or environmental change – within reach.

Overall, the foundational work on parasite interactions and community dynamics in African buffalo we describe in this chapter helps sets the stage for future studies in this model system addressing what is one of the central challenges in disease ecology: how to predict and mitigate infectious disease threats during a time of unprecedented, rapid environmental change.

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